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ENVIRONMENTAL FATE STUDIES ON CERTAIN MUNITION WASTEWATER CONSTITUENTS

Phase III, Part II--Laboratory Studies

Final Report

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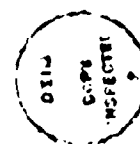
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EXECUTIVE SUMMARY

This report describes the results of three subtasks under the Phase III-Model Validation task of Contract DAMD 17-78-C-8081.

In Part A, the biotransformation pseudo-first order and second order rate constants were determined for six isomeric dinitrotoluenes and five isomeric trinitrotoluenes. Orientation of the nitro groups para or ortho to each other accelerated the transformation rate which is primarily reduction.

In Part B, Henry's Law constant for TNT, 2,4-DNT, RDX, and HMX were measured so that the volatilization rate constants for these chemicals could be estimated. The data suggest that volatilization will be a very slow process for all of the chemicals investigated.

In Part C, the photochemical behavior of TNT in natural waters was investigated. The results of this study suggest that the indirect photolysis of TNT can occur by several mechanisms. In one mechanism, light is absorbed by natural organics and the resultant energy is transferred to TNT. In a second mechanism, TNT is complexed with natural organics (humic acids) and the complex readily absorbs light leading to the phototransformation of TNT.

PART A

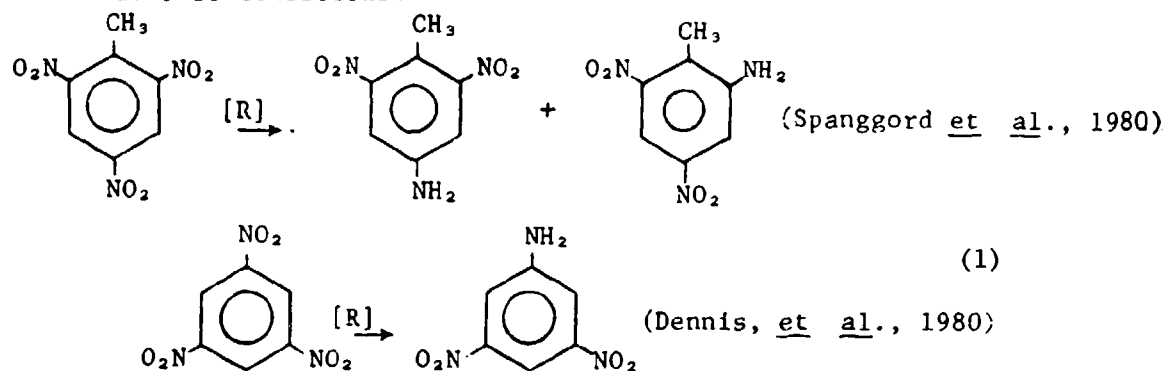
BIOTRANSFORMATION OF NITROAROMATIC
COMPOUNDS AS A FUNCTION OF STRUCTURE

By

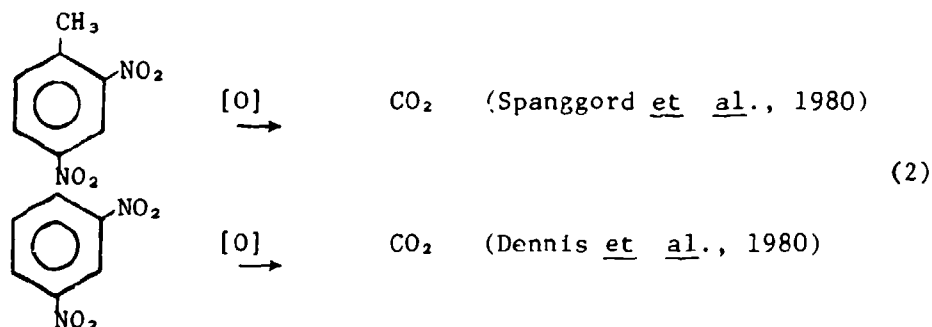
Tsong-Wen Chou
Ronald J. Spanggord

I INTRODUCTION

Polynitroaromatic compounds represent a class of electron-deficient compounds that can undergo several modes of biotransformation. The most common mode is reduction, examples of which are shown for 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitrobenzene (TNB) in Equation 1. This reduction can occur readily under anaerobic conditions and even under aerobic conditions.



The other mode of biotransformation is oxidation. While there is no evidence that TNT or TNB undergoes microbial oxidation, their counterparts, 2,4-dinitrotoluene and 1,3-dinitrobenzene will undergo microbial oxidation that can proceed to complete mineralization as shown below.



However, when we examined the biotransformation of 2,6-dinitrotoluene in the presence of both oxidative and reductive microorganisms, no oxidation was observed and reduction proceeded very slowly.

These results indicate that unique structural features exist that can assist or retard biotransformation in structurally related polynitroaromatic compounds, or that highly specific enzymes exist in some organisms that would allow them to use only certain structures of polynitroaromatic compounds.

To study these possibilities, we investigated the biotransformation of the isomeric dinitro (DNT)- and trinitrotoluenes (TNT) using natural water microorganisms to determine if the rates of transformation could be correlated with structural or electronic features that allow biotransformation to proceed in these classes of compounds.

II MATERIALS AND METHODS

A. Chemicals

The chemicals used in this study were either obtained from commercial sources or were synthesized in our laboratory. Each chemical was found to be more than 98% pure by gas chromatographic analysis. The chemicals 3,6-; 2,3-; and 3,4-dinitrotoluene were obtained from Aldrich Chemical Co., Milwaukee, Wisconsin; 2,5- and 2,4-dinitrotoluene were obtained from ICN Pharmaceuticals, Plainview, New York; and 3,5-dinitrotoluene was obtained from the synthetic deamination of 4-amino-3,5-dinitrotoluene (Cohen et al., 1905).

All trinitrotoluene isomers were synthesized using the method of Dennis et al. (1975) except for 2,4,6-trinitrotoluene, which was obtained from E. I. DuPont, Wilmington, Delaware.

B. Water Samples

Water samples were collected from two sources: a pond near Searsville Lake in Woodside, California, and Coyote Creek in San Jose, California. The waters were collected after the bottom sediment was stirred so that some sediment microorganisms were included. The water was placed in a 20-liter sterile glass reservoir bottle, mixed well, and the sediment allowed to settle by gravity. The supernatant was siphoned and filtered through a fine mesh polyester cloth to remove insects and unsettled particles.

C. Biotransformation Screening Test

Biotransformation screening tests were conducted using 2 liters of the natural water sample in sterile 4-liter glass bottles or flasks. Twenty ml of potassium phosphate buffer solution (pH 7.5, 100 g liter⁻¹) was added to the water to make a final buffer concentration of 1g liter⁻¹.

The chemical under investigation was dissolved in dimethylsulfoxide (DMSO) at 40 mg ml^{-1} and was added to the natural water to yield a 10 ppm final concentration. Each bottle contained one test chemical. For Coyote Creek water, 20 mg of each chemical was weighed and dissolved directly into 2 liters of the natural water using a Waring blender. Yeast extract was added to the water from a 100 mg ml^{-1} sterile aqueous solution.

The bottles were closed with cotton plugs and incubated in the dark at 25°C . The bottles were hand-shaken at the beginning of the experiment and before every sampling. Red lamps were used during the periodic withdrawal and handling of the samples to limit photochemical transformation. About 4 ml of sample was pipetted into a glass vial containing 1 drop of 4% HgCl_2 . The vial was capped with a teflon liner cap, wrapped with aluminum foil, and frozen until analyzed. Autoclaved waters in bottles were used as sterile controls.

D. Biotransformation Investigations Using 2,4-DNT Utilizing Mixed Culture

In Phase II of our environmental fate studies (Spanggord et al., 1980), we isolated an enriched mixed culture, capable of utilizing 2,4-DNT as a sole carbon and energy source, from Waconda Bay near Chattanooga, Tennessee. Frozen cells from this isolate were thawed at room temperature in a water bath and inoculated into shaker flasks containing 100 ppm of 2,4-DNT and basal salts medium (BSM). The BSM contained, per liter: 0.7 g of K_2HPO_4 , 0.3 g of KH_2PO_4 , 0.5 g of $(\text{NH}_4)_2\text{SO}_4$, 0.5 g of NaCl , 0.05 g of $\text{MgSO}_4 \cdot 7 \text{ H}_2\text{O}$, 0.1 g of $\text{CaCl}_2 \cdot 2 \text{ H}_2\text{O}$, 0.003 g of $\text{FeSO}_4 \cdot 7 \text{ H}_2\text{O}$, and 1 ml of trace-elements solution. The trace-elements solution contained, per liter: 0.1 g of H_3BO_3 and 0.05 g each of $\text{CaSO}_4 \cdot 5 \text{ H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7 \text{ H}_2\text{O}$, and $\text{Na}_2\text{MoO}_4 \cdot 6 \text{ H}_2\text{O}$. The flask was incubated on a rotary shaker incubator at 25°C in the dark for 3 days. The microorganisms from this flask were inoculated into flasks, each containing 30 ppm of one DNT or TNT isomer in BSM. The degradation of DNT or TNT isomer was determined by broth turbidity and by UV scanning (190-400 nm) of a hexane extract of the broth.

E. Biotransformation Rate Study with High Cell Population

A mixed culture of Waconda Bay water organisms acclimated to 2,4,6-TNT plus yeast extract in BSM, and a mixed culture of Searsville Lake pond water organisms grown in 0.5 g liter⁻¹ yeast extract, were used for a high cell population biotransformation study.

The mixed cultures were grown in 1 to 2 g liter⁻¹ yeast extract in BSM for 16 to 19 hours and harvested near the end of the growth phase. The cells were centrifuged at 5000 × g, washed with BSM, centrifuged again and resuspended in 1/5 volume of BSM. The organisms were diluted with BSM and transferred to Erlenmeyer flasks and DNT or TNT isomers (40 mg ml⁻¹ DMSO) were added with the aid of a Hamilton syringe, to make the final concentration 10 ppm. The flasks were gently shaken on a rotary shaker (100 rpm) in the dark. Aliquots were withdrawn periodically for microbial plate counts and chemical analyses.

Bacterial counts were made by serial dilution of the water sample followed by the growing of the organisms on Difco Plate Count Agar. After 3 days of incubation, the organisms were counted with aid of a colony counter and the total counts were calculated from the dilution factor.

Chemical analyses were performed by high-pressure liquid chromatography (HPLC) after filtering the water sample through a 0.45-μ filter. The following conditions were employed for all DNT and TNT isomers:

Instrument:	Spectra-Physics Model 3500B Liquid Chromatograph
Column:	Water's C ₁₈ μ-Bondapak column in a Water's Radial Compression Module
Solvent:	Methanol:water (60:40)
Flow Rate:	2 ml min ⁻¹
Detector:	UV @ 254 nm

Quantitation was achieved by the internal standard method. For the DNT's and 2,3,4-TNT, benzophenone was used as the internal standard. For the remaining TNT isomers, 3,5-DNT was used as the internal standard.

F. Metabolite Studies

For the metabolite study, media, containing 30 ppm of DNT or TNT and exposed to high cell populations, were extracted with two volumes of ethyl acetate. The aqueous phase was acidified to pH 1-2 by HCl, and the extraction was repeated. The extracts were combined, dried over Na_2SO_4 , and concentrated by rotary evaporation. The samples were analyzed by thin-layer chromatography (TLC), HPLC, and gas chromatography-mass spectrometry (GC-MS). TLC was performed on precoated EM-silica gel 60-F-254 plates and developed in hexane:benzene (1:1) or chloroform.

HPLC was performed using a C_{18} radial compression model and a gradient program starting from 35:65 methanol-water to 75:25 methanol:water over 20 min with detection at 254 nm.

GC-MS was performed using an LKB 9000 GC-MS equipped with a 6ft $\times$ 2 mm glass column packed with 10% DC-200 on Gas Chrom-Q. The oven was temperature programmed from 100 to 220°C at 4° min⁻¹.

III RESULTS

A. Screening for Biotransformation of DNT and TNT Compounds with Local Water

None of six DNT isomers (2,3-; 2,4-; 2,5-; 2,6-; 3,4-; and 3,5-) or five TNT isomers (2,3,4-; 2,3,6-; 2,4,5-; 2,4,6-; and 3,4,5-) added to Searsville pond waters were mineralized by the microorganisms during six weeks of incubation. Losses of approximately 30% of 2,4,5-T and 3,4,5-T were observed, but loss of these chemicals was also observed in sterile pond water; therefore the loss appeared to be due to a chemical transformation.

The screening test was also conducted with Coyote Creek water. Again, microorganisms capable of using any DNT or TNT as the sole carbon and energy source were not obtained within six weeks.

However, when DNT or TNT compounds were added to the pond water with 500 ppm yeast extract, all of the DNT and TNT isomers decreased to non-detectable levels after five days of incubation. The local eutrophic waters do not contain microorganisms that will mineralize these compounds, but contain microorganisms that will transform DNT and TNT compounds when other metabolizable organic compounds are present for their growth (cometabolism).

B. Screening Test with Waconda Bay 2,4-DNT Transformation Organisms

When the mixed culture obtained from Waconda Bay water, capable of using 2,4-DNT as the sole carbon and energy source, was grown in 2,4-DNT/BSM and inoculated to flasks, each containing one DNT or TNT isomers, the organisms mineralized 2,4-DNT but did not mineralize other DNT or TNT compounds. The experiment was repeated by using washed and resuspended cells that were grown in 50 ppm 2,4-DNT/BSM. Again, no DNT or TNT compound other than 2,4-DNT was utilized. This mixed culture was unique in its ability to mineralize 2,4-DNT, but it was unable to utilize other DNT or TNT compounds.

C. Screening Test with Waconda Bay 2,4,6-TNT:Yeast Extract Transformation Organisms

The mixed culture obtained from Waconda Bay and shown to reductively transform 2,4,6-TNT in the presence of yeast extract was grown in Trypticase Soy broth, and the washed and resuspended cells were tested for biotransformation of DNT and TNT compounds. All 11 compounds were transformed in the presence of a high population of washed cells. This result is similar to that occurring with microorganisms from Searsville pond water. The organisms from these natural waters apparently can nonspecifically biotransform DNT and TNT isomers when sufficient organic nutrients are present to support microbial growth.

D. Metabolites

The compounds 2,4-DNT, 3,4-DNT, and 2,3,6-TNT were biotransformed by high populations (10^9 CFU ml⁻¹) of the pond microorganisms in BSM, and their metabolites were analyzed.

The TLC plates of broth extracts showed that each compound produced several metabolites. By co-chromatography, some spots had the same R_f value and yellowish color as 2-amino-4-nitrotoluene in 2,4-DNT extracts; 4-amino-3-nitrotoluene in 3,4-DNT extracts; and 3-amino-2,6-dinitrotoluene and 6-amino-2,3-dinitrotoluene in 2,3,6-TNT extracts.

By HPLC and GC-MS analyses, 3-amino-2,6-dinitrotoluene was confirmed to be a major transformation product of 2,3,6-TNT.

The metabolism study indicated that the biotransformation of these compounds occurred mainly through the reduction of nitro group.

E. DNT Biotransformation Rate Constant

Although DNT and TNT isomers were not utilized as sole carbon and energy sources, they could be reductively biotransformed when microorganisms were grown on other organic nutrients. The biotransformation rates of DNT isomers by yeast extract/BSM grown cells of Waconda Bay and 2,4,6-TNT/yeast extract/BSM acclimated microorganisms were compared by mixing the DNT isomer and the cell suspension and by monitoring the

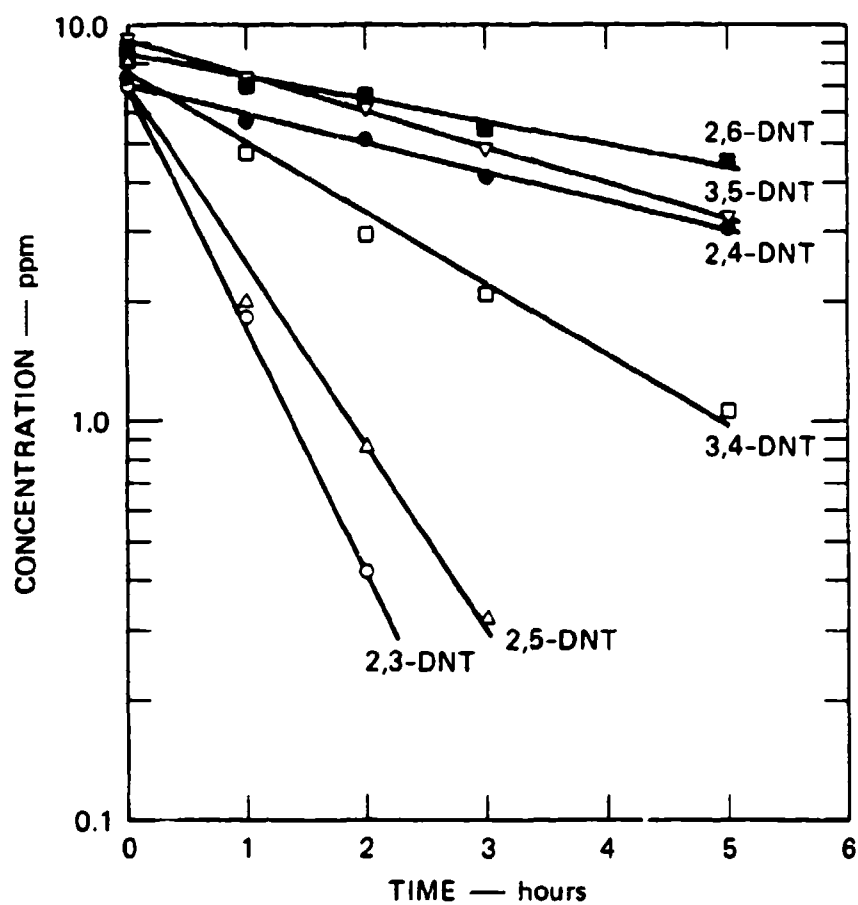
disappearance of the compounds. Each compound appeared to be transformed by a pseudo-first-order rate process. The biotransformation of DNTs by 4.6×10^9 cell ml^{-1} of Waconda Bay organisms as a function of time is shown on a semilogarithmic graph in Figure 1. The first-order rate constants, k_b' , for DNT isomers are calculated from the data by a least-square method and are listed in Table 1, along with their calculated second-order rate constants, k_{b2} .

Table 1

PSEUDO-FIRST-ORDER AND SECOND-ORDER RATE CONSTANTS OBSERVED
FOR ISOMERIC DINITROTOLUENES WITH WACONDA BAY ORGANISMS

Compound	k_b' (h^{-1})	$k_{b2} \times 10^{10}$ ($\text{ml cell}^{-1} \text{h}^{-1}$)
2,3-DNT	1.41	3.1
2,5-DNT	1.05	2.3
3,4-DNT	0.41	0.89
3,5-DNT	0.21	0.46
2,4-DNT	0.17	0.37
2,6-DNT	0.13	0.28

The biotransformation rate was also investigated with Searsville pond water organisms, using a cell suspension of 9.6×10^8 cell ml^{-1} and 10 ppm of DNT isomers. The pseudo-first-order and second-order rate constants are listed in Table 2.



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FIGURE 1 BIOTRANSFORMATION OF DNT ISOMERS BY A MIXED CULTURE OF WACONDA BAY WATER MICROORGANISMS GROWN IN YEAST EXTRACT-BASAL SALTS MEDIUM

Table 2

PSEUDO-FIRST-ORDER AND SECOND-ORDER RATE CONSTANTS OBSERVED
FOR ISOMERIC DNTs WITH SEARSVILLE POND ORGANISMS

Compound	k_b' (h^{-1})	$k_{b2} \times 10^{10}$ ($ml\ cell^{-1}\ h^{-1}$)
2,5-DNT	1.15	12.
3,4-DNT	0.59	6.1
2,3-DNT	0.57	5.9
3,5-DNT	0.49	5.1
2,4-DNT	0.33	3.4
2,6-DNT	0.008	0.093

The average of the second-order rate constants and their standard deviations from the above two studies are shown in Table 3.

Table 3

AVERAGE BIOTRANSFORMATION SECOND-ORDER RATE CONSTANTS
FOR ISOMERIC DINITROTOLUENE

Compound	$k_{b2} \pm S.D. \times 10^{10}$
2,5-DNT	7.2 $\pm$ 6.9
2,3-DNT	4.5 $\pm$ 2.0
3,4-DNT	3.5 $\pm$ 3.7
3,5-DNT	2.8 $\pm$ 3.3
2,4-DNT	1.9 $\pm$ 2.1
2,6-DNT	0.18 $\pm$ 0.14

The observed rate constants indicate that although there were slight differences in rate constants of the two waters, isomers with the nitro groups oriented ortho and para to each other (2,5-DNT, 2,3-DNT, 3,4-DNT) were transformed at a faster rate than isomers with the nitro groups oriented meta to each other (3,5-DNT, 2,4-DNT, and 2,6-DNT). This may reflect an ease in reduction of a nitro group when oriented para or ortho to a neighboring nitro group. In both studies, 2,6-DNT was found to be transformed the slowest, indicating that possibly the methyl group was hindering the reduction process.

F. TNT Biotransformation Rate Constants

The rate of biotransformation of 10 ppm of each TNT isomer was determined using a high cell population (7.5×10^8 cells ml^{-1}) of washed and resuspended Waconda Bay and Searsville Pond organisms exposed to TNT and yeast extract in BSM. The pseudo-first-order rate constants, k_b' , were obtained by a least square method, and the calculated second-order rate constants, k_{b2} , for the transformation with Waconda Bay organisms appear in Table 4. The reference compound used was 3,5-DNT.

Table 4

PSEUDO-FIRST-ORDER AND SECOND-ORDER RATE CONSTANTS OBSERVED FOR ISOMERIC TNT WITH WACONDA BAY ORGANISMS

Compound	k_b' (h^{-1})	$k_{b2} \times 10^{10}$ ($\text{ml cell}^{-1} \text{h}^{-1}$)
2,3,6-TNT	1.70	22.0
2,4,5-TNT	1.58	21.0
2,3,4-TNT	0.36	4.6
3,4,5-TNT	0.22	2.9
2,4,6-TNT	0.10	1.3
3,5-DNT	0.044	0.59

The rates of transformation of 10 ppm each of TNT isomers were also compared using 8.3×10^8 cell ml^{-1} of Searsville pond organisms. The pseudo-first-order and calculated second-order rate constants are listed in Table 5.

Table 5

PSEUDO-FIRST-ORDER AND SECOND-ORDER RATE CONSTANTS OBSERVED
FOR ISOMERIC TNT WITH SEARSVILLE POND ORGANISMS

Compound	k_b' (h^{-1})	$k_{b2} \times 10^{10}$ ($\text{ml cell}^{-1} \text{h}^{-1}$)
2,3,6-TNT	2.24	27.
2,4,5-TNT	2.00	24.
2,3,4-TNT	1.35	16.
3,4,5-TNT	1.23	15.
2,4,6-TNT	0.57	6.9
3,5-DNT	0.15	1.8

The average second order rate constants and the standard deviations appear in Table 6.

Table 6

AVERAGE SECOND-ORDER BIOTRANSFORMATION RATE CONSTANTS
FOR ISOMERIC TRINITROTOLUENES

Compound	$k_{b2} \pm 5.0 \times 10^{10}$
2,3,6-TNT	25 ± 4
2,4,5-TNT	23 ± 2
2,3,4-TNT	10 ± 8
3,4,5-TNT	9.0 ± 8.6
2,4,6-TNT	4.1 ± 4.0

The observed rate constants indicate that TNT isomers generally transform faster than DNT isomers. The transformation rates are greater with those compounds possessing a para orientation of the nitro groups (2,3,6-TNT and 2,4,5-TNT). The compounds with the nitro groups oriented ortho to each other (2,3,4-TNT and 3,4,5-TNT) were transformed slower, and the compound with nitro groups oriented meta to each other (2,4,6-TNT) was transformed the slowest. The molecular planarity induced by the para nitro/group orientation and the increased positive charge on the nitrogen atom possibly facilitates the reduction of the nitro group.

G. Isolation of Pure Cultures Of 2,4 DNT That Can Mineralize 2,4-DNT

The mixed culture of organisms obtained from Waconda Bay and acclimated to grow on 2,4-DNT was used to isolate a pure culture of 2,4-DNT-utilizing organisms. The colonies of mixed culture grown on Difco plate count agar and 200 ppm 2,4-DNT in basal salts agar were isolated and tested in liquid medium containing 100 ppm 2,4-DNT in BSM. After several trials, we were able to isolate several strains that can grow on 2,4-DNT agar or 2,4-DNT liquid medium. Each strain can grow alone in 2,4-DNT/BSM, and observation of the isolates growing on plate count agar showed there are at least two different types of microorganisms that are capable of utilizing 2,4-DNT as a sole carbon and energy source.

IV. DISCUSSION

It was unexpected that none of the DNT or TNT isomers was mineralized by the local eutrophic water microorganisms under our test conditions. Only the Waconda Bay mixed culture utilized 2,4-DNT as its sole carbon and energy source out of the 11 nitroaromatic compounds tested.

The oxidative ring cleavage of 2,4-DNT appears to be site-specific and chemical-specific. Ring-opening by a catechol mechanism (hydroxylation of adjacent ring hydrogens) was not evident. We would expect 2,3-; 3,4-; and 2,6-DNT to be mineralized if this mechanism were operative. It is not clear from this study whether the initial 2,4-DNT metabolic step involves methyl group oxidation, hydroxylation of the benzene ring, or elimination or reduction of the nitro group.

The observation that 2,4-DNT bio-oxidation microorganisms were not found elsewhere but were found in Waconda Bay water, and that two or more different strains of pure culture were isolated from this mixed culture, led us to speculate that the genes for enzymes responsible for the initial 2,4-DNT biotransformation may be encoded in a plasmid, a small DNA molecule outside the chromosome in the cell. This plasmid may be transferred from one strain of bacteria to another. Many enzymes involved in biodegradation of synthetic compounds are found to be genetically encoded in plasmids (Chakrabarty, 1976).

All of the 11 DNT and TNT isomers tested, including 2,4-DNT, undergo nitro group reduction when natural water microorganisms are grown on other organic nutrients. It appeared that many natural microorganisms possess enzyme(s) that can reduce organonitro groups without apparent induction periods. The reductive system may include several different enzymes such as hydrogenases, xanthine oxidases, and nitro reductases. The biotransformation rate observed may be the manifestation of all enzyme activities combined.

The transformation rate constants of the compounds by the grown microorganisms from two water sources are slightly different. This may be because the organisms are mixed cultures and represent different distributions of microbial populations. The population distribution and physiological state of the cells may also differ at the time the cells are harvested for the cell suspension preparation. However, the rate constant difference for the compound between two experiments is normally within one order of magnitude, and the order of decreasing rate among the isomers is fairly similar between two water source organisms.

The results of this study indicate that the TNT isomers are more readily reduced than the DNT isomers. This may be a result of the increased electropositive character created in the molecule by the presence of an additional nitro group. However, the orientation of the nitro groups relative to each other in the molecules was critical to the rate of transformation. The relative transformation rates in both TNTs and DNTs followed the order of para > ortho > meta.

The reduction of nitroaromatic compounds by hydrogen in the presence of enzyme preparation from Veillonella alcalescens was studied by McCormick et al. (1976). The specific activities of the enzyme on 2,4,6-TNT, 2,4-DNT, and 2,6-DNT are 147, 58, and 39 nmol H₂ min⁻¹ mg protein⁻¹, respectively. They also reported that specific activities to para, ortho, and meta dinitrobenzene (DNB) are 210, 79, and 43 nmole H₂ min⁻¹ mg protein⁻¹ respectively. These data correlate fairly well with the biotransformation rate constants determined in this study and the effect of orientation on nitro-group reduction. *V. alcalescens* is an anaerobe and is found, among other places, in the gastro intestinal tract.

The electrolytic reduction potentials of various nitro compounds were measured by Pearson (1948) using polarography. The half-wave potentials for 2,4,6-TNT, 3,4-DNT, 2,3-DNT, 2,4-DNT, and 2,6-DNT were reported to be -0.31, -0.36, -0.40, and -0.46 volts at pH 7.4. These values also correlate with the observed biotransformation rate constants. Thus, biotransformation by a reductive process can be related to the electrophilic nature of reactive sites within the nitroaromatic molecule. Since there

is a wealth of information on the reduction potentials of nitroaromatic compounds, the biotransformation rates should be predictable by comparative analysis.

The reductive process appears to be a very general property possessed by a number of natural water organisms. The so-called "nitroreductase" enzymes are believed to be responsible for the observed transformations. The mode of action of these enzymes appears to be directly dependent on the electropositive character of the nitrogen atom within the nitro group or the electropositive character of the carbon atom bearing the nitro group. Steric interactions appear to play some role in the transformation rate (i.e., 2,6-DNT transforms at a slower rate than 2,4-DNT). Out-of-plane twisting of the nitro group may reduce the electropositive character of the nitrogen atom through reduced resonance interactions with the benzene ring. This effect, however, should be reflected in the polarographic half-wave potential.

The structure-activity relationships developed in this study can serve as a model to predict the fate of other nitroaromatic compounds in the presence of microbial populations in natural waters to sewage treatment ponds. Although the transformations are dependent on additional organic nutrient, the relative ease of transformation can still be estimated.

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PART B

DETERMINATION OF HENRY'S LAW CONSTANTS

By

Daniel L. Haynes

James H. Smith

I. INTRODUCTION

The volatilization rate of chemical C from water can be written as

$$\frac{-d[C]}{dt} = k_v^C [C] \quad (1)$$

where k_v^C is the volatilization rate constant. Based on a classical two-film mass-transfer model, an expression for k_v^C can be written in terms of the mass transfer rates of chemical C across liquid-and gas-phase boundary layers (Mackay, and Leinonen 1975).

$$k_v^C = \frac{1}{L} \left[\frac{1}{k_l^C} + \frac{RT}{H_c^C k_g^C} \right]^{-1} \quad (2)$$

where:

- k_v^C = volatilization rate constant (h^{-1})
- L = depth (cm)
- k_l^C = liquid-film mass-transfer coefficient ($cm\ h^{-1}$) for chemical C
- R = gas constant (liter torr $K^{-1}\ mol^{-1}$)
- T = temperature (K)
- H_c^C = Henry's law constant (torr liter mol^{-1} = torr M^{-1}) for chemical C
- k_g^C = gas film mass-transfer coefficient for chemical C ($cm\ h^{-1}$)

Equation 2 indicates that the Henry's law constant is a key factor in estimating the volatilization of a chemical from water. If $H_c > 3500$ torr M^{-1} , then mass transfer resistance in the liquid phase controls at least 95% of the volatilization rate constant. If $10 < H_c < 3500$ torr M^{-1} , then mass transfer resistance in both the liquid and gas phases are significant. Mass transfer resistance is only significant in the gas phase if $H_c < 10$ torr M^{-1} .

We have proposed that the value of H_c is the only physical property that is needed to estimate the volatilization rate of a chemical in the environment (Smith, 1981). We have estimated the volatilization rate constants and half-lives in ponds and lakes or rivers for 114 organic chemicals on the EPA Priority Pollutant list using Equation 3,

$$k_v^C = \frac{1}{L} \left[\frac{1}{k_l^C (D_l^C/D_l^O)^m} + \frac{RT}{H_c^C k_g^W (D_g^C/D_g^W)^n} \right]^{-1} \quad (3)$$

where k_l^O and k_g^W are the liquid and gas phase mass transfer coefficients for oxygen and water, respectively, and D_l^C , D_l^O , D_g^C , and D_g^W are the diffusion constants of C and O in water and C and W in air. The derivation of Equation 3 may be found in Smith (1981). The relationship of the volatilization rate constants and half-lives to Henry's law constant for the 114 priority pollutants is plotted in Figures 1A and 1B. Selected values of L , k_l^O , k_g^W , n , and m used in Equation 3 are indicated in Figures 1 and 2.

In Phase I of this project (Spanggord et al., 1980) we found no literature values for H_c for the studied munition compounds. However, H_c can be estimated from vapor pressure and solubility data. The estimated values of H_c that were reported earlier (Spanggord et al., 1980a) are summarized in Table 1.

Table 1
ESTIMATED HENRY'S CONSTANTS

Chemical	Estimated Value of H_c (torr M^{-1})
2,4-DNT	3.4
TNG	< 0.06
2,4,6-TNT	0.18
RDX	2×10^{-5}

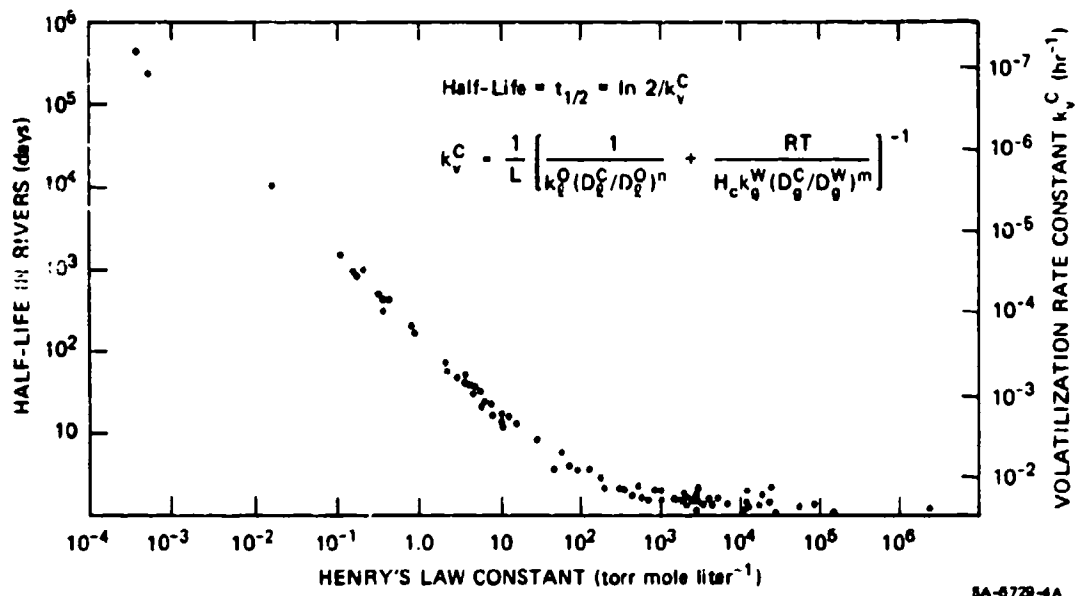


FIGURE 1(a) ESTIMATED HALF-LIVES VERSUS HENRY'S CONSTANT FOR THE PRIORITY POLLUTANTS IN RIVERS

(Values used: $L = 200$ cm, $k_g^0 = 8.0$ cm hr⁻¹, $k_g^W = 2100$ cm hr⁻¹, $n = m = 0.7$)

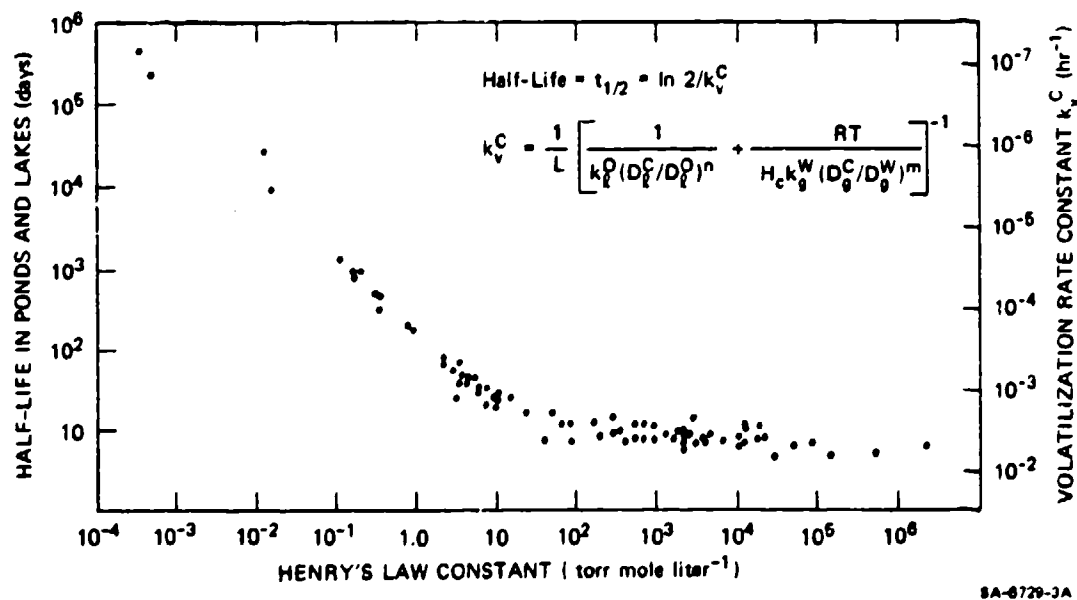


FIGURE 1(b) ESTIMATED HALF-LIVES VERSUS HENRY'S CONSTANT FOR THE PRIORITY POLLUTANTS IN LAKES OR PONDS

(Values used: $L = 200$ cm, $k_g^0 = 1.8$ cm hr⁻¹, $k_g^W = 2100$ cm hr⁻¹, $n = 1$; $m = 0.7$)

However, there was some question about the validity of these estimates, due to questionable vapor pressure data. Also, preliminary results (Spanggord et al., 1978) suggested that 2,4-DNT volatilized rapidly from solution.

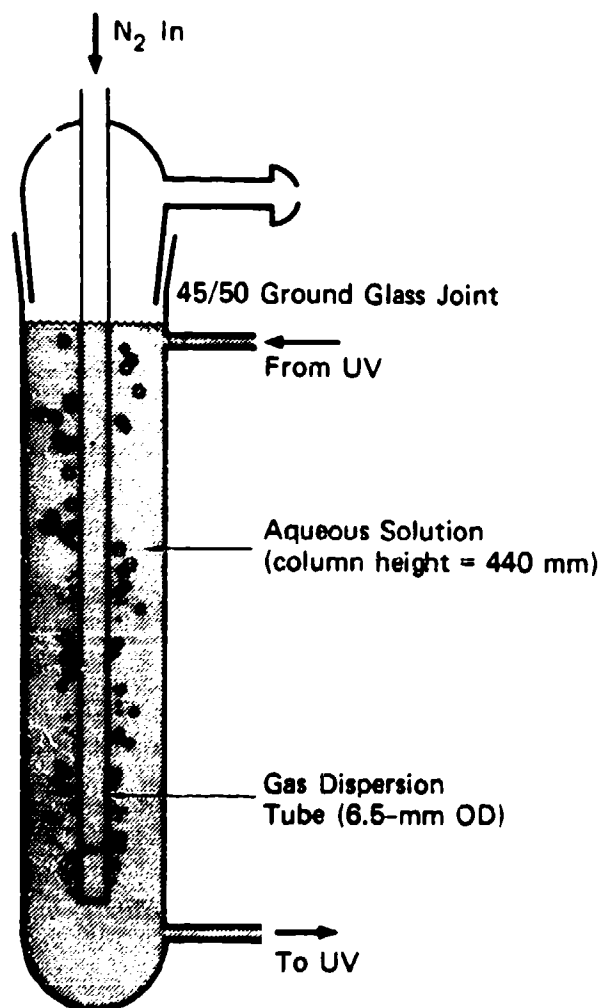
During Phase III we measured H_c for these munitions in order to determine the importance of volatilization to their aquatic fate. We also compared our experimentally determined values of H_c with those calculated from solubility and vapor pressure data.

II MATERIALS AND METHODS

The method used to measure H_c was reported by Mackay et al. (1979). A solution of the chemical of interest is sparged with water-saturated nitrogen, and the concentration of that chemical and the nitrogen flow rate are measured periodically. The H_c apparatus, which is shown in Figure 2, consists of a glass column fitted with a gas dispersion tube with a nitrogen inlet and outlet and a liquid inlet and outlet. Nitrogen from a compressed gas cylinder fitted with a pressure-reducing regulator is passed through a bubbler filled with water and then to the apparatus shown in Figure 2. The water bubbler saturates the nitrogen with water vapor and prevents water evaporation from the H_c apparatus. The thermostats of both the water bubbler and H_c apparatus were set at $25.0 \pm 0.2^\circ\text{C}$. The flow of nitrogen was regulated by a fine needled valve and was measured periodically using a bubble meter. Typical flow rates were between 65 to 85 ml min^{-1} .

Solutions of the munitions were made from 99+% purity chemicals. The chemical was dissolved in Milli-Q^{*} water near saturation. After equilibrating for several hours, the solution was filtered through a Millipore 0.45 μm mixed cellulose ester filter (MCEF). The solution was then diluted by about half or to a convenient working concentration and was then placed in the H_c apparatus. TNG solutions were prepared by water dilution of a stock solution made from the acetone extract of TNG from cellulose. The purity of the solutions was checked by HPLC (before and after completion of the H_c measurements), except for 2,4-DNT, which was checked by gas chromatography (GC). The volume of solution was also measured after the H_c measurement. The concentrations of 2,4-DNT, TNG, and 2,4,6-TNT were measured by UV absorption using a Spectra Physics SP

*Millipore Corp. trademark.



SA-8386-3A

FIGURE 2 SAMPLING SYSTEM FOR DETERMINATION
OF HENRY'S LAW CONSTANTS

8200 high-pressure liquid chromatographic (HPLC) detector with a 254 nm filter. A Milton Roy Co. minipump circulated the solution between the H_c apparatus and the HPLC detector. Two H_c measurements were made concurrently using a Rheodyne model 7010 six-port switching valve that alternatively connected one or the other H_c apparatus to the UV detector. The concentrations of RDX and HMX were measured in the same H_c apparatus. A Waters HPLC consisting of two model 6000A pumps, a Model 660 solvent programmer, a Model U6K injection valve, and a Model 440 detector with a 254nm filter were used to measure the concentration of RDX and HMX. The HPLC column was a Waters Associates μ -Bondapak C_{18} (30 cm $\times$ 4.4 cm ID), and 2,4,6-TNT was used as an internal standard. A flow rate of 2.0 ml min⁻¹ of 30% acetonitrile:70% water was used to separate all three components.

Samples of the solution in the H_c apparatus were analyzed by GC or HPLC before and after each experiment to determine if losses were from causes other than volatilization. If degradation or contamination of the solution in the H_c apparatus occurred, then the degradation products or contaminants would likely be found. No such degradation products or contaminants were found at any significant levels. Also, the water level in the H_c apparatus was monitored, and the apparatus was refilled, if necessary, to keep it at a constant volume. Saturation of the nitrogen carrier gas with water vapor kept water evaporation at a minimum.

III RESULTS AND DISCUSSION

H_c^C can be calculated from the experimental data using the following equation as reported by Mackay et al. (1979).

$$\ln C/C_0 = - \frac{H_c^C}{VRT} V_N \quad (4)$$

where:

C = the concentration of the chemical at a given time

C_0 = the initial concentration of the chemical

V = volume of solution in H_c apparatus

V_N = volume of water-saturated nitrogen passed through the solution at a given time.

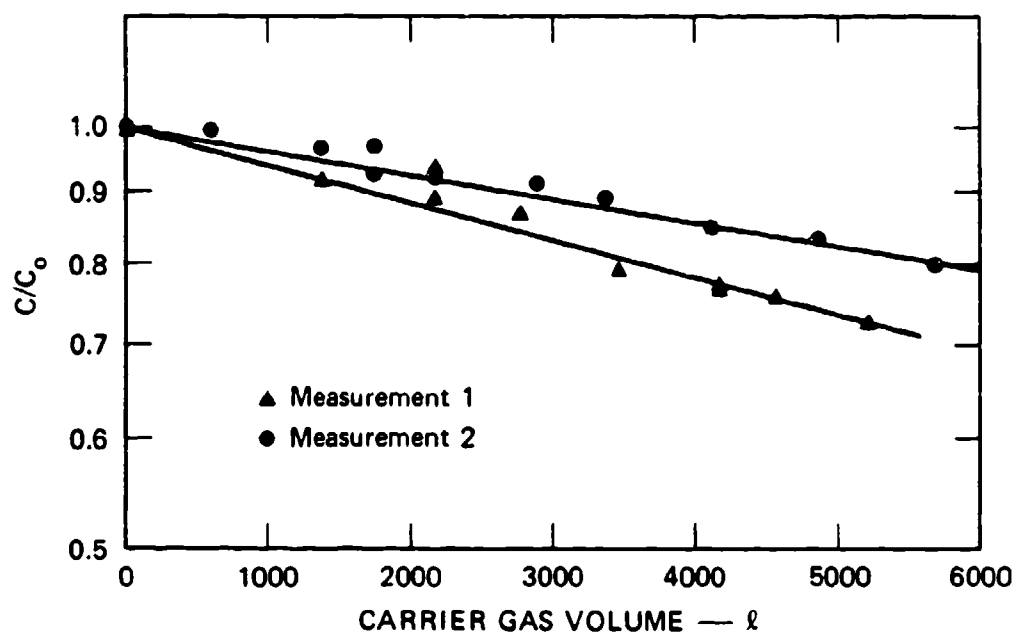
A plot of $\ln C/C_0$ versus V_N should be a straight line with a slope of $-H_c^C /VRT$. Since the quantities R and T are known and V can be measured, H_c^C can be calculated. Table 2 and Figure 3 contain the experimentally measured volatilization data.

Only 2,4-DNT has a slope significantly different from zero. The other compounds have slopes that are not significantly different from zero except for RDX, which has a slightly positive slope. Loss of either water or the internal standard (2,4,6-TNT) would result in a positive slope. However, both RDX and HMX were measured concurrently in the same H_c apparatus, and HMX does not show a significant positive slope. A slope of zero was assigned to those compounds having a slightly positive slope such as that of RDX. The minimum possible negative slope was calculated by adding the 95% confidence limit error to the slope, and an upper limit of H_c was calculated.

Measured values of H_c are reported in Table 3, along with values calculated from vapor pressure and solubility data. The two experimental values for 2,4-DNT are in good agreement. Comparison of

Table 2
VOLATILIZATION DATA

TNG		2,4,6-TNT		RDX		HMX	
V_N (liters)	C/C ₀	V_N (liters)	C/C ₀	V_N (liters)	C/C ₀	V_N (liters)	C/C ₀
0	1.00	0	1.00	1762	1.00	1762	1.00
687	1.01	727	1.00	2501	1.00	2501	0.90
1190	1.01	1478	1.01	3220	1.03	3220	0.95
1426	1.01	2178	1.00	3937	1.04	3937	0.94
2145	1.01	2919	1.00	4550	1.05	4550	1.03
2861	1.03	3651	0.99	5664	1.09	5664	0.98
3596	1.03	4374	1.00	6061	1.12	6061	1.02
4296	0.99	5093	1.00	6785	1.11	6785	0.99
4615	0.96	5844	0.99	7522	1.14	7522	1.05
5342	1.00	6556	1.00				
6065	1.01	7185	1.00				
		7950	1.01				
		8626	1.00				
		9343	0.98				



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FIGURE 3 VOLATILIZATION DATA FOR 2,4-DNT

Table 3

EXPERIMENTAL AND CALCULATED VALUES OF H_c

Chemical	Estimated value* of H_c (torr M^{-1})	Measured value of H_c (torr M^{-1})	Predicted Environmental half-life (days)
2,4-DNT	3.4	0.45 ± 0.04 0.34 ± 0.05	600
TNG	<0.06	<0.06	> 5,000
2,4,6-TNT	0.18	<0.02	>10,000
RDX	2×10^{-5}	<0.04	> 6,000
HMX	---	<0.15	> 1,000

*See Final Report, Phase I - Literature Review for references.

the calculated and experimental values of H_c for 2,4-DNT and 2,4,6-TNT show the experimental values to be at least an order of magnitude lower than the calculated value. For TNG the experimental value is in agreement with the calculated value of H_c . The lowest H_c that we could measure with reasonable accuracy and precision was about 0.1 torr M^{-1} . No vapor pressure or solubility data were available for a calculation of H_c for HMX.

Mackay et al. (1979) compared values of H_c calculated, using Equation 4, with values experimentally determined by the method we have used and found good agreement. All of the compounds had relatively high vapor pressures and aqueous solubilities ($> 0.09 \text{ torr}$ and $> 31 \text{ ppm}$ at 25°C). The aqueous solubilities of the munitions covered in this report are all 4.5 ppm or more. Measurement of solubilities of this order should be very accurate. However, the H_c values of these munitions are an order of magnitude or more lower than those of the chemicals used by Mackay. Therefore, it is likely that the error in the calculated H_c (Table 3) values is most probably in the vapor pressure data, probably due to the difficulty in measuring such low vapor pressures. Another source of error may result from extrapolating measured vapor pressure data to temperatures outside the range used to measure them.

In conclusion, the H_c values were experimentally determined for several munitions and compared to calculated values from solubility and vapor pressure data. The experimental values of H_c were consistently lower than those calculated. Some or all of these differences may be due to errors in the vapor pressure data. Volatilization should be a very slow process for all of these munitions, and it will not be a major aquatic fate for 2,4-DNT, TNG, RDX, HMX, and 2,4,6-TNT.

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PART C

PHOTOCHEMICAL STUDIES OF 2,4,6-
TRINITROTOLUENE IN NATURAL WATERS

By

William R. Mabey
Theodore Mill
Doris S. Tse
John S. Winterele

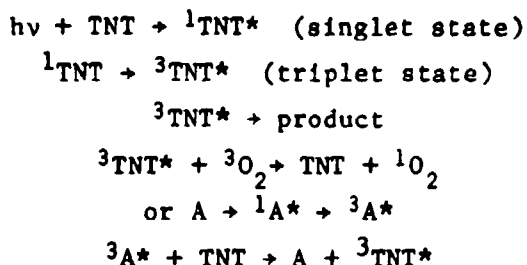
INTRODUCTION

Previous work in our laboratories has shown that the photolysis of TNT has a half-life of less than a day in sunlit pure water, with much shorter half-lives in several natural waters ($t_{1/2} < \text{hour}$, Spanggard et al. 1980). This report discusses studies directed toward understanding and predicting the photolysis rate of TNT in natural waters and elucidating the mechanism of the photolysis.

In these investigations, we performed experiments to establish a quantitative relationship to describe the indirect photolysis rate of TNT in aquatic environments. Since the literature provides evidence that nitroaromatic chemicals can be complexed by other organic substrates, we also conducted some experiments to determine whether TNT may be complexed with humic substances present in natural waters. Such complexes are not allowed for in current models that describe environmental processes (i.e., chemicals are either dissolved in solution or sorbed to particulates/sediment), and complexed TNT may exhibit reactivity different from that of TNT in the true solution or sorbed state. Finally, as support for the larger effort to model the fate of TNT in the Holston River, we conducted some photolysis experiments in sunlight to determine a photolysis rate constant for use in the computer modeling.

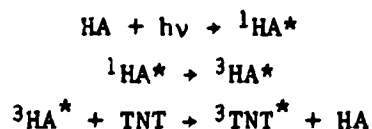
BACKGROUND

Our previous studies found that the photolysis reaction quantum yield (ϕ) for TNT in pure water was $\sim 3.3 \times 10^{-3}$, and that even at low conversions of TNT at 1 ppm concentrations in water the photolysis products accelerated the photolysis rate of TNT. We also found that TNT was photolyzed approximately twice as fast in water purged with argon (i.e., oxygen-free water) than in air-saturated water, and that acetone in pure water also promoted the photolysis of TNT. These results are consistent with a photolysis process occurring via reaction(s) of a TNT-excited triplet state that is quenched by oxygen and that can be generated indirectly by a sensitizer such as acetone (A):



Our photolysis experiments using TNT solutions in filtered natural waters showed that substances present in natural waters promoted the rate of photolysis of TNT by up to 70-fold; we refer to these substances as humic acids in the generic sense, and without reference to any more defined classification of natural substances (e.g., humic acid fraction, fulvic acid fraction, etc.). Although the natural waters used in these studies were not characterized (i.e., in terms of organic carbon, dissolved solids, and other water quality parameters), nor were their spectra accurately measured and analyzed, we observed that the more

colored and more UV-absorbing waters did give the more rapid TNT photolysis rates. We also found that irradiation of TNT in natural water solutions at wavelengths above 400 nm, where TNT does not absorb light, led to the transformation of TNT, presumably via initial light absorption by humic acids, which then induced reaction(s) of TNT. Although both singlet oxygen and free radicals reaction(s) have been reported to occur in natural waters as a result of irradiation of air-saturated humic acid solutions (Mill et al., 1981; Zepp et al., 1978), we judged that TNT would not readily react with such electrophilic oxidants. As with the pure water solutions, the results of TNT photolysis experiments in natural waters can also be explained by reaction of the triplet excited state of TNT. In the natural water, the triplet species is formed by energy transfer from another light-absorbing species, which, if we assume it to be humic acid (HA), follows the scheme:



The ability of humic substances to act as a triplet sensitizer has been demonstrated in one chemical system (Zepp et al., 1981), and therefore they may act in a similar role in TNT photolysis. Because humic acids have no well-defined chromophores nor unique molecular structures or properties (i.e., molecular weight, molar absorption coefficient, etc.), we have characterized humic acids in natural waters by their UV absorbances in a 1-cm cell at a specific wavelength, λ ; this absorbance is denoted as α_λ . Our use of α_λ values assumes that this parameter is at least proportional to the concentration of chromophores responsible for promoting photolysis of TNT.

III EXPERIMENTAL METHODS

The following sections describe the experimental methods used to study the photochemical processes. Also described are the analytical methods used for each fate assessment.

A. Photochemical Studies

1. Laboratory Photolyses

Reaction mixtures of the chemical (4 ml) were placed in 10 mm (OD) borosilicate tubes (Pyrex 7740) and photolyzed on a merry-go-round reactor (Ace Glass). The irradiation source was a Hanovia 450-watt, medium-pressure Hg lamp in a borosilicate immersion well. The distance between the irradiation source and the tubes was about 10 cm. The reaction temperature was at the ambient operating temperature of the system (~ 28°C).

The photolyses were carried out using several different filter systems, which were placed between the Hg lamp and reaction mixtures. In all laboratory photolyses, the borosilicate glass immersion well (8 mm total glass path length) served as a filter to screen out all light below 290 nm. Other filter systems used in the photolyses are described below.

- Filter system for 313 nm: Corning CS 7-54 glass filter with a 0.001 M potassium chromate solution in 3% aqueous sodium carbonate circulated in the immersion well. This system transmitted primarily the 313.2 and 312.6 nm Hg lines, which represented more than 95% of the light incident on the reaction solution (a small Hg line at 302.2 nm was also present).

- Filter system for 366 nm: Corning Glass CS 0-52 and CS 7-60 glass filters. This pair of glass filters transmitted only the 364.1, 365.6, and 366.4 nm lines of the Hg lamp, with no other lines observed (less than 1% of the light outside the 366 nm band). Sample tubes were removed from the merry-go-round reactor at appropriate times and analyzed immediately.

2. Sunlight Photolyses

Outdoor photolyses using sunlight were carried out with solutions of each chemical in natural waters, as described in the discussion section. Photolyzed solutions were placed in a location free of excessive reflections from walls and windows and without morning and afternoon shadows. We used 11 mm (OD) borosilicate tubes held in a rack at a 60° angle to the horizon; the tubes are were made from the same glass stock used in the laboratory photolyses.

The outdoor photolyses, performed in dishes, were conducted in black-painted, lined tubs containing water. The blackened liner minimized reflections back into the dish, and the water acted as a temperature bath to keep the photolyzed solution at or near constant temperature.

B. Preparation of Solutions

Natural water samples were filtered through 0.45 μ filters and subsequently through 0.20 μ filters before use in photolysis experiments. Pure water was obtained from a Milli-Q water purification system, which consists of a reverse-osmosis filter, followed by two ion-exchange columns an activated charcoal column, and a final 0.22 μ filter. Aliquots of TNT in acetonitrile were added to the pure or natural waters to obtain 1% acetonitrile in water solutions. Although TNT is quite soluble in pure water, this procedure was convenient for preparation of TNT solutions, since the addition of TNT dissolved in acetonitrile did not require time for dissolution.

C. TNT Analysis

Aqueous TNT samples were analyzed by direct injection into the HPLC column.

Instrument: Waters Associates Model 6000A.

Column: μ -Bondapak C₁₈, 30 cm x 4 mm.

Solvent: Kinetic experiments in natural waters and in pure water using 50% methanol in water at isocratic conditions.

Flow rate: 1.6 ml min⁻¹.

Detector: UV @ 254 nm.

D. Polarography

Direct current (DC) polarography on a dropping mercury electrode was performed using a Par Model 174 polarographic analyzer. The drop time of the electrode was set at 2.0. For the analysis, the analytic vessel was immersed in a water bath maintained at 25 ± 0.1 °C. The cyclic voltametric analysis was performed using an IBM Model EC 225-2A voltametric analyzer. The sweep rate was 100 mv sec⁻¹, and the pulse height was 20 mv.

IV RESULTS

A. TNT Photolysis in Natural Waters

After reviewing the literature concerning indirect photolysis processes and our previous work on the photolysis of TNT, we decided to more precisely establish the role of TNT during its photolysis in natural waters. For instance, while the photolysis of TNT is clearly promoted by humic substances in natural waters, it has not been quantitatively shown whether photolysis occurs because these substances initially absorb the light and then induce TNT transformation (probably by a triplet-photosensitized process as discussed above), or whether TNT itself initially absorbs the light and the excited-state TNT then reacts with the substances in the natural water. Although our previous studies did show that light absorption by the natural waters at wavelengths above 400 nm will promote the photolysis of TNT, our studies did not determine whether both of the above mechanisms could be occurring at the lower, more energetic wavelengths, and if either could be the dominant process that would occur in the environment. Our previous work found that the photolysis rates of TNT were different in different natural waters. We therefore needed to determine the relationship or correlation between the concentration of humic substances present in the natural water and the resulting TNT photolysis rate constants; the most obvious properties of the natural water for correlation are the absorbances at specific wavelengths.

If initial light absorption by the natural water is responsible for transformation of TNT, the simplest relation to evaluate photolysis of TNT in natural waters is to measure a reaction quantum yield, ϕ'_0 defined as the ratio of the rate of loss of TNT to the rate of light absorption by the natural water (Equation 1)

$$\frac{k_p' [\text{TNT}_0]}{I_\lambda (1 - 10^{-\alpha_\lambda \ell})} = \phi_0' \quad (1)$$

where k_p' is the measured first-order rate constant for loss of TNT, I_λ is the light flux from a source of light of wavelength λ , α_λ is the absorbance of the natural water in a 1 cm cell, and ℓ is the cell pathlength (cm). A value for $\ell = 1.12$ cm has been previously determined for our borosilicate reaction tubes using the method of Zepp (1979). It should be noted that in Equation 1, the quantum yield depends on the concentration of TNT, which obviously decreases as the photolysis proceeds; therefore, the initial concentration of TNT (TNT_0) has been used to calculate ϕ_0' . We have also evaluated our photolysis data using the value $\phi_0'/[\text{TNT}]$, which is the quantum yield corrected for the TNT concentration.

Photolyses of TNT were performed in natural waters at two wavelengths and at several concentrations of TNT to evaluate the influence of α_λ , I_λ , and $[\text{TNT}_0]$ on k_p' and subsequently on ϕ . The photolyses were performed in water samples from the South Fork of the Holston River and from Searsville Pond; experiments were also conducted in water from Searsville Pond diluted with an equal amount of pure water to test the effect of humic substances on photolysis rates without any ambiguities caused by a different water source. The values of $[\text{TNT}_0]$, α_λ , I_λ , and k_p' obtained in these experiments and the ϕ_0' and $\phi_0'/[\text{TNT}]$ values are listed in Table 1.

If Equation 1 applies to a quantitative description of the photolysis of TNT in natural waters, then the quantum yield ϕ_0' should be constant when $[\text{TNT}_0]$ values are the same; however, the ratio $\phi_0':[\text{TNT}_0]$ should be constant throughout. In fact, ϕ_0' and $\phi_0'/[\text{TNT}]$ both vary by factors of up to three. The least precise value used in the calculation of ϕ is α_λ , which several measurements show is reliable to within 10%. We conclude that other, more subtle factors affect the photolysis rates of TNT in these natural water solutions, giving a moderate variation in the ϕ value.

Table 1

PHOTOLYSIS OF TNT IN NATURAL WATERS

Water Samples	$[\text{TNT}]_0 \text{ M} \times 10^6$	$\lambda(\text{nm})$	$\alpha_\lambda \text{ l}^d$	$k_p \text{ (s}^{-1}\text{)} \times 10^5$	$\phi \times 10^4$	$\phi/[\text{TNT}]_0 \times 10^{-2}$
Holston River, South Fork	2.79	313 ^a	0.043	6.29 ± 0.48	14.	4.8
Searsville Pond	2.79	313 ^a	0.119	10.4 ± 0.48	8.8	3.2
Searsville Pond	2.79	366 ^b	0.049	18.8 ± 0.8	5.0	1.8
Searsville Pond, ^e	2.79	313 ^a	0.065	3.27 ± 0.31	4.8	1.7
Searsville Pond	1.26	313 ^c	0.119	9.66 ± 0.28	6.6	5.3
Searsville Pond	2.52	313 ^c	0.119	8.32 ± 0.52	11.	4.5
Searsville Pond	4.46	313 ^c	0.119	7.16 ± 0.29	17.	3.9
Pure water	2.79	313 ^a	0.0033	0.711	19.	---

^a $I_\lambda = 1.37 \times 10^{-6}$ Einsteins⁻¹l-s at 313 nm.

^b $I_\lambda = 9.85 \times 10^{-6}$ Einsteins⁻¹l-s at 366 nm.

^c $I_\lambda = 7.67 \times 10^{-7}$ Einsteins⁻¹l-s at 313 nm.

^d $l = 1.12$ cm.

^eDiluted with equal amount of pure water.

Reasons why ϕ is not constant could be that (1) α_λ is only an approximation of the absorbance of the active chromophores that promote TNT photolysis, and the proportion of active and inactive chromophores may change with wavelength or natural waters; (2) the ability of humic substances to promote or quench TNT photolysis may vary slightly with concentration of TNT or of humic substance; or (3) TNT is complexed with humic substances in natural water, and therefore the photochemistry may involve both complexed and uncomplexed forms of TNT. Although we could not perform enough experiments to determine the influence of complexation on the photolysis rates of TNT in natural waters, we did obtain some evidence that a complex between TNT and humic substances may exist (see following discussion). It is also important to note that Equation 1 does not take into account that TNT is lost by both indirect and direct photolysis when k_p' is measured; however, since the photolysis of TNT in natural waters is at least 10 times faster than in pure water, correction of k_p' to subtract out the direct photolysis process contribution will not significantly affect the 2- to 3-fold differences we calculate in ϕ_0' or $\phi_0'/[\text{TNT}_0]$ values.

B. Evidence for Triplet-Sensitized Mechanism in TNT Photolysis

The above observation that the photolysis rate constant of TNT in a natural water can be roughly correlated with the absorbance of the natural water is consistent with humic acid acting as a triplet sensitizer, as previously discussed. Further evidence for this mechanism was obtained in experiments where 0.60 ppm TNT was photolyzed in Searsville Pond (SP) water and in pure water containing p-acetobenzene sulfonic acid (ABSA). ABSA is a water soluble form of acetophenone, which is well known to sensitize photolyses via triplet excited-state processes. The absorbances of the SP water and ABSA solution were matched so that the amounts of irradiating light absorbed by the solutions at 313 nm were the same, and therefore the photolysis rate constants for TNT in these solutions could be directly compared. Table 2 lists the data from these experiments, along with ϕ_0' values previously discussed. These data clearly show that ABSA is more

Table 2

PHOTOLYSIS OF TNT IN NATURAL WATER AND IN THE
PRESENCE OF A TRIPLET SENSITIZER AT 313 AND 366 nm

Water	$k'_p (s^{-1}) \times 10^5$	$\phi'_o \times 10^3{}^a$
At 313 nm		
Searsville Pond (SP)	7.87 ± 0.29	1.2
$1/2$ SP	4.98 ± 0.20	1.4
ABSA ^b	31.9 ± 0.3	4.9
$1/2$ ABSA	19.8 ± 1.2	5.7
At 366 nm		
SP	26.1 ± 0.8	0.75
$1/2$ SP	15.0 ± 0.9	0.82
ABSA	15.8 ± 1.3	12.9

^a ϕ'_o values are directly comparable because the same initial
TNT concentrations were used (2.6×10^{-6} M TNT)

^b 1.01×10^{-3} M ABSA.

efficient than SP water in promoting photolysis of TNT. From these results we may conclude that humic acid may indeed be acting as a triplet sensitizer similar to acetone, as discussed earlier. It should be noted that acetophenone (and therefore ABSA) is an optimal sensitizer for such sensitized reactions, since its quantum yield (or efficiency) for intersystem crossing from singlet to triplet state is nearly one, and its triplet state is relatively long lived. If the photolysis of TNT in the natural water had been more rapid than in the ABSA-pure water solutions, this would have been strong evidence for a photolysis mechanism other than the simple sensitized process discussed above.

C. TNT Photolysis in Natural Waters - Sunlight Experiments

The sunlight photolysis rate constants for TNT in natural water solutions were also measured in outdoor experiments (Table 3). The experiments using Holston River (South Fork) and Searsville pond water were performed in several types of reaction vessels (or cells) to determine what effect the geometry of the cell has on the measured photolysis rate constant of TNT; the experiments in uncovered 10 cm (OD) dishes are considered closest to an environmental situation, where sunlight irradiates a water body only through the surface of the water. An experiment was also conducted in a dish containing unfiltered Holston River water, in which the sediment collected with the water was maintained in suspension by stirring.

The data in Table 3 show several significant features regarding the photolysis of TNT, the most notable being the effect of cell geometry on the sunlight photolysis rate of TNT. The experiments in Holston River water and in Searsville pond water showed that TNT photolyzed about 3.4 times faster in the borosilicate tube than in the open dish. This is due in part to the fact that direct light, as well as skylight (or scattered light), enters the dish cell from only the top, whereas skylight enters the tube from all directions (360°). Therefore the total light flux per unit volume passing through the tube is greater than that entering the dish. Although it cannot be shown quantitatively

Table 3

MEASURED SUNLIGHT PHOTOLYSIS RATE CONSTANTS FOR TNT

<u>Water Sample</u>	<u>Date and Time of Expt.</u>	<u>Type of Cell</u>	<u>Rate Constant $k_p(s^{-1}) \times 10^4$</u>
Holston River, North Fork	Late afternoon, 5/12/81	Tube	4.97 ± 0.21
Holston River, South Fork	Late afternoon,	Tube	4.88 ± 0.02
Pure water	Late afternoon,	Tube	0.462 ± 0.029
Holston River, South Fork	Midday, 7/17/81	Tube	10.3 ± 0.3
Holston River, South Fork	Midday, 7/17/81	Dish	2.97
Holston River, with sediment in suspension	Midday, 7/17/81	Dish	2.09 ± 0.09
Searsville Pond 7/15/81	Midday	Tube	16.7 ± 0.7
Flask		Dish	5.0 ± 0.2 8.4 ± 0.4
Pure water 7/15/81	Midday	Tube	1.9

by these experiments, the optical pathlength per unit of solution volume is possibly greater in the tube than in the dish because of internal reflection within the cylindrical tube. Whatever the reasons for the differences in rates, the photolysis rate constants of TNT in natural water solution measured in tubes should be divided by about 3 to obtain rate constants for use in environmental assessments.

The data for photolysis of TNT in filtered and unfiltered Holston River water (South Fork) also show that the particulates (mainly suspended sediment) slow the photolysis of TNT in natural waters. The suspended sediment in Holston River water measured at the time of sampling was about 100 ppm, and we assume that a similar concentration was present in our experiment. The slower rates are due mainly to light absorption by these particulates rather than to light-scattering effects, which also occurs; we did not determine from our experiment how important this process is in comparison to light absorption.

D. Investigations of the Complex Formation Between TNT and Humic Substances

Our review of the literature regarding the photolysis and other chemistry of nitroaromatics found little information useful for evaluating the reactions that occur in aqueous solutions. The literature does show, however, that complexation of nitroaromatics with electron-rich substrates has been extensively studied in organic solvent media. Although trinitrobenzene (TNB) has been the most commonly used nitroaromatic for such studies, some papers did report complexation of TNT and DNT. Zeichmann (1972) discussed the formation of charge-transfer complexes of humic acids with organic chemicals, and cites humic acid as an electron donor that can interact with TNB as the acceptor. Although several authors (Zepp, et al., 1979) claimed to show evidence for complexation between humic acid and organic chemicals, the interpretation of the evidence has been the subject of debate.

The issue of whether TNT is complexed to humic acids in aquatic systems is relevant to understanding the indirect processes that may occur for TNT in the aquatic environment. Thus, a fundamental question

is whether TNT is "free" in solution (only solvated by water) or associated with humic substances in some kind of complex (i.e., charge-transfer, etc.). This phenomenon can be represented as Equation 2:



The presence of such a complex would be a complicating factor in evaluating the photolysis of TNT in aquatic systems (Zepp et al., 1981) since complexation may affect the UV absorbances responsible for light absorption and subsequent reactions of TNT, as discussed previously.

To test for TNT-humic acid complex formation, we employed a polarographic approach. Our rationale for polarographic experiments is that the complexed TNT and the "free" TNT should have different reduction potentials if the time scale for the electrochemical experiment is more rapid than the equilibrium time scale. For these experiments we used 10 ml solutions of 4.4×10^{-5} M TNT at pH 7.2, to which 0.1 ml of 1 M NaCl was added as a supporting electrolyte. Solutions were prepared using pure water or Searsville Pond water. Data from these experiments for the reduction half-wave potentials and their differences are listed in Table 4.

Table 4
POLAROGRAPHY OF TNT IN PURE AND NATURAL WATER

Wave Reduction	Half-wave Potentials (volts)	
	Pure Water	90% Natural Water 10% Pure Water
First	-0.334	-0.267
Second	-0.464 (-0.130) ^a	-0.423 (0.156) ^a
Third	-0.670 (-0.336) ^b	-0.567 (-0.300) ^b

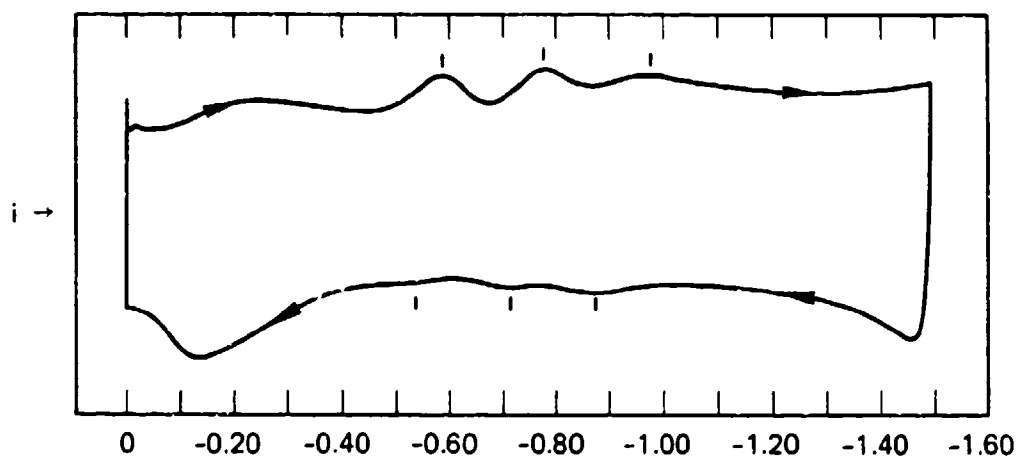
^a Difference between second wave and first wave.

^b Difference between third wave and first wave.

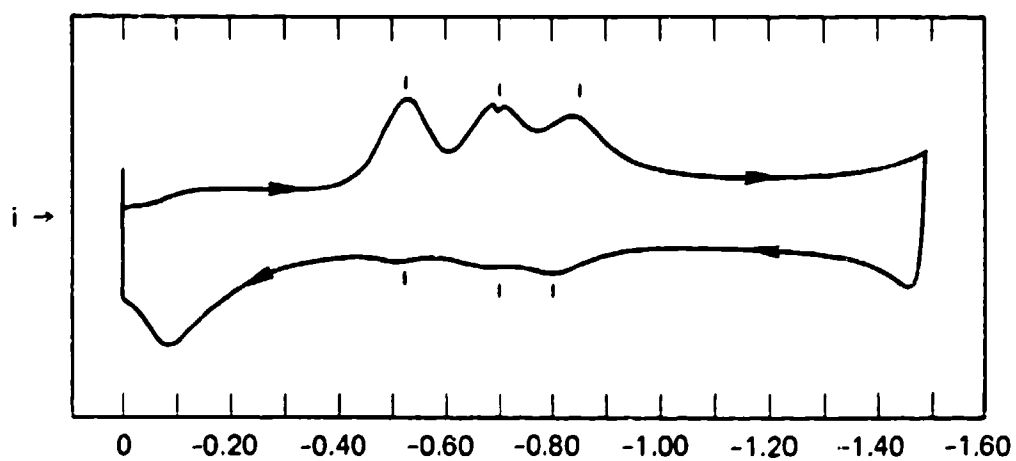
These data show that the reduction polarograms of TNT in pure water and in natural water are different, both in absolute values of the half-wave potentials and in the difference between these potentials. We also found that the TNT polarograms in the natural waters were better resolved than those in the pure water solutions. This better resolution is attributed to humic substances acting as surfactants, since the polarogram in pure water was also better resolved when Triton surfactants were added. The addition of synthetic surfactants to improve polarographic resolution is a common practice; since humic acids are polymeric materials containing phenolic and carboxylic acid groups, it is possible that they would exhibit surfactant properties in our experiments. It is important to note, however, that while surfactants do improve polarographic resolution, they do not affect the half-wave potential values observed in our experiments.

In a second set of experiments, we sought to obtain conditions in which both sets of half-wave potentials were observed by using diluted solutions of the Searsville Pond Water (SPW) and 10 ppm TNT. Such conditions were not found: using 90% SPW we observed the "complexed TNT" polarogram (as judged by half-wave potential values), whereas in 50% SPW/50% pure water we found the same half-wave potentials as TNT in pure water. We conclude that the two sets of polarograms might be observed only between 50% and 90% SPW. Alternatively these results may indicate that micelle formation occurs at some concentration of humic acid in this range.

In another brief electrochemical study to investigate the possible complexation of TNT and humic acids, we obtained cyclic voltamograms on a carbon electrode for TNT in pure water and in the natural water. A differential pulse technique was used in these voltammetric experiments to better locate the half-wave potential peaks in the traces. The voltamograms in pure water and in natural water are shown in Figures 1A and 1B.



(a)

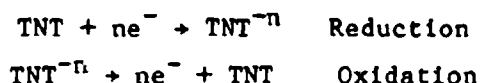


(b)

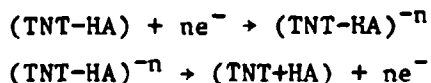
LA-7934-18

FIGURE 1 CYCLIC VOLTAMMOGRAM OF TNT IN PURE WATER (a)
AND IN NATURAL WATER (b)

The upper curves in each figure are plots of differential ion current as a function of voltage for reduction of species in solution, and the lower curves are for oxidations. Maxima on the reduction cycle and minima on the oxidation cycle indicate reduction or oxidation, respectively.



As discussed for the half-wave potentials, these figures also show that the onset of each reduction wave occurs at a lower voltage for TNT in the natural water than for TNT in the pure water. More significantly, the oxidation potentials for TNT in the natural water (Figure 1B) occur at the same position for two reduction waves and about 0.05 volts off for the third wave; this behavior is contrasted to that of TNT in pure water, where no such matching is observed. These curves do indicate that reduction-oxidation of TNT in the natural water is more reversible than that in pure water. Such results can be explained by the existence of a TNT-humic acid complex (TNT-HA) in the natural water, with the oxidation-reduction of the complex



more reversible than that for TNT in pure water alone.

While the electrochemical experiments support the idea that TNT exists in complexed form in natural waters, more extensive experimental studies are required. These studies should be conducted at lower TNT concentrations that approximate environmental levels and using more sophisticated polarographic and voltammetric techniques; it is also possible that micelle formation may account for the observed results. These issues will be pursued in further research.

The effect of possible complexation processes on the photolysis of TNT, compared to an "uncomplexed" TNT, cannot be calculated at this time. Attempts to identify a complexed TNT species by UV-visible spectroscopy were unsuccessful, possibly because of the low concentrations of TNT and humic substances in solution and the low sensitivity of the spectrophotometer used.

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