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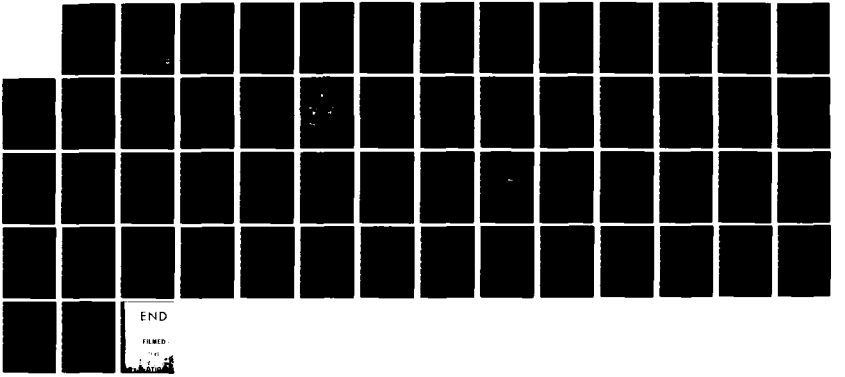

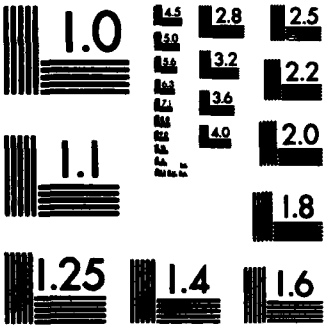

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TECHNICAL REPORT 8202

THE ENVIRONMENTAL FATE OF 2,4,6-TRICHLOROANILINE CHEMICAL AND PHYSICAL PATHWAYS

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JANUARY 1983

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Analysis	Pyrolysis
Environment	Solubility
Fate	Trichloroaniline
N,N'-bis(trichlorophenyl) urea	Vapor pressure
Photolysis	2,4,6-Trichloroaniline

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

2,4,6-Trichloroaniline (2,4,6-TCA) is not a contaminant of the estuarine sediments of the Edgewood Arsenal area of the Aberdeen Proving Ground (APG), MD. This conclusion was reached through a study of the environmental fate of 2,4,6-TCA and by direct analysis of sediment samples. 2,4,6-TCA is soluble in water (mg/L) to the extent of 32 mg/L at 19°C, and is degraded by sunlight. The compound is also lost rapidly by evaporation from water. Analysis of sediments by gas chromatography/mass spectrometry showed two major substances;

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20. Abstract (continued)

2,4,6-trichloroaniline and 2,4,6-trichlorophenylisocyanate, in nearly equal concentrations. It was found that these substances arise by pyrolysis of N,N'-bis(2,4,6-trichlorophenyl) urea. This occurs when the urea is injected into the hot (250°C) injection port of the gas chromatograph. The presence of N,N'-bis(2,4,6-trichlorophenyl) urea was also confirmed by high pressure liquid chromatography.

ACKNOWLEDGMENTS

The authors wish to acknowledge the technical assistance of Mr. Alan B. Rosencrance and Ms. Theresa Trybus for the gas chromatographic analyses and of Mr. Ernst Bruggemann for his development of a high pressure liquid chromatographic analysis of N,N'-bis(2,4,6-trichlorophenyl) urea. We also thank Dr. Wayne Mitchell for obtaining the Canal Creek (APG) mud sample that led to our discovery of N,N'-bis(trichlorophenyl) urea in this sediment. Finally, we acknowledge the help of Dr. David H. Rosenblatt in initiating this work and in acting as liaison between USATHAMA and ourselves during this study.

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INTRODUCTION

The US Army Toxic and Hazardous Materials Agency (USATHAMA) requested that the US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) undertake an environmental fate study of the compound 2,4,6-trichloroaniline in October 1980. Evidence from contractors[1] for USATHAMA indicated that the aquatic sediments in certain areas of the Gunpowder River, adjacent to Aberdeen Proving Ground, MD (APG), were contaminated with this compound. The level of 2,4,6-trichloroaniline was believed to range from 1 to 40 parts per million and to contaminate an area of 15 to 20 square miles (Fig. 1). Our task was to determine the physical properties of 2,4,6-trichloroaniline and to determine the chemical changes it might be expected to undergo in the aqueous environment. This environmental fate study of 2,4,6-trichloroaniline included the assessment of photolysis and oxidation as possible pathways of chemical transformation. It also addressed physical properties such as solubility in water, octanol/water partition coefficient, volatility (or partial pressure) of the substance in water and its affinity for aquatic sediments.

Late in this study, during an analysis of sediments from the contaminated area, we found that N,N′-bis(2,4,6-trichlorophenyl) urea, not 2,4,6-trichloroaniline, was a major organic component of the sediments. Evidence for the true contaminant in the sediments, in addition to our increased understanding of the volatility of 2,4,6-trichloroaniline in water and its susceptibility to photodegradation, led us to abandon the pursuit of 2,4,6-trichloroaniline as an environmental pollutant, and to embark on the study of N,N′-bis(2,4,6-trichlorophenyl) urea.

OBJECTIVE

The purpose of this report is to present the chemical and physical behavior of 2,4,6-trichloroaniline in the role of a pollutant in the aqueous environment, and to present evidence showing that N,N′-bis(trichlorophenyl) urea, not 2,4,6-trichloroaniline, is present in the estuarine sediments of Canal Creek, APG, and probably in sediments of the Gunpowder River, into which Canal Creek discharges.

RESULTS

PURIFICATION AND CHARACTERIZATION OF 2,4,6-TRICHLOROANILINE (2,4,6-TCA)

One hundred grams of 2,4,6-TCA were obtained from the Aldrich Chemical Company. The compound, recrystallized from hot methanol, had mp 76-77°C and was pure by gas chromatography (Fig. 2). The ultraviolet (UV) spectrum of 2,4,6-TCA in water showed two maxima, 245 nm ($\log \varepsilon = 3.932$) and 306 nm ($\log \varepsilon = 3.446$) (Fig. 3). The NMR spectrum in $CDCl_3$ (Fig. 4) showed a singlet at δ 6.9 (aromatic H) and a broad absorption at δ 4.0 to 4.2 ($-NH_2$); the ratio of these was 1:1. The IR spectrum (Fig. 5, KBr pellet) matched a reference spectrum. The mass spectrum of the recrystallized compound is shown in Figure 6.

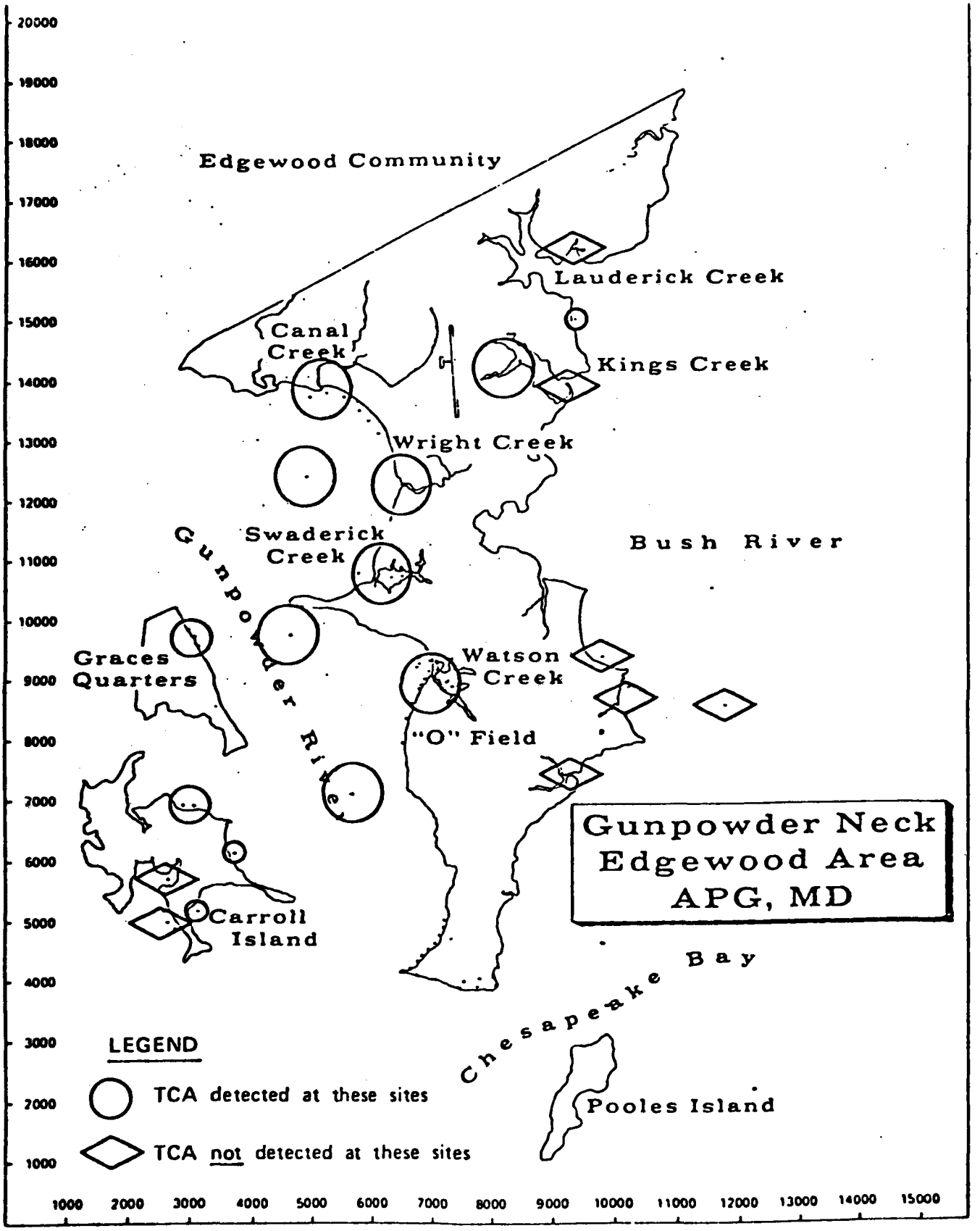


Figure 1. Area believed to be contaminated by 2,4,6-TCA.

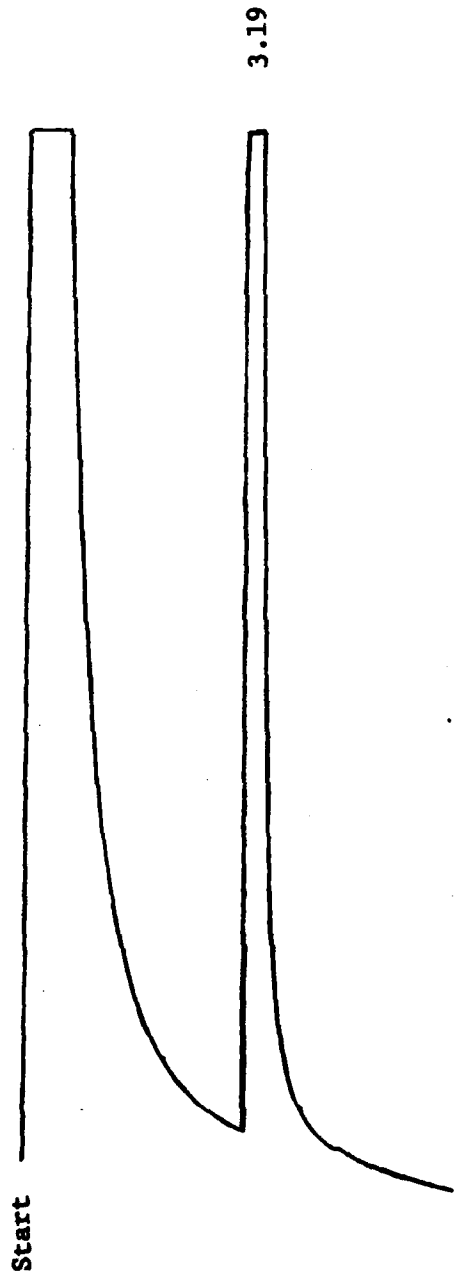


Figure 2. Gas chromatogram of recrystallized 2,4,6-trichloroaniline. GC conditions: 3% OV-1 on Gas CHROM Q, 120° to 150°C at 10°/min.

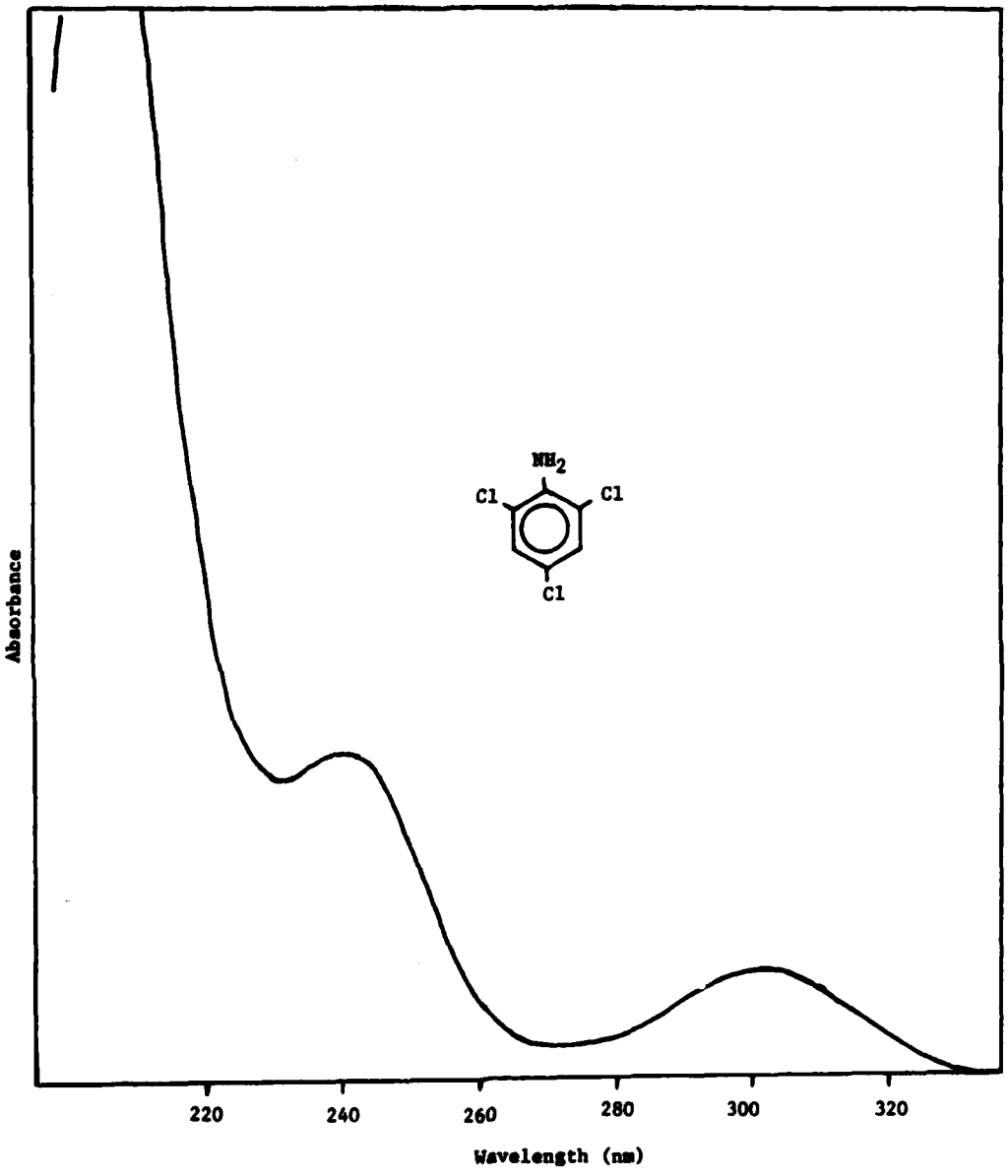


Figure 3. UV spectrum of aqueous 2,4,6-TCA.

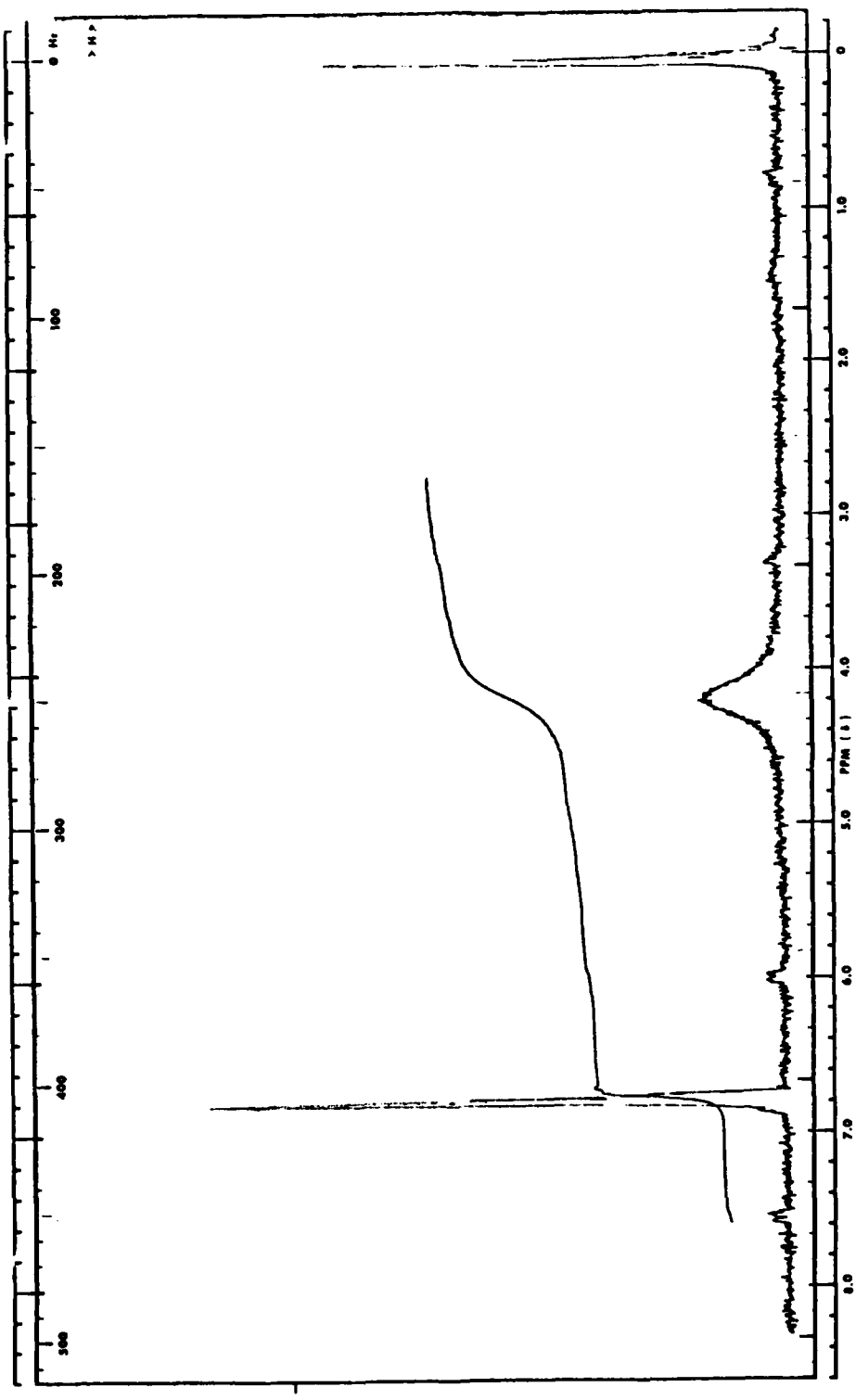


Figure 4. NMR Spectrum of 2,4,6-TCA.

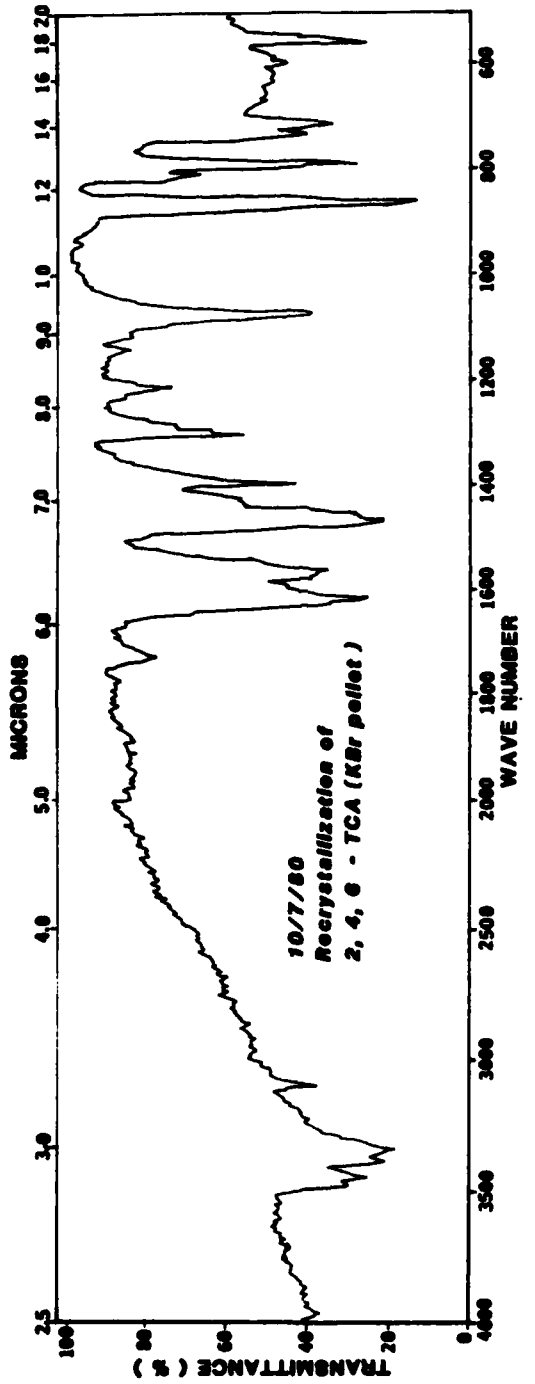
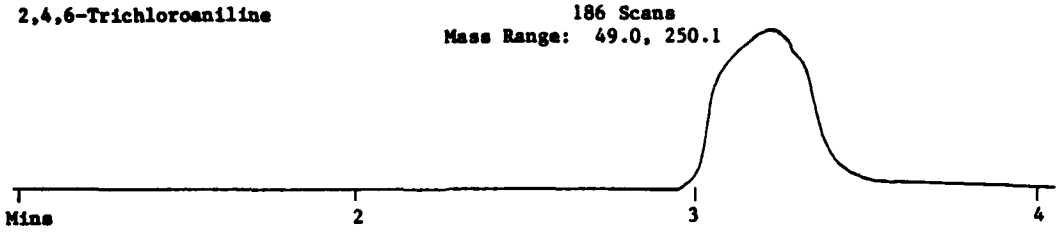
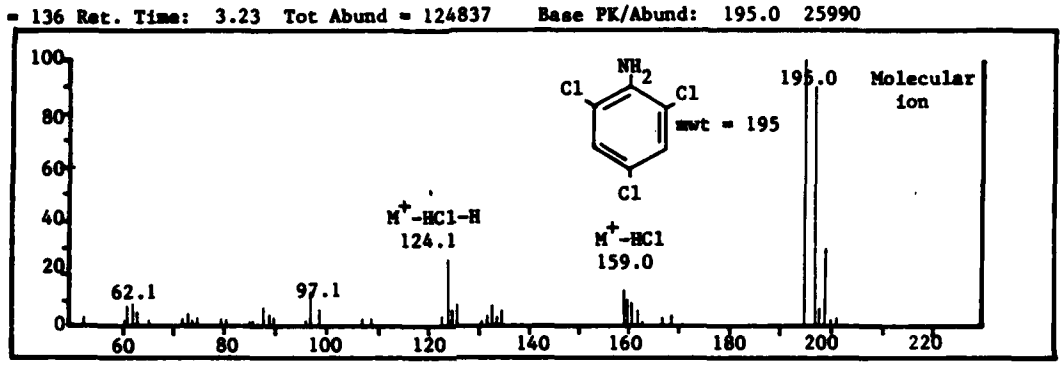


Figure 5. Infrared spectrum of purified 2,4,6-TCA.



= 136 Ret. Time: 3.23 Tot Abund = 124837 Base PK/Abund: 195.0 25990



FRN 7001, Spectrum # 136 Ret. Time: 3.23, 134 Peaks

M/Z	REL ABUND	M/Z	REL ABUND	M/Z	REL ABUND	M/Z	REL ABUND
61.1	6.9	124.1	25.5	159.0	13.8	197.0	92.2
62.1	8.4	125.1	6.3	160.0	10.8	198.0	7.4
88.1	7.8	126.1	8.5	161.0	9.3	199.0	30.1
97.1	12.6	133.1	8.9	162.0	7.0		
98.9	6.4	135.0	6.1	195.0	100.0		

Figure 6. Mass spectrum of purified 2,4,6-TCA.

ANALYSIS OF 2,4,6-TRICHLOROANILINE IN WATER

A gas chromatographic method (FID) was devised for the analysis of aqueous solutions of 2,4,6-TCA. The method is simple, rapid and sensitive for aqueous solutions as low as 1 ppm. Figure 7 shows an example of such a chromatogram, and the conditions for analysis. Analysis was carried out isothermally at 165°C with pentadecane as an internal standard. To determine the concentration of 2,4,6-TCA in water, a standard curve was prepared from aqueous solutions of 2,4,6-TCA. Response (peak area TCA/peak area internal standard) was plotted versus 2,4,6-TCA concentration. A more detailed description of this procedure is given in Appendix A.

The characteristic UV spectrum of TCA (see above) also allowed us to determine its concentration in aqueous solutions where there were no interfering substances. Ultraviolet analysis was of particular use in determining the solubility of 2,4,6-TCA in water. The absorptivity at 240 nm was a linear function of concentration between 0 and 30 mg/L TCA.

STABILITY OF 2,4,6-TRICHLOROANILINE IN WATER

Three aqueous solutions of 2,4,6-trichloroaniline (30 mg/L) were separately buffered to pH 5, 7, and 9 (10^{-3} M acetate, phosphate and borate, respectively) and stored in stoppered volumetric flasks in the dark at room temperature. Ultraviolet scans on the day of preparation and at intervals of 1 and 2 weeks showed no significant changes. This indicates that 2,4,6-TCA in water is not hydrolyzed or oxidized over a 2-week period under ambient conditions in the absence of light.

SOLUBILITY OF 2,4,6-TCA IN WATER

Excess 2,4,6-TCA was added to flasks of buffered, glass-distilled water (10^{-3} M at pH 5, 7, and 9). The mixtures were stirred 24 hr in a constant temperature bath, filtered quickly by suction and the filtrates analyzed in a 1 cm quartz cuvette for 2,4,6-TCA by UV scans at 244 nm on a Beckmann Acta CV spectrophotometer. For a given temperature, the solubility was nearly the same at each pH. Table 1 shows the solubility of 2,4,6-TCA measured at various temperatures.

TABLE 1. SOLUBILITY OF 2,4,6-TCA IN WATER AT FOUR TEMPERATURES

Temperature	Solubility of 2,4,6-TCA in water in mg/L		
	pH 5.0	pH 7.0	pH 9.0
32°C	45.9	46.1	46.2
19°C	31.2	31.7	33.2
10°C	22.4	22.5	22.7
5°C	19.8	20.5	21.5

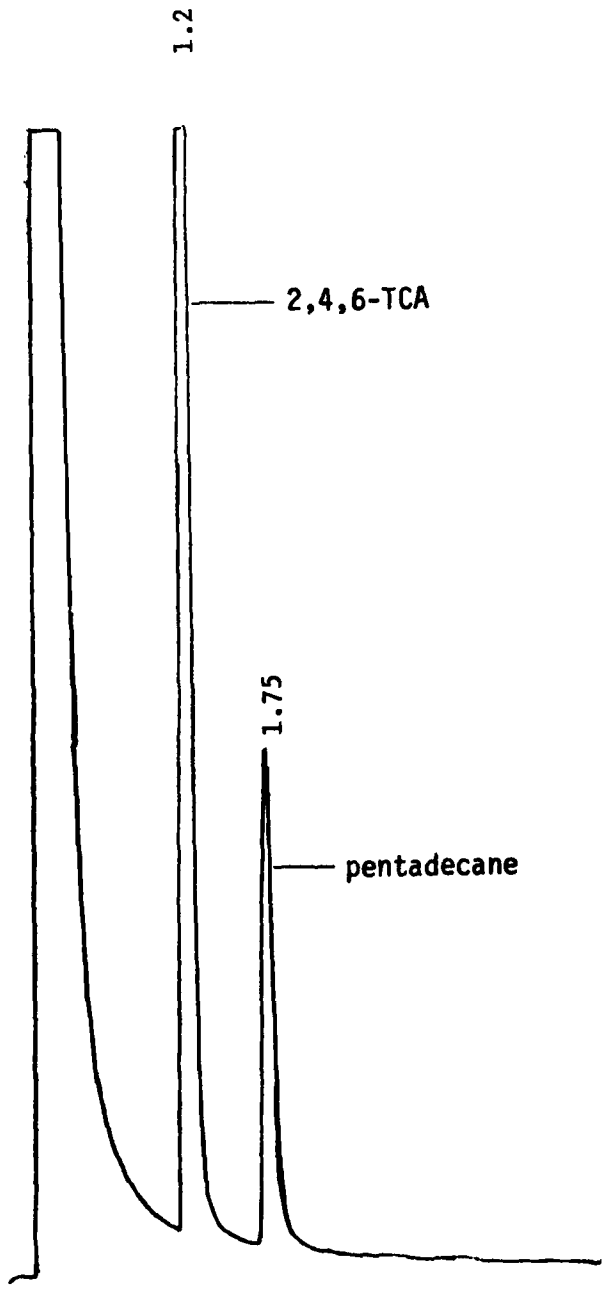


Figure 7. Gas chromatogram of a CH_2Cl_2 extract of an aqueous solution of 2,4,6-TCA. Pentadecane is the internal standard and the GC conditions are: 6′, 3% OV-1 on 80/100 mesh GAS CHROM Q, 165°.

PHOTOLYSIS OF 2,4,6-TRICHLOROANILINE IN WATER WITH A FILTERED UV LAMP

A preliminary experiment was performed to determine the behavior of a 10 mg/L aqueous solution of 2,4,6-TCA on exposure to artificial sunlight. The solutions were sealed in 5 mL Vacules® * and exposed to a Pyrex-filtered 450 watt mercury-vapor lamp for 20, 45, 69, and 141 hours. After 20 hours, the 2,4,6-TCA had disappeared (GC analysis) and the solution had become yellow. Between 45 and 69 hours, the yellow color had faded and the solution became colorless after 141 hours. None of the photolyzed samples contained 2,4,6-TCA. Photolysis of an unbuffered solution of 2,4,6-TCA showed an increase in alkalinity (pH 8).

A more definitive study was made with a 30 mg/L solution of 2,4,6-TCA buffered at pH 7 with 10^{-3} M phosphate. Aliquots (5 mL) were again sealed in Vacules® and placed in a "Merry-Go-Round" apparatus (see Fig. 8). Accompanying these samples were sealed solutions (10 mg/L) of 2,4,6-trinitrotoluene (2,4,6-TNT), which is known to photolyze in water. Thus the rate of photolysis of 2,4,6-TCA relative to that of 2,4,6-TNT was determined. Exposed solutions were removed from the apparatus at different times and placed in a dark refrigerator. All samples were analyzed by gas chromatography at the termination of the experiment. Figure 9 shows the rate of degradation of 2,4,6-TCA relative to that of 2,4,6-TNT.

PRODUCT STUDY OF 2,4,6-TCA PHOTOLYSATE

An unbuffered aqueous solution (1 L) of 2,4,6-trichloroaniline (15 mg/L) was exposed overnight to the pyrex-filtered UV lamp. The aqueous photolysate (orange) was extracted with methylene chloride. The extract was concentrated by evaporation and analyzed by gas chromatography using 3 percent OV-1 at 240°C. Three major peaks were found, as shown in Figure 10. Gas chromatography/mass spectrometry (GC/MS) was used to further characterize the extract, and revealed four substances. Figures 11 through 14 show their mass spectra. The GC/MS results are summarized in Table 2.

TABLE 2. GC/MS ANALYSIS OF COMPOUNDS ISOLATED BY METHYLENE CHLORIDE EXTRACTION OF 2,4,6-TCA PHOTOLYSATE

Peak	M.W.	Formula
1	246	$C_{12}H_7ClN_2O_2$
2	246	$C_{12}H_7ClN_2O_2$
3	280	$C_{12}H_6Cl_2N_2O_2$
4	280	$C_{12}H_6Cl_2N_2O_2$

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

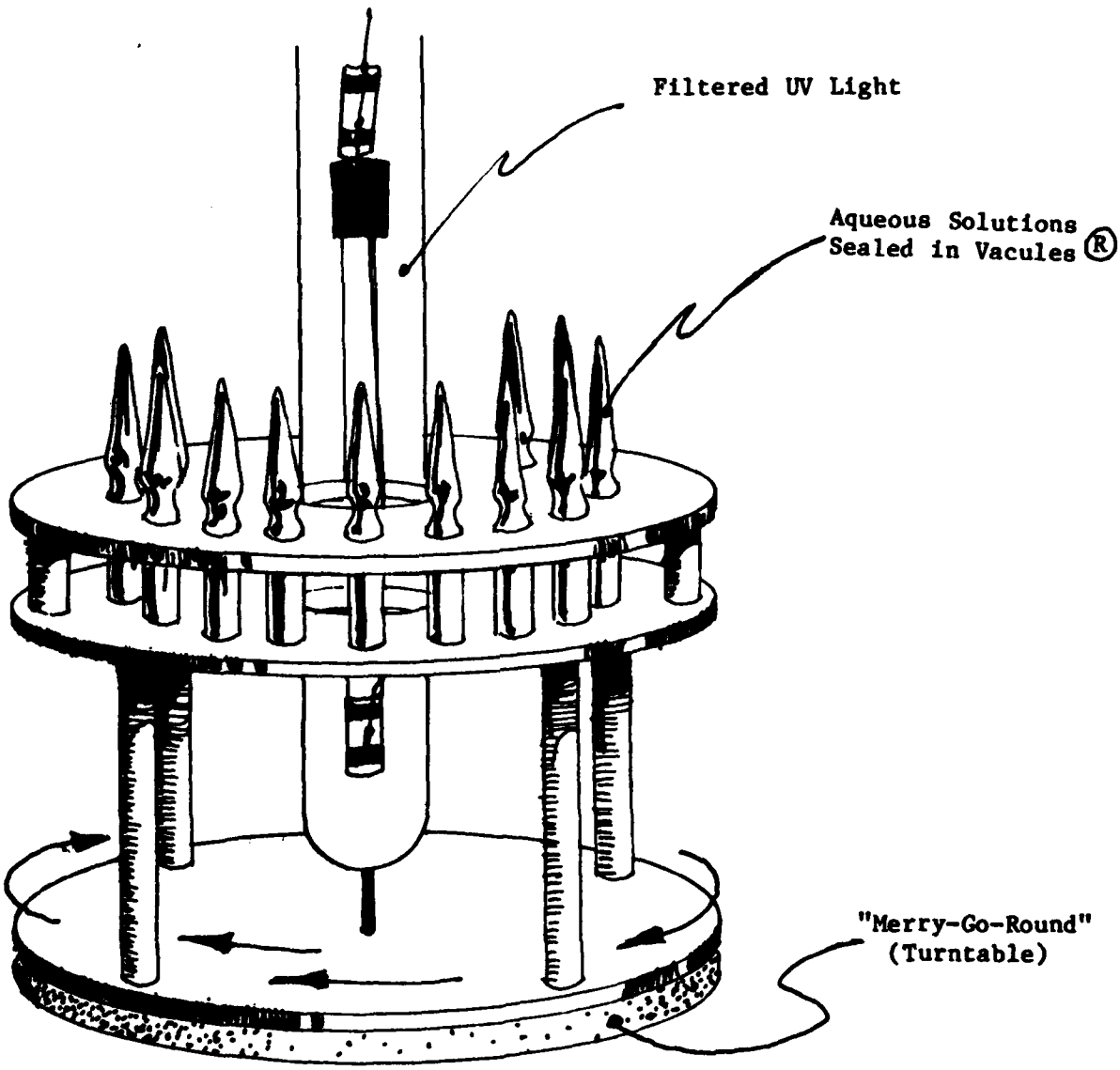


Figure 8. "Merry-Go-Round" apparatus for exposing sealed aqueous solutions to filtered UV light.

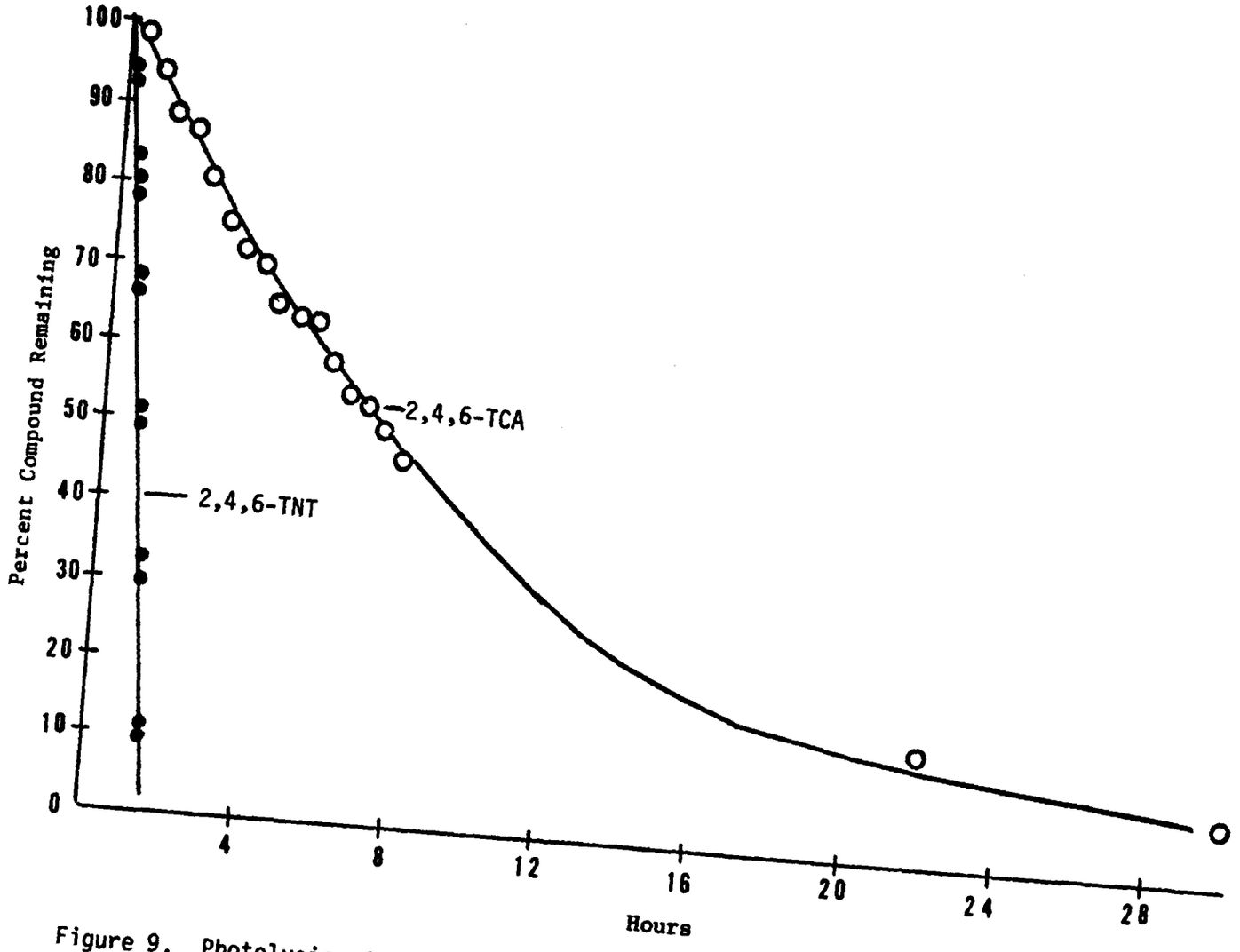


Figure 9. Photolysis of aqueous 2,4,6-TCA and 2,4,6-TNT at pH 7.

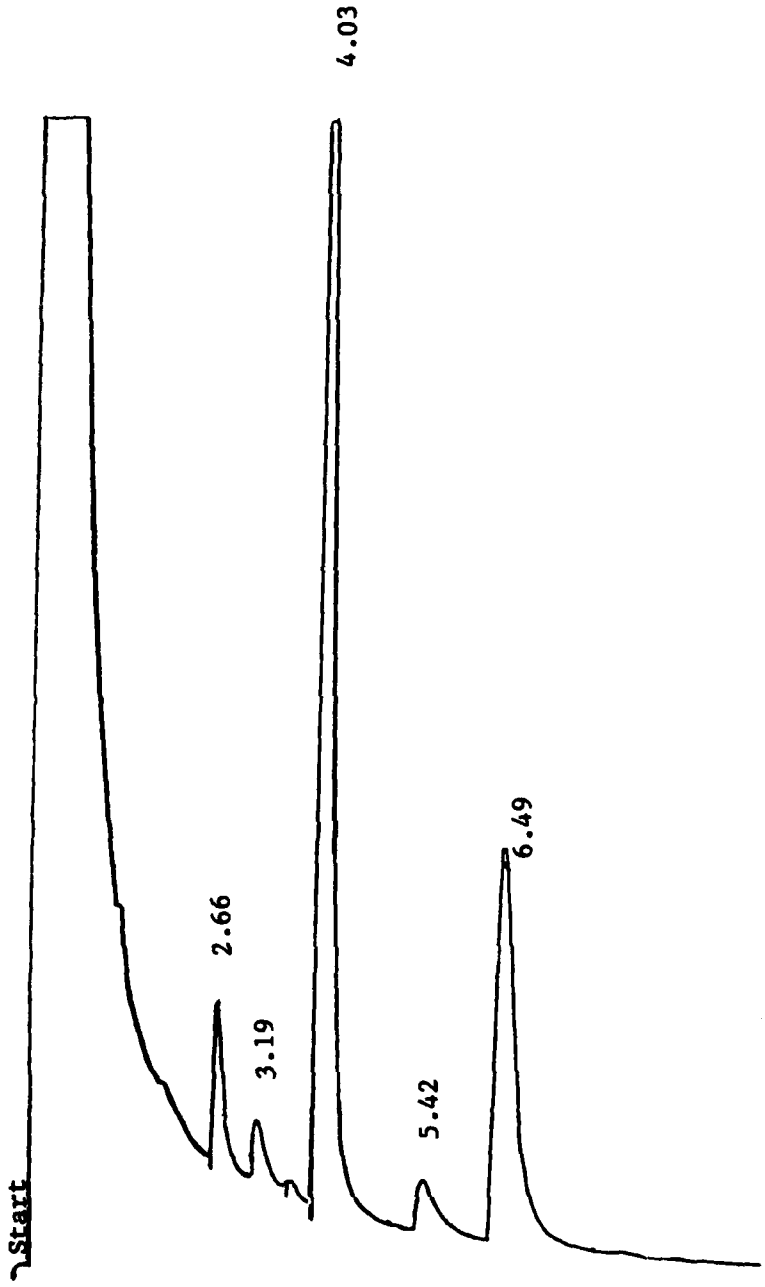


Figure 10. Gas chromatogram of methylene chloride extract of aqueous 2,4,6-TCA photolysate. 6', 3% OV-1 on 80/100 mesh GAS CHROM Q at 240°.

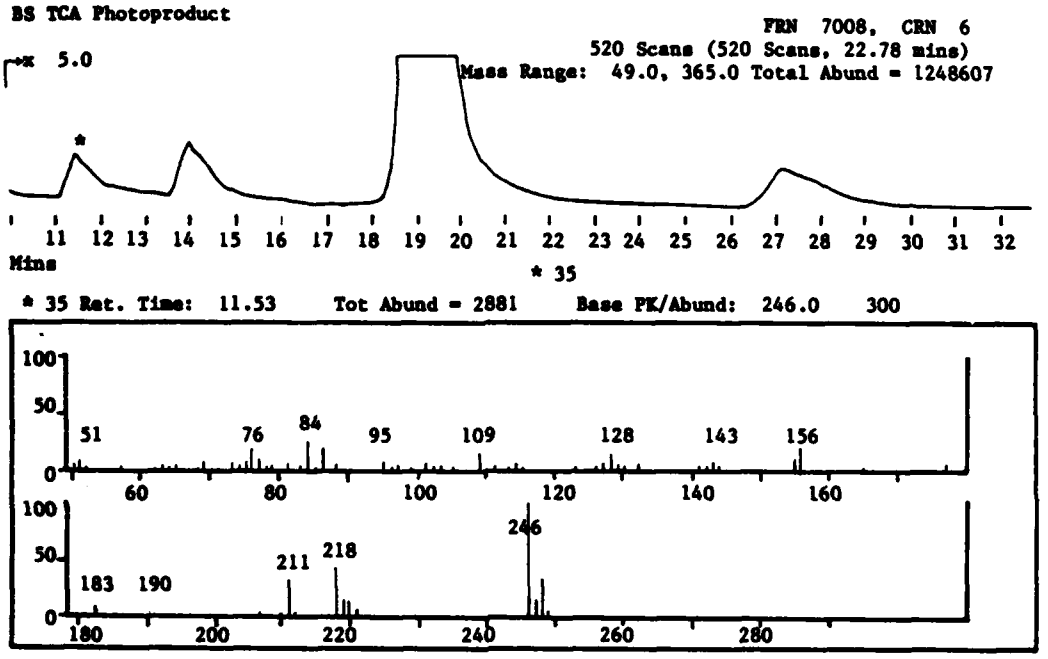


Figure 11. Mass spectrum of GC peak 1 from aqueous 2,4,6-TCA photolysate.

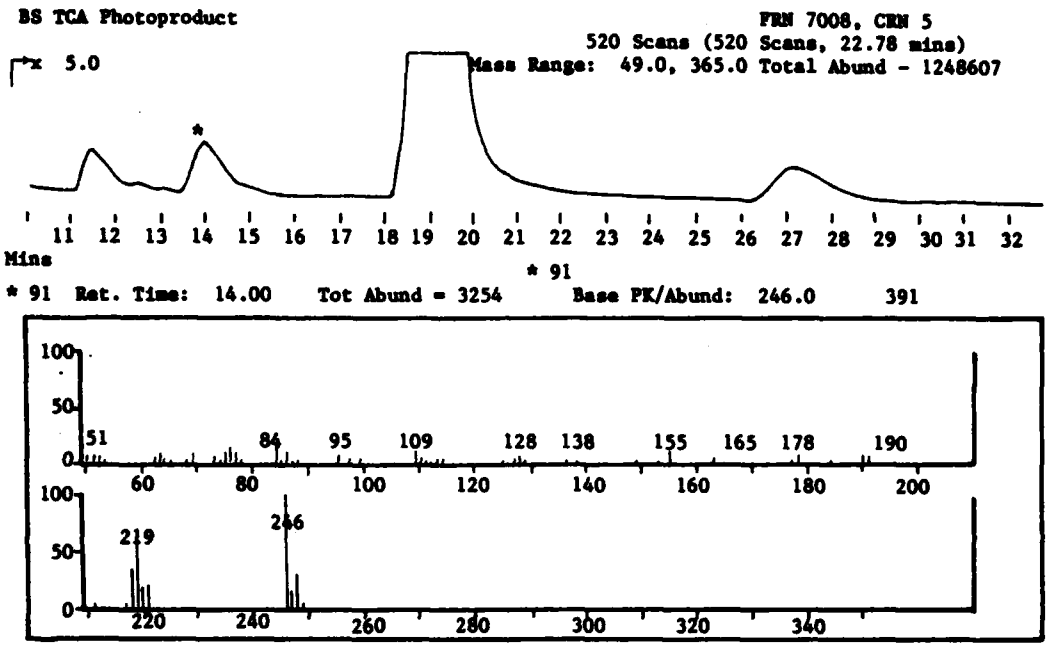


Figure 12. Mass spectrum of GC peak 2 from aqueous 2,4,6-TCA photolysate.

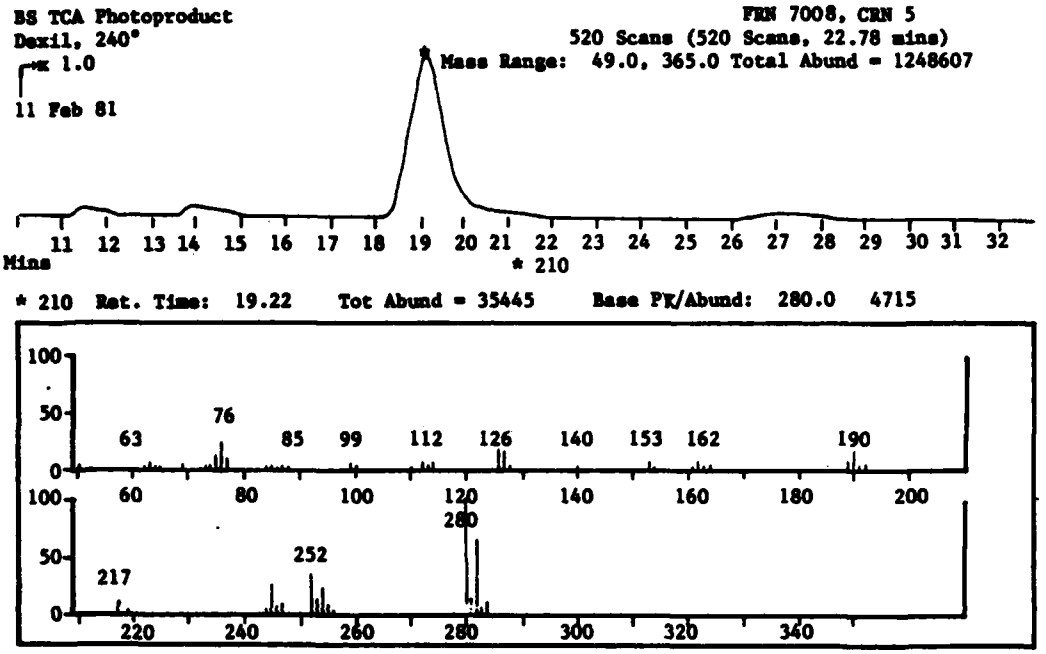


Figure 13. Mass spectrum of major GC peak 3 from aqeuous 2,4,6-TCA photolysate.

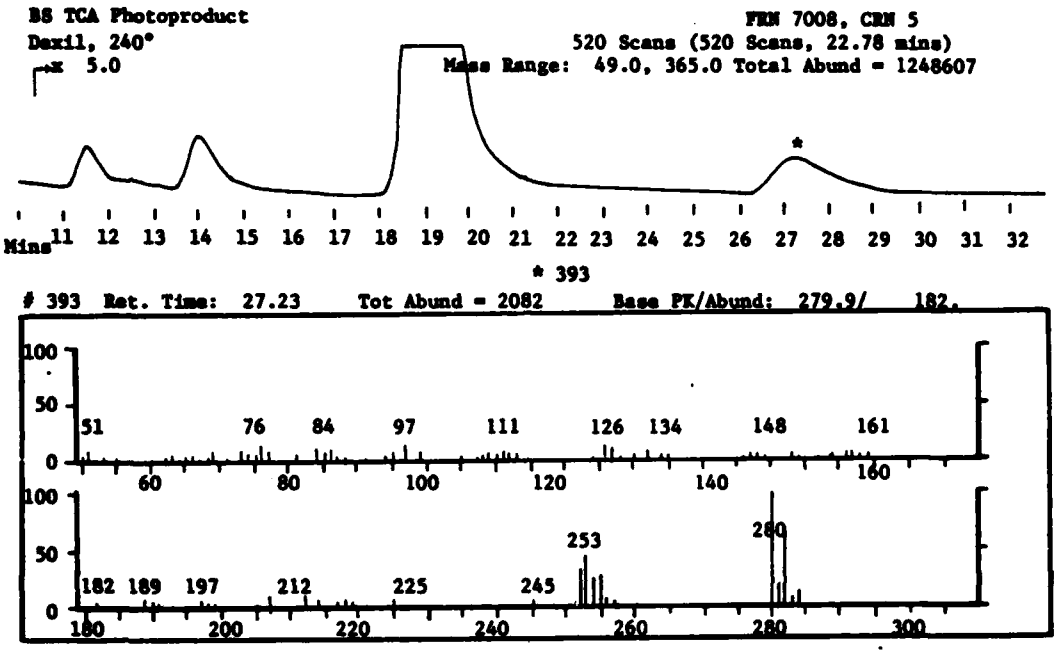


Figure 14. Mass spectrum of GC peak 4 from aqueous 2,4,6-TCA photolysate.

To isolate these compounds for further study, thin-layer chromatography (TLC) was used. The solvent system was acetone/hexane (45/55:v/v) on silica gel. Figure 15 shows a xerographic image of such a TLC plate. Four major bands were scraped from the silica gel plate and the scrapings extracted with ethyl acetate. After solvent evaporation, four red substances were isolated. Direct inlet probe mass spectra were run on each compound (see Figures 16 and 17); this data is summarized in Table 3.

TABLE 3. MASS SPECTROMETRIC ANALYSIS OF SUBSTANCES ISOLATED FROM TLC PLATE

Band	R_f	M.W.	Formula
1	0.76	314	$C_{12}H_5Cl_3N_2O_2$
2	0.70	280	$C_{12}H_6Cl_2N_2O_2$
3	0.68	280	$C_{12}H_6Cl_2N_2O_2$
4	0.45	246	$C_{12}H_7Cl\ N_2O_2$

In comparing Tables 2 and 3, it is evident that there are at least five compounds. The primary feature of all the mass spectra is m/e M-28; in some cases M-35 ions are observed. The M-35 ion is due to the loss of chlorine and the M-28 to loss of nitrogen. Structure II is a likely representation of the compound in Band 2 or 3.

The compound phenazine (I) contains two nitrogens and shows a M-28 peak on MS analysis (Fig. 18). This loss of nitrogen in phenazine and the observed photolysis products leads to a conclusion that the photolysis products may be substituted phenazines.

PHOTOLYSIS OF AQUEOUS 2,4,6-TCA IN SUNLIGHT

A pyrex volumetric flask containing 1 liter of 2,4,6-TCA (18.5 mg/L) in glass-distilled water was exposed to bright sunlight (mid-June) for a period of 42 hours. Samples of the solution were withdrawn at various times during this period and analyzed by gas chromatography. A sample withdrawn at zero time and stored in the dark served as control. Figure 19 shows the disappearance of 2,4,6-TCA from the solution as a function of time. After 42 hours, the solution was orange in color and contained a brown floc. The colored material could be extracted into ethyl acetate. Thin-layer chromatographic analysis of this extract showed it to be identical to the photolysate obtained from exposure of aqueous 2,4,6-TCA to a pyrex-filtered Hanovia lamp.

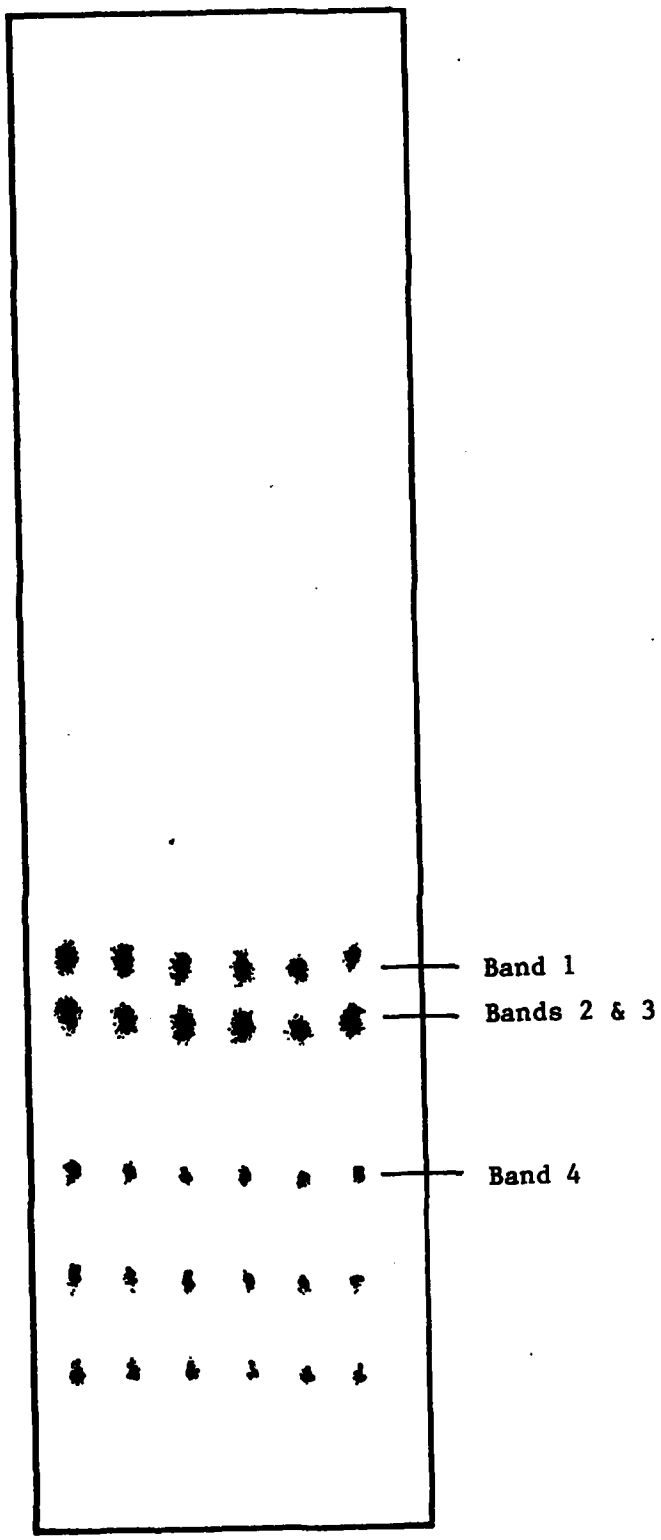


Figure 15. Xerox image of silica gel TLC plate after separation of aqueous photolysate.

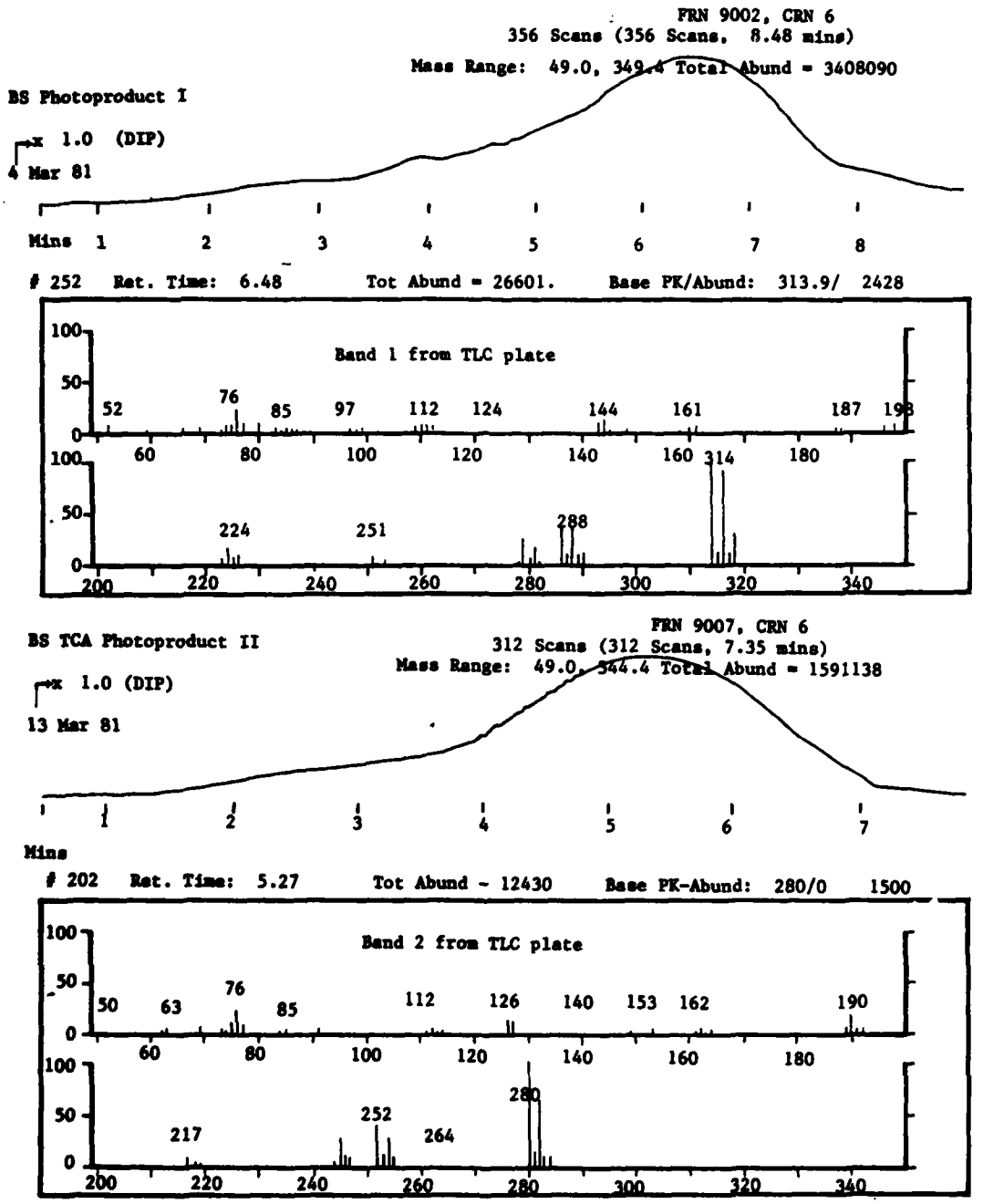


Figure 16. Direct inlet probe mass spectra of bands 1 and 2 scraped from TLC plate.

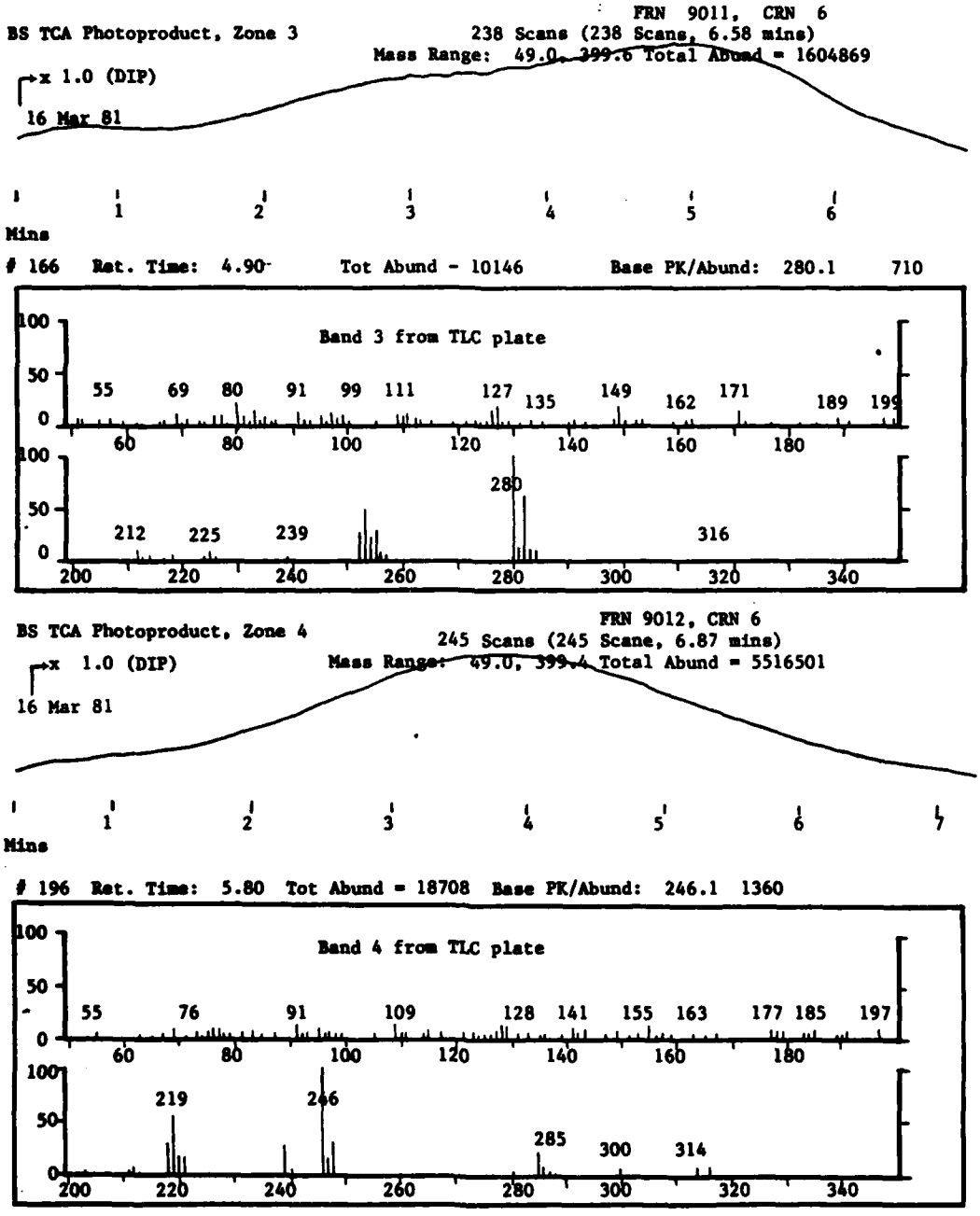
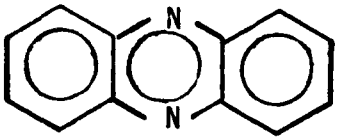
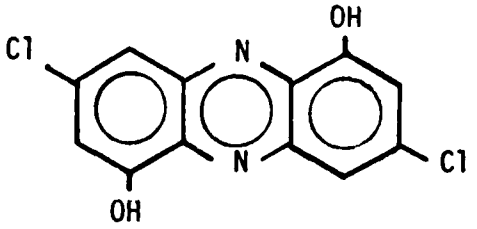


Figure 17. Direct inlet probe mass spectra of bands 3 and 4 scraped from silica gel TLC plate.



Phenazine (I)



m.wt. 280 (II)

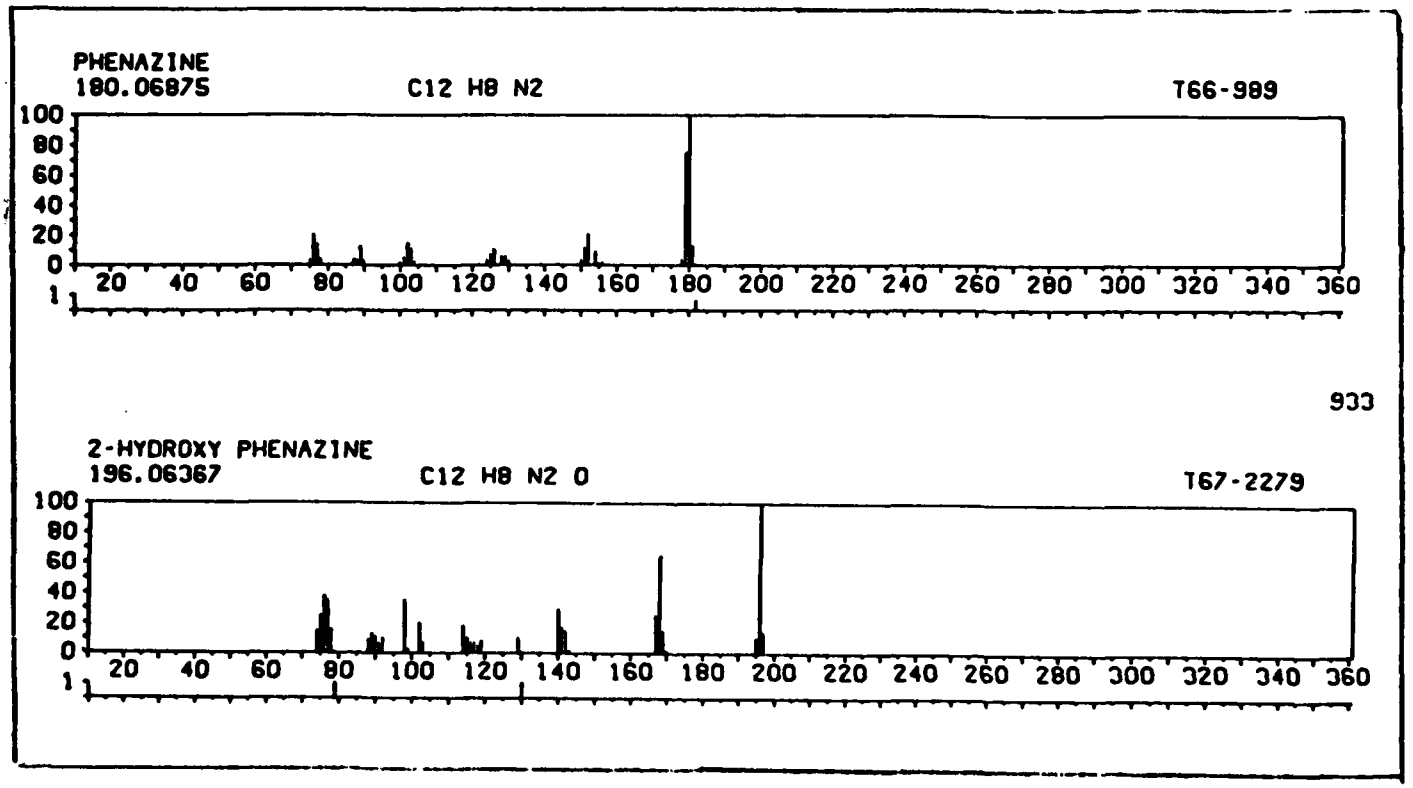


Figure 18. Mass spectra of phenazine and 2-hydroxyphenazine.

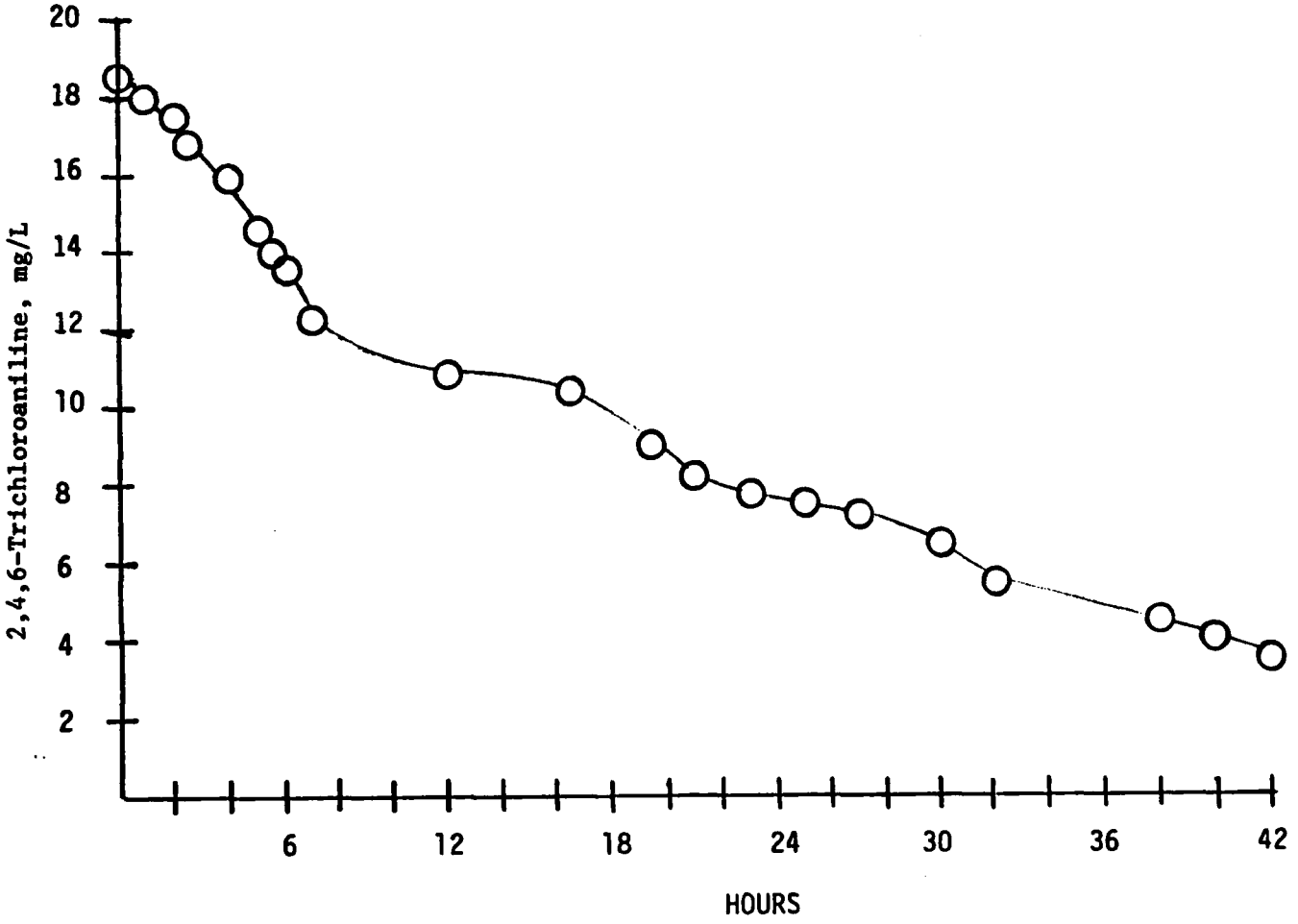


Figure 19. Loss of 2,4,6-TCA from an aqueous solution by photolysis in sunlight. Exposed intermittently to sunlight over a 7-day period.

OCTANOL/WATER PARTITION COEFFICIENT OF 2,4,6-TCA

Solutions of 2,4,6-TCA were prepared in aqueous buffers at pH 5, 7, and 9 and placed in separatory funnels. The partition was carried out between 1 mL of octanol and 200 mL of the aqueous 2,4,6-TCA solution. The flasks were shaken every 5 minutes for 1 hour, then allowed to separate into two phases. The resultant aqueous phase was assayed for 2,4,6-TCA by UV spectrometry. A blank of octanol-saturated water was used as a reference.[3]

In addition to this direct measurement, the octanol/water partition coefficient, K_p, for 2,4,6-TCA can be estimated in two ways: from the Hansch fragment constants and log K_p for other chloroanilines and chlorobenzenes,[4] and by empirically determined relationships between K_p and solubility in water.[5,6] A third estimation was made from the established linear correlation of log K_p with log HPLC retention time.[3] Table 4 shows a summary of the K_p values obtained by each of these methods.

TABLE 4. OCTANOL/WATER PARTITION COEFFICIENT OF 2,4,6-TCA

pH	Method	K_p	Avg K_p
7	Direct	1.09×10^4	
7	Direct	6.76×10^3	7.89×10^3
7	Direct	5.98×10^3	
9	Direct	5.20×10^3	
9	Direct	2.51×10^3	3.55×10^3
9	Direct	2.95×10^3	
5	Direct	4.90×10^3	
5	Direct	5.41×10^3	5.16×10^3
—	HPLC[3]	2.39×10^3	
—	(Ref 4)	$2.8\text{-}7.1 \times 10^3$	
—	(Ref 5)	6.1×10^2	
—	(Ref 6)	3.3×10^3	

VAPOR PRESSURE OF 2,4,6-TCA IN AQUEOUS SOLUTION

Our approach to measuring the partial pressure of organic compounds in water is based upon the absorptive character of TENAX, a porous polymer based on 2,6-diphenyl-p-phenylene oxide. Zlatkis, et al.[7] used TENAX for trapping volatile organic constituents of urine. We expected that 2,4,6-TCA could also be trapped by TENAX.

Two glass tubes (30 x 4 mm, 1.5-mm ID) were filled with TENAX ($^{60}/_{80}$ mesh) held in place by glass wool plugs and connected by a short piece of tygon tubing. The second tube was connected to a longer tube leading to an autoanalyzer pump. The train of tubes was placed into a glass sleeve and the sleeve inserted into a 250-mL jacketed flask (see Fig. 20) which contained 10 mL of an aqueous solution of ^{14}C-2,4,6-TCA (11.3 mg/L). The synthesis of the ^{14}C-TCA is described in Appendix B. Water from a constant temperature

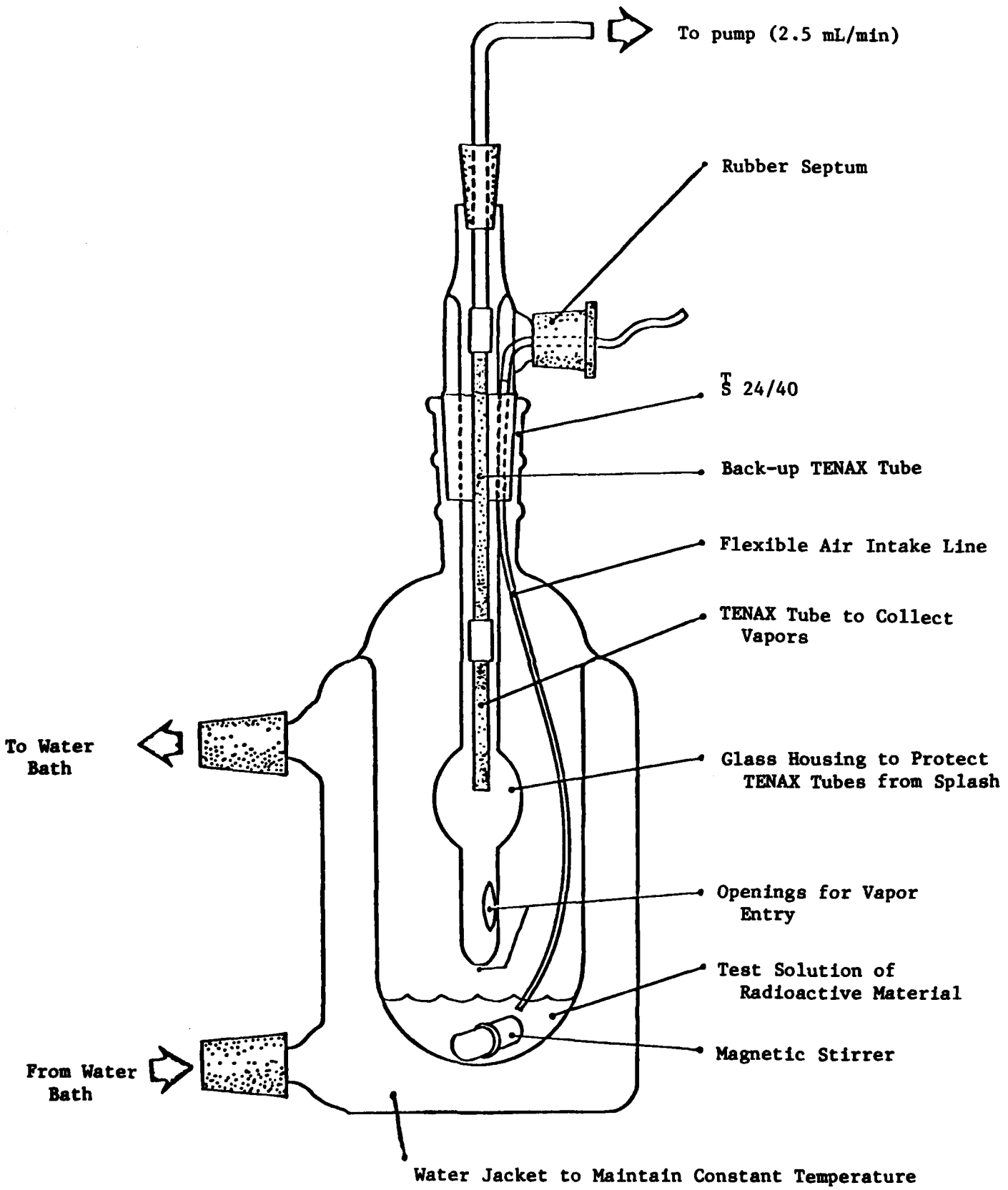


Figure 20. Apparatus designed for trapping solute vapor onto TENAX polymer from air. Solute in air is in equilibrium with aqueous solute.

bath was passed through the jacket of the flask to maintain constant temperature. Air was slowly withdrawn from the flask through the TENAX tubes (~1.95 mL/min) and was replaced with air from the outside via a flexible plastic tube. The flow of air from the apparatus was determined by displacement of water from an inverted volumetric flask. Flow was monitored at the beginning and end of each experiment. After overnight operation, the train of tubes was withdrawn and the contents of each tube placed in a scintillation vial with 10 mL of scintillation fluid (Instagel) for determination of radioactivity as counts per minute (CPM). All ^{14}C activity was found in the first tube exposed to the vapors within the flask; the back-up trap contained no ^{14}C. From the ^{14}C activity absorbed on TENAX and the volume of air withdrawn, the partial pressure of 2,4,6-trichloroaniline was calculated and was found to be 4.48×10^{-7} atm at 20.5°C. This yielded a Henry's Law constant of 4.1×10^{-1} atm. A duplicate determination at 20.0°C gave a partial pressure of 3.88×10^{-7} atm.

A sample calculation is shown below:

1. Concentration of ^{14}C-2,4,6-TCA in solution = 9.11 mg/L at 20.5°C

2. Activity of ^{14}C-2,4,6-TCA was 1 µg = 1,286 DPM

3. Flow rate through apparatus was 1.95 mL/min

4. Running time was 16 hours

	CPM	Counting Efficiency	DPM
TENAX Tube 1	8,230	0.919	8,956
TENAX Tube 2	22	0.733	30
Background	20	0.880	23

5. µg ^{14}C-2,4,6-TCA in TENAX Tube 1 = $\frac{8,933 \text{ DPM}}{1,286 \text{ DPM/µg}}$ = 6.95 µg

6. Liters of air drawn through the TENAX trap = (0.00195 L/min) (16 hr) (60 min/hr) = 1.872 L

7. Moles of 2,4,6-TCA trapped from 1.872 L air =

$$\frac{6.95 \times 10^{-6} \text{ g TCA}}{196.5 \text{ g/mole}} = 3.54 \times 10^{-8} \text{ mole}$$

8. Moles of TCA in 1 L of air would be:

$$\frac{3.56 \times 10^{-8} \text{ mol}}{1.872 \text{ L air}} = 1.9 \times 10^{-8} \text{ mole TCA/L air}$$

9. Assume that 1 mole TCA vapor = 23.6 L, then:

$(1.9 \times 10^{-8}$ mole/L air) (23.6 L TCA vapor/mole) = 4.48×10^{-7} L TCA vapor per L air

10. The partial pressure of TCA at 20.5°C is therefore 4.26×10^{-7} atm

11. The mole fraction of TCA (X_{TCA}) in solution is:

$$X_{TCA} = \frac{\text{moles TCA}}{\text{moles water}} = \frac{5.8 \times 10^{-5} \text{ mole/L}}{55.55 \text{ moles/L}} = 1.04 \times 10^{-6}$$

12. Henry's Law states:[8]

$$H = \frac{P_{TCA}}{X_{TCA}} = \frac{4.26 \times 10^{-7} \text{ atm}}{1.04 \times 10^{-6}} = 0.41 \text{ atm}$$

2,4,6-TCA has considerable volatility in water. In fact, it was possible to observe visually the disappearance of 2,4,6-TCA from a dilute solution in the following experiment:

A 10 mg/L aqueous solution of 2,4,6-TCA was placed in a crystallizing dish (18.4 cm diameter) at a depth of 1 cm. The dish and contents were weighed and then placed in a fume hood. A gentle breeze over the uncovered dish kept the solution temperature at 17°C. The liquid was not stirred. Periodically, the dish was removed and weighed. Water loss due to evaporation was replaced with distilled water. After reconstitution, a portion of the solution was removed and scanned in a UV spectrophotometer to determine the concentration of 2,4,6-TCA. After each UV scan, the dish and contents were returned to the fume hood. Figure 21 shows the superimposed scans of this solution taken over an 8-hour period. This experiment was <u>not</u> performed to determine partial pressure, but only to demonstrate clearly how rapidly 2,4,6-TCA is lost from an aqueous solution.

DISCOVERY OF N,N'-BIS(2,4,6-TRICHLOROPHENYL) UREA IN APG SEDIMENTS

One of the last tasks scheduled was to measure the adsorption isotherm of 2,4,6-TCA between water and sediments from the Canal Creek area of APG. The sediment examined was collected as a grab sample for use in a planned microbial degradation study.[9] In preparation for the adsorption isotherm, a portion of the mud was dried at 105°C. The dry material was broken up with a steel spatula and passed through a 20-mesh screen. Only the material under 20 mesh was characterized.

To assess the organic content of the sediment, three 1-gram samples were fired in a muffle furnace at 500°C for 30 minutes. The loss in weight is considered a rough measure of organic content. The dry sediment lost 7.27 percent (mean) in weight (std. dev. = 0.16%).

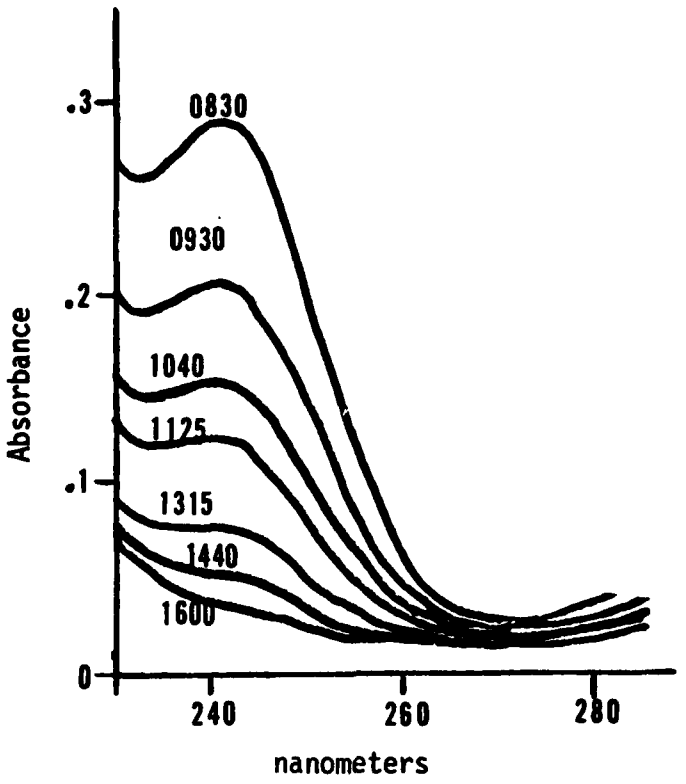
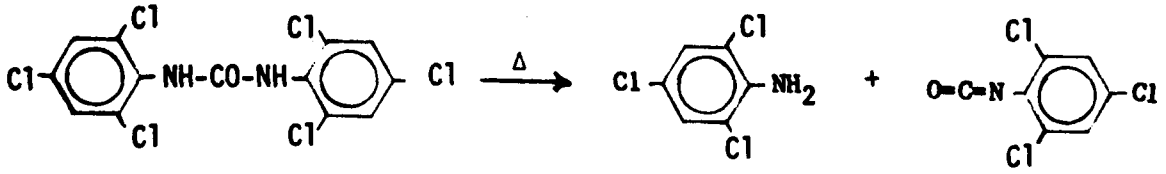


Figure 21. UV scans of a solution of 2,4,6-TCA exposed to air for 8 hr.

A 1.367 g sample of sediment (dried at 105°C) was also shaken for 30 minutes (ultrasonic bath) with 5 mL of chloroform. The extract was centrifuged and the supernatant $CHCl_3$ analyzed by GC and by GC/MS. GC showed the presence of two major peaks of nearly equal peak area (see Fig. 22). These substances proved to be 2,4,6-TCA and 2,4,6-trichlorophenylisocyanate (Fig. 23). Since the latter compound is readily hydrolyzed in water, it could not have been present as such in the wet sediment. Our interpretation of this finding is that N,N'-bis(2,4,6-trichlorophenyl) urea is actually present in the mud, and was extracted into chloroform. Upon injection of the extract onto the GC column (injection port temperature 250°C) this substance would pyrolyze as follows:



The pyrolysis of N,N'-disubstituted urea derivatives is known to occur.[10]

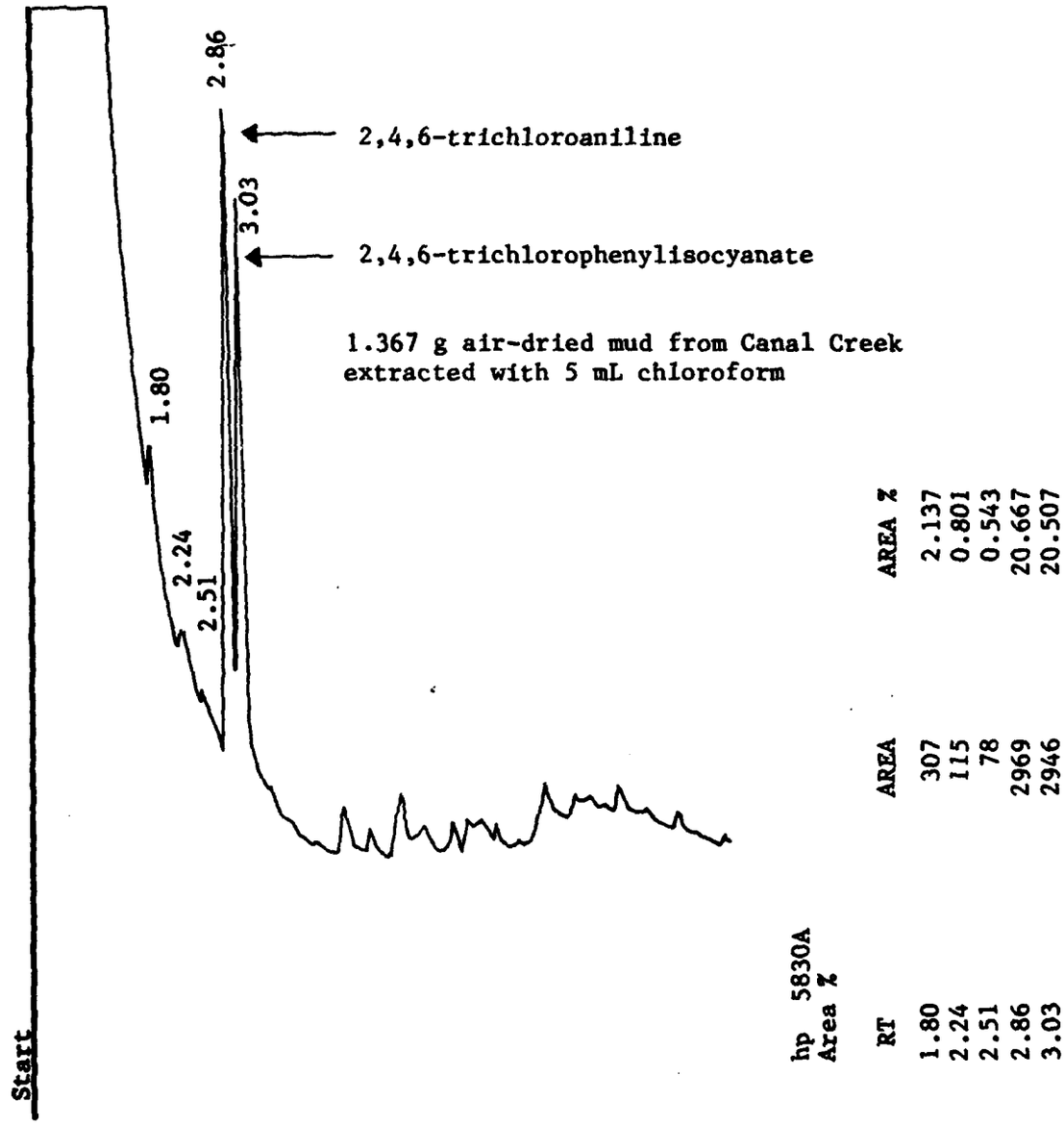


Figure 22. Gas chromatogram of a chloroform extract of Canal Creek mud. GC conditions: 6', 3% OV-1 on 80/100 mesh GAS CHROM Q, 110-240°C at 15°/min.

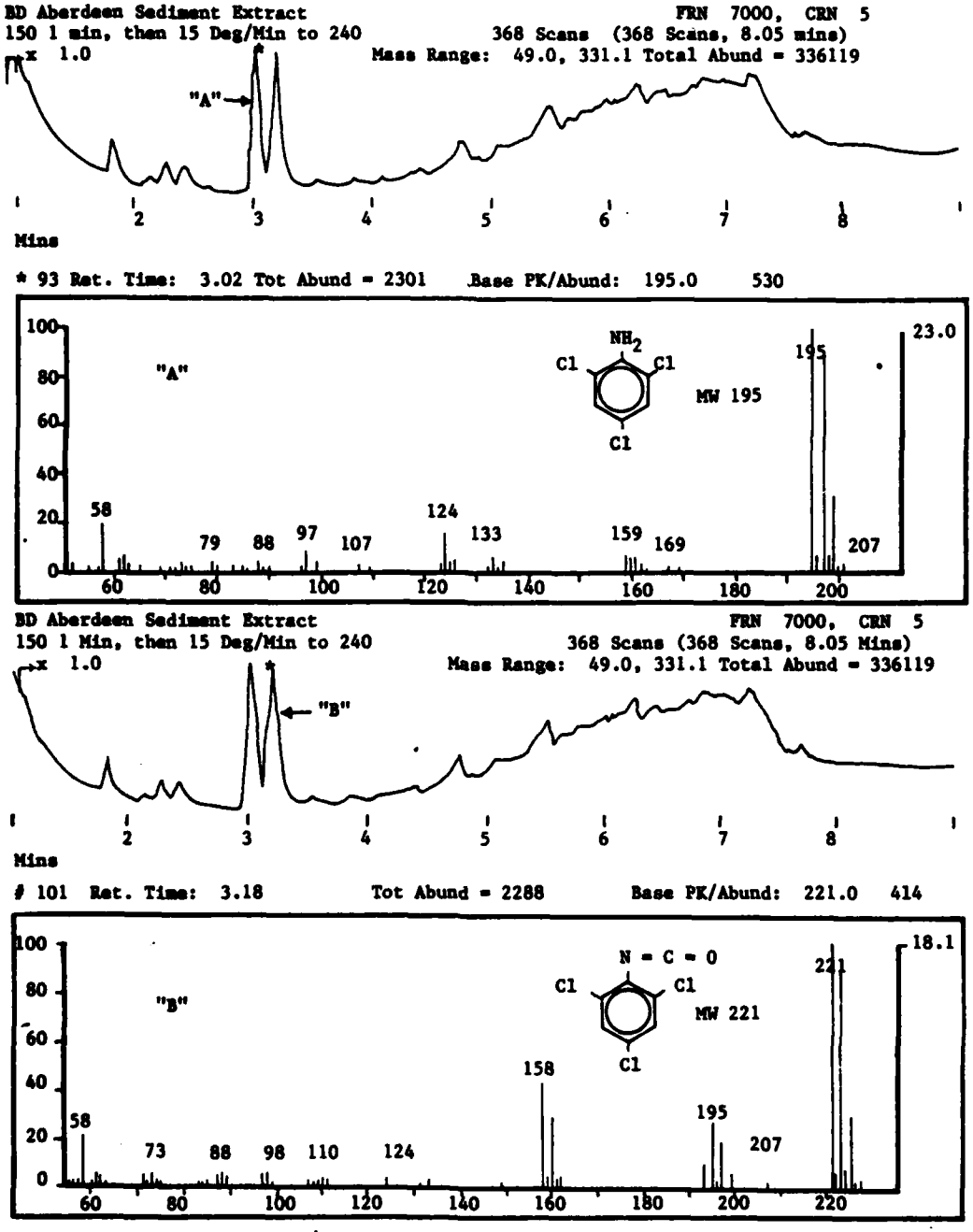


Figure 23. GC/MS analysis of chloroform extract of Canal Creek sediments.

In order to show on-column pyrolysis, the temperature of the GC injection zone was varied from 100° to 250°C and the same extract analyzed. Figure 24 shows the effect of changing the injection zone temperature. When the extract was injected into a zone below 170°C little or no 2,4,6-TCA or 2,4,6-trichlorophenylisocyanate was detected. However, at 220°C and above, substantial amounts were detected by gas chromatography. This indicates that these substances are produced by pyrolysis of a precursor.

The presence of N,N'-bis(2,4,6-trichlorophenyl) urea in the sediment was confirmed in two ways:

1. A sample of N,N'-bis(2,4,6-trichlorophenyl) urea was synthesized by a method[10] described in Appendix C. This compound is slightly soluble in CH_2Cl_2, $CHCl_3$, benzene or methanol. Its melting point was 330° to 340°C (decomp.) (reported 347°C[11] and 326° to 327°C[12]). The mass spectrum is shown in Figure 25. On injection of this compound onto a GC column, two peaks were found which correspond to those observed on GC analysis of the $CHCl_3$ extract of APG sediment.

2. Since gas chromatography leads to the pyrolysis of N,N'-bis(2,4,6-trichlorophenyl) urea, a nondegradative method was sought for analysis of the sediment sample. High-pressure liquid chromatography (HPLC) met this need. Conditions were found for the separation of 2,4,6-TCA from N,N'-bis(2,4,6-trichlorophenyl) urea. The HPLC chromatogram showing this separation is presented in Figure 26. The same figure also shows HPLC analysis of an acetonitrile extract of the sediment. The sediment clearly shows the presence of N,N'-bis(2,4,6-trichlorophenyl) urea, but <u>not</u> 2,4,6-TCA.

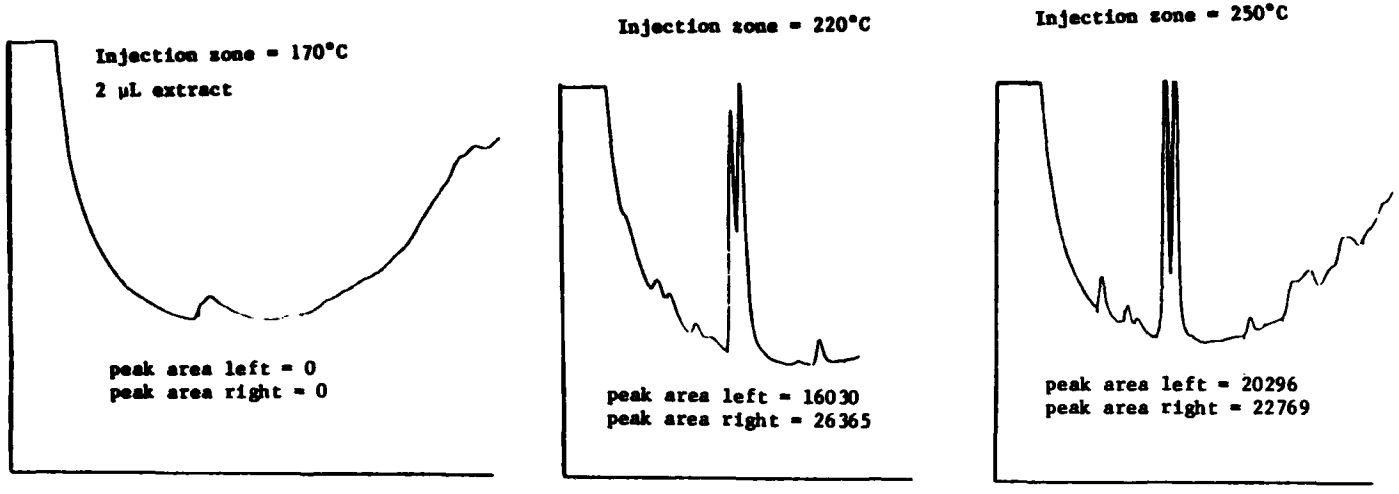


Figure 24. GC analysis of chloroform extract of Canal Creek mud using three different injection zone temperatures.

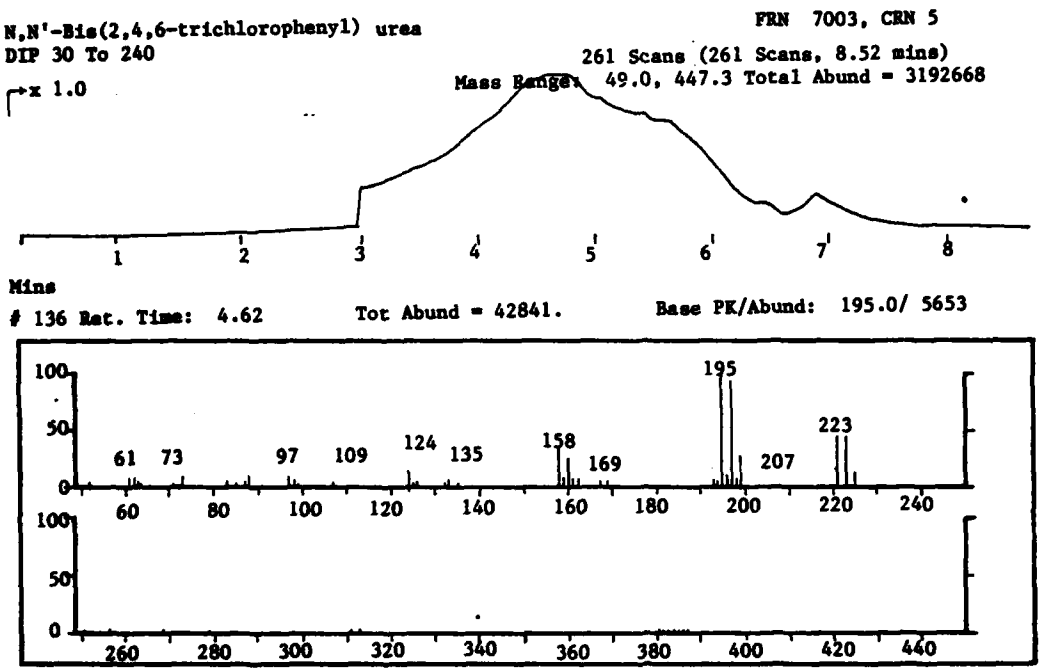


Figure 25. Mass spectrum of synthesized N,N'-bis(2,4,6-trichlorophenyl) urea.

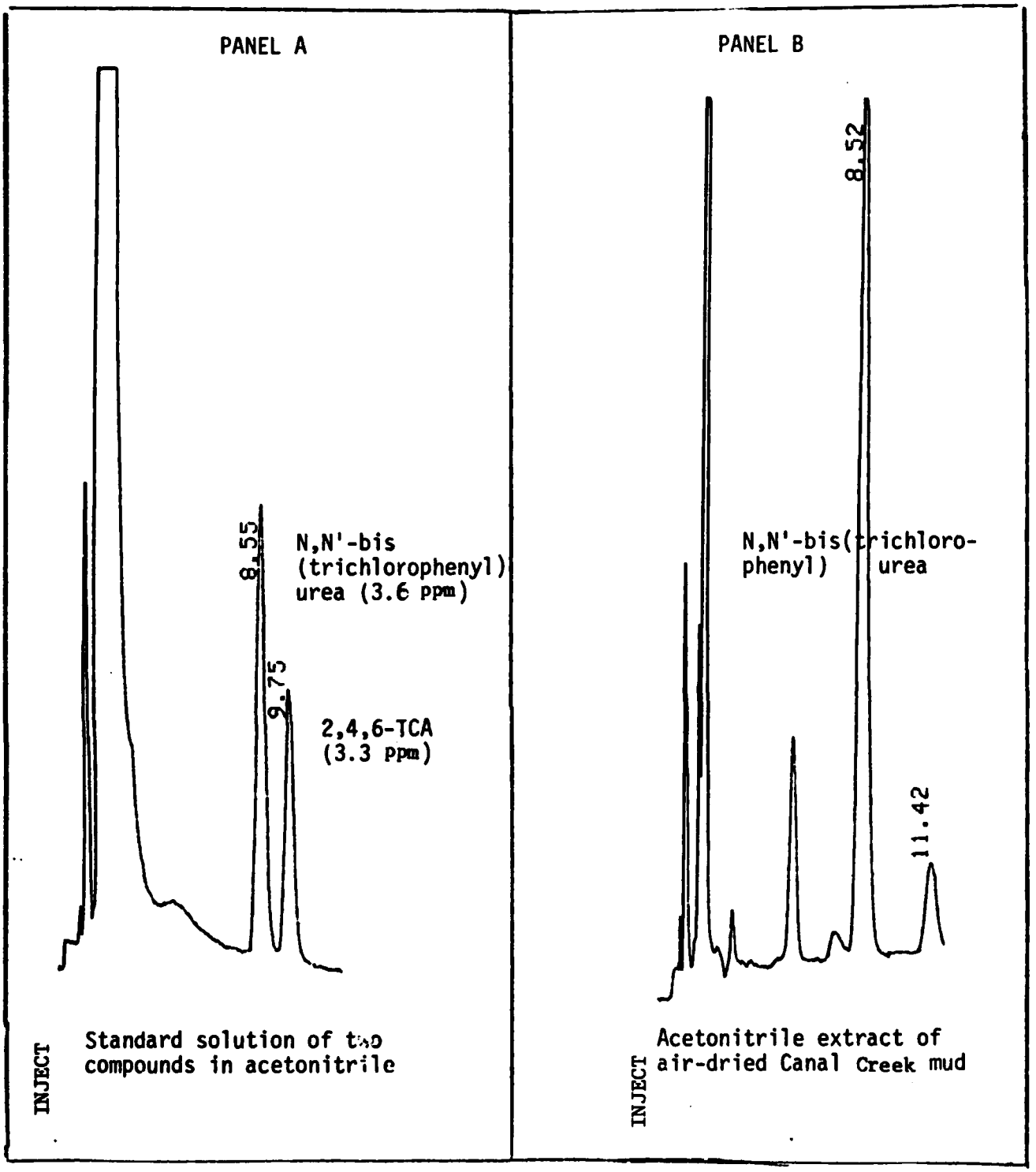


Figure 26. HPLC chromatogram showing separation of N,N'-bis(2,4,6-trichlorophenyl) urea and 2,4,6-TCA.

DISCUSSION

2,4,6-Trichloroaniline (2,4,6-TCA) does not appear to be a contaminant of the estuarine sediments of the Edgewood Arsenal area of Aberdeen Proving Ground (APG), MD. We have reached this conclusion, which contradicts that of earlier investigators, by approaches from two different directions. The first approach was an environmental fate study of 2,4,6-TGA; the second was a direct analysis of a sediment from Canal Creek.

From the environmental fate approach we found that 2,4,6-TCA is an active substance in the aqueous environment. It is soluble in water to the extent of 32 mg/L at 19°C, and is easily degraded by sunlight. Competing with photo-degradation is the pathway of vaporization from water; 2,4,6-TCA was found to be quite volatile.

Our second and most direct proof of the absence of 2,4,6-TCA in APG sediments came from a chemical analysis of the sediments of Canal Creek in Edgewood Arsenal. The sediment sample was found by gas chromatography to yield two major substances. These substances were identified by GC/MS as 2,4,6-TCA and 2,4,6-trichlorophenylisocyanate. Since the latter compound is readily hydrolyzed in water, it could not have been present in the wet sediment. We deduced that N,N'-bis(2,4,6-trichlorophenyl) urea was present in the sediment and that the compound pyrolyzed upon injection onto the GC column to give the observed products. This pyrolysis, occurring during the analytical process, had undoubtedly misled previous investigators into believing that 2,4,6-TCA was present in the sediments. Examination of the GC/MS data of sediment extracts produced by Calgon Environmental Systems revealed the same twin GC peaks of 2,4,6-trichlorophenyl isocyanate and 2,4,6-TCA that we observed, in at least three Calgon analyses. Their use of the Mass Spectral Search System to identify major peaks failed to find a good match to 2,4,6-trichlorophenylisocyanate. This compound was not in the computer data bank, and was therefore not identified by them.

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APPENDIX A

DETERMINATION OF 2,4,6-TRICHLOROANILINE (2,4,6-TCA) IN WATER BY GC/FID

1. APPLICATION AND SCOPE

This procedure was developed for determining the concentration of 2,4,6-TCA in water in order to monitor the loss from water through microbial, physical and chemical pathways.

a. Tested Concentration Range: 0.1 to 30 µg/mL in water.

b. Detection Limits: Lower detection limit in water is 0.2 µg/mL

2. SUMMARY OF METHOD

Water samples are extracted with methylene chloride (CH_2Cl_2) containing an internal standard. The CH_2Cl_2 extract is analyzed by gas chromatography using a flame ionization detector.

3. HAZARDS

Trichloroaniline shows possible carcinogenicity in chronic feeding studies with rats. Analyst should avoid direct contact with solid or solutions of this compound.

4. INTERFERENCES

Organic substances with similar GC retention times could interfere. However, in present applications, no interferences were found.

5. APPARATUS AND MATERIALS

a. Instrumentation

(1) A Hewlett-Packard Model 5710A Gas Chromatograph with a flame ionization detector.

(2) Column is 6' x 1/4" OD x 2 mm ID glass column packed with 3% OV-1 on 80/100 mesh GAS CHROM Q.

(3) Hewlett-Packard Model 7671A automatic sampler.

(4) Hewlett-Packard 3390A Integrator.

(5) Carrier gas - Helium

(6) AADCO hydrogen generator

b. Operating Parameters

(1) Flow rates: 30 mL/min Helium
30 mL/min H_2
240 mL/min Air

(2) Oven temperature: 165°C isothermal

(3) Injection port: 250°C

(4) Detector: 250°C

c. Glassware and Hardware

(1) Glass vials with screw caps and teflon liners sizes 45 x 15 mm (5 mL) and 65 x 14 mm (12 mL)

(2) Volumetric pipets (1 mL, 5 mL and 10 mL sizes)

(3) Pasteur pipets

(4) 1.5 mL screw cap vials (Wheaton) with screw cap and teflon-faced silicone septa (Supelco 3-3210) (optional - for use with auto sampler).

(5) 100 mL volumetric flasks

(6) 10 µL Hamilton syringe

d. Chemicals

(1) Methylene chloride, nanograde or equivalent.

(2) n-Pentadecane

(3) Anhydrous sodium sulfate (AR)

(4) Methanol

6. STANDARDS

a. Stock 2,4,6-trichloroaniline: Dissolve 100 mg of pure 2,4,6-trichloroaniline in 100 mL of methanol to prepare 1000 mg/L of stock A. Dilute 3 mL of stock A to 100 mL with glass-distilled water to give stock B at 30 mg/L.

b. Working Stocks: Prepare 100 mL of each solution as follows:

Concentration	mL Stock B
20.0 mg/L	66.7
10.0 mg/L	33.3
5.0 mg/L	16.7
2.5 mg/L	8.3
1.0 mg/L	3.3
0.5 mg/L	1.65
0.2 mg/L	0.66

Stock A is stored in refrigerator. Stock B and working stocks are stored at room temperature in the dark. Working stocks are prepared every month or when necessary.

7. PROCEDURE

a. Sample Handling: Samples are received in Teflon-lined screw-cap vials. Aliquots of 5 mL or 10 mL are premeasured by the requestor and analyzed on day of receipt. If the concentration range is 20 to 1 mg/L, 5 mL samples are used. If the range is from 2 to 0.1 mg/L, 10 mL samples are used. The pH of the sample should be above 6.5.

b. Extraction: One mL of methylene chloride containing 5 mg/L of n-pentadecane is added to each sample and to each of five aliquots of the working stocks in vials. Volume of the working stocks are the same as that of samples. All vials are capped tightly and shaken vigorously by hand for 1 minute, then allowed to stand for 5 minutes to allow layers to separate. In event that an emulsion results, about 100 mg of anhydrous Na_2SO_4 should be added to break the emulsion. The CH_2Cl_2 layer (bottom) is ready for GC analysis.

c. GC Analysis: Two options are available to the analyst; manual injection or auto sampler.

(1) For manual injection, dip the tip of microliter syringe into the CH_2Cl_2 layer, withdraw 1 µL and inject this into the injection port of the 5710 GC.

(2) To use the automatic sampler, the CH_2Cl_2 layer must be withdrawn from the extraction vial with a pasteur pipet and transferred into a 1.5 mL vial (Wheaton) and sealed with a screw cap containing a septum.

d. Calibration: Prior to analysis of samples, a standard solution containing a known concentration of 2,4,6-TCA and pentadecane in CH_2Cl_2 is injected onto the GC column. The resulting chromatogram should show two peaks with different retention times. This is then compared with chromatogram of the same standard made at the initiation of this method in the Lab. From this comparison the analyst can detect the following:

(1) Loss of detector sensitivity (decrease in detector response - smaller peaks).

(2) Change in Helium flow rate (shift in retention times).

(3) Column deterioration (poor resolution).

(4) Column contamination (poor baseline).

(5) Any combination of the above.

Every 3 months, a standard column calibration mixture will be injected onto the column. This mixture consists of a homologous series of n-alkanes in chloroform. Analysis is always made under the same comditions of flow rates, temperature and recorder sensitivity. The chromatogram will be compared with previous analyses in order to detect changes in column or detector.

8. CALCULATIONS

a. Identification of GC Peaks: The retention time of each peak is the identifier for the analyst. Any deviation ± 0.1 min from this time is considered reason for further examination. Any deviation is also examined relative to the internal standard (if the 2,4,6-TCA peak is earlier or later than expected, so should be the internal standard).

b. Calculation of Peak Ratios: When the 2,4,6-TCA peak and internal standard peak are identified, the peak areas are converted to a ratio thus:

$$\text{(R) Ratio} = \frac{\text{peak area of 2,4,6-TCA}}{\text{peak area of pentadecane}}$$

These calculations will be made on the chromatographic print-out. The R-value is identical to instrumental response (R).

$$R = \frac{\text{area 2,4,6-TCA}}{\text{area internal standard}} = \text{Instrument Response}$$

c. Plot of Standard Curve: The ratios, R, for all working stocks (6.b.) are plotted against the concentration of 2,4,6-TCA in the working stocks, to give the standard curve. With the computer regression fit, the values of 2,4,6-TCA concentration can be determined from R values of unknown samples.

9. PRECISION

Precision of method was determined by analyzing the same sample five times and determining the mean (μ) and standard deviation (σ). This was done for low and high concentrations. The limit of detection is defined as the concentration where the relative standard deviation exceeds 10%. The precision of 2,4,6-TCA analysis is shown at the 10 mg/L and 1.0 mg/L levels in the table below:

Precision of 2,4,6-TCA Analyses at the 1 mg/L and 10 mg/L Levels

Target Concentration	Concentration Found	Mean	Std. Dev.	% Coefficient of Variation
10.30 mg/L	10.35	10.41	0.10	0.96
	10.31			
	10.35			
	10.50			
	10.51			
	10.32			
1.03 mg/L	1.03	1.01	0.02	2.0
	1.01			
	1.01			
	0.98			
	1.01			

10. REPRODUCIBILITY

The day-to-day consistency of standard curves is shown below by the equations generated by the regression lines. The concentrations of standards were 0, 1, 2, 5, and 10 mg/L and GC response (Y axis) was plotted against TCA concentration in the aqueous sample (X axis).

Date	Equation of Straight Line	Correlation (R^2)
Nov 18, 1981	Y = 0.062 X - 0.005	1.000
Nov 20, 1981	Y = 0.060 X - 0.001	1.000
Dec 1, 1981	Y = 0.060 X - 0.003	1.000
Dec 2, 1981	Y = 0.061 X - 0.004	1.000

APPENDIX B

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

The synthesis of 2,4,6-TCA was based on the description by J. Erdelyi (C.A. 1929. 23:4937). ^{14}C-(U) Aniline hydrochloride (250 µCi) in ethanol (0.8 mL) was mixed with aniline (reagent grade, 0.25 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, (Na_2SO_4) 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-TCA (fractions #12-#20) were combined to yield 33 mg of ^{14}C-2,4,6-TCA, 99.8 percent pure, with a specific activity of 0.58 µCi/mg.

APPENDIX C

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

Urea (9.0 g), 2,4,6-trichloroaniline (9.2g), glacial acetic acid (80 mL) and water (0.8 mL) were placed in a 250 mL round-bottom flask and allowed to 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, then 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 had mp 330-340°(d), was not sufficiently soluble for purification by recrystallization from benzene, but was pure by HPLC analysis. The mass spectrum of the synthesized material is shown in Figure 25.

APPENDIX D

Determination of 2,4,6-Trichloroaniline in Moist Sediments

1. APPLICATION AND SCOPE

Environmental fate studies carried out for USATHAMA on 2,4,6-TCA indicated that the subject compound was very volatile from water. Due to the volatility of 2,4,6-TCA, losses would be incurred on drying a sediment sample. Therefore, this method must address analysis of a wet or moist sediment. The method must also distinguish N,N'-bis(2,4,6-trichlorophenyl) urea, known to be present in these sediments, from 2,4,6-TCA. Gas chromatography as developed for USATHAMA by contractor (Calgon Environmental Systems) does not clearly distinguish these substances. The present approach uses HPLC.

a. Tested Concentration Range: 1 µg to 16 µg 2,4,6-TCA per gram of sediment (on dry basis).

b. Detection Limit: 1 µg 2,4,6-TCA in 2 grams of moist sediment.

2. SUMMARY OF METHOD

Moist sediment sample is extracted with acetonitrile and the extract analyzed by HPLC.

3. HAZARDS

None

4. INTERFERENCES

UV-absorbing organics that have same HPLC retention time. N,N'-bis (2,4,6-trichlorophenyl) urea, if present in sediments, does not interfere.

5. APPARATUS AND MATERIALS

a. Instrumentation

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

(2) Ten cm RAD-PAK reverse phase C_{18} column

b. Operating Parameters

(1) Mobile phase: acetonitrile/water = 60/40

(2) Flow rate: 2 mL/min

(3) UV detector: 230 nm

(4) Injection volume: 50 µL

c. Glassware and Hardware

(1) Culture tubes (15 mL) with teflon-lined screw caps

(2) COREX^{R} centrifuge tubes

(3) 20 mL glass vials (scintillation type) or equivalent

(4) Low speed centrifuge (2,0000 rpm)

(5) High speed centrifuge - SORVALL^{R}

(6) 100 µL Hamilton syringe

(7) Volumetric pipets and pasteur pipets

(8) Sonic bath

d. Chemicals

(1) Acetonitrile, HPLC grade

(2) Water, glass-distilled

6. STANDARDS

a. Stock 2,4,6-trichloroaniline: dissolve 100 mg of pure 2,4,6-trichloroaniline in 100 mL of methanol to prepare 1000 mg/L of stock A. Dilute 3 mL of stock A to 100 mL with glass-distilled water to give stock B at 30 mg/L.

7. PROCEDURE

a. Sample Handling: Since 2,4,6-TCA is known to disappear from water through volatilization, samples should arrive at the laboratory in sealed jars and be processed immediately.

b. Extraction: A fresh, wet sediment sample is poured onto a disc of filter paper resting in a Büchner funnel fitted to a filter flask with a rubber stopper. The filter flask is connected to a suction pump. If the sample is fine-grained the use of a rubber dam* is recommended. To do this, a piece of thin latex rubber sheeting is placed over the top of the Büchner funnel and held in place with a rubber band. Due to the suction, the rubber sheet compresses the wet sediment, driving out the excess water. A few minutes of compression is sufficient. The moist cake of sediment is then removed and a portion (2 grams) is placed into a clean 15-mL screw-top tube and weighed. Three milliliters of acetonitrile are added to the tube. Teflon-lined screw caps are placed on the tubes and the contents agitated for 15 min at room temperature in a sonic bath. Following this, the tubes are

*A description of the rubber dam can be found in Experiments in Organic Chemistry, 3rd edition, L.F. Fieser, D.C. Health & Co., 1957, p. 258.

centrifuged at 2000 rpm for 10 min. The resulting clear supernatant liquid is ready for direct HPLC analysis.

c. Moisture Determination: Another portion of the moist sediment is weighed and dried at 105°C to constant weight. From this the percent moisture in the sample can be determined. This is needed since final results are reported as µg TCA/gram of dry sediment.

d. Calibration: Prior to analysis, a standard solution containing a known concentration of 2,4,6-TCA is injected onto the HPLC. The resulting peak area and retention time should be the same as that determined at an earlier time using the same parameters. From the comparison the analyst may detect the following:

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

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

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

8. CALCULATIONS

a. Plot of Standard Curve: Peak areas for all working standards are plotted against their concentrations. From the regression fit, the equation of a straight line is obtained, and the concentration of 2,4,6-TCA in sediment sample x is

$$(\mu g/mL)C_{\text{sample x}} = \frac{(A_x - b)}{m}, \text{ where } A_x = \text{peak area.}$$

The concentration of compound in the dry sediment is

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

where C_{sample} is in µg/mL and weight of sediment is in grams.

9. ACCURACY AND PRECISION

a. Reproducibility of standard curves: Dry sediment samples of one gram were spiked with 1 mL of aqueous 2,4,6-TCA at levels of 0, 0.5X, 2X, 5X, and 10X, where X is the estimated detection limit. One sample at each concentration level was analyzed in one day, for four separate days. Table A below presents these data. The peak area (x axis) was plotted against the concentration of 2,4,6-TCA (y axis) in the sediment. The sediment sample used in this procedure did not contain N,N'-bis(2,4,6-trichlorophenyl) urea.

TABLE D-1. PEAK AREAS FOR 2,4,6-TCA

Concentration of TCA (µg/g Soil)	Day 1	Day 2	Day 3	Day 4
0	0	0	0	0
0.25	46,958	60,106	62,996	58,828
0.5	120,085	129,399	136,483	125,423
1.0	300,959	305,897	296,105	292,562
2.5	624,632	674,747	660,391	610,260
5.0	1,332,810	1,217,646	1,240,716	1,203,818

Regression Curves*

Day 1	Y = 265,496 X - 5,067	0.998
Day 2	Y = 244,775 X + 20,634	0.995
Day 3	Y = 248,045 X + 17,044	0.998
Day 4	Y = 239,477 X + 12,621	0.998

* Where X = µg TCA/g
Y = HPLC peak area

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