Environ. Sci. Technol. **2005**, *39,* 9446−9451

Identification of Brominated Carbazoles in Sediment Cores from Lake Michigan

LINGYAN ZHU AND RONALD A. HITES*

School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana 47405

During the analysis of brominated flame retardants in sediment cores taken from Lake Michigan, a group of unknown brominated compounds were observed. To identify their chemical structures, a variety of gas chromatographic mass spectrometric techniques were used. With the help of high-resolution and positive chemical ionization mass spectrometry, the molecular formula of the most abundant congener was established to be $C_{12}H_5Br_4N$. On the basis of the electron impact and electron capture negative ionization mass spectra, this chemical was suspected of being a tetrabromocarbazole. The structure was confirmed by comparing the mass spectra and gas chromatographic retention times of an authentic standard of 1,3,6,8-tetrabromocarbazole with those of the most abundant unknown. The other unknown compounds may be other bromocarbazole congeners. The time trend determined by measuring these compounds as a function of depth in the Lake Michigan sediment cores indicated that the concentration of 1,3,6,8-tetrabromocarbazole peaked around 1920−1935. This is the first time that bromocarbazoles have been found in the environment.

Introduction

Anthropogenic brominated organic compounds accounted for ~80% of bromine's global production in 2002 (*1*). These compounds include flame retardants, biocides, gasoline additives, halons, bromobutyl rubber, pharmaceuticals, and dyes. Among these, the manufacturing of brominated flame retardants used 38% of the global production of bromine. Brominated flame retardants, such as polybrominated biphenyls, polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A, and hexabromocyclododecanes, have been identified in a variety of environmental compartments (*2*, *3*). Recently, other brominated flame retardants have been found in the environment. For example, Hoh and co-workers (*4*) discovered 1,2-bis(2,4,6-tribromophenoxy)ethane and 2,3,4,5,6-pentabromoethylbenzene in U. S. atmospheric and sediment samples, and Kierkegaard and co-workers discovered decabromodiphenylethane in Swedish sediment, sewage sludge, and air samples (*5*−*7*).

Not all brominated compounds in the environment have anthropogenic sources; in fact, on the order of 2000 naturally produced brominated compounds have been reported (*8*, *9*). Generally, these compounds are produced by marine animals (sponges, corals, sea slugs, tunicates, and sea fans) and by seaweed, plants, fungi, lichen, algae, bacteria, and microbes. For example, methoxylated polybrominated diphenyl ethers were found to be naturally produced in certain whales and in other aquatic animals (*10*). Many of these brominated compounds are used as chemical defense agents or as hormones (*11*). Recently, a family of hexahalogenated heterocyclic compounds, based on a dimethyl bipyrrole skeleton and containing both chlorines and bromines, was discovered in fish, mammals, and seabirds in the Pacific and Atlantic Oceans (*12*−*14*). Some evidence indicates that these compounds are of biogenic origin; for example, one of the congeners, hexabromo-2,2′-bipyrrole, is a known natural product. These compounds are persistent and are able to bio-accumulate in biota (*14*, *15*). Radiocarbon evidence suggests that at least one of these compounds, DBP−Br_4Cl_2, has a natural source (*16*).

In 2004, we took two sediment cores from Lake Michigan to study the historical distribution of brominated flame retardants (BFRs) in the Great Lakes. To make these measurements, we used electron capture negative ionization (ECNI) gas chromatographic mass spectrometry and selected ion monitoring of the bromide ion at m/z 79 and 81. Under these conditions, we detected relatively high levels of several unknown compounds containing bromine. The retention times of these unknown peaks did not match any of the most common polybrominated diphenyl ethers (PBDEs). We report here the identities of these previously unknown compounds and give some information on their abundances in the sediment of Lake Michigan.

Experiment Section

Sampling Sites. Two sediment cores were taken from northern and southern Lake Michigan at the end of April 2004. At the northern site (MI-47), the water depth was 197 m, and at the southern site (MI-18), the water depth was 161 m. In both cases, a 30 cm × 30 cm × 52 cm box corer was deployed from the U. S. EPA's research vessel, the *Lake Guardian*. Once the box core was back on the deck, three subcores were taken by carefully inserting subcore tubes (10 cm i.d. × 60 cm) into the sediment box in such a way as to avoid distortion of the sediment. These cores were 51 cm long. The cores were cut into 0.5-cm intervals down to 10 cm and in 1-cm intervals below. The samples were immediately frozen, stored at −30 °C, and returned to our laboratory.

Sediment Dating. The core from site MI-47 was dated by measuring the specific activities of ^{210}Pb as a function of depth in one of the three subcores. These samples were air-dried for 2 weeks, ground in a mortar, and sieved through a 2-mm mesh screen. Each sample was loaded into a Petri dish (50 mm o.d. × 9 mm), and the ^{210}Pb activities were measured by gamma spectrometry at 46.54 keV, using a high-purity germanium detector (Canberra GL 2820R, Canberra Industries, Meriden, CT). The core from MI-18 was not dated in this study, but the sedimentation rate is available in the literature (*17*).

Materials. Most PBDE congeners were purchased in nonane solution from Cambridge Isotope Laboratories (Cambridge, MA). The standard compound, 2,3′,4,4′,5-PeBDE (BDE-118), was donated to us by Cambridge Isotope Laboratories. This compound is not present in the ambient environment. Decabromobiphenyl (BB-209) was purchased from Dr. Ehrenstorfer, GmbH, Augsburg, Germany. The 1,3,6,8-tetrabromocarbazole standard was purchased from the Florida Center for Heterocyclic Compounds, Department of Chemistry, University of Florida. The NMR spectrum we obtained of the authentic compound matched well with literature data (*18*). The measured ^1H spectrum (in $CDCl_3$,

* Corresponding author e-mail: HitesR@Indiana.edu.

10.1021/es0515248 CCC: $30.25 © 2005 American Chemical Society
Published on Web 11/09/2005

400 MHz): δ 7.26 (1H, s, N−H), 7.75 (2H, d, H4 and H5), 8.05 (2H, d, H2 and H7). The measured ^{13}C spectrum (in $CDCl_3$, 400 MHz): δ 105.114 (s), 113.160 (s), 122.868 (s), 124.895 (s), 131.651 (s), and 136.987 (s). All the solvents used for the extraction and cleanup procedures were residue-analysis grade.

Sample Preparation. Approximately 15 g of wet sediment was well-mixed with 100 g of pre-cleaned, anhydrous Na_2SO_4 (to remove water) and with 20 g of pre-cleaned granular copper (20−30 mesh, J. T. Baker, NJ), which was used to remove elemental sulfur (*19*). After spiking with a known amount of the standards $^{13}C_{12}$-2,3,3′,4,4′,5-hexachlorodiphenyl ether (CDE-156) and $^{13}C_{12}$-2,2′,3,3′,4,4′,5,5′-octachlorodiphenyl ether (CDE-194) (Cambridge Isotope Laboratories), the samples were Soxhlet extracted for 24 h with 1:1 acetone/hexane. Throughout the extraction and analysis procedure, the analytes were protected from light by wrapping the containers with aluminum foil or by using amber glassware.

After evaporating most of the solvent, ~4 mL of concentrated H_2SO_4 (EM Science, Gibbstown, NJ) was added to remove fat and organic polymers from the extracts. After centrifugation for 10 min, the hexane layer was recovered; 5 mL of fresh hexane was used to wash the sulfuric acid residue; and the two hexane extracts were combined. After reducing the extract volume to ~1 mL under a clean N_2 flow, the samples were loaded onto a 3% water deactivated silica (Grace Davison, Columbia, MD) column (1.9 cm i.d. × 20 cm). One fraction of 150 mL dichloromethane was collected. The solvent was blown down with clean N_2 to 500 μL, and the samples were fractioned on an alumina (ICN Biomedicals GmbH, Eschwege, Germany) column (0.6 cm i.d. × 6 cm). The column was first eluted with 8 mL of hexane followed by 8 mL of 2:3 dichloromethane/hexane. The PBDEs and the unknown peaks eluted in the second fraction. After solvent exchange to hexane, BDE-118 and BB-209 were added as standard compounds, and the samples were analyzed by GC/MS.

Sample Cleanup. To obtain full scan electron impact (EI) mass spectra of the unknown compounds, the extracts of five samples were combined, and the solution was fractioned on a 3% water deactivated silica column (0.6 cm i.d. × 6 cm). The first fraction was 8 mL of hexane, the second fraction was 8 mL of 1:4 dichloromethane/hexane, and the third fraction was 8 mL of 2:3 dichloromethane/hexane. Most of the unknown peaks were in the second fraction, which was used for the mass spectrometry.

Low-Resolution GC/MS. Low-resolution, electron capture negative ionization (ECNI), positive chemical ionization (PCI), and electron impact (EI) mass spectra were obtained on an Agilent 6890 series gas chromatograph coupled to an Agilent 5973 mass spectrometer with helium as the carrier gas. For ECNI and PCI spectra, methane was used as the reagent gas. For ECNI spectra, the ion source temperature was 150 °C; for PCI spectra, the ion source temperature was 250 °C. EI was operated at 70 ev. The 2-μL injections were made in the pulse splitless mode, with a purge time of 2.0 min. The GC injection port was held at 285 °C.

High-Resolution GC/MS. The molecular formulas of the unknown compounds were determined using a Trace gas chromatograph (Thermo Electron Corp.) coupled to a MAT 95XP Trap high-resolution mass spectrometer (Thermo Electron Corp., Waltham, MA). The mass spectrometer was operated in full scan, electron impact ionization mode (electron energy set at 70 ev) with resolution 18 000. Internal mass scale calibration was based on high-boiling-point perfluorokerosene (Scientific Instrument Services, Ringoes, NJ). Masses were recorded from 400 to 625 Da at a scan rate of ~1 scan/sec.

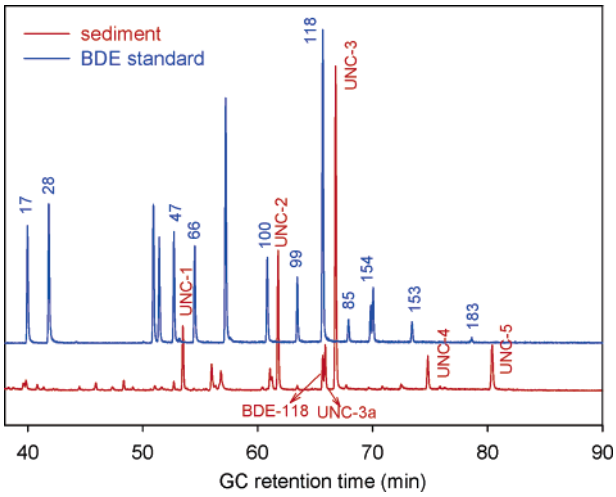


FIGURE 1. Gas chromatograms of an extract of a sediment sample at depth 17 cm from Lake Michigan at site MI-47 and of a PBDE standard solution obtained by monitoring *m/z* 79 (Br^-) with a 60-m DB-5-MS column. The response of the major UNC-3 peak was higher than those of major BDEs in this sample.

Gas Chromatography. For all GC/MS work, the GC column was a 60 m × 250 μm (i.d.) fused silica capillary tube coated with DB-5-MS (0.25 μm film thickness; J&W Scientific, Folsom, CA). The GC oven temperature program was as follows: isothermal at 110 °C for 1.90 min, 15 °C/min to 180 °C, 1.85 °C/min to 300 °C, and held at 300 °C for 45 min. The GC to MS transfer line was held at 285 °C. Two other capillary columns were used to compare the retention times of the unknown peak with that of the standard. One such column was a BETA DEX 120 column (30 m × 250 μm i.d × 0.25 μm film thickness) from Supelco (Bellefonte, PA). In this case, the GC oven temperature program was as follows: starting at 110 °C, 30 °C/min to 225 °C, and held at 225 °C for 100 min. The other column was a DB-35-MS column (30 m × 250 μm i.d × 0.25 μm film thickness) from J&W Scientific (Folsom, CA). In this case, the oven temperature program was as follows: isothermal at 110 °C for 1 min, 15 °C/min to 300 °C and held at 300 °C for 17 min, then 10°C/min to 320 °C and held at 320 °C for 2 min.

Quantification of 1,3,6,8-Tetrabromocarbazole. The concentrations of 1,3,6,8-tetrabromocarbazole were measured on the DB-35-MS column described above. Calibration standard solutions were prepared gravimetrically from the neat standard. Quantitation was done using selected ion monitoring in the ECNI mode. The ions at *m/z* 79 and 81 were used for quantitation, and the ions at *m/z* 482.8 and 484.8 were used for identification. BDE-118 was used as the standard to quantitate 1,3,6,8-tetrabromocarbazole and as the GC retention time reference. All concentrations are given on a dry weight of sediment basis. The recovery of 1,3,6,8-tetrabromocarbazole was 80−99%, and it was not affected by the concentrated H_2SO_4 that was used to treat the samples. The chromatogram of the standard indicated that it had some minor impurities; therefore, the concentrations of 1,3,6,8-tetrabromocarbazole in the sediment are somewhat overestimated.

Results and Discussion

During the analysis of brominated flame retardants in sediment core samples from Lake Michigan, several large, unknown GC peaks were found in the deeper layers; see Figure 1. Low-resolution ECNI and EI mass spectra of these peaks indicated that they were a series of brominated compounds, likely sharing the same carbon skeleton but having different bromine numbers and substitution patterns.

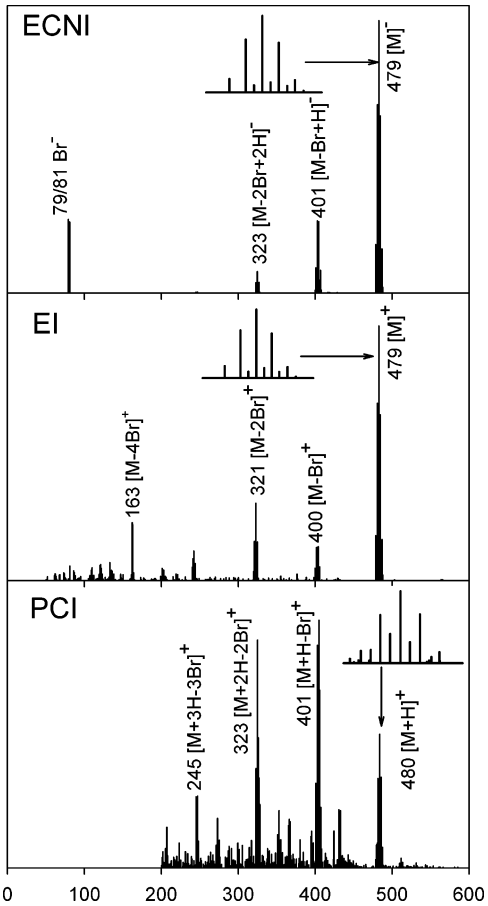


FIGURE 2. ECNI, EI, and PCI mass spectra of UNC-3 in pooled sample extracts from Lake Michigan sediments at site MI-47. The conditions for the ECNI, EI, and PCI mass spectrometry are described in the text.

Since UNC-3 was the most abundant congener, it will be used as an example to illustrate the structural identification strategy.

Figure 2 shows the ECNI, EI, and PCI mass spectra of UNC-3. The ECNI mass spectrum shows the successive losses of two bromines (with the addition of an hydrogen each time). The EI mass spectrum shows similar losses of two bromines (but without the hydrogen additions). The PCI mass spectrum shows a protonated molecular ion at m/z 480, a small C_2H_5 adduct ion at 508, and other ions due to the loss of one, two, or three bromines from the molecule. These observations suggest that the unknown compound has a stable chemical structure without side chains. The isotopic pattern of the molecular ion cluster suggests that UNC-3 has exactly four bromines. The GC retention time on this unknown compound was similar to that of BDE-118, which has five bromines. However, because of its odd molecular weight (suggesting the presence of an odd number of nitrogen atoms), UNC-3 was clearly not a PBDE or a polybrominated dibenzofuran.

High-resolution mass spectra of the UNC-3 were obtained to determine its molecular composition. Four ions from the most abundant ion clusters in the EI mass spectra of the unknown peaks were analyzed. The most probable elemental composition of each ion was the composition which gave a theoretical mass with the smallest difference from the experimental mass and indicated exactly four bromines. The experimental and theoretical masses are listed in Table 1. These data suggest that UNC-3 has a molecular composition of $C_{12}H_5Br_4N$. Only four compounds are listed in Chemical Abstracts with this composition, and three of them are

TABLE 1. Elemental Composition of Ions from the Unknown Compounds Found in Lake Michigan Sediment Cores

UNC		measured mass	theoretical mass[a]	difference (mmu)	composition
2	M+2	402.7958	402.8030	+7.2	$C_{12}H_6Br_3N$
	M+4	404.7996	404.8010	+1.4	
3a	M	478.7121	478.7156	+3.5	$C_{12}H_5Br_4N$
	M+4	482.7085	482.7116	+3.1	
3	M	478.7130	478.7156	+2.6	$C_{12}H_5Br_4N$
	M+4	482.7078	482.7116	+3.8	
4	M	556.6266	556.6260	−0.6	$C_{12}H_4Br_5N$
	M+4	560.6216	560.6220	+0.4	

[a] Calculations are based on the following exact masses C (12.000000), H (1.007825), Br (78.918348), Cl (34.968854), F (18.998405), N (14.003074), and O (15.994915).

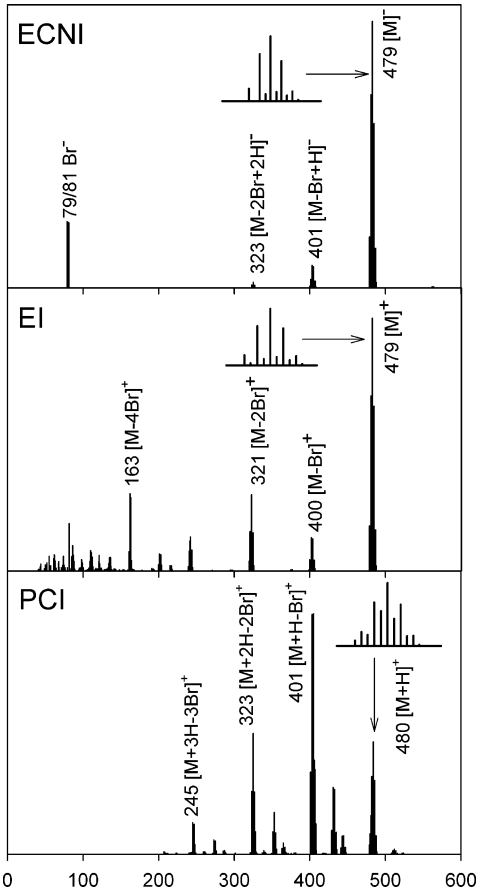
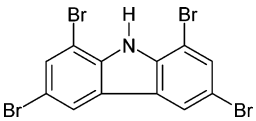


FIGURE 3. ECNI, EI, and PCI mass spectra of an authentic sample of 1,3,6,8-tetrabromocarbazole. The conditions for the ECNI, EI, and PCI mass spectrometry are described in the text.

tetrabrominated carbazoles. Of these, 1,3,6,8-tetrabromocarbazole is the most cited.



An authentic sample of 1,3,6,8-tetrabromocarbazole was purchased from the Department of Chemistry at the University of Florida. Mass spectra of the authentic compound were obtained under ECNI, EI, and PCI conditions, and these spectra are shown in Figure 3. These spectra agree with those of UNC-3 (see Figure 2) almost exactly.

Interestingly, as illustrated in the chromatogram shown in Figure 4, the standard bought from the University of Florida

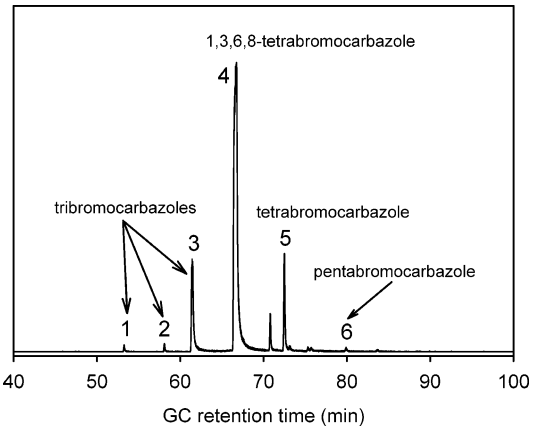


FIGURE 4. Gas chromatogram of the authentic sample of 1,3,6,8-tetrabromocarbazole obtained by monitoring *m/z* 79 (Br^-) with a 60-m DB-5-MS column.

TABLE 2. Comparison of the Absolute GC Retention Time (min) between the Samples and Authentic Standard Compounds

	peak	DB-5-MS	DB-35-MS	Beta DEX 120
sample	UNC-1	53.24	13.59	32.41
standard	tribromo-1	53.24	13.56	32.41
difference		0.00	0.03	0.00
sample	UNC-2	61.46	15.14	
standard	tribromo-2	61.47	15.14	
difference		0.01	0.00	
sample	UNC-3	66.70	15.93	77.90
standard	1,3,6,8-tetrabromo	66.73	15.94	78.02
difference		0.03	0.01	0.12
sample	UNC-4	79.89	20.30	92.08
standard	pentabromo	79.87	20.25	91.97
difference		0.02	0.05	0.11

contained some impurities in addition to the major component. The retention times of some of these peaks matched those of the other unknown compounds in the Lake Michigan sediment cores. Peaks 1, 3, and 6 in the chromatogram of the standard matched the retention times of UNC-1, UNC-2, and UNC-4, respectively, in the sediment. From the mass spectra of these impurities (see Supporting Information), we determined that they are most likely tri- to pentabrominated carbazole congeners. For example, peaks 1−3 are tribromocarbazoles, peak 5 is a tetrabromocarbazole, and peak 6 is a pentabromocarbazole. These mass spectra also are very similar to those of unknown compounds in the sediment cores. These data suggest that other unknown peaks are also brominated carbazoles; specifically, we suggest that UNC-1 and UNC-2 are tribromocarbazoles, UNC-3a is a tetrabromocarbazole, and UNC-4 is a pentabromocarbazole.

To confirm the assignment of UNC-3 to 1,3,6,8-tetrabromocarbazol, we measured the GC retention times of the unknown and authentic compounds on three different capillary GC columns. Table 2 lists these retention times on these columns. The retention times of UNC-3 and other peaks in the sediment samples matched very well with those of 1,3,6,8-tetrabromocarbazole and with other minor peaks, respectively, on the three kinds of columns. To further prove the identical retention time, one sediment sample was spiked with the standard and was run on two GC columns. An increase of the peak height of UNC-3 was observed on both columns (see Supporting Information). On the basis of the evidence provided here, we conclude that UNC-3 is 1,3,6,8-tetrabromocarbazole. The other peaks are likely bromocarbazole congeners of unknown substitution patterns. This is the first time that this compound has been detected in the environment.

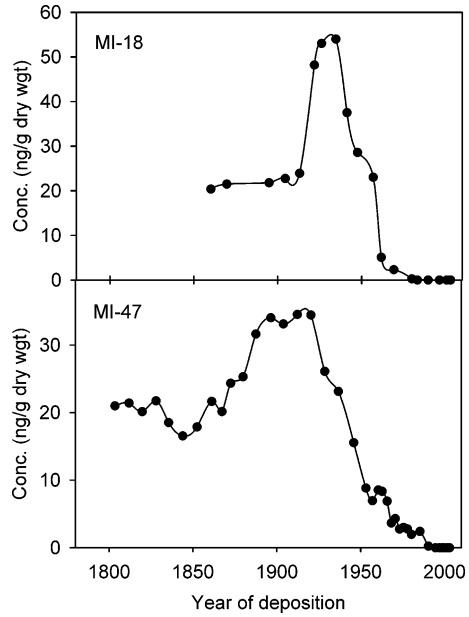


FIGURE 5. Time trend of 1,3,6,8-tetrabromocarbazole concentrations in the two sediment cores from Lake Michigan.

Using the authentic sample of 1,3,6,8-tetrabromocarbazole as a standard, we measured the concentrations of this compound in the sediment core samples for Lake Michigan by GC-MS-ECNI selected ion monitoring. These concentrations are shown in Figure 5. At site MI-47, the highest concentration (35 ng/g dry weight) occurred in ~1920. This concentration is similar to the highest PCB concentration (20 ng/g dry weight in ~1970) (*17*) and the highest BDE-209 concentration (115 ng/g dry weight in ~1996) (*19*) found in the same Lake. The time trend of 1,3,6,8-tetrabromocarbazole in the sediment core at MI-47 shows the concentration increased from the early 19th century, peaked at around 1920, then declined rapidly after that time. After 1992, the concentration was under the detection limit (0.1 ng/g dry weight). The time trend of 1,3,6,8-tetrabromocarbazole in the MI-18 sediment core is also shown in Figure 5. The concentrations and time trend are similar to that of MI-47. The highest concentration, 54 ng/g dry weight, happened in 1935 at this site and afterward the concentrations decreased to undetectable levels. The date of maximum deposition of 1,3,6,8-tetrabromocarbazole in the sediment cores may be affected by porewater diffusion. For example, PCBs were observed to move downward in a sediment core due to binding of the PCB to colloidal organic matter in the sediment porewater (*20*). The hydrogen attached to the nitrogen makes this molecule more polar than PCBs, and as a result, bromocarbazoles might be more affected by porewater diffusion than PCBs.

During the study of PBDEs in these Lake Michigan sediment cores, another core was collected from Lake Erie in the summer of 2003. The bromocarbazoles were not found in this core; however, the bottom layer represented 1972 (*19*) due to the high sedimentation rate at this site. It is possible that this core was not deep enough to find the bromocarbazoles.

The physicochemical properties of 1,3,6,8-tetrabromocarbazole was estimated by EPI Suite software provided by EPA. The estimated log K_{ow}, log K_{oc}, and water solubility are 6.79, 4.91, and 1.14×10^{-4} mg/L, respectively. These properties are similar to those of BDE-47 and some of the halogenated dimethyl bipyrroles (*21*). The high log K_{ow} and log K_{oc} values

and the low water solubility suggest that this chemical has a high binding affinity to particles and a tendency to accumulate in sediments.

The source of these compounds to Lake Michigan's sediment is unclear. Brominated carbazoles are not industrial chemicals, and they have not been used as such in the past. For example, the EPA Toxic Substances Control Act Inventory and the EPA Inventory Rule Update do not show any record of the production of these compounds. On the other hand, bromocarbazoles have been synthesized under laboratory conditions (*18*, *22*−*24*). For example, 1,3,6,8-tetrabromocarbazole was synthesized by heating carbazole and bromine overnight at 60 °C in glacial acetic acid (*24*) and by treating carbazole with hydrobromine acid and hydrogen peroxide under microwave irradiation (*18*).

While it is not possible to rule out a natural source for these compounds, it is likely that they are, in fact, anthropogenic. Four reasons support this suggestion: First, most naturally produced brominated organic compounds are found in marine systems, but the Great Lakes contain freshwater. Second, the impurities in the chemical standard match the distribution of the chemicals found in the sediment cores. Third, these compounds have a clear time trend showing a peak in 1920−1935. This implies that there may have been a large amount of production and usage of these compounds in the past. If they were biogenic, their concentrations would probably have remained constant. Fourth, there were no bromochlorocarbazoles detected in the sediment. Generally, chemicals from natural sources have mixed halogenation (both bromine and chlorine atoms), while industry products almost never have mixed halogenation.

Instead of the direct production of these compounds, it is also possible that they have been made as byproducts of some other synthetic process. For example, 2,4,6-tribromoaniline (TBA) has been widely used in industry for a number of years. It is used in the preparation of various dyes and polymers, and in combination with antimony trioxide, TBA is used as a flame retardant in the textile and plastic industry (*25*). Studies indicate that bromocarbazoles, mostly tetrabromocarbazoles, can be produced during the combustion of TBA (*26*).

One argument in favor of a natural source is the mere presence of these compounds in sediment layers dating from before 1900. There is little chance that any chemical industry could have produced the brominated carbazoles or their precursors at that time. Further study is needed to clarify the source of these compounds.

Acknowledgments

We thank Jonathan A. Karty and Xingfeng Gao at Indiana University for high-resolution mass spectrometric and NMR analyses, respectively; and the U.S. Environmental Protection Agency for allowing us to collect the sediment cores using the Research Vessel *Lake Guardian* and for funding through Grant R-83039701.

Supporting Information Available

Mass spectra of UNC-2, UNC-3a, UNC-4, a tribromocarbazole in standard, ENCI mass spectrum of a pentabromocarbazole, time trend of 1,3,6,8-tetrabromocarbazole, and sample chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Alaee, M.; Arias, P.; Sjodin, A.; Bergman, A. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* **2003**, *29*, 683−689.

(2) de Boer, J. Brominated flame retardants in the environment − The price for our convenience? *Environ. Chem.* **2004**, *1*, 81−85.

(3) Watanabe, I.; Sakai, S. I. Environmental release and behavior of brominated flame retardants. *Environ. Int.* **2003**, *29*, 665−682.

(4) Hoh, E.; Zhu, L.; Hites, R. A. Novel flame retardants, 1,2-Bis-(2,4,6-tribromo-phenoxy)ethane and 2,3,4,5,6-pentabromoethylbenzene, in United States' environmental samples. *Environ. Sci. Technol.* **2005**, *39*, 2472−2477.

(5) Kierkegaard, A.; Bjoerklund, J.; Friden, U. Identification of the flame retardant decabromodiphenyl ethane in the environment. *Environ. Sci. Technol.* **2004**, *38*, 3247−3253.

(6) Kierkegaard, A.; Bjoerklund, J. The presence of a "new" flame retardant, decabromodiphenyl ethane, in environmental samples. *Organohalogen Compd.* **2003**, *61*, 183−186.

(7) McCrindle, R.; Chittim, B.; Konstantinov, A.; Kolic, T.; McAlees, A.; MacPherson, K.; Reiner, E.; Potter, D.; Tashiro, C.; Yeo, B. Native and mass labeled [$^{13}C_{14}$]-decabromodiphenylethane (DBDPE): Characterization and use in determination of DBDPE in sewage sludge. *Organohalogen Compd.* **2004**, *66*, 3793−3798.

(8) Gribble, G. W. The diversity of naturally produced organohalogens. In *Handbook of Environmental Chemistry*; Springer: Berlin, 2003; pp 1−15.

(9) Gribble, G. W. Naturally occurring organohalogen compounds. In *2002 Annual Conference Proceedings*; American Water Works Association: Denver, CO, 2002; pp 479−496.

(10) Teuten, E. L.; Xu, L.; Reddy, C. M. Two abundant bioaccumulated halogenated compounds are natural products. *Science (Washington, DC, United States)* **2005**, *307*, 917−920.

(11) Gribble, G. W. The natural production of organobromine compounds. *Environ. Sci. Pollut. Res. Int.* **2000**, *7*, 37−49.

(12) Tittlemier, S. A.; Norstrom, R. J. Halogenated dimethyl bipyrroles − naturally produced, persistent, bioaccumulative, toxic compounds? In *EnviroAnalysis 2002, Proceedings of the Biennial International Conference on Monitoring and Measurement of the Environment, 4th*, Toronto, ON, Canada, May 27−30; EnviroAnalysis 2002 Conference Secretariat: Ottawa, ON, 2002; pp 65−70.

(13) Tittlemier, S. A.; Simon, M.; Jarman, W. M.; Elliott, J. E.; Norstrom, R. J. Identification of a novel $C_{10}H_6N_2Br_4Cl_2$ heterocyclic compound in seabird eggs. A bioaccumulating marine natural product? *Environ. Sci. Technol.* **1999**, *33*, 26−33.

(14) Tittlemier, S. A.; Blank, D. H.; Gribble, G. W.; Norstrom, R. J. Structure elucidation of four possible biogenic organohalogens using isotope exchange mass spectrometry. *Chemosphere* **2002**, *46*, 511−517.

(15) Tittlemier, S. A.; Fisk, A. T.; Hobson, K. A.; Norstrom, R. J. Examination of the bioaccumulation of halogenated dimethyl bipyrroles in an Arctic marine food web using stable nitrogen isotope analysis. *Environ. Pollut. (Oxford, United Kingdom)* **2001**, *116*, 85−93.

(16) Reddy C. M.; Xu, L.; O'Neil G. W.; Nelson R. K.; Eglinton T. I.; Faulkner, D. J.; Norstrom, R.; Ross P. S.; Tittlemier S. A. Radiocarbon evidence for a naturally produced, bioaccumulating halogenated organic compound. *Environ. Sci. Technol.* **2004**, *38*, 1992−1997.

(17) Song, W.; Li, A.; Ford, J. C.; Sturchio, N. C.; Rockne, K. J.; Buckley, D. R.; Mills, W. J. Polybrominated diphenyl ethers in the sediments of the Great Lakes. 2. Lakes Michigan and Huron. *Environ. Sci. Technol.* **2005**, *39*, 3474−3479.

(18) Smith, K.; James, D. M.; Mistry, A. G.; Bye, M. R.; Faulkner, D. J. A new method for bromination of carbazoles, b-carbolines and iminodibenzyls by use of *N*-bromosuccinimide and silica gel. *Tetrahedron* **1992**, *48*, 7479−7488.

(19) Zhu, L. Y.; Hites, R. A. Brominated flame retardants in sediment cores from Lakes Michigan and Erie. *Environ. Sci. Technol.* **2005**, *39*, 3488−3494.

(20) Eisenreich, S. J.; Capel, P. D.; Robbins, J. A.; Bourbonniere, R. Accumulation and diagenesis of chlorinated hydrocarbons in lacustrine sediments. *Environ. Sci. Technol.* **1989**, *23*, 1116−1126.

(21) Tittlemier, S. A.; Braekevelt, E.; Halldorson, T.; Reddy, C. M.; Norstrom, R. J. Vapor pressures, aqueous solubilities, Henry's Law constants, and octanol/water partition coefficients of a series of mixed halogenated dimethyl bipyrroles. *Chemosphere* **2004**, *57*, 1373−1381.

(22) Bogdal, D.; Lukasiewicz, M.; Pielichowski, J. Halogenation of carbazole and other aromatic compounds with hydrohalic acids and hydrogen peroxide under microwave irradiation. *Green Chem.* **2004**, *6*, 110−113.

(23) Lao, W.; You, J.; Yu, Z.; Ou, Q. Synthesis of bromocarbazoles and their separation by normal phase HPLC. *Huaxue Shiji* **2000**, *22*, 260−261.
(24) Chestand, A.; Yang, L.; Walter, R. I., Preparation of carbazole-3,6-d2 and carbazole-1,3,6,8-d4. *J. Labelled Compd. Radiopharm.* **1990**, *28*, 1239−1242.
(25) Bindra, S. K.; Narang, R. S. Combustion of flame retardants. II. *Chemosphere* **1996**, *33*, 1981−1996.
(26) Bindra, S. K.; Narang, R. S. Combustion of flame retardants. *Chemosphere* **1995**, *31*, 4413−4425.

Received for review August 2, 2005. Revised manuscript received September 28, 2005. Accepted October 7, 2005.

ES0515248