

AD-A031 067

STANFORD RESEARCH INST MENLO PARK CALIF
TOXICITY OF TNT WASTEWATER (PINK WATER) TO AQUATIC ORGANISMS.(U)
JAN 76 D H LIU, R J SPANGGORD, H C BAILEY

F/G 19/1

DAMD17-75-C-5056

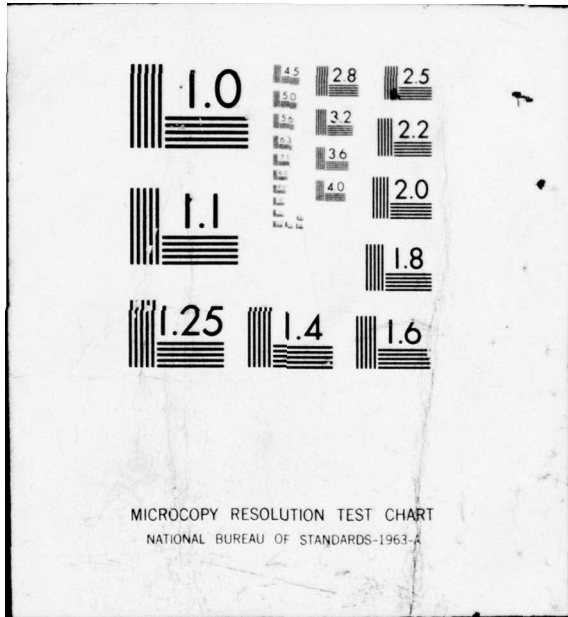
NL

UNCLASSIFIED

| OF |
AD
A031067



END
DATE
FILMED
11-76



AD A 031 067

12

AD

16.

TOXICITY OF TNT WASTEWATER (PINK WATER) TO AQUATIC ORGANISMS

Annual Report

By

DAVID H. W. LIU
RONALD J. SPANGGORD
HOWARD C. BAILEY

January 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D.C. 20314

Contract DAMD17-75-C-5056

STANFORD RESEARCH INSTITUTE
MENLO PARK, CALIFORNIA 94025

DoD DISTRIBUTION STATEMENT

DDDC
OCT 20 1976
C

Approved for public release: distribution unlimited.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Approved for public release; distribution unlimited.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM	
1. REPORT NUMBER AD	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) ⑥ TOXICITY OF TNT WASTEWATER (PINK WATER) TO AQUATIC ORGANISMS. ✓		5. TYPE OF REPORT & PERIOD COVERED Annual Report 4/15/75 - 12/31/75	
7. AUTHOR(s) ⑩ David H.W. Liu, Ronald J. Spanggord Howard C. Bailey		6. PERFORMING ORG. REPORT NUMBER	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Stanford Research Institute ✓ 333 Ravenswood Avenue Menlo Park, California 94025		8. CONTRACT OR GRANT NUMBER(s) ⑮ DAMD17-75-C-5056 NEW	
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Washington, D.C. 20314		12. REPORT DATE January 1976	13. NO. OF PAGES 31
14. MONITORING AGENCY NAME & ADDRESS (if diff. from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED ⑫ 29p.	
16. DISTRIBUTION STATEMENT (of this report) Approved for public release; distribution unlimited. ⑨ Annual rept. 15 Apr - 31 Dec '75		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from report)			
18. SUPPLEMENTARY NOTES			
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Acute toxicity, TNT wastewater, α-TNT, 2,4-DNT, photodegradation, pH, fish, <u>Daphnia magna</u> .			
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The acute toxicity was determined of aqueous solutions of α-TNT and 2,4-DNT and three types of TNT wastewater from the Joliet Army Ammunition Plant to the fathead minnow (<u>Pimephales promelas</u>) and the aquatic invertebrate <u>Daphnia magna</u> . The toxicity tests were conducted on materials that had been adjusted to pH 5, 7, and 9.4 and exposed to ultraviolet light. All tests were conducted under static conditions without aeration. →			

DD FORM 1473
1 JAN 73
EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

3325001

JB

19. KEY WORDS (Continued)

20 ABSTRACT (Continued)

alpha-

The pH of the material during irradiation had little effect on its toxicity. Ultraviolet irradiation of LAP and condensate wastewater and of α -TNT and 2,4-DNT, the respective major constituents of the two wastewaters, reduced their toxicity, however, this effect was not statistically significant unless the α -TNT or 2,4-DNT content was reduced to zero. The toxicity of red water was not affected by irradiation.

The acute toxicity of benzene and aqueous fractions of nonirradiated wastewater and of 50% photolyzed aqueous solutions of α -TNT and 2,4-DNT was also determined. The benzene fractions were more toxic than the aqueous fractions. Evidence was obtained suggesting that α -TNT is probably the most toxic ingredient of LAP wastewater. The study did not identify the most toxic component of condensate water or red water.

alpha

The minnow and Daphnia magna were equally sensitive to 2,4-DNT, but the latter was more tolerant of α -TNT.

ADDRESS ON FILE	
NRIS	White Section <input checked="" type="checkbox"/>
U.S.	Duff Section <input type="checkbox"/>
UNANNOUNCED	<input type="checkbox"/>
JUSTIFICATION	
BY	
DISTRIBUTION/AVAILABILITY CODES	
DEPT.	ATTN. NUMBER/SPECIAL
A	

SUMMARY

The acute toxicity was determined of aqueous solutions of α -TNT and 2,4-DNT and three types of TNT wastewater from the Joliet Army Ammunition Plant to the fathead minnow (Pimephales promelas) and the aquatic invertebrate Daphnia magna. The toxicity tests were conducted on materials that had been adjusted to pH 5, 7, and 9.4 and exposed to ultraviolet light. All tests were conducted under static conditions without aeration.

The test results indicated that the pH of the material during irradiation had little effect on its toxicity. Ultraviolet irradiation of LAP and condensate wastewater and of α -TNT and 2,4-DNT, the respective major constituents of the two wastewaters, reduced their toxicity; however, this effect was not statistically significant unless the α -TNT or 2,4-DNT content was reduced to zero. The toxicity of red water was not affected by irradiation.

The acute toxicity of benzene and aqueous fractions of unirradiated wastewater and of 50% photolyzed aqueous solutions of α -TNT and 2,4-DNT was determined, and the benzene fractions were found to be more toxic than the aqueous fractions. Evidence was obtained suggesting that α -TNT is probably the most toxic ingredient of LAP wastewater. The study did not identify the most toxic component of condensate water or red water.

The minnow and Daphnia magna were equally sensitive to 2,4-DNT, but the latter was more tolerant of α -TNT.

CONTENTS

DD FORM 1473	1
SUMMARY.	3
INTRODUCTION	7
METHODS.	9
Bioassay Procedures	9
Materials Tested.	9
Sources of the Test Material.	12
Test Organisms.	12
Photoirradiation Procedures	12
Extraction Procedures	12
Chemical Analytical Procedures.	13
Water Quality Conditions.	13
RESULTS.	15
Toxicity of α -TNT and 2,4-DNT To The Fathead Minnow	15
Toxicity of α -TNT and 2,4-DNT to Daphnia magna.	17
Toxicity of Authentic TNT Wastewater to the Fathead Minnow.	17
Toxicity of the Aqueous and Benzene Fractions	20
Relative Toxicity of Authentic Wastewater and Selected Components	23
Miscellaneous Observations.	25
CONCLUSIONS.	27
RECOMMENDATIONS.	29
REFERENCES	31
DISTRIBUTION LIST.	33

INTRODUCTION

TNT wastewater is a product of plants that manufacture and handle the explosive 2,4,6-trinitrotoluene (α -TNT). Several types of TNT wastewater exist, and the chemical composition of each kind depends on the point in the manufacturing or handling process at which the waste effluent is produced as well as on the explosive formulation being prepared.

Two kinds of TNT wastewater are normally discharged into natural bodies of water. One of these contains a high percentage of α -TNT and is often called pink water because of the reddish color that develops when it is exposed to sunlight at pH 7 or above. The other is called condensate wastewater and consists of condensed steam distillates from the Sellite manufacturing process and wastewater generated from the continuous TNT manufacturing process. The major militarily unique chemical component of condensate wastewater is 2,4-dinitrotoluene (2,4-DNT).

Little is known about the toxicity of TNT wastewater to aquatic organisms. Most of the published information concerns the acute toxicity of certain chemical constituents--primarily α -TNT and 2,4-DNT.

α -TNT can be toxic to fish at concentrations of less than 10 ppm. In work with the bluegill sunfish, Pederson¹ reported 96-hour TLm values that ranged from 2.3 to 2.8 ppm. Gring² reported a 96-hour TLm of 2.0 ppm for the same species. LeClerc³ reported the 96-hour minimum lethal dose of α -TNT for unidentified minnows to be 4 to 5 ppm in soft and hard water.

Certain freshwater algae appear to be more tolerant of α -TNT than fish. Fitzgerald et al.⁴ reported that 8 ppm is lethal to the bluegreen alga Microcystis aeruginosa. In the same species, Smock⁵ and co-workers found only a slight inhibition of growth at 15 ppm of α -TNT; marked inhibition was not observed until the concentration reached 50 ppm. Smock and co-workers found the green alga Selenastrum capricornutum to be much more sensitive to α -TNT than M. aeruginosa--5 ppm strongly inhibited the growth of the former. Another green alga, Chlamydomonas reinhardtii, showed a toxic response at 3 ppm.²

Less is known about the toxicity of 2,4-DNT. According to Burton,⁶ the 24- and 96-hour TL50's for bluegill sunfish are 50 and 16 mg/liter, respectively.

This report describes an investigation of the acute toxicity of three authentic TNT wastewaters to the fathead minnow (Pimephales

promelas) and a freshwater invertebrate (Daphnia magna). In the investigation, we determined the influence of ultraviolet irradiation at three pH levels on the toxicity of the wastewaters and compared the results with those obtained on similarly treated aqueous solutions of pure α -TNT and 2,4-DNT. We also determined the acute toxicity of benzene and aqueous fractions of some of the solutions in a preliminary attempt to identify the toxic agent(s).

This investigation is part of a multiphased program, sponsored by the U.S. Army Medical Research and Development Command, to assess the potential hazards to aquatic life of wastewater from TNT manufacturing and processing plants. The objective of this phase of the program was to obtain preliminary information on factors that might affect wastewater toxicity so that the experimental approach used in subsequent phases could be designed effectively. The ultimate objective of the program is to develop a portion of the necessary data base from which environmental quality standards can be established for militarily unique wastewaters from Army munitions plants.

METHODS

Bioassay Procedures

The toxicity tests were conducted as described in Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians.⁷ The test containers used in the minnow tests were 19-liter glass jars filled with 15 liters of test solution. Tests on D. magna were conducted in 250-ml beakers filled with 200 ml of test solution. All tests were conducted under static conditions without aeration. Dechlorinated tap water was used as the diluent water. Table 1 presents a partial chemical characterization of this water. All tests were conducted at a nominal temperature of 20°C, maintained by use of thermostatically controlled water baths. The animals were not fed during the tests and for 48 hours before initiation of the tests.

We monitored the temperature, dissolved oxygen content, and pH of representative solutions in each test series daily. Each week, before tests were initiated, the diluent water was analyzed for hardness, alkalinity, and conductivity.

Temperature was determined continuously through thermocouples attached to a 24-channel temperature recorder (Honeywell, Electronic 112); dissolved oxygen was measured with a YSI, Model 54, oxygen meter; and pH was determined with a Radiometer, Model 26, pH meter. Water hardness and alkalinity were determined by titration, using the reagents and instructions provided by Delta Scientific. Conductivity was measured with a conductivity meter (YSI, Model SC-T).

Before we prepared the test concentrations, we analyzed authentic LAP and red water for α -TNT and condensate water for 2,4-DNT. Stock solutions of pure α -TNT and 2,4-DNT were analyzed for their respective compounds before and after uv irradiation. We did not analyze the test material after dilution.

We estimated the LC50 values and their 95% confidence limits by the method of Litchfield and Wilcoxon.⁸ The confidence limits were used to judge the significance of differences between LC50 values.

Materials Tested

Table 2 shows the materials tested. In the tests on the unfractionated wastewaters and solutions of α -TNT and 2,4-DNT, we used the minnow as the test organism. The minnow and D. magna were used in all tests on the aqueous and benzene fractions; these tests were exploratory and were not designed to provide LC50 estimates. We also determined the toxicity of unirradiated α -TNT and 2,4-DNT to D. magna.

Table 1

CHEMICAL ANALYSIS OF SRI DECHLORINATED TAP WATER

Except Where Noted, All Analyses Were Conducted by
International Nutronics, Inc., Palo Alto, California

<u>Analysis</u>	<u>Concentration (mg/liter)</u>
Calcium (as Ca)	8.4
Magnesium (as Mg)	2.5
Potassium (as K)	0.40
Sulfate (as SO ₄)	9.2
Nitrate (as NO ₃ -N)	0.00
Nitrite (as NO ₂ -N)	0.001
Free ammonia	0.060
Organic ammonia	0.375
Phenol	0.000
Free chlorine	0.00
Chloride	4.04
Fluoride	0.30
Cyanide	< 0.01
Iron	0.08
Copper	0.0041 (SRI)
Zinc	0.0026 (SRI)
Cadmium	0.00012 (SRI)
Chromium	0.008 (SRI)
Nickel	< 0.050
Lead	0.0007 (SRI)
Total alkalinity (as CaCO ₃)	23.3
Total hardness (as CaCO ₃)	31.2
Total dissolved solids	48.0

Table 2
MATERIALS TESTED FOR TOXICITY

Test Material	0% Photodegradation			50% Photodegradation			100% Photodegradation		
	pH 5	pH 7	pH 9.4	pH 5	pH 7	pH 9.4	pH 5	pH 7	pH 9.4
LAP wastewater	X	X	X	X	X	X		X	
Aqueous fraction		X							
Benzene fraction		X							
Condensate wastewater		X			X			X	
Aqueous fraction		X							
benzene fraction		X							
Red water		X			X			X	
Aqueous fraction		X							
Benzene fraction		X							
α -TNT	X	X	X	X	X	X		X	X
Aqueous fraction					X			X	
Benzene fraction					X			X	
2,4-DNT					X			X	
Aqueous fraction					X			X	
Benzene fraction					X			X	

Sources of the Test Material

We collected the authentic wastewater samples from the Joliet Army Ammunition Plant (JAAP) in Joliet, Illinois, and shipped them in polyethylene-lined, 55-gallon metal drums to the SRI aquatic toxicology laboratory in Menlo Park, California, via air freight. The three wastewater samples were (1) LAP wastewater--a washdown effluent from the load and pack operation, which involves the filling of ammunition cases with TNT and other munitions compounds; (2) condensate wastewater; and (3) red water--a high-solids effluent from the Sellite process. Red water is not released from the plant but is steam distilled along with other types of wastewater and may contribute chemical components to condensate wastewater. We purchased α -TNT from K & K Laboratories, Inc.; the chemical was shipped with 20% water. 2,4-DNT was purchased from Matheson, Coleman, and Bell.

Test Organisms

The fathead minnows and D. magna were from breeding colonies maintained at SRI. The original stock of minnows was obtained from the EPA laboratory in Newtown, Ohio, and the original stock of D. magna was obtained from the EPA laboratory in Duluth, Minnesota.

The minnows used were juveniles with an average standard length of 3.3 cm and wet weight of 0.6 g. The D. magna used were up to 12 hours old.

Photoirradiation Procedures

All photoirradiations were performed in a water-jacketed, 3.2-liter Pyrex reaction vessel with an immersion well and a 1200-watt Hanovia medium-pressure mercury lamp.

Solutions of pure chemicals and wastewaters were irradiated until the concentration of the major component (α -TNT for LAP water and red water and 2,4-DNT for condensate water) had decreased to 50% and 100% degradation levels. For saturated α -TNT and 2,4-DNT solutions, 100% degradation was achieved after 24 hours of irradiation. A 50% degradation level was achieved for α -TNT between 30 minutes and 2 hours; 2,4-DNT took 10 hours to achieve the same level.

When pH adjustments were necessary to bring solutions to neutrality, 0.1N and 6N HCl solutions or 0.1N and 5N NaOH solutions were used. pH was measured on an Orion Model 610 digital pH meter with a combination electrode.

Extraction Procedures

To prepare the aqueous and benzene extracts for toxicological evaluation, we mixed an aliquot of the test material with an equal volume of benzene and separated the benzene and aqueous phases. The

benzene fraction was evaporated to dryness before it was submitted for toxicological evaluation. The aqueous fraction was heated to 60°C, swirled under aspirator vacuum, and finally purged with nitrogen gas to remove all traces of benzene. All aqueous samples submitted for toxicological evaluation were without benzene, as determined by gas chromatography using phenol as an internal standard.

Chemical Analytical Procedures

Determination of α -TNT and 2,4-DNT

After irradiation, 20 ml of the wastewater solution was extracted with an equal volume of diethyl ether. The ether solution was dried over anhydrous magnesium sulfate and rotary-evaporated to dryness. The residue was dissolved in acetone and an internal standard was added (m-dinitrobenzene for α -TNT and for 2,4-DNT). The solutions were analyzed by gas chromatography under the following conditions:

Instrument: Varian 2700 equipped with an HP Model 3380A integrator-recorder.

Column: 6' x 2 mm glass column packed with 10% DC-200 on 80/100 mesh Gas-Chrom Q.

Temperature: 150 to 250°C at 4°C/min.

Flow rate: 20 ml/min N₂.

Detector: Flame ionization.

Determination of Total Dissolved Solids

We determined the amount of dissolved solids in the unfractionated wastewater and aqueous solutions of α -TNT and 2,4-DNT as well as in the aqueous and benzene fractions by freezing samples of each in a dry ice-acetone bath; the frozen sample was then lyophilized to dryness, using a Welch Scientific Co. lyophilizer (Model 1402). The weight of the dried sample represented total dissolved solids.

Water Quality Conditions

During all toxicity tests, the temperature of the test solutions averaged 20 ± 1°C. Dissolved oxygen ranged from 2.0 to 9.8 ppm; we observed the low values toward the end of the tests, particularly in the longer tests involving the minnow. A reduction in dissolved oxygen can occur in static toxicity tests in which the test solutions are not aerated. We did not observe any signs of stress among the test organisms in the presence of low oxygen; however, in the definitive phases of the overall project, we recommend use of the flow-through technique to minimize this problem.

Water hardness varied from 31 to 45 ppm (as CaCO_3). Alkalinity ranged from 25 to 40 ppm (as CaCO_3). Conductivity ranged from 66 to 80 μmhos , and pH ranged from 8.0 to 9.5.

RESULTS

Toxicity of α -TNT and 2,4-DNT to the Fathead Minnow

Table 3 shows for the fathead minnow estimates of the 24- and 96-hour median lethal concentration (LC50) for α -TNT and 2,4-DNT before and after exposure to uv irradiation at various pH's. These estimates are based on the initial concentration of these compounds, that is, the concentration before irradiation. The unirradiated solutions did not contain any degradation products when the tests were initiated, but degradation products could have formed during the tests. Degradation products were present in the irradiated solutions; however, because we were comparing the effect of irradiation on the toxicity of the solutions and all their component parts rather than the effect of the amount of α -TNT and 2,4-DNT remaining after irradiation, we did not distinguish between the primary chemical and its degradation products in preparing the table.

pH Effects

The toxicity of unirradiated α -TNT decreased with increasing pH; however, the difference was significant only between the 96-hour LC50 values for the pH 5 and pH 9.4 solutions. Although the 95% confidence limits for the pH 5 and pH 9.4 LC50 values do not overlap and therefore indicate that the values are different, we believe the zone of nonoverlapping is too small to enable us to conclude that the toxicity of the pH 5 solution is indeed greater than that of the pH 9.4 solution. It should be emphasized that these pH values apply only to the irradiation process, not to the pH of the solutions used in the toxicity tests.

Tests on α -TNT solutions, irradiated until 50% of the initial α -TNT was degraded, were performed on a logarithmic series of concentrations ranging up to 3.2 mg/liter. Only with the pH 7 solution did we obtain mortality data sufficient for estimating the 96-hour LC50. With the pH 5 solution, we observed 17% mortality at 3.2 mg/liter and no mortality at 2.4 mg/liter, which was the next lowest test concentration. The pH 9.4 solution was not lethal at any concentrations tested. These results indicate that α -TNT and the degradation products produced during irradiation at pH 7 are more toxic than α -TNT and the degradation products produced during irradiation at pH 5 or pH 9.4.

Irradiation Effects

Exposure of aqueous solutions of α -TNT or 2,4-DNT to uv irradiation reduced their toxicity to the fathead minnow. Although we did not obtain sufficient data to estimate the 96-hour LC50 for the 50% degraded pH 5 solution of α -TNT, the data indicate that this solution is considerably less toxic than its nonirradiated counterpart. The

Table 3
 ACUTE TOXICITY OF α -TNT AND 2,4-DNT BEFORE AND AFTER ULTRAVIOLET IRRADIATION
 Test Animal - Fathead Minnow

Compound	Percent Degradation	pH	LC50 (mg/liter)*		95% Confidence Limits (96-hr)	Highest Nonlethal Concentration
			24-hr	96-hr		
α -TNT	0	5	4.2	1.2	0.8-1.9	0
	50	5	> 3.2	> 3.2	---	2.4
	0	7	> 3.2	2.0	1.9-2.2	0
	50	7	> 3.2	3.0	2.1-4.2	0
	100	7	48.5	44.1	40.5-48.1	37.0
2,4-DNT	0	9.4	3.0	2.4	2.0-3.0	1.0
	50	9.4	> 3.2	> 3.2	---	3.2
	0	7	33.0	31.0	28.4-33.8	25.0
	50	7	> 45.0	44.0	38.3-50.6	25.0
	100	7	< 75,	< 75,	---	56.0
			> 56	> 56		

* Expressed in terms of the concentration of the primary chemical before irradiation.

percentage mortality at 3.2 mg/liter was 17% for the 50% degraded solution, whereas the same concentration of the unirradiated solution killed all exposed fish, as shown in Table 4. In addition, we observed no mortality at 2.4 mg/liter or less of the 50% degraded solution, but approximately 38 to 62% mortality occurred in fish exposed to 0.75 to 1.8 mg/liter of nonirradiated α -TNT after 96 hours of exposure.

Exposure of α -TNT at pH 7 to uv light until 50% degradation had little effect on its toxicity. At this pH, the 96-hour LC50 was 2.0 mg/liter for the nonirradiated solution and 3.0 mg/liter for the 50% degraded solution. Comparison of the 95% confidence limits indicates no significant difference between these two values at the 5% level. Complete degradation of α -TNT resulted in a considerable and statistically significant reduction in toxicity. The 96-hour LC50 of the 100% degraded solution was 44.1 mg/liter. We observed no mortality at 37 mg/liter.

α -TNT, 50% degraded and irradiated at pH 9.4, was not lethal at 3.2 mg/liter, the highest concentration tested. The same concentration of nonirradiated α -TNT, prepared at a pH of 9.4, killed 90% of the test population in 96 hours. This solution had a 96-hour LC50 of 2.4 mg/liter.

Ultraviolet irradiation of aqueous pH 7 solutions of 2,4-DNT also reduced the toxicity of the solutions. The 96-hour LC50 of the 50% degraded solution was approximately 1.4 times greater (less toxic) than the 96-hour LC50 of the nonirradiated solution; this difference was statistically significant ($p = 0.05$). At 56 mg/liter, the 100% degraded solution was not lethal, whereas 75 mg/liter killed all the test animals.

Toxicity of α -TNT and 2,4-DNT to *Daphnia magna*

We conducted 48-hour exposure tests on nonirradiated, pH 7 solutions of α -TNT and 2,4-DNT using *D. magna*. The estimated 48-hour LC50 and 95% confidence limits for α -TNT were 6.6 mg/liter and 4.5 to 9.7 mg/liter, respectively. For 2,4-DNT, these values were 35.0 mg/liter and 22.5 to 54.2 mg/liter, respectively. These results indicate that *D. magna* is more tolerant than the minnow to α -TNT, but equally susceptible to 2,4-DNT.

Toxicity of Authentic TNT Wastewater to the Fathead Minnow

Table 5 presents the estimated 24- and 96-hour LC50 values for LAP, condensate, and red water effluents before and after uv irradiation at various pH values.

Table 4

MORTALITY IN MINNOWS EXPOSED TO DIFFERENT CONCENTRATIONS
 OF α -TNT, IRRADIATED AND UNIRRADIATED AT pH 5
 (Percent)

<u>Concentration*</u> <u>(ppm)</u>	<u>0% Degraded</u>	<u>50% Degraded</u>
0	0	0
0.75	37.5%	--
1.0	37.5	0
1.4	--	0
1.8	62.5	0
2.4	--	0
3.2	100	17%
5.6	100	--

* Expressed in terms of concentration of α -TNT before irradiation.

Table 5
 ACUTE TOXICITY OF AUTHENTIC TNT WASTEWATER BEFORE AND AFTER ULTRAVIOLET IRRADIATION

Test Animal - Fathead Minnow

Wastewater	Percentage of Degradation*	pH	LC50 (% Wastewater)		95% Confidence Limits (96-hr)	Highest Nonlethal Concentration
			24-hr	96-hr		
LAP	0	5	4.0	2.7	2.4-3.0	1.8
	50	5	4.0	3.1	2.8-3.4	2.3
	0	7	4.2	2.7	2.4-3.1	1.2
	50	7	5.0	3.5	---	2.3
	100	7	> 9.5	> 9.5	---	7.1
	0	9.4	4.0	3.0	2.7-3.3	2.3
Condensate	50	9.4	< 7.1, > 5.3	< 5.3, > 3.0	---	3.0
	0	7	> 18.3	13.1	12.1-14.2	10.2
	50	7	25.0	15.4	14.2-16.7	10.0
	100	7	> 35.0	25.0	20.2-31.0	20.0
	0	7	0.7	0.47	0.38-0.59	0.23
	50	7	0.4	0.30	0.15-0.58	0.00
100	7	0.8	0.53	0.43-0.62	0.23	

* Of the α -TNT component of LAP wastewater and red water; of the 2,4-DNT component in condensate wastewater.

pH Effects

The pH of LAP wastewater during irradiation had no effect on the toxicity of the wastewater to the minnow. The 96-hour LC50 of nonirradiated LAP wastewater at pH 5 and 7 was 2.7%. For nonirradiated LAP wastewater at pH 9.4, the 96-hour LC50 was not significantly higher (3.0%). The 96-hour LC50's of the 50%-degraded wastewater irradiated at pH 5 and pH 7 were 3.1% and 3.6%, respectively. We did not have sufficient data to estimate the LC50 of wastewater irradiated at pH 9.4. We observed no deaths in the 3.0% solution and 100% mortality in the next highest dilution of 5.3%, indicating that the 96-hour LC50 is between 3.0 and 5.3%.

Irradiation Effects

Exposure of the three types of wastewater to uv irradiation until the initial content of α -TNT (LAP wastewater and red water) or 2,4-DNT (condensate wastewater) was reduced by 50% had no effect on toxicity. However, when these major components were reduced by 100%, toxicity was reduced significantly for LAP and condensate wastewater. Irradiation did not affect the toxicity of red water.

Toxicity of the Aqueous and Benzene Fractions

Our experiments were limited to the aqueous and benzene fractions of 50% degraded solutions of α -TNT and 2,4-DNT at pH 7 and to nonirradiated samples of the three authentic wastewaters at pH 7. We designed the tests to obtain a rough approximation of the relative toxicity of the two fractions.

We used nonirradiated samples of the authentic wastewaters because they were more toxic than the irradiated samples. We tested the 50%-degraded solutions of α -TNT and 2,4-DNT because they had about the same toxicity as the nonirradiated solutions and contained degradation products similar to those found in authentic LAP and condensate wastewater.

Table 6 shows the range of concentrations that bracket the 48-hour (D. magna) or 96-hour (minnow) LC50 for each fraction. Where we observed greater than 50% response at the lowest test concentration or less than 50% response at the highest test concentration, we provided an estimate of the corresponding bracketing concentration. These estimated values are shown in parentheses.

Table 7 shows the concentration of total dissolved solids (TDS) in the aqueous and benzene fractions before they were diluted for toxicity evaluation. TDS was determined by weighing the lyophilized residue from known volumes of each fraction. Table 7 also shows the concentration of α -TNT and 2,4-DNT in the fractions before lyophilization.

Table 6

TOXICITY OF AQUEOUS AND BENZENE FRACTIONS
OF α -TNT, 2,4-DNT, AND AUTHENTIC TNT WASTEWATER SAMPLES
TO THE FATHEAD MINNOW AND DAPHNIA MAGNA

Sample	Concentrations* that Bracket the LC50			
	Aqueous		Benzene	
	Minnow	D. magna	Minnow	D. magna
α -TNT, 50% degraded	17-30	(50)†-93	3.2-10	7.5-10
2,4-DNT, 50% degraded	25-50	> 50	10-50	> 50
LAP wastewater	> 100	> 1150	3.2-5.6	10-(100)
Condensate wastewater	> 100	142-(200)	7.5-(20)	18-32
Redwater	100-500	> 500	10-50	10-50

* In mg/liter total dissolved solids.

† Values in parentheses are estimated.

Table 7

TOTAL DISSOLVED SOLIDS AND α -TNT OR 2,4-DNT IN AQUEOUS AND BENZENE FRACTIONS OF SELECTED SYNTHETIC AND AUTHENTIC TNT WASTEWATER

α -TNT and 2,4-DNT Concentrations are Shown in Parentheses

Sample	Concentration (ppm)		TDS Ratio (Aq:Benz)
	Aqueous Fraction	Benzene Fraction	
α -TNT, 50% degraded	207.5 (12.3) [†]	57.1 (63.3) [†]	3.6:1
2,4-DNT, 50% degraded	50 (6.7) [*]	22.3 (79.9) [*]	2.2:1
LAP wastewater	1150 (3.7) [†]	275 (78.5) [†]	4.2:1
Condensate wastewater	142 (1.1) [*]	83.7 (46.5) [*]	1.7:1
Red water	76,197 (<1) [†]	296 (4) [†]	258:1

* 2,4-DNT.

[†] α -TNT.

As expected, a large amount of α -TNT or 2,4-DNT was found in the benzene fraction; however, we also found significant quantities of these compounds in the aqueous fraction, indicating incomplete extraction. We also noticed a discrepancy in the amount of TDS and α -TNT or 2,4-DNT in the benzene fraction of the 50%-degraded synthetic wastewaters. We believe that lyophilization caused a loss of some of the more volatile organics resulting in unreliable TDS values, not only for the synthetic wastewaters but also for the authentic ones. We believe, however, that the values for α -TNT and 2,4-DNT are reliable because they were determined before lyophilization.

Relative Toxicity of Authentic Wastewater and Selected Components

Our analysis of LAP and condensate wastewater showed that α -TNT and 2,4-DNT are the largest single organic components. We found 84 mg/liter of α -TNT in LAP wastewater and 62 mg/liter of 2,4-DNT in condensate wastewater. Red water contained 4 mg/liter of α -TNT, which is only 0.005% of the total amount of dissolved solids in red water.

To determine whether α -TNT or 2,4-DNT was responsible for the toxic action of LAP or condensate wastewater, we compared the 96-hour LC50 values of the pure compounds and the two wastewaters, using only the values obtained for the nonirradiated and 50%-degraded, pH 7 solutions. To make the comparison, we first converted the values for the authentic wastewaters from percentage of dilution to mg/liter total dissolved solids. This conversion made the values equivalent to those shown in Table 3 for the pure compounds. Then we multiplied the values by the amount of the pure compound found in the tested solutions and divided by 100. The resulting values express toxicity only in terms of α -TNT or 2,4-DNT. Although α -TNT is not a major component of red water, the amount found in the undiluted effluent exceeds the 96-hour LC50 of pure α -TNT; hence, we included red water in the evaluation. The results are shown in Table 8.

In terms of α -TNT content, no significant difference existed between the toxicity of LAP wastewater and pure α -TNT before and after uv irradiation. The LC50's for the nonirradiated solutions were 2.0 mg/liter for α -TNT and 2.3 mg/liter for LAP wastewater, and the LC50's for the 50%-degraded counterparts were 1.5 and 1.4 mg/liter, respectively. The similarity of the LC50 values suggests that the most toxic component of LAP wastewater is α -TNT. On the other hand, the LC50's for red water before and after irradiation were 0.02 and 0.006 mg/liter, respectively, indicating that α -TNT is not the most toxic component of that effluent.

Although 2,4-DNT is the most abundant nitrobody in condensate wastewater, it does not appear to be the most toxic component. The 96-hour LC50 of pure, nonirradiated 2,4-DNT was 31 mg/liter; however, the 96-hour LC50 of nonirradiated condensate wastewater was only 9.5 mg/liter. The LC50 of 50%-degraded condensate wastewater was also approximately three times less than the LC50 of 50%-degraded 2,4-DNT.

Table 8

COMPARISON OF THE LC50 VALUES OF THE AUTHENTIC
WASTEWATERS AND SOLUTIONS OF α -TNT OR 2,4-DNT IN MG/LITER
OF α -TNT OR 2,4-DNT

<u>Toxicant</u>	<u>Measured Component</u>	<u>96-Hour LC50</u>	
		<u>Unphotolyzed</u>	<u>50% Photolyzed</u>
α -TNT	α -TNT	2.0	1.5
LAP wastewater	α -TNT	2.3	1.4
Red water	α -TNT	0.02	0.006
2,4-DNT	2,4-DNT	31.0	14.0
Condensate wastewater	2,4-DNT	9.5	4.9

Miscellaneous Observations

We observed behavioral changes in fish exposed to lethal concentrations of the unfractionated toxicants. Fish exposed to red water became highly excited and stayed near the surface of the water, gasping. We observed the opposite effect in fish exposed to lethal concentrations of the other toxicants. Compared with controls, the affected fish showed diminished random movement. They swam about listlessly and eventually exhibited tail drop. This progressed to a more severe loss of equilibrium, which they evidenced by swimming upsidedown or sideways. At later stages of toxicity, voluntary movement appeared to cease; the fish lay almost motionless on the bottom. The only discernible movement was shallow and rapid opercular movement. In the most severely affected fish, prodding produced rapid muscular movement, but the fish did not swim. These symptoms usually developed within the first 24 hours. Most of the fish that exhibited these symptoms died.

Fish exposed to lethal concentrations of the unfractionated toxicants except red water developed deep hemorrhagic swellings. These swellings occurred most frequently in the spinal area below the dorsal fin and sometimes at the caudal peduncle. Hemorrhaging usually occurred within the first 24 hours. Hemorrhagic spots appeared in a few fish between the 24th and 48th hour but not thereafter. Only fish with these red spots died.

The benzene fractions produced symptoms similar to those caused by the parent solution. The symptoms were not observed in fish exposed to the aqueous fraction except for those exposed to lethal concentrations of the aqueous fraction of α -TNT. We analyzed that fraction and found about 1 mg/liter of α -TNT.

CONCLUSIONS

This study of the acute toxicity of TNT wastewater to the fathead minnow and D. magna provided evidence for the following conclusions:

- The 96-hour LC50 of α -TNT for the minnow ranges from 1.2 to 2.4 mg/liter. These LC50 values are about the same as those reported by Smock⁵ and co-workers for the same species and by Pederson¹ and Nay⁹ for bluegills.
- The 96-hour LC50 of 2,4-DNT for the minnow is 31 mg/liter. This value is higher than the 16 mg/liter reported by Burton⁶ for an unspecified mixture of DNT isomers.
- The 48-hour LC50's of α -TNT and 2,4-DNT for D. magna are 6.6 and 35.0 mg/liter, respectively. These values indicate that D. magna is more tolerant than the minnow to α -TNT but is about as sensitive to 2,4-DNT.
- The 96-hour LC50 of LAP wastewater for the minnow ranges from 2.7 to 3.0%. These percentages are equivalent to 2.3 and 2.5 mg/liter of α -TNT, respectively.
- The 96-hour LC50 of condensate wastewater for the minnow is 13.1%, equivalent to 9.5 mg/liter of 2,4-DNT.
- The 96-hour LC50 of red water for the minnow is 0.47%.
- Exposure of LAP and condensate wastewater to uv irradiation reduces their toxicity. The same is true for aqueous solutions of α -TNT and 2,4-DNT. The toxicity of red water is not affected by irradiation. Reduction by 50% of the α -TNT content of LAP wastewater and of aqueous solutions of α -TNT does not significantly reduce their toxicity; however, complete degradation of α -TNT reduces their toxicity markedly. Although the toxicity of condensate wastewater is reduced significantly only upon complete photodegradation of 2,4-DNT, the toxicity of 2,4-DNT is reduced significantly upon 50% and 100% photodegradation of 2,4-DNT.
- The pH of LAP wastewater and aqueous solutions of α -TNT during uv irradiation has little or no effect on the toxicity of these materials.

- The primary toxic components of all of the materials evaluated are benzene-extractable. The most toxic component of LAP wastewater appears to be α -TNT. The most toxic component of condensate wastewater and red water was not identified.

RECOMMENDATIONS

In this study, we determined the toxicity to a fish and an aquatic invertebrate of three types of TNT wastewater and of α -TNT and 2,4-DNT, which are the most abundant organic components of two of the wastewaters. We found that the most toxic component(s) of these wastewaters is benzene-extractable. We found evidence that α -TNT is probably the most toxic ingredient in LAP wastewater. We found that, although the most toxic factor is benzene-extractable, the aqueous fraction is not without toxicity.

Our study showed that treatment of LAP and condensate wastewater with uv light reduces their acute toxicity but that significant changes in toxicity do not occur unless α -TNT or 2,4-DNT is completely photolyzed. Our study also showed that, although the reddish color characteristic of wastewater containing α -TNT does not develop unless exposure to sunlight or uv light occurs at pH 7 or higher, the toxicity of α -TNT-containing water is not affected significantly by the pH under which photoirradiation occurs.

The change in the color of the TNT solutions indicates the formation of photolytic degradation products, which could be more or less toxic than α -TNT. However, the colored components have been identified as highly polar organic acids that remain in the aqueous fraction. Because the benzene fraction was shown to be much more toxic than the aqueous fraction, the colored components appear not to contribute significantly to the toxicity of the wastewater.

The information gained from this study, in addition to information already published, is insufficient for establishing TNT effluent limits that will ensure that aquatic organisms in streams or lakes receiving such effluents will not be harmed. Therefore, we recommend that the USAMRDC continue its sponsorship of research on the environmental hazard of TNT wastewater. The ultimate goal of such research is to provide information on which meaningful effluent standards may be based. To this end, we recommend that the experiments in the next phase of the study be designed to obtain the following kinds of information:

- The acute toxicity of TNT wastewater to a larger variety of aquatic organisms from different trophic levels.
- The acute toxicity to selected aquatic organisms of other components found in significant quantities in TNT wastewater.
- The effect of water quality on wastewater toxicity.

- The effect of exposure to natural sunlight on wastewater toxicity.
- The acute toxicity of TNT wastewater to fish eggs and fry.
- The extent to which selected components of TNT wastewater are taken up, distributed, and excreted by fish.

We believe that ultimately it will be necessary to conduct long-term exposure studies using selected aquatic species and wastewater types, and possibly selected wastewater components, to determine the concentrations that will and will not adversely affect survival, growth, or reproduction.

REFERENCES

1. G. L. Pederson. Evaluation of Toxicity of Selected TNT Wastes on Fish: Phase I - Acute Toxicity of Alpha-TNT to Bluegills. Sanit. Eng. Sp. Study No. 24-007-70/71, U.S. Army Envir. Hyg. Agency, Edgewood Arsenal, Maryland (1971).
2. D. M. Gring. 1971. Biological Effects of Trinitrotoluene (TNT). Ph.D. Thesis, Department of Zoology, Indiana University.
3. E. LeClerc. The self-purification of streams and the relationship between chemical and biological tests. Proc. 2nd Symp. Treatment of Wastewaters, 281-317 (1960).
4. G. P. Fitzgerald, G. C. Gerloff, and F. Skoog. Studies on chemicals with selective toxicity to bluegreen algae. Sewage and Ind. Wastes 24, 888-896 (1952).
5. L. A. Smock, D. C. Stoneburner, and J. R. Clark. Unpublished report, U.S. Army Envir. Hyg. Agency, Aberdeen Proving Ground, Maryland.
6. D. T. Burton. Evaluation of Radford Army Ammunition Plant's major water outfalls by acute bioassay procedures. In Biological and Engineering Investigation to Develop Optimum Control Measures to Prevent Water Pollution. L. L. Smith and W. I. Dickerson (eds.), Radford Army Ammunition Plant, Final Engineering Report, Production Engineering Project PE-249 (Phase I), Propellant Plant Pollution Abatement (1972), Appendix A, 51-67 (1971).
7. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series, EPA-660/3-75-009 (1975).
8. J. T. Litchfield and F. A. Wilcoxon. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Therap. 96, 99-113 (1949).
9. M. W. Nay. 1971. A Biodegradability and Treatability Study of TNT Manufacturing Wastes with Activated Sludge Systems. Ph.D. Thesis, Virginia Polytechnic Institute.

DISTRIBUTION LIST

<u>Copies</u>	<u>Address</u>
4	HQDA (SGRD-RP) Washington, D.C. 20314
25	Environmental Protection Department ATTN: SGRD-UBG U.S. Army Medical Bioengineering Research & Development Laboratory Fort Detrick, Frederick, MD 21701
12	Defense Documentation Center (DDC) ATTN: DDC-TCA Cameron Station Alexandria, VA 22314
1	Superintendent Academy of Health Sciences, U.S. Army ATTN: AHS-COM Fort Sam Houston, TX 78234
1	Dean School of Medicine Uniformed Services University of the Health Sciences Office of the Secretary of Defense 6917 Arlington Road Bethesda, MD 20014

