

Tetrachloro-9H-Carbazole, a Previously Unrecognized Contaminant in Sediments
of the Buffalo River

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Running Head: Tetrachloro-9H-Carbazole in Sediments

ABSTRACT

Three sediment samples taken from the Buffalo River, New York, have been analyzed for polychlorinated planar molecules similar in structure to the highly toxic 2,3,7,8-TCDD. Although no TCDD was found, residues of chlorinated dibenzofuran, fluorene, biphenylene, phenanthrene (anthracene), naphthalene, and 9H-carbazole were identified. 1,3,6,8-tetrachloro-9H-carbazole was identified as the major chemical of this type in the sediments, and quantified at levels as high as 25 ng/g at one site.

Additional Index Words: Toxic substances, dioxin, chlorinated hydrocarbons,

GC/MS

INTRODUCTION

The Buffalo River in New York, which receives both industrial and municipal sewage discharges, is recognized as one of the most heavily polluted river systems in the United States. Fishes in the Buffalo River were found to exhibit a high incidence of tissue lesions, which led investigators to correlate high levels of mutagenicity (Ames Test) to polynuclear aromatic hydrocarbons in proximity to a dye manufacturing plant (Black et al. 1980). Other studies showed that sediments from this section of the river also contained high levels of aromatic amines (Diachenko 1979, Nelson and Hites 1980). Many textile dyes containing amino groups have been shown to be mutagenic by the Ames Test (Friedman et al. 1980).

For the past several years, our lab has been involved in the analysis of Great Lakes fish and sediment samples for unusual polychlorinated chemicals that are not routinely monitored, such as polychlorinated styrenes (Kuehl et al. 1981). These studies are an essential part of hazard assessment for the environmental regulation of industrial activity, and can be used for establishing priorities in regulatory and health related research. This work has continued with exploratory studies similar to those we have previously published (Kuehl et al. 1980).

This paper reports GC/MS analysis of a previously unrecognized environmental contaminant identified in a sample extract that contains a very select class of chemicals, polychlorinated planar aromatic compounds. This class of chemicals contains the very toxic chlorinated dibenzo-p-dioxins and dibenzofurans, which have previously been shown to cause edemas in fish (Helder 1981). This study examines sediments from three sites in the Buffalo River near Lake Erie, and is a result of a continued concern over high levels of potentially hazardous chemicals in sediments and fish from this river.

MATERIALS AND METHODS

Sample Collection

Core samples were collected by field crews of the U.S. Environmental Protection Agency, Great Lakes National Program Office, Chicago, Illinois, and the top 6 in. were shipped frozen in glass jars to the Environmental Research Laboratory-Duluth (ERL-D), where they remained frozen at -20°C .

Sample Preparation

Two sets of samples were prepared for GC/MS analysis, A) a composite of each of three sediments was used for qualitative analysis, and B) a set of individual sediments was used for quantitative analysis.

Each jar of sediment was allowed to thaw, stirred 5 minutes and transferred to a funnel where the excess water was allowed to drain off (5% moisture). Subsamples (10 g) of each sediment were combined and blended to yield the composite sample used for qualitative GC/MS analysis. In addition, a 10 g subsample to be used for quantitative analysis of each sediment was spiked with 1.4 ng 1,2,3,4-tetrachlorodibenzo-p-dioxin (1,2,3,4-TCDD) as a surrogate spike used to evaluate the efficiency of the sample preparation method. The samples were then thoroughly blended with an equivalent weight of glass beads (140 mesh), and soxhlet extracted for 24 hours with hexane/acetone (1:1) (Burdick and Jackson). The soxhlet extraction round bottom flask contained 1-g freshly cleaned copper for sulfur removal.

The samples were then reduced to a volume of 5 mL in the round bottom flask fitted with a three-ball Snyder column. Each sample was transferred to a 2 cm x 50 cm glass column containing 20 g Celite 545 (Baker Chemical Co.) coated with 10 mL sulfuric acid (Ultrex, Baker Chemical Co.) and eluted with 200 mL hexane. The eluent continued to flow through a second column, 9 mm x 25 cm, containing 1 g cesium silicate (Stalling et al. 1981), and was

collected in a Kuderna-Danish (K-D) apparatus. The samples were again reduced in volume to 5 mL for chromatography on the carbon-glass system.

Carbon-Glass Column Chromatography

The carbon-glass column (CGC) was prepared by blending (Waring blender) AMOCO PX-21 carbon (50 mg) with a shredded glass filter pad (600 mg) in methylene chloride (100 mL) for two min. The carbon coated glass fibers were slurry packed into a 1 cm x 4 cm glass column fitted with glass fiber pads and teflon end fittings.

The sample was pumped (FMI Corp., G-50) through an all teflon 8-way valve (Rainin) onto the column at a rate of 4 mL/min (Figure 1). Non-planar molecules were eluted with methylene chloride (50 mL). Loosely bound planar molecules were eluted with methylene chloride/benzene (1:1, 50 mL) while tightly bound planar molecules were eluted with toluene (50 mL) flowing in the reverse direction to the previous fractions. Both the benzene and toluene fractions were reduced in volume on a rotary vacuum evaporator to ≈ 0.5 mL. Samples were concentrated to 100 μ L with dry $N_2(g)$ prior to GC/MS analysis.

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Gas Chromatography - Mass Spectrometry

Identification of polychlorinated planar chemical residues in the composite sample was done by computer library search and manual interpretation of GC/MS data. Spectra were generated on a Finnigan-MAT 4500-INCOS GC/MS/computer system in the electron impact ionization mode at 70 eV, by scanning m/z 50-550 every second. Samples were chromatographed on a 30 m x 0.25 mm SE-54 fused silica capillary column temperature programmed from 80° to 250°C at 4°/min. Helium was used as the carrier gas at a linear velocity of 35 cm/sec.

Quantification of 1,3,6,8-tetrachloro-9H-carbazole (1,3,6,8-TCC), and determination of the percent recovery of the surrogate spike, 1,2,3,4-TCDD were done on the same system using the multiple ion detection (MID) mode with dibromobiphenyl as an internal standard, and using a set of external standards of 1,2,3,4-TCDD and 1,3,6,8-TCC. Because of potential interferences in the analysis of 1,3,6,8-TCC from the molecular ions of isomers of tetrachlorodibenzofurans (TCDF), the molecular ions of TCDF were also monitored. The following nominal masses were monitored during a 2 sec. scan period at the appropriate retention time window: dibromobiphenyl, 310; 1,2,3,4-TCDD, 320, 322; 1,3,6,8-TCC, 303, 305; and TCDF, 304, 306. Quantification of 1,2,3,4-TCDD was based upon the response of m/z 320 and quantification of 1,3,6,8-TCC was based upon the response of m/z 303.

Synthesis of 1,3,6,8-Tetrachloro-9H-Carbazole

9H-carbazole (100 mg) (Chemical Service, Inc.) was added to a 500 mL round bottom flask and dissolved in methylene chloride (100 mL). To this, methylene chloride saturated with chlorine (100 mL) was slowly added over 30 minutes. The reaction was allowed to stir for 24 hours at room temperature. The solution was transferred to a 500 mL beaker and the solvent was allowed to evaporate off to yield light brown crystals. The product was transferred to a filter funnel and washed with cold iso-octane to yield 165 mg white crystals, m.p. 221-222°C uncorrected. GC/MS analysis showed the product to be 95% tetrachloro-9H-carbazole, 3% trichloro-9H-carbazole and 2% pentachloro-9H-carbazole. Nuclear magnetic resonance (NMR) showed protons at H_A , 8.25; H_B , 7.60; and H_N , 1.95 ppm down field from tetramethyl silane and integrating 2:2:1, respectively.

RESULTS AND DISCUSSION

A number of polychlorinated chemicals frequently reported in environmental sample monitoring studies were identified by mass spectral library search (INCOS FIT >850 of 1,000) in the planar molecular fraction of the Buffalo River composite sediment extract. These chemicals include pentachlorophenol, pentachlorotoluene, and various isomers of dichloro- through hexachlorobenzene, dichloro- through octachloronaphthalene, trichloro- through pentachlorodibenzofuran, and octachlorodibenzofuran. No isomers of TCDD were found by plotting mass chromatograms of the molecular ions m/z 320, 322, 324 and evaluating for a tetrachloro isotope pattern. However other planar compounds were tentatively identified based upon molecular ion data and retention time window data (Table 1). These include isomers of trichloro- and tetrachlorophenanthrene (anthracene), pentachlorobiphenylene, pentachlorofluorene, and pentachloro-9H-carbazole as minor components. Based upon the GC/MS total ion current peak area of molecular ions (m^+ , $m+2^+$), a tetrachloro-9H-carbazole (TCC) isomer was found at the highest level of all of the chlorinated planar chemicals identified.

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Because the major peak in the chromatogram was tentatively identified as a chemical analogous in structure (and in potential biological activity) to 2,3,7,8-TCDD and 2,3,7,8-TCDF, an attempt was made to confirm its structure. This was accomplished by chlorinating 9H-carbazole in an organic solvent, and identifying the stable products by GC/MS (Figure 2) and NMR. It was observed that 9H-carbazole very easily added four chlorine to yield one major product. Because of the ortho-para directing power of the nitrogen, one would predict the structure to be 1,3,6,8-TCC, which was confirmed by NMR. Moreover, the 1,3,6,8-TCC standard and the major TCC peak in the sediment samples had

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identical capillary column GC retention time (+2 sec). These findings strongly indicate that the major TCC in the sediment appears to be 1,3,6,8-TCC. This cannot be completely established, however, until all 38 TCC congeners have been synthesized and characterized by GC retention time (a task beyond the scope of this study).

Figure 3 is an example of the ion current profile for the analysis of TCDFs and TCCs in one of the sediment samples. The peak at spectrum 703 is the internal standard dibromobiphenyl. Major TCDF peaks are observed at spectra 882 and 916, and 1,3,6,8-TCC is at spectrum 979. Minor peaks between scan number 900 and 1200 appear to be a mixture of TCDFs and TCCs. Table 2 lists the sample identification, percent recovery of 1,2,3,4-TCDD, and quantification of 1,3,6,8-TCC. The recovery of 1,3,6,8-TCC from each step of the sample cleanup procedure was >95%. The recovery of the surrogate spike, 1,2,3,4-TCDD, was ≈80% for Casenovia Creek and Black Rock Canal samples, however the determination of percent recovery of TCDD in the "dye plant" sample was not possible because of a large interference at m/z 322. The levels of 1,3,6,8-TCC were 0.30 ng/g at Casenovia Creek, 0.60 ng/g at Black Rock Canal and 25 and 22 ng/g in duplicate analysis of the Dye Plant site sample. No TCC was found in the procedural blank above 0.10 ng/g.

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SUMMARY

Sediments from the Buffalo River have been found to contain a large number of polychlorinated planar chemicals similar in structure to TCDD and TCDF. These residues may be partially responsible for the unusual effects upon Buffalo River fish. Also, in agreement with other published data, levels of chemical residues found in the river sediment appear to be highest near an area where a dye manufacturing plant has previously discharged into the river (map included in reference 3).

Future studies should include the synthesis of all 38 TCC isomers to verify isomer identification, an evaluation of acute and chronic toxicity of these chemicals to freshwater fish, and a monitoring program to determine the source and distribution of these chemicals.

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Table 1. GC Retention Time and Major Characteristic Ions of Polychlorinated Planar Molecule Isomers in Buffalo River Sediment.

Chemical	Relative Retention Time*	Major Ions
Hexachlorobenzene	1.00	Cl ₆ , m/z 282
Pentachlorobiphenylene	1.87	Cl ₅ , m/z 322
Tetrachlorodibenzofuran	1.88	Cl ₄ , m/z 304
Tetrachlorodibenzofuran	1.95	Cl ₄ , m/z 304
Pentachlorofluorene	2.00	Cl ₅ , m/z 336
Trichloroanthracene/Phenanthrene	2.01	Cl ₃ , m/z 280
Tetrachloro-9H-Carbazole	2.08	Cl ₄ , m/z 303
Pentachloro-9H-Carbazole	2.37	Cl ₅ , m/z 337
Tetrachloroanthracene/Phenanthrene	2.41	Cl ₄ , m/z 314
Octachlorodibenzofuran	3.61	Cl ₈ , m/z 440

* Retention time relative to hexachlorobenzene.

Table 2. Buffalo River Sample Identification and Quantification Data

Sample Code	Sample Identification	Latitude Longitude	Percent Recovery	
			1,2,3,4-TCDD at 0.14 ng/g	1,3,6,8-TCC ng/g*
NS01S55	Casenovia Creek	42°51'13" 78°48'48"	77	0.30
NS01S79	Black Rock Canal	42°54'34" 78°54'08"	82	0.60
NS03S66	Dye Plant	42°51'43" 78°50'42"	X**	25,22

* detection limit 0.10 ng/g

**not determined

FIGURE CAPTIONS

Figure 1. Carbon-glass chromatographic system.

Figure 2. Electron impact mass spectrum of 1,3,6,8-tetrachloro-9H-carbazole.

Figure 3. GC/MS total ion current chromatogram for sample NS03566, "Dye Plant" site.





