Determination and Mass Spectrometric Investigation of a New Mixed Halogenated Persistent Component in Fish and Seal

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An unknown component that caused an intense signal in sample extracts of fish tissue was enriched and investigated using a variety of mass spectrometric techniques coupled to gas chromatographic separation. With the help of electron capture negative ion mass spectrometry (ECNI-MS) and electron impact mass spectrometry (EI-MS) it was established that the component carries 2Br and 3Cl atoms and forms a molecular ion at *m/z* 396. A concentrated solution of this mixed halogenated compound (MHC-1) was investigated by gas chromatography interfaced to electron impact high-resolution mass spectrometry (GC/EI-HRMS). Using full scan and SIM techniques, the molecular formula of MHC-1 was established to be $C_{10}H_{13}Br_2Cl_3$. This points toward MHC-1 having a monoterpene backbone. No chemical with this molecular formula has been synthesized, but two components with this composition have been earlier isolated from marine algae.

The concentrations of MHC-1, and other halogenated compounds, were estimated with GC/ECD in different fish samples. Using the ECD response of *trans*-chlordane as a reference, the concentrations of MHC-1 in pollack (*Polachus polachus*) and salmon (*Salmo salar*) reached up to 0.9 mg/kg lipid weight. MHC-1 was also detected in several species of commercial sea- and farmed freshwater fish. We also detected MHC-1 in the blubber of monk seals (*Monachus monachus*) with the help of GC/ECNI-MS. The concentrations in these samples were estimated to be 0.006−0.033 mg/kg. Higher MHC-1 levels (0.034−0.059 mg/kg) were determined in the blubber of two hooded seals (*Cystophory cristata*) from Northern Europe and two harp seals (*Phoca groenlandica*) from Greenland, while the concentrations in Antarctic Weddell seals (*Leptonychotes Weddelli*) were ∼0.001 mg/kg. For identification of MHC-1 in sample extracts, we suggest GC/ECNI-MS and monitoring *m/z* 79/81 and *m/z* 158/160 as bromine selective SIM masses, along with *m/z* 114/116, which corresponds to $[BrCl]^-$, and indicates the presence of both bromine and chlorine in a compound.

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Introduction

Compounds such as PCBs, DDT, and other chloropesticides are ubiquitous environmental contaminants. Residues of these organohalogen compounds belong to the most serious threats of marine environments. Several organohalogen compounds thus appear on lists with the most hazardous environmental chemicals (*1*). Repeatedly, diseases and biological malfunctions have been traced back to intoxication with organohalogen compounds. Nevertheless, the real impact of organohalogen compounds on various organisms can only be understood if all halogenated contaminants in the environment are known and investigated. Therefore, in addition to the well-investigated compounds, such as DDT and PCBs, new types of halogenated compounds have gained more attention in the scientific literature (*2*). Among them are brominated flame-retardants, whose concentrations are increasing in several regions of the world (*3*). Despite these efforts, a recent study of dolphins from Australia demonstrated that the four most abundant signals, by electron capture detection, originated from unknown substances (*4*). Other previously unknown organohalogen compounds, whose origins may be associated with natural sources (*5*−*7*), have also been detected in top predators of marine food webs, i.e., seabirds and marine mammals. In the present study we report a new compound (hereafter abbreviated as MHC-1) that was initially detected in selected sample extracts of fish. The goals of our study were to provide mass spectrometric data, to elucidate the molecular formula, and to determine initial environmental levels of MHC-1 in wild marine mammals and fish grown up for commercial use. Furthermore, we aimed to develop an analytical protocol that enables other researchers to identify MHC-1 with high selectivity in sample extracts.

Material and Methods

Chemicals. Purity of solvents used for fish analysis (*n*-hexane, acetone, cyclohexane, ethyl acetate, isooctane, toluene) was "zur Rückstandsanalyse" (Promochem, Wesel, Germany). PCB and pesticide reference standards were from Dr. Ehrenstorfer (Augsburg, Germany). Sodium sulfate and sea sand were heated for at least 5 h at 650 °C. Silica (Kieselgel 60, 70−230 mesh; Merck, Germany) was activated for 16 h at 130 °C. Origin and quality of solvents and chemicals used for analysis of marine mammals were published in detail elsewhere (*8*).

Sample Cleanup. Twenty grams of fish fillet (for species see Table 3, below) was ground with sodium sulfate (∼250 g) and sea sand (∼50 g) to obtain dry and homogeneous material. Column extraction of lipids and lipophilic substances was performed with 300 mL of *n*-hexane/acetone (2:1, v/v) according to Ernst et al. (*9*). Following this procedure, 0.5 g of the resulting fish oil and 50 ng of isodrin (internal standard) were dissolved in 5 mL of ethyl acetate/cyclohexane (1:1, v:v). Sample cleanup was performed according to the official German procedure, developed for the determination of chloropesticides and PCBs in fish (*10*). This method included gel-permeation chromatography (GPC) with bio-beads S-X3 and elution with ethyl acetate/cyclohexane (1:1, v:v). After GPC, the solvent was changed to isooctane and concentrated to 1 mL, and some of the samples were treated with 2 mL of concentrated sulfuric acid. The samples were further purified on 1 g of silica deactivated with 1.5% water (w:v). Elution with 8 mL of *n*-hexane provided a solution with PCBs and other, mainly aromatic organo-

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TABLE 1. Important Fragment Ions and Intensity in the EI-MS of MHC-1

fragment	*m/z*[a]	abundance[b]	halogen isotope pattern	mass eliminated from the molecular ion	suggested composition of mass ion
1	396	12.0 (398)	Br_2Cl_3	M	M
2	381	1.3 (383)	Br_2Cl_3	M − 15	M − CH_3
3	361	11.0 (363)	Br_2Cl_2	M − 35	M − Cl
4	347	2.2 (349)	Br_2Cl_2	M − 49	M − CH_2Cl
5	333	1.4 (333)	Br_2Cl_2	M − 63	M − C_2H_4 − Cl
6	325	8.8 (327)	Br_2Cl	M − 71	M − Cl − HCl
7	313	44.6 (315)	Br_2Cl	M − 83	M − $CHCl_2$
8	301	2.4 (303)	*c*	M − 95	*c*
9	281	25.5 (283)	$BrCl_2$	M − 115	M − Cl−HBr
10	265	3.7 (267)	Br_2Cl	M − 131	M − 2Cl − 61
11	253	4.9 (255)	Br_2	M − 143	M − Cl_3 − 38
12	245	56.8 (247)	BrCl	M − 151	M − Cl − HCl − HBr
13	233	20.7 (235)	BrCl	M − 163	M − CH_2 − Cl − HCl − HBr
14	229	*c*	*c*	*c*	*c*
15	217	9.2 (219)	Br_2Cl	M − 179	M − Cl_2 − 109
16	201	40.7 (201)	Cl_2	M − 195	M − Cl − 2HBr
17	165	53.9 (165)	Cl_2[c]	M − 231	*c*
18	91	100 (91)	Cl	M − 305	C_4H_8Cl[d]

[a] Value is first line in isotope pattern. [b] In percent relative to *m/z* 91 (=100) based on the major abundant ion in fragment pattern which is listed in parentheses. [c] Not unequivocally identified; halogen isotope pattern interfered. [d] And/or C_7H_7 (*21*).

TABLE 2. Calculation of Exact Masses[a] for Theoretically Possible Elemental Compositions of the Molecular Ion of MHC-1 Based on LRMS Results that Confirmed Presence of 2Br and 3Cl

	additional atoms next to 2Br and 3Cl	contribution of atoms mentioned in left column	sum of atoms other than Br and Cl	addition of 262.743258 (Br_2Cl_3)[b]	δ
#1	C_7HO_3	84 + 1.007825 + 47.984745	132.992570	395.736	
#2	$C_{11}H$	132 + 1.007825	133.007825	395.751	0.015
#3	C_8H_5S	96 + 5.039125 + 31.972074	133.011199	395.754	0.003
#4	C_8H_6P	96 + 6.046950 + 30.973763	133.020713	395.764	0.010
#5	$C_8H_5O_2$	96 + 5.039125 + 31.989830	133.028955	395.772	0.008
#6	C_9H_6F	108 + 6.046950 + 18.998405	133.045355	395.789	0.017
#7	C_9H_9O	108 + 9.070425 + 15.994915	133.065340	395.809	0.020
#8	$C_8H_9N_2$[c]	96 + 9.070425 + 28.006148	133.076573	395.820	0.011
#9	$C_7H_{11}F_2$	84 + 11.086075 + 37.996810	133.082885	395.826	0.006
#10	$C_6H_{13}O_3$	72 + 13.101725 + 47.984745	133.086470	395.830	0.004
#11	$C_{10}H_{13}$	120 + 13.101725	133.101725	395.845	0.015
#12	$C_7H_{17}S$	84 + 17.133025 + 31.972074	133.105099	395.848	0.003

[a] Calculations are based on the lowest mass isotope using the exact masses C (12.000000), H (1.007825), Br (78.918348), Cl (34.968854), F (18.998405), N (14.003074), O (15.994915), P (30.973763), and S (31.972074) [b] Rounded at three significant figures after the period. [c] Structural variants with odd number of N do not appear in the table because they are impossible as this would cause odd (rounded) molecular weights

halogen compounds (fraction A). Subsequent elution with 8 mL of *n*-hexane/toluene (65:35, v:v) yielded other, more polar organohalogen compounds (e.g. aliphatic/alicyclic chloropesticides, brominated compounds) including MHC-1 (fraction B). The fractions were concentrated to 2 mL and analyzed by GC/ECD. MHC-1 eluted quantitatively in fraction B. For GC/MS investigation, an enriched solution was obtained by repeated cleanup of several sample aliquots (30 times 0.5 g of salmon oil) and combination and concentration of the extracts.

Seal blubber samples were available from monk seals (*Monachus monachus*) that died during the mortality at the western Saharan coast of Africa (Mauretania) in 1997 (*11*). Approximately 1 g of tissue was weighed into a 50 mL microwave extraction tube, and an internal standard of perdeuterated α-HCH (α-PDHCH) and 10 mL of ethyl acetate/cyclohexane (1:1, v/v) was added. Microwave-assisted extraction, followed by gel-permeation chromatography and cleanup on 3 g of deactivated silica, was performed as earlier described in detail (*12*). Following this stage, PCBs, and other less-polar organohalogens, were separated from more polar compounds, such as CTTs, using 8 g of activated silica (*8*). Both fractions were concentrated and adjusted to 1.0 mL and then investigated by GC/ECD and GC/MS. MHC-1 eluted into fraction 2 together with toxaphene, chlordane, and other alicyclic chlorinated pesticides. Additionally, blubber extracts of hooded seals (*Cystophory cristata*) from Northern Europe, harp seals (*Phoca groenlandica*) from Greenland, and Weddell seals (*Leptonychotes Weddelli*) from Antarctica were analyzed in this study. These sample extracts were available from earlier studies and have been cleaned with another method. Details on this method as well on the origin of the samples were published by Hummert et al. (*13*).

Gas Chromatography/Electron Capture Detection and Gas Chromatography/Mass Spectrometry. Fish sample extracts were analyzed with a GC/ECD system consisting of a Hewlett-Packard 6890 gas chromatograph equipped with a split/splitless injector. A t-piece after the injector in the oven divided the gas flow onto two capillary columns which both ended in electron capture detectors. Two microliters were injected (splitless time 1 min) at 285 °C. Helium (5.0 quality) was used as carrier gas at a constant pressure of 1.5 bar. Argon/methane (90/10) was used as makeup gas at a flow of 40 mL/min. The ECD temperatures were set at 300

TABLE 3. Concentrations of MHC-1 in Fish [mg/kg Lipids] and Blubber of Seals [mg/kg Wet Weight]

species	origin	concentration [mg/kg]; (n = sample size)
	Marine Mammals	
Monk seal (*Monachus monachus*)	Mauretania (Northwestern Africa)	0.006−0.033 ($n = 14$)
Weddell seal (*Leptonychotes Weddelli*)	Weddell Sea (Antarctica)	0.001 ($n = 1$)
Hooded seal (*Cystophory cristata*)	Jan Mayen, North Sea (Europe)	0.058; 0.059 ($n = 2$)
Harp seal (*Phoca groenlandica*)	Greenland Sea, Westice region	0.034; 0.035 ($n = 2$)
	Sea Fish	
Pollack (*Pollachus pollachus*)	from Denmark	0.940 ($n = 1$)
Salmon (*Salmo salar*)	farmed in Norway	0.007−0.700 ($n = 8$)
Salmon (*Salmo salar*)	from Chile	0.001 ($n = 1$)
Salmon (*Salmo salar*)	origin Ireland, U.K. or unknown	0.001−0.029 ($n = 11$)
Mackerel (*Scomber scombrus*)	origin unknown	0.004−0.027 ($n = 16$)
	Freshwater Fish	
Trout (*Salmo trutta*) (1999, 2000)	farmed in Bavaria (Southern Germany)	0.003−0.017 ($n = 13$)
Saibling (*Salvelinus alpinus*), Carp (*Cyprinus carpio*) (2000, 2001)	farmed in Bavaria (Southern Germany)	0.001−0.006 ($n = 3$)

°C. The GC capillary columns were both 30 m long and of 0.25 mm internal diameter (Hewlett-Packard). One column was coated with 0.25 μm of 95% dimethyl, 5% methylphenyl polysiloxane (HP-5), and the other with 0.25 μm of dimethyl polysiloxane (HP-1). The GC oven was programmed as follows. After 2 min at 90 °C, the temperature was raised at 30 °C/min to 150 °C, then at 3 °C/min to 204 °C (hold time 3 min), and finally at 8 °C/min to 280 °C (hold time 10 min). The run time was 44.5 min. Marine mammal samples were analyzed using a GC/ECD system equipped with two capillary columns (CP-Sil 2 and CP-Sil 8/C18, Chrompack, The Netherlands) and two ECDs in parallel (*12*). Quantification of MHC-1 in fish and seal blubber was carried out relative to the peak height of the external standard *trans*-chlordane, as in the method suggested for Q1 (*8*). Limit of detection (LoD) in samples was depending on the sample weight and finale volume as well as origin of the sample (number and abundance of peaks in neighborhood of MHC-1). Typical LoDs of MHC-1 were 0.1−1 μg/kg lipids.

GC/EI-MS analysis were performed with a Hewlett-Packard 6890 gas chromatograph coupled to a 5973 mass selective detector. A 30 m × 0.25 mm × 0.25 μm HP5-MS column was installed in the GC oven. Helium was used as the carrier gas at a column head pressure of 1.1 bar. The split/splitless injector (splitless time 2 min) and the interface transfer line temperature were set at 250 °C and 280 °C, respectively. The GC oven was programmed in the following way: 70 °C for 2 min, then at 25 °C/min to 150 °C, at 3 °C/min to 200 °C, and finally at 8 °C to 280 °C, which was held for 10 min. Two microliters were injected.

GC/ECNI-MS investigation was performed with a Hewlett-Packard 5890 series II gas chromatograph interfaced to a 5989B mass spectrometer. A capillary column coated with 25% *tert*-butyldimethylsilylated β-cyclodextrin diluted in PS086 (β-BSCD) was installed in the GC oven (*7*). In the full scan mode m/z 30 to m/z 550 or 650 were recorded at a scan time of 0.8 or 0.7 scans/s. The GC oven temperature started at 80 °C (hold time 4 min) and then was raised at 20 °C/min to 160 °C (hold time 15 min), 180 °C (hold time 25 min), and 215 °C (hold time 15 min).

Electron impact high-resolution mass spectrometry was performed on a Micromass MasSpec (Micromass, Manchester, U.K.) double-focusing magnetic sector mass spectrometer (geometry EBE) connected to a Hewlett-Packard 6890 gas chromatograph, equipped with a DB-5 (J&W Scientific) nonpolar capillary column (30 m × 0.25 mm × 0.25 μm). The GC injection port and transfer line were operated at 280 °C. The oven temperature was programmed from 70 °C to 280 °C at 10 °C/min, with an initial isothermal time of 2 min and a final isothermal time of 5 min. Helium was used as a carrier gas at 1 mL/min, and the samples (2 μL) were injected splitless. Mass spectra were measured in electron impact (EI) mode at 70 eV, with a source temperature of 200 °C, and an acceleration voltage of 8 kV. For full scan measurements, the instrument was scanned between m/z 100 and m/z 450 at 1 scan/s and at a resolution of 3500. For selective ion monitoring (SIM) measurements, the resolution was increased to 10 000. Perfluorokerosene (PFK, Aldrich, Deisenhofen, Germany) was used as a calibration gas. For SIM experiments, PFK ion 392.9761 was used as the lock mass.

Results and Discussion

Mass Spectrometric Characterization of MHC-1. Figure 1 shows the GC/ECD chromatogram of a Norwegian salmon extract (recombined fractions A and B), which is dominated by the peak of MHC-1. Hexachlorobenzene, tribromoanisole, congeners PCB 138, PCB 153, and PCB 180, p,p′-DDE, and the toxaphene component B9-1679 (P-50) were detected in the range of 0.01−0.09 mg/kg lipids, whereas the signal of MHC-1 in the ECD was an order of magnitude higher (Figure 1). The retention time of MHC-1 relative to B9-1679 ($rrt_{B9-1679}$) was 0.7125 (DB-1) and 0.7463 (DB-5). MHC-1 eluted between *trans*- and *cis*-chlordane from both columns. Aside from MHC-1, the pattern was typical of salmon samples from Northern Europe, both in terms of organohalogen concentrations and component pattern.

The GC/ECNI-MS full scan spectrum provided first information on the identity of MHC-1 (Figure 2). The most abundant fragment ions were detected at low m/z values. The ions at m/z 79 and 81 unequivocally prove the presence of bromine in MHC-1. Furthermore, the intense cluster starting at m/z 158 indicates at least two bromines. Between the $[Br]^-$ and $[Br_2]^-$ there is a fragment ion cluster starting at m/z 114 that exhibits the typical isotope ratio of $[BrCl]^-$. Detection of m/z 35 and 37 in the typical ratio of ∼3:1 confirmed the presence of chlorine in MHC-1 (mixed halogenated compound 1). At high mass, two intense fragment ions were detected at m/z 317 and 361, respectively. The ion cluster at m/z 317 has a $BrCl_3$ pattern, and, therefore, the molecular ion of MHC-1 must be higher than m/z 361, because the pattern of m/z 361 indicates the presence of 2Br and only 2Cl. Addition of 79 amu to m/z 317 and 35 amu to m/z 361 results in m/z 396. The ion at m/z 396 was of very low abundance in the ECNI-MS spectrum, but enlargement of the relevant mass range enabled detection of a clear Br_2-Cl_3 pattern starting at m/z 396 (Figure 2, upper right). Injection of more concentrated solutions resulted in the detection of $[M + Br]^-$ and $[M + Cl]^-$ adducts (data not shown). These

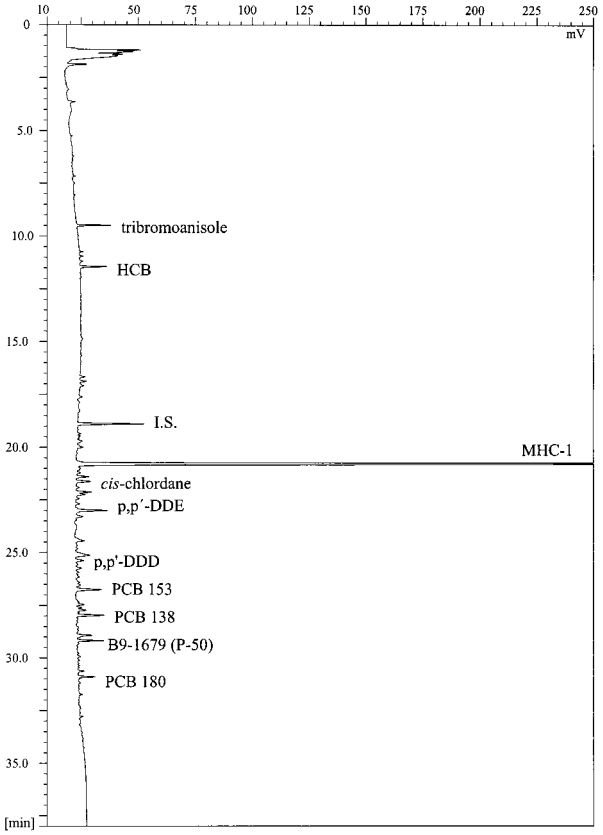


FIGURE 1. GC/ECD chromatogram (HP-1) of a Norwegian salmon extract (combined fractions A and B).

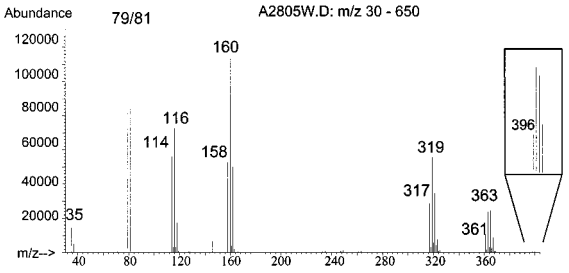


FIGURE 2. GC/ECNI-MS full-scan mass spectrum of MHC-1. Frame in right corner shows an enlargement of the molecular ion at *m/z* 396.

investigations clearly indicate that the molecular ion of MHC-1 as defined in MS is *m*/*z* 396.

Confirmation of the ECNI-MS data was obtained with GC/EI-MS (Figure 3). A solution produced by repeated cleaning and concentration of sample aliquots (see above) was used for these investigations. Typical fragment ions of brominated compounds in ECNI-MS (*m*/*z* 79/81; *m*/*z* 158/160/162) were not clearly detected in the EI-MS spectrum. However, like ECNI-MS, the EI-MS spectrum showed the highest mass ion at *m*/*z* 396. There is also an ion at *m*/*z* 361, corresponding to $[M - Cl]^+$ fragment ion, while a $[M - Br]^+$ fragment ion (assumed at *m*/*z* 317) was not detected. At *m*/*z* 313 (M − 83 amu), a fragment ion was identified that showed a halogen isotope pattern corresponding to Br_2Cl (Table 1). Loss of 83 amu from the molecular ion can be explained with the elimination of a $\cdot CHCl_2$ fragment. In addition, the Br_2Cl isotope cluster at *m*/*z* 325 is formed by elimination of Cl and HCl from the molecular ion. This means that chlorine is more easily lost from the molecular ion than bromine to form stable fragment ions at high mass. Both *m*/*z* 245 and *m*/*z* 233 which are separated by 12 amu exhibit a BrCl isotope cluster.

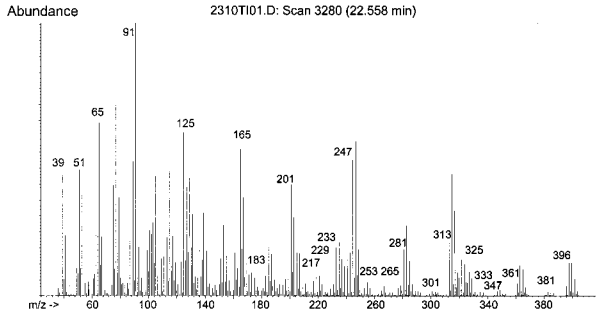


FIGURE 3. GC/EI-MS full-scan mass spectrum of MHC-1. The first ions in the respective isotope patterns are labeled.

Fragment ion *m*/*z* 245 is formed without C−C-cleavage, while the fragment ion at *m*/*z* 233 has one carbon less. Several other fragment ions were detected as well (Table 1, Figure 3) but could not be verified. Intense fragment ions at *m*/*z* 39 (41), 51 (53), 65, 77, and 89 (91) are without halogens and may correspond to $C_3H_3^+$ ($C_3H_5^+$), $C_4H_3^+$ ($C_4H_5^+$), $C_5H_5^+$, $C_6H_5^+$, and $C_7H_5^+$ ($C_7H_7^+$). The fragment ion at *m*/*z* 91 may also be interpreted as $[(CH_2)_4Cl]^+$, which is typical for aliphatic and alicyclic chlorinated compounds (*14*). By contrast, the corresponding Br-analogue at *m*/*z* 135 was not clearly identified. The lack of a doubly charged molecular ion and high-mass fragment ions in the EI-MS suggest that MHC-1 is not aromatic.

To establish the molecular formula of MHC-1 we performed selected experiments with high-resolution mass spectrometry. The data obtained from the low-resolution MS investigation confirm a presence of 3 Cl (105 amu) and 2 Br (158 amu) on MHC-1. Subtraction of these contributions to the molecular weight of 396 amu leaves 133 amu for carbon and hydrogen as well as heteroatoms other than Br and Cl. To leave all possibilities open, we allowed heteroatoms F, N, O, P, and S, in addition to C and H for EI-HRMS investigations. Table 2 lists 12 elemental compositions by increasing exact mass. Inspection of the list shows that, with minor exceptions, the exact masses increase with increasing numbers of hydrogen atoms. A scan from *m*/*z* 100 to *m*/*z* 450, at resolution 3500 gave a molecular ion at *m*/*z* 395.843—closest to #11 in Table 2. To confirm this measurement, elemental compositions whose exact masses were within 20 mamu of this value were monitored in the SIM mode at resolution 10 000. The measured ion intensities (expressed in %) for elemental compositions #9, #10, #11, and #12 were 18:35:100:95. This experiment confirmed highest abundance at 395.845 (#11), with the responses measured for the neighboring variants in good agreement with those expected at $R = 10\,000$. Note that #9, #10, and #12 are not plausible because the high number of heteroatoms and hydrogens are not consistent with six or seven carbons. Therefore, the molecular formula of MHC-1 must be $C_{10}H_{13}Br_2Cl_3$.

In a final experiment, each ion in the halogen isotope cluster was recorded and compared with the experimental finding (Figure 4). The maximum deviation of the four most abundant isotope peaks was 3 mamu. The lower abundant isotope peak showed a somewhat larger deviation. Nonetheless, this experiment further demonstrates that the molecular mass of MHC-1 is $C_{10}H_{13}Br_2Cl_3$. The general formula C_nX_{18} (X = H, Br, Cl) deviates from a saturated hydrocarbon (C_nX_{2n+2}) by four substituents which corresponds to two double bond equivalents. It is interesting to note that such a formula could be fulfilled by halogenated monoterpenes.

Chemical Behavior, Levels, and Hypotheses on the Origin of MHC-1. The chemical behavior of MHC-1, during sample cleanup procedures, was typical of halogenated compounds. MHC-1 was collected in the organochlorine fraction during GPC separation of lipids (see above). It is a

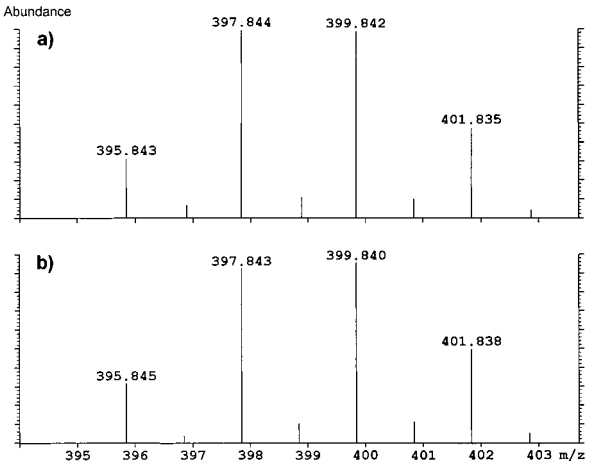
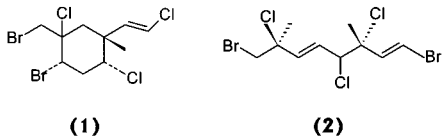


FIGURE 4. GC/EI-HRMS study of MHC-1. Exact measured (A) and calculated exact masses (B) of the isotope cluster of the molecular ion.

nonpolar component, as it is not retained on deactivated silica, and MHC-1 is stable against sulfuric acid. Separation of PCBs and toxaphene resulted in the presence of MHC-1 in the more polar fraction, which is typical of other brominated compounds. This was, however, also expected due to the high degree of saturation of the possible monoterpene backbone.

Unambiguous information on the origin of MHC-1 was not available. Mixed brominated and chlorinated compounds have scarcely been described in higher organisms. Only a few compounds have been synthesized, but they have different molecular masses and/or another ratio of Cl:Br. A known man-made source for mixed halogenated compounds may be waste combustion (*15*), but the high degree of saturation in MHC-1 makes this source less plausible. The lack of oxygen in MHC-1 also argues against a combustion product. These interpretations would seem to rule out anthropogenic sources for MHC-1. Kuehl et al. 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. Although these authors were not able to elucidate the origin of the detected component, the molecular mass and GC/MS fragmentation pattern confirm that this component and MHC-1 are not identical. Further mixed brominated/chlorinated compounds were recently described by Tittlemier et al. (*5*) and interpreted as natural organohalogen compounds having a 1,1′-dimethyl-2,2′-bipyrrole backbone with Br = 3−6 and Cl = 3−0 and Br + Cl = 6. The molecular weights of the halogenated dimethyl bipyrroles were lower than those of the compound described by Kuehl et al. (*16*). In addition, a heptachloro compound (Q1) and several brominated compounds were tentatively identified as natural and bioaccumulative compounds (*4*, *7*, *17*). Further information on natural organohalogens was available from a recent review of Gribble (*18*). More than 100 marine halogenated monoterpenes have been isolated from marine sources since the initial work of Faulkner et al. in 1973 (*19*). Marine algae are known synthesizers of mixed halogenated compounds and thus the most plausible source for MHC-1. Several monoterpenes such as telfairine (a halogenated dimethyl-vinyl-cyclohexane) have been isolated from red algae (*18*). Our molecular formula-specific search of the literature provided two mixed halogenated monoterpenes that may be identical or isomeric to MHC-1 (Figure 5). Higgs et al. isolated **1** from the red algae *Plocamium cartilagineum* collected from the British coast (*20*). **1** has the same hydrocarbon backbone as telfairine but one bromine less and a different distribution of halogens on the carbon backbone. Stierle and Sims identified **1** in the same algae



(1) (2)

FIGURE 5. Structure of two monoterpenes corresponding with the molecular formula of MHC-1: (1R,2S,4S,5S)-4-bromo-5-bromomethyl-1-(E)-chlorovinyl-2,5-dichloromethylcyclohexane (1) and 1,8-dibromo-3,4,7-trichloro-3,7-dimethyl-1,5-octadiene (2).

species collected in the Antarctic (*21*). The Antarctic algae also contained **2** (*21*). The aliphatic monoterpene **2** and stereoisomers thereof were also detected in other algae from different sites in the world (*22*−*24*).

The initial detection of MHC-1 was carried out with GC/ECD, which gives response to all halogenated compounds (see above). GC/MS investigations in the SIM mode are usually carried out with *m*/*z* values selective for the target compounds. Brominated compounds are usually identified in the ECNI mode by monitoring *m*/*z* 79/81. Additional monitoring of *m*/*z* 158/160 may help to distinguish between different types of brominated compounds since some form $[Br_2]^-$ fragment ions and others do not. Due to our full scan data (Figure 2) we recommend monitoring of *m*/*z* 114/116 for screening samples on mixed-halogenated compounds by GC/ECNI-MS in the SIM mode. The six *m*/*z* values mentioned in this paragraph were used for screening samples on MHC-1 in the SIM mode. The abundance of *m*/*z* 116 and *m*/*z* 160 relative to *m*/*z* 79 (defined as 1.0) was 0.5 and 0.95, respectively.

This SIM technique enabled unequivocal determination of MHC-1 in environmental and food samples (Table 3). MHC-1 was present in blubber of monk seals (*Monachus monachus*) from Mauretania (Africa). Fourteen individuals, which died during a mass mortality in 1997—most likely caused by a mobillivirus infection (*11*), were investigated. A sample which accumulated 0.011 mg/kg MHC-1 (based on the ECD response of *trans*-chlordane) showed p,p′-DDE, Q1, and PCB 153 levels of 0.887, 0.046, and 0.774 mg/kg, respectively. Higher MHC-1 concentrations were determined in the blubber of hooded seals (*Cystophory cristata*) from Northern Europe and harp seals (*Phoca groenlandica*) from Greenland, while the concentrations in an Antarctic Weddell seal (*Leptonychotes Weddelli*) was ∼0.001 mg/kg (Table 3). Presence of MHC-1 in seals from Africa, Europe, and the Antarctic agrees with the prediction that potential producers of MHC-1 are found at different places in the world (see above).

Additionally, results were available from a random selection of commercial fish analyzed during food-control inspection in Bavaria (Southern Germany). The origin of these fish was partly unknown. The highest concentration of MHC-1 was determined in a fillet sample of pollack (*Pollachus pollachus*) labeled "from Denmark" (Table 3); MHC-1 was more than 1 order of magnitude higher concentrated than *cis*-chlordane (0.025 mg/kg), p,p′-DDE (0.055 mg/kg), and PCB 153 (0.019 mg/kg). MHC-1 was also detected in 20 extracts of farmed salmon. A wide range in concentration was determined with the highest levels in two samples from Norway, but all other samples also contained MHC-1, although in lower quantities (Table 3). Concentration of MHC-1 in salmon fillet was at least 20% of PCB 153. MHC-1 was also detected in fillet of mackerel (Table 3).

Analysis of freshwater fish from southern Bavaria revealed the presence of MHC-1, too. It is remarkable that MHC-1 was detected in almost all samples of farmed freshwater fish but not in any free-living freshwater fish. This suggests that the source of MHC-1 in these samples is commercial fodder (e.g. fishmeal) containing sea fish. A recent examination of fishfood containing fishmeal and fish oil (Ecoline, BioMar

A/S, Denmark) confirmed this. The MHC-1 concentration in the commercial fishfood sample was about 50% of PCB 153.

No correlation between concentrations of MHC-1 and other organohalogen components was found. At this stage of the research we suggest that MHC-1 is likely to be a natural organohalogen compound that may be bioaccumulative for fish and their consumers. More research on this topic is required to fully understand this complex theme. Nevertheless, more and more natural halogenated components are found in higher organisms, something that was ruled out some 20 years ago (*25*). Our analytical methods described in this work may help to fill up gaps in the understanding of persistent organohalogen compounds in organisms that accumulate these components in a similar way as humans.

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