Reviews of Environmental Contamination and Toxicology

VOLUME 188

Reviews of Environmental Contamination and Toxicology

Continuation of Residue Reviews

VOLUME 188

Springer

Coordinating Board of Editors

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Heidelberg: Postfach 10 52 80, 69042 Heidelberg, Germany

Library of Congress Catalog Card Number 62-18595
ISSN 0179-5953

Printed on acid-free paper.

Printed in the United States of America. (BS/SBA)

ISBN-10: 0-387-31911-5 e-ISBN: 0-387-32964-1
ISBN-13: 978-0387-31911-7

springer.com

Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations. These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on "toxic" chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer triumvirate:

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (Vol. 1 in 1966) for rapid publication of short reports of significant advances and

discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (Vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for *Reviews* and the *Archives* are in identical formats and are peer reviewed by scientists in the field for adequacy and value; manuscripts for the *Bulletin* are also reviewed, but are published by photo-offset from camera-ready copy to provide the latest results with minimum delay. The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the Board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

The role of *Reviews* is to publish detailed scientific review articles on all aspects of environmental contamination and associated toxicological consequences. Such articles facilitate the often-complex task of accessing and interpreting cogent scientific data within the confines of one or more closely related research fields.

In the nearly 50 years since *Reviews of Environmental Contamination and Toxicology* (formerly *Residue Reviews*) was first published, the number, scope and complexity of environmental pollution incidents have grown unabated. During this entire period, the emphasis has been on publishing articles that address the presence and toxicity of environmental contaminants. New research is published each year on a myriad of environmental pollution issues facing peoples worldwide. This fact, and the routine discovery and reporting of new environmental contamination cases, creates an increasingly important function for *Reviews*.

The staggering volume of scientific literature demands remedy by which data can be synthesized and made available to readers in an abridged form. *Reviews* addresses this need and provides detailed reviews worldwide to key scientists and science or policy administrators, whether employed by government, universities or the private sector.

There is a panoply of environmental issues and concerns on which many scientists have focused their research in past years. The scope of this list is quite broad, encompassing environmental events globally that affect marine and terrestrial ecosystems; biotic and abiotic environments; impacts on plants, humans and wildlife; and pollutants, both chemical and radioactive; as well as the ravages of environmental disease in virtually all environmental media (soil, water, air). New or enhanced safety and environmental concerns have emerged in the last decade to be added to incidents covered by the media, studied by scientists, and addressed by governmental and private institutions. Among these are events so striking that they are creating a paradigm shift. Two in particular are at the center of ever-increasing media as well as scientific attention: bioterrorism and global warming. Unfortunately, these very worrisome issues are now super-imposed on the already extensive list of ongoing environmental challenges.

The ultimate role of publishing scientific research is to enhance understanding of the environment in ways that allow the public to be better informed, The term "informed public" as used by Thomas Jefferson in the

age of enlightenment conveyed the thought of soundness and good judgment. In the modern sense, being "well informed" has the narrower meaning of having access to sufficient information. Because the public still gets most of its information on science and technology from TV news and reports, the role for scientists as interpreters and brokers of scientific information to the public will grow rather than diminish.

Environmentalism is the newest global political force, resulting in the emergence of multi-national consortia to control pollution and the evolution of the environmental ethic. Will the new politics of the 21st century involve a consortium of technologists and environmentalists, or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls, to avoid endangering the environment, public health, and wildlife. Ensuring safety-in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, for the old, established materials are continually being displaced by newly developed molecules more acceptable to federal and state regulatory agencies, public health officials, and environmentalists.

Reviews publishes synoptic articles designed to treat the presence, fate, and, if possible, the safety of xenobiotics in any segment of the environment. These reviews can either be general or specific, but properly lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, toxicology and regulation. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems may also be appropriate.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are likely in preparation or planned. The field is so very large and the interests in it are so varied that the Editor and the Editorial Board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of foreign chemicals in our surroundings. Thus, manuscripts may encompass case studies from any country. Food additives, including pesticides, or their metabolites that may persist into human food and animal feeds are within this scope. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their purview.

Manuscripts are often contributed by invitation. However, nominations for new topics or topics in areas that are rapidly advancing are welcome. Preliminary communication with the Editor is recommended before volunteered review manuscripts are submitted.

Tucson, Arizona G.W.W.

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Marine Halogenated Natural Products of Environmental Relevance

Walter Vetter

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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.

Communicated by George W. Ware.

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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.

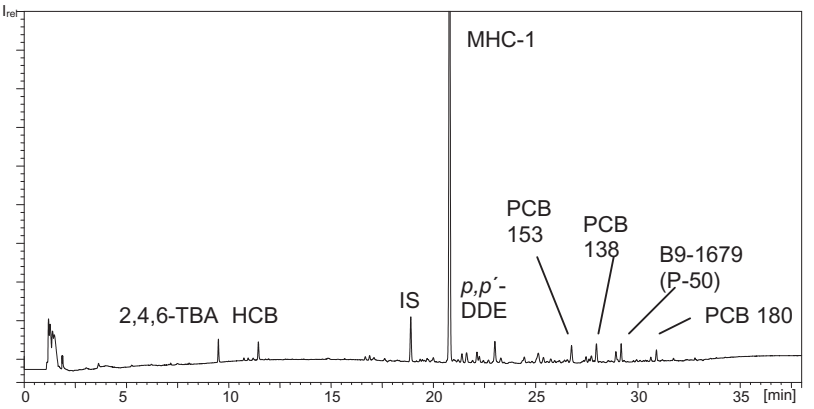

Fig. 1. GC/ECD chromatogram (HP-1) of the purified organohalogen extract of a Norwegian salmon (Adapted from Vetter et al. 2001b with permission from the American Chemical Society).

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.

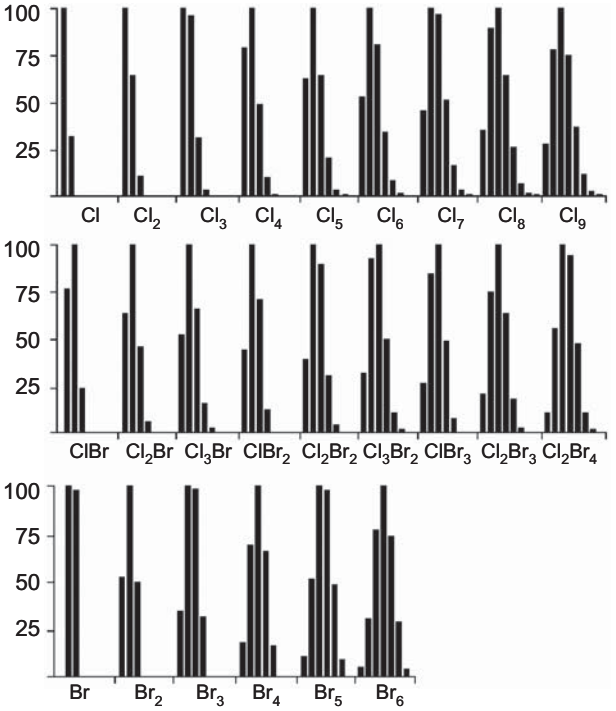

Fig. 2. Halogen isotope patterns.

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

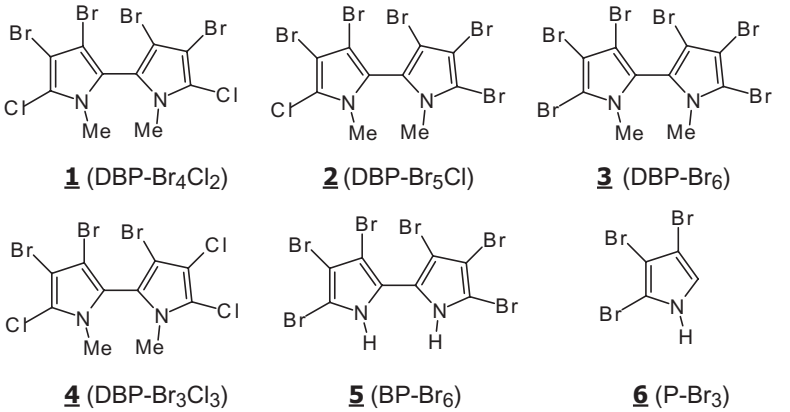

Fig. 3. Structures of halogenated dimethylbipyrroles and related compounds. **1**: 5,5′-dichloro-1,1′-dimethyl-3,3′,4,4′-tetrabromo-2,2′-bipyrrole; **2**: 5′-chloro-1,1′-dimethyl-3,3′,4,4′,5-pentabromo-2,2′-bipyrrole; **3**: 1,1′-dimethyl-3,3′,4,4′,5,5′-hexabromo-2,2′-bipyrrole; **4**: 1,1′-dimethyl-3,3′,4-tribromo-4′,5,5′-trichloro-2,2′-bipyrrole;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

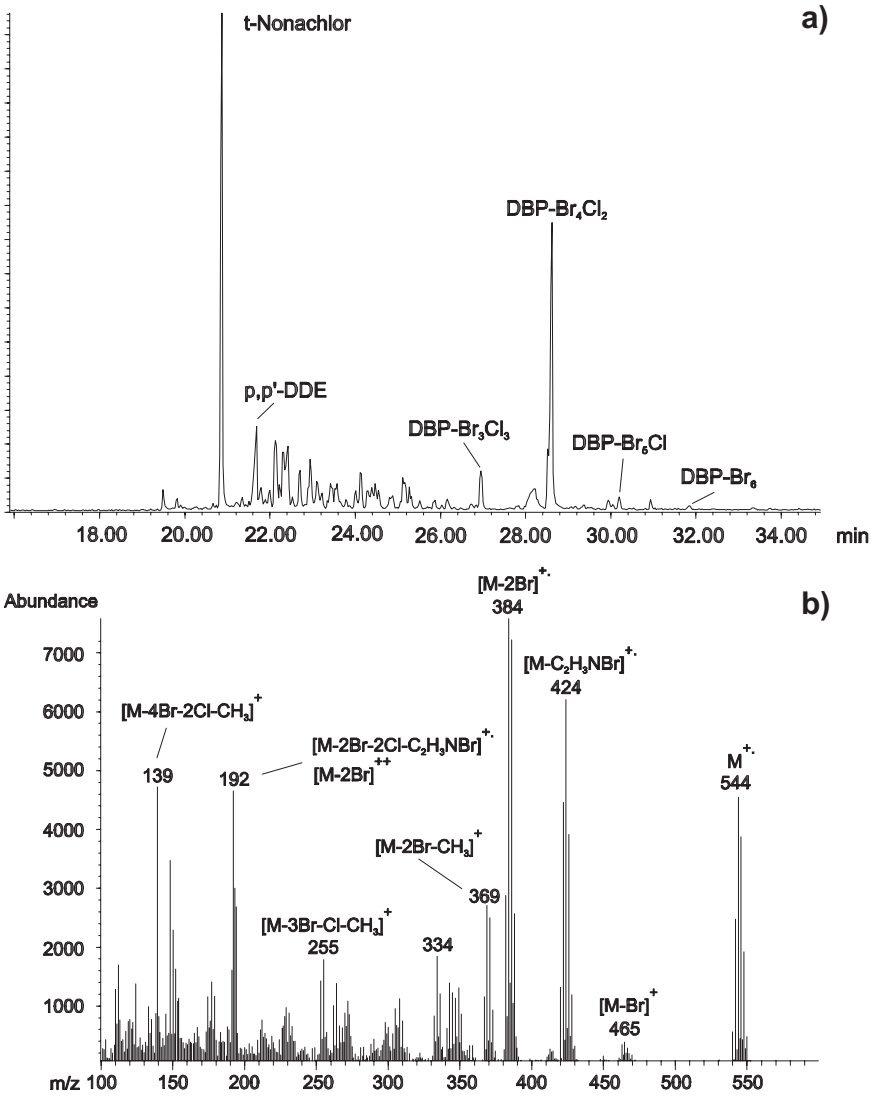

Fig. 4. GC/ECNI-MS total ion chromatogram of bald eagle liver extract (*top*) (a) and EI-MS of DBP-Br_4Cl_2 (*bottom*) (b) (Adapted from Tittlemier et al. 1999 with permission from the American Chemical Society).

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