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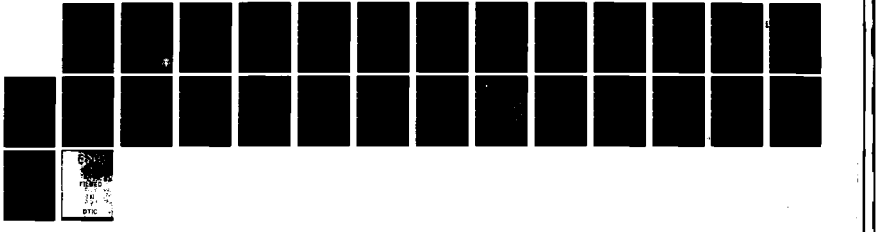

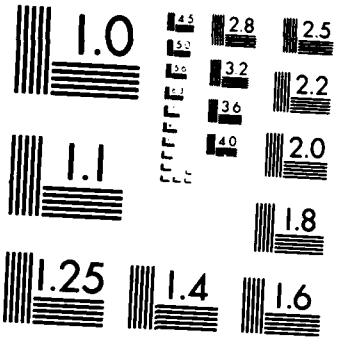

AD

TECHNICAL REPORT 8211

AN INVESTIGATION OF THE PRESENCE OF N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA IN ESTUARINE SEDIMENTS OF ABERDEEN PROVING GROUND, MD (EDGEWOOD AREA)

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APRIL 1983

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Analysis
Contamination
Hexachlorocarbanilide
N,N'-Bis(2,4,6-trichlorophenyl) urea
Properties
River
Sediments
Solubility

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

N,N'-Bis(2,4,6-trichlorophenyl)urea is present in the sediments of Canal Creek in the Edgewood area of Aberdeen Proving Ground, MD. The highest concentrations are found in the top 2 inches of sediment, (100-300 µg/g), and the concentration declines with increasing depth. Below a 6-inch depth there is little or no contamination. Although the detection limit of the HPLC method described here is 5 µg/g of dry sediment, modification of this method to lower levels is possible. The chemical literature gives little information concerning this material, but does indicate a very high stability to chemical

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20. Abstract (continued)

hydrolysis. The solubility in water is quite low (82 µg/L at 22°) and the estimated octanol/water partition coefficient is high (log K >5). The conjunction of these properties points to long persistence of the compound in the environment.

ACKNOWLEDGMENTS

The author wishes to acknowledge the technical assistance of Ms. Theresa Trybus and Mr. Ernst Brueggemann for their development of a high pressure liquid chromatographic method for analysis of N,N'-bis(2,4,6-trichlorophenyl)urea. I also thank Dr. Elizabeth Burrows for obtaining the mass spectra of this compound and its pyrolysis products.

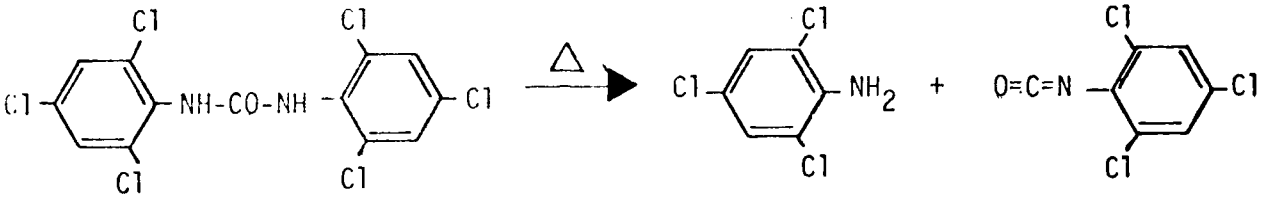
TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

In FY81, the US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) undertook an environmental fate study of 2,4,6-trichloroaniline (TCA).[1] During this work, a sediment sample taken from the bottom of Canal Creek in the Edgewood area of Aberdeen Proving Ground was shown to contain two major substances by gas chromatographic analysis. These substances proved to be TCA and 2,4,6-trichlorophenyl isocyanate. Since the latter compound is readily hydrolyzed in water, it would not have been present in the wet sediment. It was concluded that N,N'-bis(2,4,6-trichlorophenyl)urea and not TCA was present in the mud. The urea was pyrolyzed upon injection onto the GC column (injection port of 250°C) to give the observed products, TCA and 2,4,6-trichlorophenyl isocyanate, as shown below:



Thus, this pyrolysis had misled previous investigators into believing that TCA was present in the sediments. A manual reexamination of four GC/mass spectra compiled by Calgon Environmental Systems[2] did show the presence of both TCA and 2,4,6-trichlorophenyl isocyanate in at least three sediment analyses, the fourth being uncertain. Identification of 2,4,6-trichlorophenyl isocyanate was not originally made because its mass spectrum is not included in the NBS library of ≥30,000 spectra accessible for computer-based search.

OBJECTIVES

It was the objective of this work to carry out a literature survey on N,N'-bis(2,4,6-trichlorophenyl)urea, synthesize the substance, develop a high performance liquid chromatography (HPLC) method to detect the compound in sediments and determine its solubility in water and its octanol/water partition coefficient.

LITERATURE SURVEY OF N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA

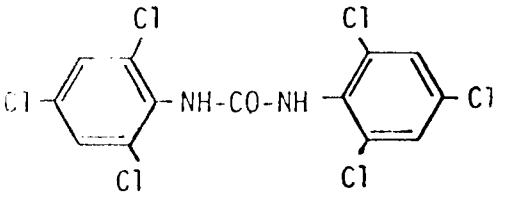
A manual literature search of Chemical Abstracts was conducted covering the period 1937 to Dec 1980. No citations were found prior to 1956. The information base of this compound is sparse.

ALTERNATIVE NAMES

N,N′-bis(2,4,6-trichlorophenyl) urea
sym-bis(2,4,6-trichlorophenyl) urea
2,2′,4,4′,6,6′-hexachlorocarbanilide
sym-hexachlorodiphenyl urea

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry Number: 20632-35-3
Chemical Formula: $C_{13}H_6Cl_6N_2O$
Structural Formula:



One of the few physical properties listed in the literature is the melting point: 295°C,[3,4] 326-327°C,[5] and 330-340°C.[6] Other properties that have been addressed are the UV spectrum[3,7] and dipole moment.[8]

N,N′-Bis(2,4,6-trichlorophenyl)urea may be synthesized by phosgenation of a solution of 2,4,6-trichloroaniline,[5,9,10] by the condensation of 2,4,6-trichloroaniline with 2,4,6-trichlorophenyl isocyanate;[4] or by refluxing 2,4,6-trichloroaniline with urea in glacial acetic acid containing sulfuric acid.[11,12] The latter process[12] was used in commercial manufacture of the compound. Chlorination of N,N′-bis(2,4,6-trichlorophenyl)urea[13] leads to N,N′bis(2,4,6-trichlorophenyl)-N,N′-dichlorourea which has antivesicant properties.[14] Chemical hydrolysis of N,N′-bis(2,4,6-trichlorophenyl)urea requires drastic conditions.[15] For example, even with 85% H_2SO_4 at 150°C in an autoclave, 30% of the urea remains unchanged after 3 hours. Likewise, with 5% NH_4OH at 150°C, only 83 percent of the urea is hydrolyzed after 5 hours.

ANALYTICAL METHODS

No information was found in the scientific literature dealing with the chemical analysis of N,N′-bis(2,4,6-trichlorophenyl)urea.

MAMMALIAN TOXICOLOGY

No information was found in the scientific literature dealing with human exposure to N,N′-bis(2,4,6-trichlorophenyl)urea. The oral toxicity of this compound to non-fasted Wistar albino rats was carried out in a screening test.[16] Ten female rats, weighing 200-500 g were dosed at 100 mg/kg and 10 at 50 mg/kg of the compound. After 7 days of observation, no animals had died from the higher dose, whereas one died at the lower dose. Such results are inconclusive; the LD_{50} is obviously greater than 100 mg/kg, indicating that the compound should not be classified among those of extreme acute toxicity. To say more than that would be going beyond the significance of the data. No information dealing with other mammalian species was found.

PLANTS

In a study of 500 urea derivatives, about one half of the N,N'-disubstituted type possessed cytokinin activity.[17] However, N,N'-bis(2,4,6-trichlorophenyl)urea was not among those tested. According to this source, diphenylurea, a coconut milk cytokinin, stimulates cell division in the tobacco pith assay at levels as low as 0.5 ppm, and several substituted diphenylureas at levels as low as 0.1 ppm. The presence of chlorines in both rings of diphenylurea caused loss of activity to varying degrees; the most active compound in this class of chlorinated ureas, N,N'-bis(3,4-dichlorophenyl)urea, was effective at 2 ppm.[17] This level is considerably higher than the solubility of N,N'-bis (2,4,6-trichlorophenyl)urea, determined as described elsewhere in this paper. As to the potential for human health effects, we have no reason to believe that cytokinin activity, per se, indicates any corresponding degree of mammalian toxicity.

EXISTING STANDARDS

No standards exist, to our knowledge.

METHODS

SYNTHESIS OF N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA[6]

Urea (9.0 g), TCA (9.2 g), glacial acetic acid (80 mL) and water (0.8 mL) were placed in a 250 mL round-bottom flask and heated at reflux while 15 g of concentrated H_2SO_4 was slowly added (over a 2-hour period). The hot mixture became cloudy. Reflux was continued another 3 hours, and the mixture was allowed to cool. The white product, N,N'-bis(2,4,6-trichlorophenyl)urea, was collected by suction filtration and washed with hot water. The resulting 0.8 g melted at 330-340°(d), was not sufficiently soluble for purification by recrystallization from benzene, but was pure by HPLC analysis. A direct inlet probe mass spectrum of the synthesized material showed no molecular ion, and its appearance was that of superimposed mass spectra of TCA and 2,4,6-trichlorophenyl isocyanate, i.e., the most prominent fragments were the trichloro isotope clusters at 195/197/199 and 221/223/225 amu.

DETERMINATION OF N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA IN SEDIMENTS BY HPLC

Application and Scope

In October 1981, work by USAMBRDL showed that N,N'-bis(trichlorophenyl)urea was present in the sediments of Canal Creek located on Edgewood Arsenal. During January 1982, USAMBRDL was given the task of developing an analytical method to determine this urea derivative in sediments. Since GC was inconclusive, HPLC was chosen as the method for development.

1. Concentration range tested: 0.5 µg/gram to 10 µg/gram of dry sediment.

2. Detection limit: 1 µg/gram of dry sediment

Summary of Method

A sample of dried sediment is leached with a mixture of acetonitrile and dimethylformamide in a sonic bath. Following centrifugation, the supernatant phase is subjected to direct analysis by HPLC.

Hazards

Both acetonitrile and dimethylformamide are toxic. The analyst should avoid direct contact with these solvents. Little is known about the toxicity of N,N'-bis(2,4,6-trichlorophenyl)urea.

Interferences

Other organic substances with similar HPLC retention times could interfere. No interferences were observed in the present application. In case of doubt, mass spectrometry may be employed to resolve any uncertainty.

Apparatus, Materials, and Operating Parameters

1. Instrumentation

   a. Waters High Pressure Liquid Chromatograph (HPLC) with variable wavelength detector.

   b. Ten cm RAD-PAK ^{R} reverse phase C_{18} column

   c. Packard Model 3385 Liquid Scintillation Spectrometer

2. Operating Parameters for HPLC

   a. Mobile phase: acetonitrile/water = 60/40

   b. Flow rate: 2 mL/min

   c. UV detector: 230 nm

   d. Injection volume: 50 µL

3. Glassware and Hardware

   a. Culture tubes (15 ml) with Teflon-lined screw caps

   b. COREX^{R} centrifuge tubes

   c. Twenty-milliliter glass vials (scintillation type) or equivalent.

   d. Low speed centrifuge (2,0000 rpm)

   e. High speed centrifuge - SORVALL^{R}

Use of trademarked name does not imply endorsement by the US Army, but is used only to assist in identification of a specific product.

f. Hamilton syringe (100 µL)

g. Volumetric pipets and Pasteur pipets

h. Sonic bath, Bransonic 52

4. Chemicals

a. Acetonitrile, HPLC grade (Burdick & Jackson)

b. Water, glass-distilled

c. Dimethylformamide (Burdick & Jackson)

d. ^{14}C-(u)Aniline hydrochloride (99% radiochemical purity, lot No. 1310-179, New England Nuclear)

e. 2,4,6-Trichlorophenyl isocyanate (ICN-K&K)

f. Instagel scintillation cocktail (Packard)

Standards

Stock N,N'-bis(2,4,6-trichlorophenyl)urea: Dimethylformamide (DMF) is the best solvent for the urea derivative. About 8 mg could be dissolved in 10 mL of DMF at room temperature. This 800 µg/mL solution was identified as stock A. It was stored at room temperature in a glass volumetric flask. Part of stock A was diluted 1:10 with DMF; this was designated stock B (80 µg/mL).

Working stocks: Dilute solutions of the urea derivative were prepared from stocks A and B in the following way:

Concentration	mL Stock A (800 µg/mL)	mL Stock B (80 µg/L)	mL Acetonitrile
1 µg/mL	-	0.125	9.875
2 µg/mL	-	0.25	9.75
4 µg/mL	-	0.50	9.50
8 µg/mL	-	1.00	9.00
20 µg/mL	0.25	-	9.75
40 µg/mL	0.5	-	9.50

These solutions were used to prepare a standard curve.

Procedure

1. Sample Handling

The urea derivative is very stable chemically and of low volatility. All samples that came into the laboratory were wet estuarine sediments in glass jars. On arrival, a portion of a sample (50 grams) was placed on a clean glass petri dish and allowed to air-dry for 2 days. The remainder of wet sample was retained in a refrigerator. The dry sample was manually

crushed in the glass dish with a metal tamp and passed through a 20-mesh screen to separate stones, twigs, and leaves from sediment. The tamp was flamed between samples to destroy organic residues. Sieved, dry soils were placed in 3-oz glass jars with screw caps, coded, and stored at room temperature away from direct sunlight. Prior to analysis, samples were dried for 1 hour at 105°C and cooled in a desiccator before weighing.

2. Extraction

Half-gram quantities of dried sediment samples were put in 15 mL screw cap tubes with 5 mL of 5 percent DMF in acetonitrile (v/v). Teflon-lined screw caps were placed on the tubes and the contents agitated for 15 min at room temperature in a sonic bath. Following this, all tubes were centrifuged at 2,000 rpm for 10 min. For each tube, 4 mL of the supernatant liquid was then centrifuged again at 10,000 rpm for 10 min in COREX^R tubes with a SORVALL^R centrifuge and SS-34 head. The resulting clear supernatant liquid was analyzed by HPLC.

3. HPLC Analysis

Instrument and parameters are described above in Apparatus, Materials Operating Parameters. Typically, the retention time was 8.03 minutes.

4. Calibration

Prior to analysis, a standard solution containing a known concentration of N,N'-bis(2,4,6-trichlorophenyl)urea was injected onto the HPLC. The resulting peak area and retention time was supposed to be the same as that determined at an earlier time using the same parameters. From the comparison the analyst could detect the following:

(a) Loss of detector sensitivity (indicated by a peak smaller than expected). A variation of 10% is not uncommon.

(b) Column deterioration (indicated by a large change in retention time). A variation of 0.2 min for retention time is not uncommon.

This check was made every day during operation of the HPLC.

5. Calculations

Peak areas for all working standards were plotted against their concentrations to give a standard curve, which was used to determine concentration (µg/mL) of the urea in the extract of each sediment sample. Concentration of the urea in the dry sediment was therefore

$$\mu g \text{ urea derivative/g sediment} = \frac{C_{extract} \times 5}{\text{weight of sediment}}$$

Where: $C_{extract}$ is in µg/mL and weight of sediment is in grams.

6. Accuracy, Precision, and Sensitivity

a. Recovery and Precision

To determine the efficiency of extraction of N,N'-bis(2,4,6-trichlorophenyl)urea from dry sediments by 5% DMF in acetonitrile, a sediment that contained no measurable urea derivative was spiked with stock A at three levels as shown in the table below. Twelve tubes, each containing 0.5 g of dry sediment were spiked with stock A (see Standards) in quadruplicate. The spiked sediments were subjected to reduced pressure (165 mm Hg) for 2 days to remove the DMF. All 12 spiked samples were analyzed for the urea derivative by the above procedure (see Procedure 2, Extraction). The results follow:

TABLE 1. RECOVERY OF N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA FROM DRIED SEDIMENTS

Number of Samples	Spiked Concentration of Urea Derivative on Sediment (µg/0.5 g)	Expected Concentration in 5 mL Solvent (µg/mL)	Concentration Found in Extraction Solvent (µg/mL)		Mean Recovery
			μ	σ	
4	3.6	0.72	0.67	0.03	93.1%
4	21.7	4.34	4.22	0.28	97.3%
4	43.4	8.68	8.48	0.16	97.7%

b. Reproducibility of Standard Curve

Precision and accuracy data were generated by the following procedure: Dry sediment samples were spiked with N,N'-bis(2,4,6-trichlorophenyl)urea at the levels of 0, 0.5X, X, 2X, 5X and 10X, where X is the estimated detection limit. One sample at each concentration level was analyzed on any one day, for four separate days. Table 2 presents these data. Peak areas (y axis) were plotted against the concentrations of urea derivative (x axis).

c. Sensitivity

Under the analytical conditions used, a concentration of 5 µg of N,N'-bis(2,4,6-trichlorophenyl)urea per gram of dry sediment produced an easily distinguishable HPLC peak, about 12 mm high; this was well above the noise level. This is considered the sensitivity of the method, although it is possible to distinguish lower peaks.

TABLE 2. HPLC STANDARD CURVES FOR N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA ON FOUR DIFFERENT DAYS

Sample (µg/0.5 g dry sediment)	Peak Areas					
	March 8, 1982	March 12, 1982	March 15, 1982	March 16, 1982	Mean	Standard Deviation
Blank	0	0	0	0		
2	138912	142188	144344	118559	136001	11840
4	278685	231844	269412	247250	256798	21229
8	520384	579803	536965	503073	535056	32884
20	1317176	1310651	1240189	1113480	1245374	94586
40	2590093	2663638	2760739	2523391	2611865	69398
Regression Curve R^2	0.999	0.999	0.998	0.996		

Equations[a]

March 8 $y = 64,665x + 10,001$

March 12 $y = 66,444x + 1,873$

March 15 $y = 66,023x - 4,010$

March 16 $y = 66,223x - 16,469$

a. y = peak area; x = sample concentration in µg/0.5 g dry sediment.

SYNTHESIS OF ^{14}C-2,4,6-TRICHLOROANILINE

The synthesis of 2,4,6-trichloroaniline was based on the description by Erdelyi.[18] ^{14}C-(u)-Aniline hydrochloride (4 mg, 8.1 μCi/mmol, or 250 μCi) in ethanol (0.5 mL) was mixed with aniline (reagent grade, 0.15 mL) in a test tube. Five milliliters of concentrated HCl was added to dissolve the aniline. Chlorine gas was passed into the acid solution for about 1 hour. During this time the solution became dark and turbid. Thin-layer chromatography of a small portion of this mixture showed the presence of 2,4,6-trichloroaniline. The turbid solution was then diluted with 50 mL of water, and $NaHCO_3$ and Na_2SO_3 were added to neutralize the acid and destroy excess chlorine, respectively. This mixture was then extracted with 20 mL of CH_2Cl_2. The CH_2Cl_2 layer was removed, dried, and evaporated. The residual solid was dissolved in 3 mL of hexane and placed at the top of a small (8 x 0.8 cm) column of silica gel. The column was eluted with hexane and 25 2.5-mL fractions were collected. The fractions shown by GC to contain 2,4,6-trichloroaniline (fractions #9-#13) were combined to yield 41 mg (12.5%) of ^{14}C-2,4,6-trichloroaniline, 99.8 percent pure, with a specific activity of 0.58 μCi/mg. Theoretically, the activity should have been 0.76 μCi/mg.

SYNTHESIS OF ^{14}C-N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA

2,4,6-Trichlorophenyl isocyanate (50 mg) was mixed with ^{14}C-TCA (23 mg, specific activity 0.58 μCi/mg, prepared as described earlier)[6] in a 15 mL pyrex culture tube. The tube was capped (not tightly) with a Teflon^{R}-lined screw cap, placed inside a steel test tube wrapped with heating tape, heated from room temperature to 180°C during 10 minutes, and held at 180°C for 30 minutes. The temperature was controlled by a variable transformer. When the tube and its contents had cooled to room temperature, 2 mL of methanol was added to the residue in the tube and the mixture was agitated for 2 minutes in a sonic bath to disperse the insoluble residue. The mixture was centrifuged at 2,000 rpm to separate the insoluble urea derivative from the red supernatant methanol solution. The supernatant methanol solution was withdrawn and replaced with 3 mL of hexane. The white residue was agitated again, centrifuged, and the supernatant hexane removed. This washing procedure was continued until the supernatant hexane had reached a constant ^{14}C level. This process effectively removed unreacted trichloroaniline and trichlorophenyl isocyanate. The product weighed 20 mg and had a specific activity of 0.3 μCi/mg. Analysis of this material by HPLC showed a purity of 99 percent (1 percent 2,4,6-trichloroaniline present).

DETERMINATION OF THE SOLUBILITY OF N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA IN WATER

Preliminary attempts to determine the water solubility of the urea derivative showed it to be too low for detection by HPLC. For this reason the ^{14}C-labeled urea derivative was made.

A small amount of the ^{14}C-N,N'-bis(2,4,6-trichlorophenyl)urea (1.1 mg) was placed in a test tube with 10 mL of 10^{-3}M phosphate buffer (pH 7.0) and agitated in a sonic bath for 15 minutes, equilibrated in a water bath for 15 minutes, then centrifuged for 5 minutes at 2,000 rpm. Two mL of the clear supernatant solution was transferred to a scintillation vial with 10 mL of Instagel and this counted for ^{14}C on a Packard Model 3385 Liquid Scintillation

Spectrometer. The remaining aqueous phase was removed from the tube and the remaining urea derivative treated with a fresh 10 mL of 10^{-3}M buffer. The same process was repeated for several days through a total of 16 cycles at 22°C to 23°C. Because the product contained 1% of ^{14}C-2,4,6-trichloroaniline, the early cycles showed an initial high ^{14}C level in the supernatant phase that rapidly declined, since 2,4,6-trichloroaniline is fairly soluble in water (32 mg/L at 19°C). From the known specific activity of the urea (0.3 μCi/mg = 6.6×10^5 DPM/mg = 660 DPM/μg) and the experimentally observed ^{14}C-activity (DPM/mL) after background subtraction, the solubility (μg/mL) was calculated. The results for cycles 8 through 16 are summarized in Table 3. Statistical interpretation indicates that there is 95 percent confidence that the best estimate of solubility lies between 0.053 and 0.127, with a geometric mean of 0.082.

TABLE 3. ^{14}C-ACTIVITY OF AQUEOUS PHASE IN EQUILIBRIUM WITH SOLID ^{14}C-N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA AT 22°C

Cycle	DPM[a]	Concentration of Urea Derivative (μg/mL)
8	184	0.138
9	162	0.123
10	157	0.118
11	61	0.046
12	216	0.162
13	50	0.037
14	56	0.042
15	85	0.064
16	148	0.112
	geometric mean =	0.082
	geometric standard deviation =	1.77

a. Disintegrations per minute in 2 mL aqueous solution after subtracting background.

ESTIMATION OF OCTANOL/WATER PARTITION COEFFICIENT

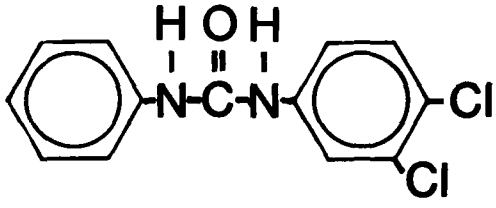
Attempted Direct Measurement

Twenty milliliters of water-saturated octanol was agitated (sonic bath) with ^{14}C-N,N'bis(2,4,6-trichlorophenyl)urea (1 mg) for 30 minutes and then allowed to stand overnight at 22°C before centrifugation. After 30 minutes of centrifugation, 0.1 mL of the clear supernatant octanol was removed and the radioactivity measured. The activity of the solution was 13,270 DPM/mL, which gives a solubility of the urea derivative in octanol of 19.9 μg/mL. When the remaining octanol (saturated with urea derivative) was shaken with 10 mL of water, no measurable ^{14}C was found in the water. The minimum detection level would be 0.03 μg/mL in water assuming a minimum significant CPM of 2X

background. Thus, direct measurement of octanol/water partition coefficient in this manner was unsuccessful. However, the attempt does show that K_p must be greater than 663 (i.e., 19.9/0.03).

Theoretical Estimation from Fragment Constants

Since the partition coefficient of N,N'-bis(2,4,6-trichlorophenyl)urea was not found in the chemical literature and the attempt to measure it directly was unsuccessful, a search was made for related compounds. The log K_p for 1-(3,4-dichlorophenyl)-3-phenyl urea (shown below) was reported[19] to be 4.70.



If the rules described by Hansch and Leo[19] for addition of substituents (f constants) to a parent structure are applied, a log K_p for the hexachloro derivative can be estimated. Starting with log K_p of 4.70 for the dichloro derivative, four H fragments (f_H value of 0.23) are subtracted and four Cl fragments (f_{Cl} value of 0.92) are added: (4.70) - (4 x 0.23) + (4 x 0.92) = 7.46. This approach gives an estimated log K_p of 7.46 for N,N'-bis(2,4,6-trichlorophenyl)urea.

Theoretical Estimation from Solubility

Chiou et al.[20] describe an empirical equation to relate log K_p to aqueous solubilities of 34 organic compounds. This regression equation is:

$$\log K = 5.00 - 0.670 \log S$$

Here, K_p = n-octanol/water partition coefficient and S = aqueous solubility in µmol/L. The solubility of N,N'-bis(2,4,6-trichlorophenyl)urea, found in this study to be 82 µg/L at 22°C, provides the following values:

$$S = \frac{82\ \mu g/L}{418\ \mu g/\mu mol} = 0.196\ \mu mol/L$$

$$\log S = -0.707$$

$$\text{hence, } \log K_p = 5.00 - (0.670)(-0.707) = 5.47$$

RESULTS

ANALYSIS OF SEDIMENT SAMPLES FROM CANAL CREEK, EDGEWOOD AREA OF APG

Soon after the discovery of the presence of N,N'-bis(2,4,6-trichlorophenyl)urea in a Canal Creek sediment grab sample collected in the summer of 1981,[6] several sediment and soil samples were obtained from Canal Creek for further analysis (Nov 1981). The purpose was to ensure that the earlier grab sample was not fortuitous. Two types of samples were gathered. The first consisted of grab samples taken from both the bottom of Canal Creek and from adjacent dry land. These grab samples comprised material from the surface to a depth of about 2 inches. The second type of sample was a vertical core. This was taken by pushing an aluminum pipe (2.25" ID, 0.049" wall, holes spaced at 6" intervals) into the sediment to a depth of 1 foot and withdrawing the sample. The sediment core was pushed from the pipe with a tamp into a cylindrical container of cardboard.

On return to the laboratory the cores were cut into 1- or 2-inch sections and air-dried. Portions of the grab samples were also dried. All dried samples were crushed in a mortar and pestle, put through a 20-mesh sieve and stored in glass jars. Table 4 gives the description of the sampling sites and Figure 1 shows their approximate location. HPLC analysis was carried out to determine the concentration of N,N'-bis(2,4,6-trichlorophenyl)urea in these samples. The results are presented in Table 5.

TABLE 4. DESCRIPTION OF SAMPLING SITES

Sample (see Fig. 1)	Description
A	Core (11.5") taken from muddy area in the creek. The core had the odor of petroleum.
B	Core (11") taken from bottom of creek 3′ from bank, 50′ from Sample A and 300′ from mouth of the creek.
C	Core (7") taken from a sandy area in the creek, 125′ from mouth of the creek. The bottom 4" of core was sand.
D	Core (10.5") taken in the marsh area adjacent to the creek. The bottom 4" of core was sand.
E	Grab sample in the woods on high ground, 5′ from the creek bank. Sample area was mostly forest litter.
F	Grab sample taken at the mouth of Canal Creek, 6′ from margin, where water depth was 2′. This was a sandy sample.
G	Grab sample in woods behind building E5879, 50′ from creek bank; mostly forest litter.
H	Grab sample taken from the side of the creek bank; mostly clay exposed to rise and fall of creek.
I	Grab sample from wet marsh area.
J	Grab sample taken near Core A.
K	Grab sample taken near J, but further into the creek.

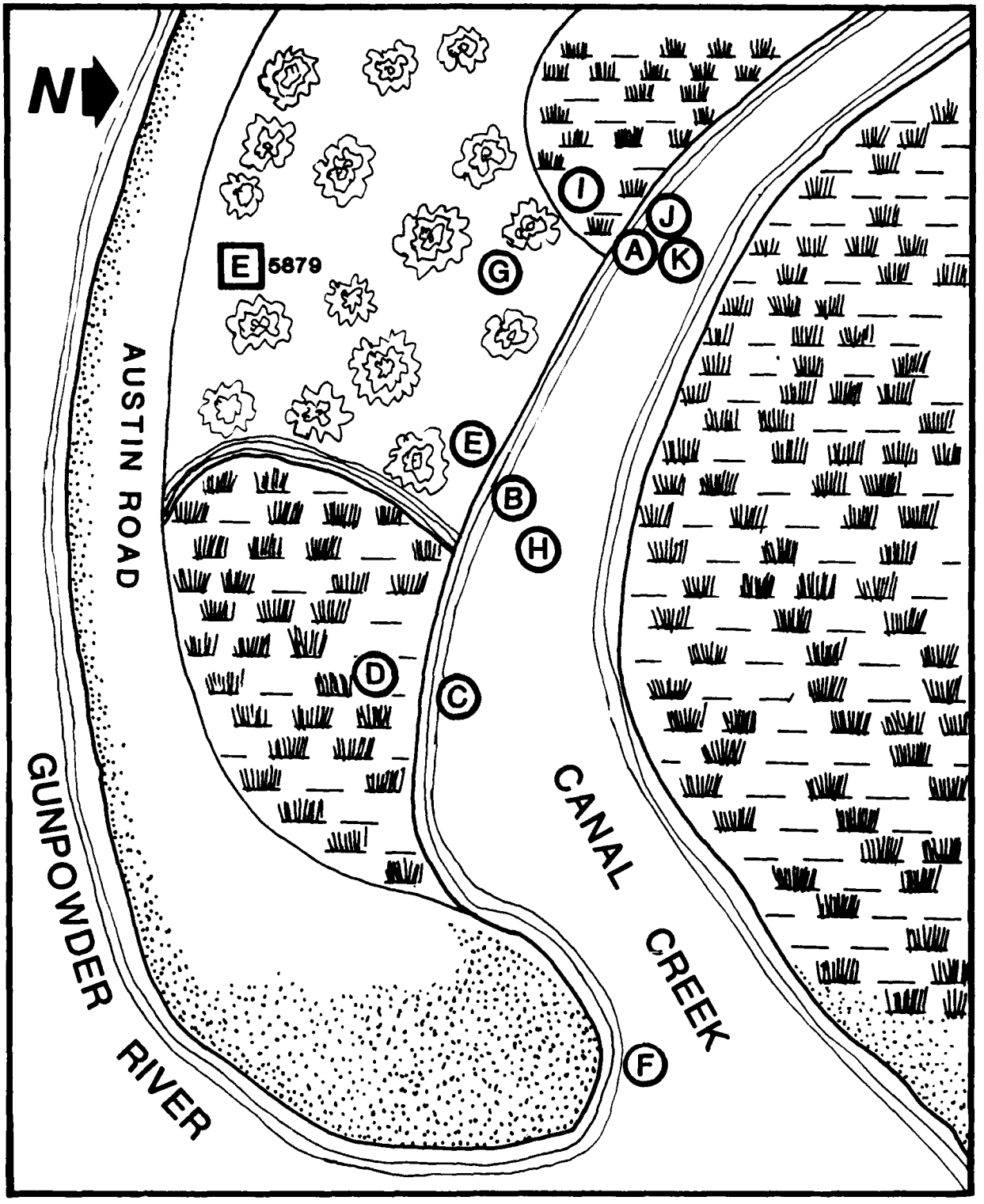


Figure 1. Map showing areas where sediment and soil samples were taken in November 1981.

TABLE 5. CONCENTRATION OF N,N'-BIS(2,4,6-TRICHLOROPHENYL)UREA FOUND IN DRY CANAL CREEK SEDIMENTS BY HPLC ANALYSIS

Sample	N,N'-Bis(2,4,6-trichlorophenyl)urea (µg/gram)
A - top 2"	314
A - 2"-3"	50
A - 3"-4"	65
A - 4"-5"	11
A - 5"-6"	<5
A - 6"-7"	<5
A - 7"-8"	<5
A - 8"-9"	<5
A - 9"-10"	<5
A - 10"-11"	<5
B	Not Analyzed
C - top 2"	118
C - 2"-3"	170
C - 3"-5"	82
C - 5"-6"	9
C - 6"-7"	<5
D - top 2"	106
D - 2"-4"	35
D - 4"-5"	5
D - 5"-7"	<5
D - 7"-9"	<5
D - 9-10.5"	<5
E	<5
F	12
G	<5
H	<5
I	83
J	139
K	213

DISCUSSION

N,N'-Bis(2,4,6-trichlorophenyl)urea is present in the sediments of Canal Creek in the Edgewood area of Aberdeen Proving Ground, MD. It appears that the highest concentrations are found in the top 2 inches of sediment, (100-300 µg/g), and that the concentration declines with increasing depth. Below a 6-inch depth there is little or no contamination. Although the detection limit of the HPLC method described here is 5 µg/g of dry sediment, modification of this method (i.e., increasing sample size or concentration of extract) to somewhat lower levels is possible. The chemical literature gives little information concerning this material, but does indicate a very high stability to chemical hydrolysis. The solubility in water is quite low (82 µg/L at 22°) and the estimated octanol/water partition coefficient is high (log K_p >5). The conjunction of these properties points to long persistence of the compound in the environment. Currently available data would not suggest that N,N'-bis(2,4,6-trichlorophenyl)urea be considered extremely toxic; nor is there a likelihood that human beings would experience heavy exposures to this compound.

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