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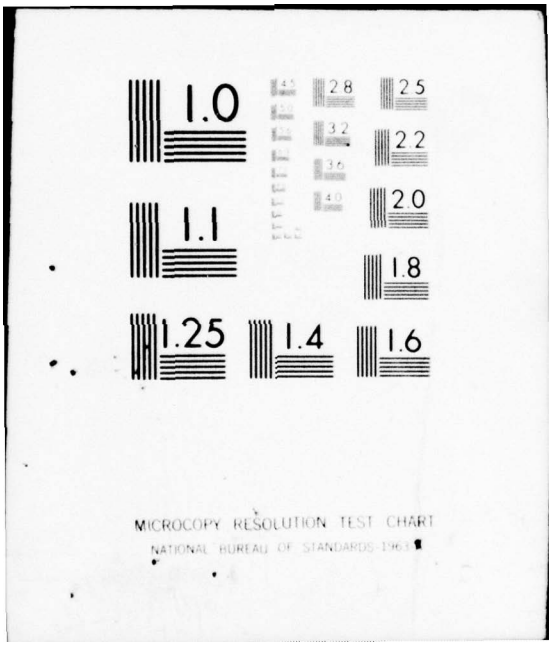
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REPORT

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MAMMALIAN TOXICOLOGICAL EVALUATION OF WASTEWATERS RESULTING FROM THE MANUFACTURE OF PRIMERS

FINAL REPORT
17 October 1975

Contract No. DAMD-17-75-C-5053
MRI Project No. 4094-B

For

Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research and
Development Laboratory
Fort Detrick, Maryland 21701

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FINAL REPORT

by

Harry V. Ellis III
Danny O. Helton

17 October 1975

Supported by

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

Contract No. DAMD-17-75-C-5053
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For

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PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri, 64110, under U.S. Department of the Army, Contract No. DAMD-17-75-C-5053, MRI Project No. 4094-B, "Mammalian Toxicological Evaluation of Wastewaters Resulting from the Manufacture of Primers." The work was supported by the Environmental Protection Research Division, U.S. Army Medical Research and Development Command, Department of the Army. Captain John P. Glennon of the Environmental Protection Research Department, U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Maryland 21701, is the contract monitor for this project.

The work was conducted in the Biological Sciences Division, under the direction of Dr. William B. House, and the Physical Sciences Division, under the direction of Dr. Harold M. Hubbard, between 15 April and 14 September 1975. The experimental work was supervised initially by Dr. James V. Dilley, Senior Toxicologist, succeeded by Dr. Harry V. Ellis III, Associate Pharmacologist and by Dr. Danny O. Helton, Senior Chemist, with the technical assistance of Bruce Anderson, Junior Biologist, Dr. Mike Harris, Associate Chemist, Bernadette Chipko, Assistant Chemist, and John Rollheiser, Junior Chemist.

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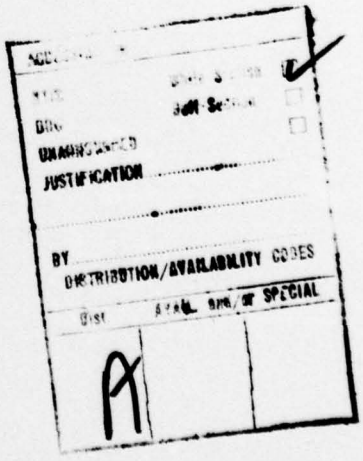

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ABSTRACT

Chemical characterization and animal toxicity studies on five desensitized primers, four wastewaters from primer production and one desensitization "blank" were performed.

High pressure liquid chromatography was most useful in characterizing the organic compounds present in the various samples. The original organic compounds were present in trace quantities or entirely absent from desensitized pure tetrazene, desensitized pure lead styphnate, desensitized pure trinitroresorcinol, tetrazene wastewater, primer mixture FA 956 wastewater, lead styphnate wastewater and trinitroresorcinol wastewater. Some PETN remained in the desensitized pure [illegible] and the desensitized primer mixture FA 956. Lead was found, as expected, in the precipitates from desensitized lead styphnate, desensitized primer mixture FA 956 and their wastewaters. Similarly, antimony was found in the precipitates from the desensitized primer mixture FA 956 and its wastewater.

Desensitized tetrazene was a mild irritant to the skin, but not the eye, of rabbits; the other samples were nonirritating in these tests. Desensitized tetrazene and desensitized primer mixture FA 956 were moderately toxic, with LD_{50}'s between 3 and 5 g solids/kg body weight in rats. Lot 3 of tetrazene wastewater was relatively toxic, with LD_{50}'s between 0.3 and 0.8 g/kg in rats and mice. This toxicity appears to be due to two characterized but unidentified organic compounds. The other samples were nontoxic in these animals.

The desensitization "blank" contained no interferring substances and was nonirritating to rabbits and nontoxic to rats or mice.

Recommendations for continued research are included in this report.

I. INTRODUCTION

The objective of this project is to characterize and determine the acute oral toxicity of some desensitized primer compounds and the wastewaters generated during the manufacture of primers. The materials studied are:

Sample No.	Sample
1	Desensitized pure tetrazene
2	Desensitized pure lead styphnate
3	Desensitized primer mixture FA 956
4	Desensitized pure PETN
5	Desensitized pure trinitroresorcinol
6	Desensitization blank
7	Wastewater from tetrazene production
8	Wastewater from primer mixture FA 956 production
9	Wastewater from lead styphnate production
10	Wastewater from trinitroresorcinol production

When received, each sample was characterized by relevant physical data, e.g., infrared spectrum, pH, etc. It was separated chromatographically, and the components characterized as much as practicable. Skin and eye irritation in rabbits and acute oral LD_{50}'s in rats and mice were determined on the mixtures and/or appropriate fractions.

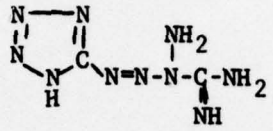
II. CHARACTERIZATION AND TOXICITY OF PRIMER SAMPLES

The characterization and toxicity of each sample are reported separately in the order mentioned above. The report on each sample is arranged by introduction, desensitization procedure or production method, sample concentration procedure, chemical assays and "fingerprinting", acute toxicity, conclusions and recommendations.

A. Densitized Pure Tetrazene

1. Introduction

a. Sample origin: Pure tetrazene (I) was desensitized by boiling in water. This procedure was carried out by the Army.



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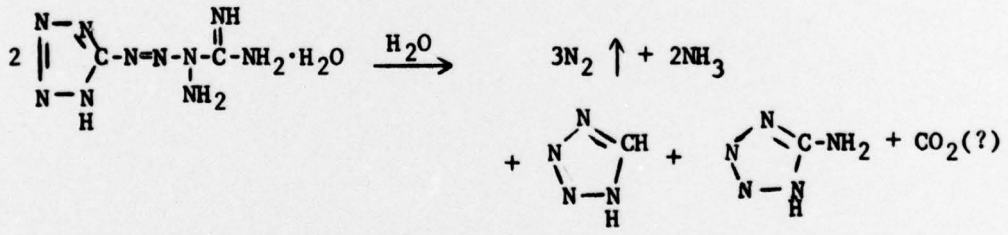
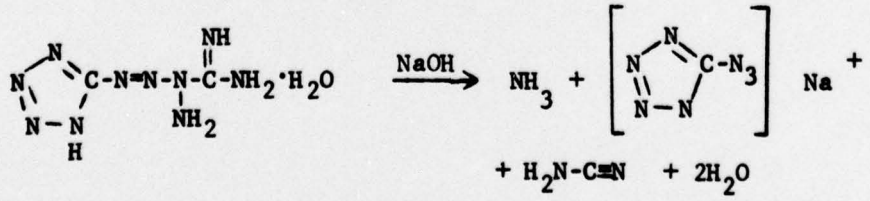
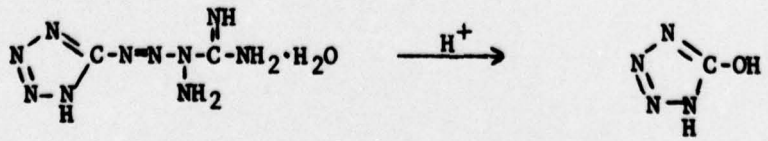
b. Chemical literature: Tetrazene (I) is an explosive which results from a complex series of reactions when aminoguanidine bicarbonate in dilute sulfuric acid is treated with sodium nitrite and is possibly the most impact-sensitive explosive used in primers.[1] The compound is stable both wet and dry at temperatures less than 75°C but hydrolyzes in boiling water to produce 1.5 to 2.0 moles nitrogen per mole tetrazene plus ammonia, guanidine 1-H-tetrazole and 5-aminotetrazole.[1]



The decomposition of tetrazene in NaOH produces ammonia, cyanamide and the sodium salt of 5-azidotetrazole,



while in dilute acid, tetrazene yields 5-hydroxytetrazole.[2]



c. Toxicological literature: No data on acute mammalian toxicity could be found.

2. Desensitization Procedure

The following procedure was used by the Army for desensitization of the tetrazene:

a. To 4 liters of water was added 298 g of tetrazene.

b. The contents were heated to 80° to 90° for 4 hours by injecting steam into the water.

c. The final volume was 7.6 liters.

d. The pH of the product was 5.8.

3. Sample Concentration for Acute Toxicity Studies

a. Sample description: The sample was a light blue liquid containing no precipitate and was used as received. The pH was 5.6.

b. Concentration: The approximate concentration of dissolved solids per ml was determined prior to concentration. A 10-ml aliquot was reduced to dryness under 20 mm Hg vacuum at 50° to 60°. The concentration of dissolved solids was 32 mg/ml, as received.

The concentration of dissolved solids was adjusted by solvent removal under 20 mm Hg vacuum at 50° to 60°. After concentration, the pH was adjusted to 7.4 by addition of 0.1 N NaOH. On addition of base, the light blue solution became green and a small amount of precipitate was noted. The concentration factor was 4.0 and the final concentration of dissolved solids was 128 mg/ml.

c. Distillate examination: The distillate resulting from sample concentration was studied by gas chromatography, thin-layer chromatography, and ultraviolet spectroscopy for the presence of components other than water. The techniques used are described under Section 4, Chemical Assays. No other components were detected in the distillate.

4. Chemical Assays and "Fingerprinting"

a. Acidity: The sample as received required 0.055 meq of sodium hydroxide per ml of sample for neutralization.

b. Metal content: The sample as received contained the following concentrations of metals:

Metal	% in Solution
Antimony	< 0.0005
Barium	0.0008
Calcium	0.0040
Lead	0.0010
Magnesium	0.0012
Sodium	0.0075

Since the sample concentration factor was 4.0, the solution used for animal toxicity studies contained four times the above concentrations of metals.

c. Reducible NO_2 content: The concentration of reducible NO_2 groups was determined by the method of Selig.[3] This procedure will determine the presence of aliphatic nitrates, aliphatic nitrites, aromatic nitro groups and inorganic nitrite and nitrate ions. Although a number of functionalities are detected simultaneously, the results are expressed in terms of mg NO_2 for comparison purposes. The nonspecificity of the assay can be considered an advantage, since when combined with the inorganic NO_2 and NO_3 assays, the covalently bound NO_2 content can be estimated.

The reducible NO_2 content when assayed on 19 May 1974, was 0.81 mg/ml. Forty-two days later the assay indicated 0.89 mg/ml.

d. Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method described by the Environmental Protection Agency.[4] The sulfate content was 0.09 mg/ml of sample as received, or 0.36 mg/ml in the sample used for animal toxicity studies.

e. Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite contents were determined by the cadmium reduction method described by the Environmental Protection Agency.[5] The nitrate content of the sample as received was 0.0018 mg/ml. The nitrite content of the sample as received was < 0.001 mg/ml.

f. Chromatographic characterization: The sample was studied by gas, liquid and thin-layer chromatography. The primary purpose of this work was to provide chromatographic "fingerprints" of the sample.

(1) Liquid chromatography: The sample supernatants were studied using the following conditions:

Instrument: Waters Associates 301 equipped with 235 nm UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 40 minutes using linear solvent program No. 6

Flow rate: 1 ml/min

Peaks were observed with retention times of 1.6, 2.76, 5.51, 23.6, 29.5, 31.5, and 32.7 minutes. Using the 235 nm detector, the peaks at 2.76 and 23.6 were large as compared to the other peaks. Tetrazene had a retention time of 1.6 minutes. Tetrazole had a retention time of 2.76 minutes, and 5-aminotetrazole had a retention time of 5.51 minutes. The peaks with retention times of 23.6 to 32.7 minutes eluted in ill-shaped bands suggesting the presence of unresolved components.

(2) Gas chromatography: The sample was studied by gas chromatography as described below:

Instrument: Bendix 2500 equipped with flame ionization detector.
Columns: (a) Glass, 6 ft x 1/4 in.; 3% OV-1 on Gas Chrom Q.
(b) Glass, 6 ft x 1/4 in.; 1.5% DC LSX-3-0295; 1.5% GE XE-60 on Gas Chrom Q.
Flow rate: 30 cc N_2/min.
Column temperature: Start at 100°C and heat at 5°/min to 250°C.
Injection port: 200°C
Detector temperature: 200°C

Using column (a), no peaks were observed. Using column (b), a single broad peak was observed at 8 minutes retention time. A reference sample of 1-H-tetrazole had a retention time of 7 minutes on column (b). No peak was observed for a reference sample of 5-aminotetrazole or tetrazene on either column. A chloroform extract of the sample showed no peaks on either column.

(3) Thin-layer chromatography: The sample was studied by thin-layer chromatography as described below:

Plate: Brinkmann Silica Gel F.
Solvent system: 95% ethanol/NH_4OH (4:1).
Material spotted: 30 µg 1-H-tetrazole (acetone).
1.5 µg 5-aminotetrazole (10% HCl).
100 µg tetrazene (10% HCl).
10 µl concentrated desensitized tetrazene.
Detection: UV - 254 nm
Ninhydrin spray.

The detection limit for 1-H-tetrazole was 30 µg (R_f 0.62). The detection limit for 5-aminotetrazole was 1 µg (R_f 0.0). Neither this solvent system nor any other system tried gave a satisfactory elution pattern for tetrazene; excessive streaking was observed. The sample showed spots at R_f 0.0, 0.45, 0.48, 0.55, and 0.62.

g. Spectral characterization: The sample was studied by infrared, ultraviolet and visible spectroscopy.

(1) Infrared spectrum: Reducing an aliquot to dryness gave a residue whose IR in KBr showed absorptions at 3300 (s), 2160 (s), 1680 (s), 1475 (s), 1410 (m), 1350 (m), 1270 (w), 1240 (m), 1200 (w), 1150 (m), 1170 (s), and 1000 (m) CM^{-1}. The band at 2160 cm^{-1} suggests the presence of a nitrile or azide.

(2) Ultraviolet spectrum: In water no peaks were observed in the range 350 to 230 nm.

(3) Visible spectrum: A broad band centered at about 650 nm was observed.

5. Acute Toxicity

Animal tests were performed on the concentrated desensitized tetrazene, prepared as described above. This material contained 128 mg solids/ml.

a. Skin and eye irritation: The primary skin and eye irritations were determined by the Draize procedure[6] using six New Zealand white rabbits. Desensitized tetrazene was scored as negative in the eye irritation test and 0.25 in the skin irritation test. This skin test score is within the "mild" class (0.2 to 2.5 score).

b. Oral toxicity: A single oral dose of 5 g/kg of desensitized tetrazene in water did not kill any of three male albino mice (Charles River CD-1® strain). The acute oral LD_{50}'s (method of Finney[7] adapted for computer) in male and female rats (Charles River CD® strain) were 3.40 g/kg and 3.30 g/kg, respectively. All deaths occurred on the first day with no remarkable symptoms. The results are summarized in Table 1.

6. Conclusions

The desensitized tetrazene sample as received was slightly acidic (pH 5.8) and contained no appreciable concentration of metals or sulfate ion. The total reducible NO_2 content was 0.81 mg/ml. The inorganic nitrate content was 1.8×10^{-3} mg/ml and the inorganic nitrite content was $<1.0 \times 10^{-3}$ mg/ml. Thus, the covalently bound NO_2 content was high relative to the inorganic NO_3 plus NO_2 content. High pressure liquid chromatography indicated the presence of at least seven ultraviolet-absorbing components, three having the retention times of 1-H-tetrazole, 5-aminotetrazole and tetrazene.

TABLE 1

ACUTE ORAL LD_{50}'s OF DESENSITIZED TETRAZENE IN RATS

Dose (g solids/kg body weight)	Mortality	
	Males	Females
4.00	5/5	5/5
3.75	5/5	ND
3.50	5/10	4/5
3.37	4/5	5/8
3.25	2/10	4/8
3.00	0/5	0/5
LD_{50} (g/kg)	3.40	3.30
95% Confidence Limits (g/kg)	3.28-3.55	3.12-3.44
Slope ± Standard Error	16.3 ± 5.2	17.4 ± 6.8

ND = Not Determined

The sample was concentrated four times for animal testing. The desensitized tetrazene was mildly irritating to the skin of rabbits and moderately toxic to rats after oral administration.

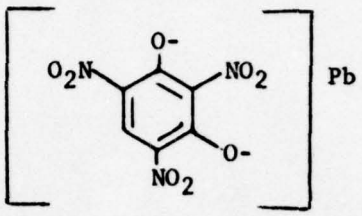
7. Recommendations

Because the sample tested has much less toxicity than the tetrazene wastewater (as discussed in Section G), no further toxicological research is warranted. However, the skin irritation test indicates that it may be necessary to protect workers from physical contact with the desensitized tetrazene. Samples should also be taken periodically and characterized by high pressure liquid chromatography to insure no significant variation in composition.

B. Desensitized Pure Lead Styphnate

1. Introduction

a. Sample origin



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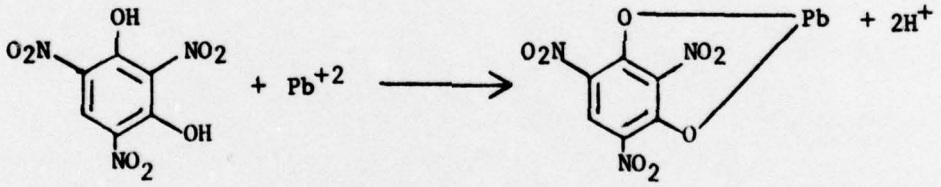
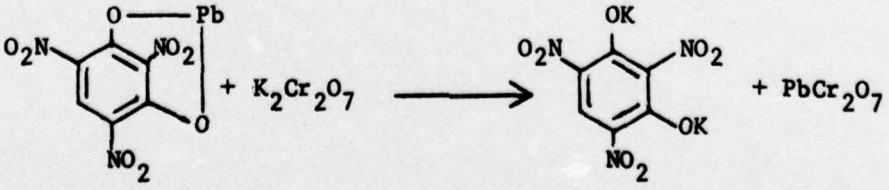
A sample of lead styphnate (I) was desensitized by heating in water with sodium hydroxide and aluminum.

b. Chemical literature: Trinitroresorcinol, or styphnic acid, is prepared by first sulfonating, then nitrating resorcinol.[8] It reacts with lead salts to form several products, the most stable of which is the 1:1 complex.[9]



Lead styphnate is quite stable as shown by vacuum stability studies at 100° and 120°C. However, it appears to undergo both hydrolysis and decomposition in water.[10] It can be decomposed by concentrated nitric or sulfuric acid. When dissolved in ammonium acetate solution, it reacts with potassium dichromate to form potassium styphnate and lead chromate.



It can also be decomposed by dissolving it in at least 40 times its weight of 20% sodium hydroxide solution.[11]

c. Toxicological literature: No data on the acute mammalian toxicology of either lead styphnate or trinitroresorcinol were found. However, lead is the best known toxic metal, well covered in all texts. The toxicity of lead styphnate is presumed due to its lead content and the solubility of the lead in the medium involved.

2. Desenitization Procedure

The following procedure was used for desensitization of the lead styphnate:

a. To a 5-liter water solution containing 157 g of NaOH was added 157 g of lead styphnate.

b. During a 20-minute period, 78.5 g of atomized aluminum were added.

c. The solution was heated at 89° to 92° for 4 hours by injecting steam into the mixture.

d. The final volume was 9.5 liters. The product pH was 11.7.

3. Sample Concentration for Animal Toxicity Studies

a. Sample description: As received, the sample was a dark green suspension with numerous small particles (< 0.028 in). Before sampling, the mixture was shaken vigorously to suspend the particles. The pH was 11.7.

b. Sample concentration: The approximate concentration of dissolved solids/ml was determined prior to concentration. A 10-ml aliquot was reduced to dryness under 20 mm Hg vacuum at 50° to 60°. As received, the concentration of dissolved solids was 0.06 mg/ml.

The concentration of dissolved solids was now adjusted by solvent removal under 20 mm Hg vacuum at 50° to 60°. After concentration, the pH was adjusted to 7.4 by addition of acetic acid. On addition of acetic acid, the solution became very turbid. The final concentration of dissolved plus suspended solids was 112 mg/ml. The concentration factor was 19.2.

c. Distillate examination: The water distillate resulting from sample concentration was studied by gas chromatography, thin-layer chromatography, and ultraviolet spectroscopy for the presence of components other than water. The techniques used are described under Section 4, Chemical Assays. No other components were detected in the distillate.

4. Chemical Assays and "Fingerprinting"

a. Alkalinity: The sample supernatant, as received, required 0.375 meq of HCl/ml of sample to neutralize it. This alkalinity corresponds to 15 mg NaOH/ml.

b. Metal content: The sample as received contained the following concentrations of metals:

Metal	% in Supernatant	% in Precipitate
Aluminum	0.9	3.35
Antimony	<0.00025	0.04
Barium	0.00002	<0.0096
Calcium	0.0001	<0.096
Lead	0.0049	59.4
Magnesium	<0.0001	<0.05
Sodium	0.88	1.7

c. Reducible NO_2 content: The concentration of reducible NO_2 groups was determined by the method of Selig.[3] This procedure determines the presence of aliphatic nitrates, aliphatic nitrites, aromatic nitro groups and inorganic nitrite and nitrate ions. Although a number of functionalities are detected simultaneously, the results are expressed in terms of mg NO_2 for comparison purposes. The nonspecificity of the assay can be considered an advantage since when combined with the inorganic NO_2 and NO_3 assays, the covalently bound NO_2 content can be estimated.

The reducible NO_2 content of the supernatant when assayed on 19 May 1975 was 0.04 mg NO_2/ml. Thirty-five days later, the assay indicated 0.10 mg NO_2/ml. The reducible NO_2 content of the precipitate when assayed on 19 May 1975 was 0.03 mg NO_2/g precipitate. Thirty-six days later, the assay indicated the same.

d. Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method described by the Environmental Protection Agency.[4] The sulfate content of the supernatant was 0.74 mg/ml of sample.

e. Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite contents were determined by the cadmium reduction method described by the Environmental Protection Agency.[5] The nitrate content of the supernatant as received was 0.0004 mg/ml. The nitrite content of the supernatant as received was 0.00168 mg/ml.

f. Chromatographic characterization: The sample was studied by gas, liquid and thin-layer chromatography. The primary purpose of the work was to provide chromatographic "fingerprints" of the sample.

(1) Liquid chromatography: The sample was studied as described below:

System 1

Instrument: Waters Associates 301 equipped with Schoeffel 770 variable wavelength. UV detector operating at 254 nm.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 30 minutes using linear solvent program No. 6.
Flow rate: 1 ml/min.

Minor peaks were observed at 9 and 13 ml elution volume, plus a major ill-defined peak at 15 to 30 ml. A peak was not observed for a reference sample of trinitroresorcinol using this system.

System 2

Instrument: Waters Associates 301 equipped with Schoeffel 770 variable wavelength UV detector operating at 254 nm.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 3% H_2O in CH_3CN.
Flow rate: 1 ml/min.

A single peak at 1.58 ml was observed. The peak was skewed suggesting the presence of more than one component. Both lead styphnate and trinitroresorcinol elute at 2.76 minutes.

(2) Gas chromatography: The sample was studied by gas chromatography as described below:

Instrument: Bendix 2500 equipped with flame ionization detector.
Column: Glass, 6 ft x 1/4 in; 3% OV-1 on Gas Chrom Q.
Flow rate: 30 cc N_2/min.
Column temperature: Start at 100°C and heat at 5°/min to 250°.
Injection port: 200°C.
Detector: 200°C.

No peaks were observed.

(3) Thin-layer chromatography: The sample was studied by thin-layer chromatography on both cellulose and silica gel.

(a) On cellulose:

Plate: Brinkmann cellulose F.
Solvent system: 95% ethanol/conc. NH_4OH (4:1)
Material spotted: 5 µg lead styphnate (10% aqueous ammonium acetate); 5, 1, 0.5 µg trinitroresorcinol; 100, 10 µl styphnate waste supernatant; 100 µl of a concentrated chloroform extract of the waste supernatant.
Detection: Visual inspection; UV-254 and 360 nm; Spray with 2% diphenylamine in 95% ethanol followed by exposure to long wave UV light for 5 minutes.

Lead styphnate has an R_f of 0.39. Trinitroresorcinol has an R_f of 0.51. Both of the waste samples had a spot at R_f 0.0, but no spot above the origin. The detection limit for lead styphnate and trinitroresorcinol was 0.5 µg.

(b) On silica gel:

Plate: Brinkmann Silica Gel F-254.
Solvent system: Acetone saturated with ammonium acetate.
Material spotted: 5 µg lead styphnate; 5 µg trinitroresorcinol; 20 µl of sample having a dissolved solids content of 112 mg/ml (i.e., 2,240 µg of solids).
Detection: (i) Visual
(ii) UV-254 nm
(iii) Spray with 2% diphenylamine in 95% ethanol followed by UV irradiation.

Lead styphnate has an R_f of 0.42 and a detection limit of 0.5 µg. Trinitroresorcinol has an R_f of 0.42 and a detection limit of 0.5 µg. The sample showed no spots above R_f 0.0.

g. Spectral characterization: The sample was studied by infrared, ultraviolet and visible spectroscopy.

(1) Infrared spectrum: A vacuum dried sample in KBr showed broad, ill-defined bands at 3300, 1620, 1400 and 1075 cm^{-1}. The C-NO_2 group normally has bands near 1500 and 1350 cm^{-1}.[12] Lead styphnate shows bands at 3570 (m), 1590 (s), 1490 (m), 1410 (m), 1315 (s), 1230 (s), 1100 (m), 790 (m), 770 (w) and 740 (w) cm^{-1}.

(2) Ultraviolet spectrum: With a 50-fold dilution, the sample shows a maximum at 328 nm (absorbance 0.43).

(3) Visible spectrum: No peaks were observed in the range 400 to 800 nm. With a 10-fold dilution, the sample showed an absorbance of 0.30 at 400 nm.

5. Acute Toxicity

Animal tests were performed on the neutralized concentrated desensitized lead styphnate, prepared as described above. The material contained 112 mg solids/ml.

a. Skin and eye irritation: The primary skin and eye irritations were determined by the Draize procedure[6] using six New Zealand white rabbits. Desensitized lead styphnate gave no eye irritation and a score of 0.16 on the skin irritation test. A score of at least 0.20 is required to classify a material as a mild irritant, so desensitized lead styphnate is nonirritating by this test.

b. Oral toxicity: Single oral doses of 5 g/kg of desensitized lead styphnate killed none of the six female and three male albino rats (Charles River CD strain) and three male mice (Charles River CD-1 strain) dosed.

6. Conclusions

The desensitized lead styphnate sample as received was basic (pH 11.7) and contained the equivalent of 15 mg NaOH/ml. The supernatant contained 0.9% aluminum and 0.88% sodium. The precipitate contained 3.35% aluminum, 59% lead and 1.7% sodium. The supernatant sulfate content was 0.74 mg/ml. The initial total reducible NO_2 content of the supernatant was 40×10^{-3} mg NO_2/ml. The inorganic nitrate content was 0.4×10^{-3} mg/ml and the nitrate content was 1.68×10^{-3} mg/ml. Thus, the covalently bound NO_2 content is high relative to the inorganic NO_3 plus NO_2 content. Gas chromatography on an OV-1 column did not indicate the presence of any volatile components. High pressure liquid chromatography (HPLC) was useful for monitoring nonvolatile ultraviolet absorbing compounds. No trinitroresorcinol ($< 0.01\%$) was observed in the sample. There are at least three uncharacterized ultraviolet absorbing compounds present by HPLC. Thin-layer chromatography also indicated the absence of trinitroresorcinol.

The homogenous concentrated neutralized sample was nonirritating to rabbits and nontoxic to rats and mice after oral administration.

7. Recommendations

Because of the lack of irritancy and toxicity, no further toxicological research is warranted. Accepted standards for lead content and worker exposure should be observed. Samples should be taken periodically and characterized by high pressure liquid chromatography for organic constituents and by elemental analysis for lead to insure no significant variation in composition.

C. Desensitized Primer Mixture FA 956

1. Introduction

a. Sample origin: A sample of primer mixture FA 956 was desensitized by the Army using sodium hydroxide and aluminum. Primer mixture FA 956 contains 37% lead styphnate, 4% tetrazene, 5% pentaerythritol tetranitrate, 32% barium nitrate, 15% antimony sulfide and 7% aluminum.

b. Chemical literature: See the sections for tetrazene (A), lead styphnate (B), and PETN (D).

c. Toxicological literature: No data on acute mammalian toxicity could be found.

2. Desensitization Procedure

The following procedure was used by the Army for the desensitization of primer mixture FA 956:

a. To 3 liters of water containing 125 g NaOH was added 150 g FA 956 priming mixture.

b. During a 46-minute period 52 g of atomized aluminum were added to the solution.

c. The contents were heated to 82° to 89° for 4 hours by injecting steam into the mixture.

d. The final volume was 5.4 liters.

e. The pH of the product was 13.2.

3. Sample Concentration for Acute Toxicity Studies

a. Sample description: The sample was a dark brown liquid containing a finely divided precipitate. The sample was shaken vigorously to suspend the particles before aliquots were removed. The pH was 11.6.

b. Concentration: The approximate concentration of dissolved solids/ml was determined prior to concentration. A 10-ml aliquot was reduced to dryness under 20 mm Hg vacuum at 50° to 60°. As received the concentration of dissolved solids was 70 mg/ml.

The concentration of dissolved solids was now adjusted by solvent removal under 20 mm Hg vacuum at 50° to 60°. After concentration the pH was adjusted to 7.8 by addition of acetic acid. The addition of acetic acid caused no change in appearance. The final concentration of dissolved plus suspended solids was 159 mg/ml. The concentration factor was 2.27.

c. Distillate examination: The distillate resulting from sample concentration was studied by gas chromatography, thin-layer chromatography, and ultraviolet spectroscopy for the presence of components other than water. The techniques used are described under Section 4, Chemical Assays. No other components were detected in the distillate.

4. Chemical Assays and Chromatographic "Fingerprinting"

a. Alkalinity: The sample supernatant as received was basic and required 0.525 meq HCl per ml of sample to neutralize it. This alkalinity corresponds to 21 mg NaOH/ml.

b. Metal content: The sample, as received, contained the following concentration of metals:

Metal	% in Supernatant	% in Precipitate
Aluminum	1.00	24.8
Antimony	0.0030	9.45
Barium	0.024	0.43
Calcium	0.0010	0.031
Lead	0.0030	9.3
Magnesium	0.0001	0.02
Sodium	1.26	1.4

c. Reducible NO_2 content: The concentration of reducible NO_2 groups was determined by the method of Selig.[3/] This procedure will determine the presence of aliphatic nitrates, aliphatic nitrites, aromatic nitro or nitroso groups and inorganic nitrite and nitrate ions. Although a number of functionalities are detected simultaneously, the results are expressed in terms of mg NO_2 for comparison purposes. The nonspecificity of the assay can be considered an advantage since, when combined with the inorganic NO_2 and NO_3 assay, the covalently bound NO_2 content can be determined.

The reducible NO_2 content of the supernatant when assayed on 20 May 1975 was 4.5 mg NO_2/ml. Forty-one days later the assay indicated 3.5 mg NO_2/ml. The reducible NO_2 content of the precipitate when assayed on 28 May 1975 was 0.06 g NO_2/g sample.

d. Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method described by the Environmental Protection Agency.[4] The sulfate content of the supernatant as received was 1.97 mg SO_4/ml of sample.

e. Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite contents were determined by the cadmium reduction method described by the Environmental Protection Agency.[5] The nitrate content of the supernatant as received was 0.76 mg/ml. The nitrite content of the supernatant as received was 1.0 mg/ml.

f. Chromatographic characterization: The sample was studied by gas, liquid and thin-layer chromatography. The primary purpose of this work was to provide chromatographic "fingerprints" of the sample.

(1) Liquid chromatography: The sample supernatant was studied as described below:

System 1

Instrument: Waters Associates 301 equipped with 235 nm Schoeffel 770 UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 30 minutes using solvent program No. 6.
Flow rate: 1 ml/min.

Peaks were observed at 7, 10, 11, 15 and 21 ml elution volume. Reference samples of pentaerythritol tetranitrate and trinitroresorcinol do not give peaks using the system.

System 2

Instrument: Waters Associates 301 equipped with 235 nm Schoeffel 770 UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 3% H_2O in CH_3CN.
Flow rate: 1 ml/min.

Trinitroresorcinol and lead styphnate elute at 2.76 ml. The sample showed peaks at 1.18, 3.15, 7.48 and 10.2 ml. The peaks at 7.48 and 10.2 ml were ill-defined.

(2) Gas chromatography: The sample supernatant and precipitate were studied by gas chromatography as described below:

Instrument: Bendix 2500 equipped with flame ionization detector.
Column: Glass, 6 ft x 1/4 in.
(a) 3% OV-1 on Gas Chrom Q.
(b) 5% OV-17 on Anakrom ABS.
Flow rate: 50 cc N_2/min.
Column temperature: (a) Start at 100°C and heat at 5°/min. to 250°.
(b) Isothermal at 130°C.
Injection port: (a) 200°C
(b) 130°C
Detector temperature: 200°C

No peaks were observed using column (a), column temperature (a), and injection temperature (a). Using the (b) conditions no peaks were observed for the supernatant, but, for the precipitate, a peak with the retention time of PETN (5.7 minutes) was observed. The concentration of the PETN was < 0.1%.

(3) Thin-layer chromatography: The sample was studied on two thin-layer chromatographic systems.

System 1 (For TNR and lead styphnate)

Plate: Brinkmann Silica Gel F-254
Solvent system: Acetone saturated with ammonium acetate.
Material spotted: 5 µg lead styphnate
5 µg trinitroresorcinol
20 µl of sample having a dissolved solids content of 112 mg/ml (i.e., 2,240 µg of solids).
Detection: (a) Visual
(b) UV-254 nm
(c) Spray with 2% diphenylamine in 95% ethanol followed by UV irradiation.

Lead styphnate has an R_f of 0.42 and a detection limit of 0.5 µg. Trinitroresorcinol has an R_f of 0.42 and a detection limit of 0.5 µg. The sample showed one spot at R_f 0.125.

System 2 (For PETN)

The precipitate (300 mg) was extracted twice with 5 ml of chloroform using sonication. The chloroform was concentrated and used for spotting in the following TLC system:

Plate: Brinkmann Silica Gel F-254
Solvent system: Cyclohexane/acetone (1:1)
Material spotted: 5 µg PETN
5 µl chloroform extract of the sample precipitate.
Detection: (a) UV-254 nm
(b) Spray with 2% diphenylamine in 95% ethanol, irradiate with UV light.

UV light indicated no spots. Diphenylamine spray indicated the presence of PETN in the sample at R_f 0.59 (gray spot).

g. Spectral characterization: The sample was studied by infrared, ultraviolet and visible spectroscopy.

(1) Infrared spectrum: A vacuum dried sample of the supernatant in KBr showed broad, ill-defined bands at 3300, 1620 and 1380 cm^{-1}. The C-NO_2 group normally has bands near 1560 and 1350 cm^{-1}.[12]

(2) Ultraviolet spectrum: With a 10-fold dilution the sample supernatant showed maxima at 355 nm (0.33 absorbance) and 270 nm (0.21 absorbance).

(3) Visible spectrum: With a 10-fold dilution the sample supernatant showed a maxima at 420 nm (0.20 absorbance).

5. Acute Toxicity

Animal tests were performed on the neutralized, concentrated, desensitized primer mixture FA 956, prepared as described above. This material contained 159 mg solids/ml.

a. Skin and eye irritation: The primary skin and eye irritation were determined by the Draize procedure[6] using six New Zealand white rabbits. Desensitized primer mixture FA 956 gave no eye irritation and a score of 0.16 on the skin irritation test. A score of at least 0.20 is required to classify a material as a mild irritant, so desensitized primer FA 956 is nonirritating by this test. The irritation noted is probably due to its content of lead styphnate and tetrazene, with a possible contribution from the inorganic constituents.

b. Oral toxicity: Desensitized primer mixture FA 956 killed none of the three male albino mice (Charles River CD-1 strain) dosed orally with 5 g/kg in water suspension. The acute oral LD_{50}'s (probit method of Finney[7] adapted for computer) in male and female albino rats (Charles River CD strain) were determined. All deaths occurred on the first day with no remarkable signs. Details are in Table 2. The LD_{50} in male rats (with 95% confidence limits) is 4.84 (4.62 to 5.19) g/kg. In females, the LD_{50} was calculated as 3.95 (3.85 to 4.04) g/kg. It is practically impossible to measure a dose between 3.8 and 4.0 g/kg, so the statistical error in the slope is large.

This sex difference is significant, and presumably reflects a difference in metabolism. In view of the known toxicity of the components, it is probable that the antimony and lead are responsible for most, if not all, of the observed toxicity. Antimony toxicity is similar to that of arsenic; lead is well known as a toxic heavy metal.

TABLE 2

ACUTE ORAL LD_{50}'S OF
DESENSITIZED PRIMER MIXTURE FA 956 IN RATS

Dose (g solids/kg body weight)	Mortality	
	Males	Females
5.5	5/5	ND
5.0	4/5	3/3
4.75	1/10	ND
4.5	3/5	ND
4.25	0/5	ND
4.0	0/5	7/8
3.8	ND	0/5
3.75	ND	0/8
LD_{50} (g/kg)	4.84	3.95
95% Confidence Limits (g/kg)	(4.64-5.19)	(3.85-4.04)
Slope ± Standard Error	12.6 ± 4.3	83.5 ± 71.8

ND = Not Determined

6. Conclusions

The sample as received was basic (pH 11.6) and contained the equivalent of 21 mg NaOH/ml. The supernatant contained 1.0% aluminum and 1.26% sodium. The precipitate contained 24.8% aluminum, 9.5% antimony, 9.3% lead and 1.4% sodium. The initial total reducible NO_2 content of the supernatant was 4.5 mg NO_2/ml. Forty-one days later the reducible NO_2 content of the supernatant was 3.5 mg NO_2/ml. The inorganic nitrate content after 41 days was 0.76 mg/ml and the nitrite content was 1.02 mg/ml. Thus the covalently bound NO_2 content is similar to the inorganic NO_3 plus NO_2 content. High pressure liquid chromatography indicated the presence of at least four uncharacterized ultraviolet absorbing components. Thin-layer chromatography indicated the presence of PETN in the precipitate. TLC did not show as many minor components as high pressure liquid chromatography. Gas chromatography indicated the presence of PETN in the precipitate in a concentration of < 0.1%. No other volatile components were detected.

The homogenous sample of neutralized, concentrated desensitized primer mixture FA 956 was nonirritating to rabbits, but moderately toxic to rats after oral administration.

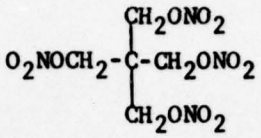
7. Recommendations

Because the toxicity is presumably due to the well-known toxic metals antimony and lead, no further toxicological research is warranted. Accepted effluent standards for these metals should be adequate. Samples should be taken periodically and characterized by high pressure liquid chromatography for organic constituents and by elemental analysis for antimony and lead to insure no significant variations in composition.

D. Desensitized Pure PETN

1. Introduction

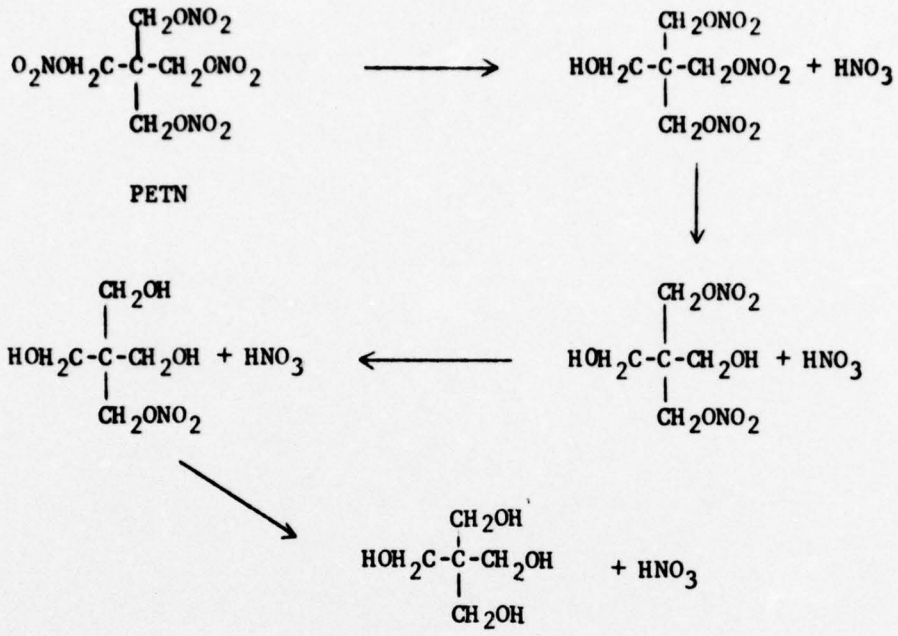
a. Sample origin: Pure pentaerythritol tetranitrate (PETN) (I) was desensitized by heating in water with aluminum and sodium hydroxide.



(I)

b. Chemical literature: PETN (I) is the nitric acid ester of pentaerythritol and is considered to be the most stable and least reactive of the explosive nitrate esters. Analytical and stability tests have indicated that its stability is increased by prolonged heating in water at 100°C.[13]

Aubertein and Rehling[14] studied the hydrolysis reactions of PETN solutions at temperatures from 90° to 125°. It was found that the rate of hydrolysis in distilled water increased exponentially with temperature and became rapid at 125°C. The rate increased considerably in dilute nitric acid and was proportional to the acid concentration. In both water and nitric acid there was less nitric acid liberated than PETN consumed, indicating only partial hydrolysis. In dilute sodium hydroxide, the rate of hydrolysis was greater than in dilute nitric acid and the amount of PETN consumed was equivalent to the amount of nitric acid liberated, indicating a total hydrolysis. Because of the poor solubility of PETN in water, almost all hydrolysis studies have been carried out in mixed solvents, namely 90% ethanol[15] and 75% dioxane[16] making them not directly applicable to the hydrolysis in wastewater. The hydrolysis is expected to occur according to the following sequence:



PETN

Pentaerythritol

Norwitz and Gordon[17] have developed a spectrophotometric method to measure the PETN in wastewater at the 2 mg/liter level. PETN can also be separated and quantitated by gas chromatography. This method provides a rapid procedure for the detection of microgram and submicrogram quantities using a flame ionization detector and nanogram to subnanogram quantities using an electron capture detector.[18]

c. Toxicological literature: Because of its widespread use as a vasodilator, and the recurrent debate over its efficacy in relieving angina pectoris, there is an immense clinical literature on PETN. Reported human toxicity is due to vasodilatation with a few incidents of dermal allergic reactions after long-term use. The metabolic fate of PETN in man and other mammals is the subject of an equally large literature. However, the only acute toxic effects mentioned are vasodilatation and its sequelae. More information is available in standard texts and previous studies.[2]

2. Desensitization Procedure

The following procedure was used by the Army for desensitization of the pentaerythritol tetranitrate.

a. To 4 liters of water containing 710 g of sodium hydroxide was added 300 g of PETN.

b. During a 107-minute period, 356 g of atomized aluminum were added.

c. The solution was heated at 89° to 98° for 4 hours by injecting steam into the solution.

d. The final volume was 6.6 liters.

e. The product pH was 13.2.

3. Sample Concentration for Acute Toxicity Studies

a. Sample description: The sample was a clear solution with a viscous, cream-colored precipitate. The sample was shaken vigorously before aliquot removal. The pH was 11.6.

b. Concentration: The approximate concentration of dissolved solids per ml was determined prior to concentration. A 10 ml aliquot was reduced to dryness under 20 mm Hg vacuum at 50° to 60°. As received, the concentration of dissolved solids was 330 mg/ml.

The concentration of dissolved solids was now adjusted by dilution with water. After dilution, the pH was adjusted to 7.5 by addition of acetic acid. No change in appearance was noted on addition of acetic acid. The final concentration of dissolved plus suspended solids was 189 mg/ml. The dilution factor was 0.57.

4. Chemical Assays and "Fingerprinting"

a. Alkalinity: The sample supernatant as received required 2.58 meq of HCl/ml of sample to neutralize it. This alkalinity corresponds to 103 mg NaOH/ml.

b. Metal content: The sample as received contained the following concentrations of metals:

Metal	% in Supernatant	% in Precipitate[a/]
Aluminum	1.0	43.0
Antimony	0.0010	<0.016
Barium	<0.0002	0.025
Calcium	0.0010	<0.063
Lead	0.0010	0.016
Magnesium	<0.0001	<0.032
Sodium	5.59	8.4

a/ The precipitate was removed by spatula and dried at 100° for 2 hours.

c. Reducible NO_2 content: The concentration of reducible NO_2 groups was determined by the method of Selig.[3] This procedure will determine the presence of aliphatic nitrates, aliphatic nitrites, aromatic nitro groups, inorganic nitrite and nitrate ions. Although a number of functionalities are detected simultaneously, the results are expressed in terms of mg NO_2 for comparison purposes. The nonspecificity of the assay can be considered an advantage since when combined with the inorganic NO_2 and NO_3 assays, the covalently bound NO_2 can be determined.

The reducible NO_2 content of the supernatant when assayed on 20 May 1975 was 9.4 mg NO_2/ml. Thirty-eight days later the assay indicated 6.9 mg NO_2/ml. The reducible NO_2 content of the precipitate when assayed on 28 May 1975 was 60 mg NO_2/g precipitate. Forty-one days later, the assay indicated 30 mg/g precipitate.

d. Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method described by the Environmental Protection Agency.[4] The sulfate content of the supernatant as received was 0.70 mg SO_4/ml of sample.

e. Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite content was determined by the cadmium reduction method described by the Environmental Protection Agency.[5]

The nitrate content of the supernatant as received was 0.082 mg/ml. The nitrate content of the supernatant as received was 0.089 mg/ml.

f. Chromatographic characterization: The sample was studied by gas, liquid and thin-layer chromatography. The primary purpose of this work was to provide chromatographic "fingerprints" of the sample.

(1) Liquid chromatography: The sample supernatant was studied using the following conditions:

Instrument: Waters Associates 301 equipped with 254 nm Schoeffel UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 30 minutes using solvent program No. 6.
Flow rate: 1 ml/min.

Using a homogenous sample dissolved in methanol, a small sharp peak was observed at 10.2 minutes and a large ill-defined peak at 19.7 to 43.3 minutes. PETN elutes at 10.2 minutes in this system.

(2) Gas chromatography: The sample was studied by gas chromatography as described below:

Instrument: Bendix 2500 equipped with flame ionization detector.
Column: Glass, 6 ft x 1/4 in.
(a) 3% OV-1 on Gas Chrom Q.
(b) 5% OV-17 on Anakrom ABS.
Flow rate: 50 cc N_2/min.
Column temperatures: 130°C.
Injection port: 200°C.
Detector temperature: 200°C.

Using Column (a) no peaks were observed. Using Column (b) PETN has a retention time of 5.7 minutes. PETN was not detected in the supernatant (< 0.01%), but was found in the precipitate in a concentration of 12% by weight.

(3) Thin-layer chromatography: The sample was extracted with chloroform and the extract studied with thin-layer chromatography.

Plate: Brinkmann Silica Gel F-254.
Solvent system: Cyclohexane/acetone (1:1).
Material spotted: 10, 5, 2.5 µg PETN (acetone).
Chloroform extract of 50 mg of the sample precipitate.
Detection: (a) UV-254 nm.
(b) Spray with 2% diphenylamine dissolved in 95% ethanol and radiate with UV light.

The sample precipitate contains 10 ± 2% PETN (R_f 0.61).

g. Spectral characterization: The sample was studied with infrared, ultraviolet and visible spectroscopy.

(1) Infrared spectrum: A vacuum dried sample of the precipitate in KBr gave an IR which was similar to that of PETN. Extraction of the precipitate with chloroform and concentration of the solvent gave a residue whose IR was identical to that of PETN.

(2) Ultraviolet spectrum: No peaks were observed above 230 nm. With a 33-fold dilution, the sample reached an absorbance of 1.0 at 233 nm.

(3) Visible spectrum: No peaks were observed in the range 400 to 800 nm. An undiluted sample showed an absorbance of 0.16 at 400 nm.

5. Acute Toxicity

Animal tests were performed on the neutralized diluted desensitized PETN, prepared as described above. The material contained 189 mg solids/ml.

a. Skin and eye irritation: The primary skin and eye irritations were determined by the Draize procedure[6] using six New Zealand white rabbits. Desensitized PETN gave no eye irritation and a score of 0.00 on the skin irritation test.

b. Oral toxicity: Desensitized PETN given orally in water at 5 g/kg killed none of the three male albino mice (Charles River CD-1 strain) and none of three male albino rats (Charles River CD strain). When this dose was given to three female rats, one died late on the first day; when given to a second group of three females, none died. Similar results were observed in other rats from the same group after treated with the sensitization blank. Therefore, the one death is an artifact.

6. Conclusions

The sample as received was basic (pH 11.6) and contained the equivalent of 103 mg NaOH/ml. The supernatant contained 1.0% aluminum and 5.6% sodium. The precipitate contained 43% aluminum and 8.4% sodium. The inorganic nitrate and inorganic nitrite content of the supernatant, as received, were both < 0.1 mg/ml. The organic NO_2 content was 9.4 mg NO_2/ml of supernatant on 20 May 1975 (sample shipped to MRI on 16 April 1975), but had decreased to 6.9 mg NO_2/ml 38 days later. The reducible NO_2 content of the precipitate was 60 mg/g of precipitate on 28 May 1975, but had decreased to about 30 mg/g of precipitate 41 days later. The PETN concentration in the precipitate when assayed shortly after arrival was 12% by weight. The supernatant PETN concentration was < 0.01%. The presence of PETN was confirmed by infrared spectrum, thin-layer chromatography, gas chromatography and high pressure liquid chromatography. High pressure liquid chromatography was the most useful chromatographic process for "fingerprinting" the sample.

The diluted, nertralized sample was ninirritating to rabbits and nontoxic to rats and mice after oral administration.

7. Recommendations

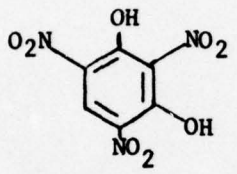
Because of the lack of irritancy and toxicity, no further toxicological research is warranted. Samples should be taken periodically and characterized by high pressure liquid chromatography to insure no significant variations in composition.

E. Desensitized Pure Trinitroresorcinol

1. Introduction

a. Sample origin: The sample was prepared by desensitizing pure trinitroresorcinol with sodium hydroxide and aluminum. The desensitization process was carried out by the Army.

b. Chemical literature: The parent compound, trinitroresorcinol (I), is a well characterized, commercially available compound.



(I)

The most sensitive and reliable assay procedure for trinitroresorcinol is by high pressure liquid chromatography.[19]

c. Toxicological literature: No data on the mammalian toxicity of trinitroresorcinol could be found.

2. Desensitization Procedure

The trinitroresorcinol (TNR) was desensitized as indicated below:

a. To a solution of 7 liters of water containing 258 g of NaOH was added 150 g of TNR.

b. During a 34-minute period, 129 g of atomized aluminum were added.

c. The solution was heated to 83° to 88° for 4 hours by injecting steam.

d. The final volume was 8 liters.

e. The pH of the product was 12.7.

3. Sample Concentration for Acute Toxicity Studies

a. Sample description: The sample as received was a dark brown turbid liquid. The pH was 11.7.

b. Concentration: The approximate concentration of dissolved solids/ml was determined prior to concentration. A 10-ml aliquot was reduced to dryness under 20 mm Hg vacuum at 50°to 60°. As received, the concentration of dissolved solids was 100 mg/ml.

The concentration of dissolved solids was adjusted by solvent removal under 20 mm Hg vacuum at 50° to 60°. After concentration, the pH was adjusted to 7.2 by addition of acetic acid. The sample remained a brown turbid liquid on addition of acetic acid. The final concentration of dissolved plus suspended solids was 170 mg/ml. The concentration factor was 1.70.

c. Distillate examination: The distillate resulting from sample concentration was studied by gas chromatography, thin-layer chromatography, and ultraviolet spectroscopy for the presence of components other than water. The techniques used are described under Section 4, Chemical Assays. No other components were detected in the distillate.

4. Chemical Assays and Chromatographic "Fingerprinting"

a. Alkalinity: The sample as received required 0.525 meq of HCl/ml of sample to neutralize it. This alkalinity corresponds to 21 mg NaOH/ml.

b. Metal content: The sample as received contained the following concentrations of metals.

Metal	% in Homogenous Aliquot
Aluminum	0.8
Antimony	<0.0005
Barium	<0.0002
Calcium	0.0010
Lead	<0.0005
Magnesium	<0.0001
Sodium	17.3

c. Reducible NO_2 content: The concentration of reducible NO_2 groups was determined by the method of Walter Selig.[3] This procedure will determine the presence of aliphatic nitrates, aliphatic nitrites, aromatic nitro or nitroso groups and inorganic nitrite and nitrate ions. Although a number of functionalities are detected simultaneously, the results are expressed in terms of mg NO_2 for comparison purposes. The nonspecificity of the assay can be considered an advantage since, when combined with the inorganic NO_2 and NO_3 assays, the covalently bound NO_2 content can be estimated.

The reducible NO_2 content of the homogenous sample when assayed on 20 May 1975 was 0.39 mg NO_2/ml. Thirty-five days later, the assay indicated 0.35 mg NO_2/ml.

d. Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method described by the Environmental Protection Agency.[4] The sulfate content of the supernatant as received was 0.85 mg SO_4/ml of sample.

e. Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite content was determined by the cadium reduction method described by the Environmental Protection Agency.[5] The nitrate content of the supernatant as received was 0.033 mg/ml. The nitrite content of the supernatant as received was 0.17 mg/ml.

f. Chromatographic characterization: The sample was studied by gas, liquid and thin-layer chromatography. The primary purpose of this work was to provide chromatographic "fingerprints" of the sample.

(1) Liquid chromatography: The sample supernatant was studied using the following conditions:

System 1

Instrument: Waters Associates 301 equipped with 254 nm UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 30 minutes using solvent program No. 6.
Flow rate: 1 ml/min.

Two small sharp peaks were observed at 9.5 and 14.6 minutes. Two large ill-defined peaks were observed at 18.9 and 25.6 minutes. Trinitroresorcinol does not give a peak using this system.

System 2

Instrument: Waters Associates 301 equipped with 235 nm Schoffel UV Detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 3% H_2O in CH_3CN.
Flow rate: 1 ml/min.

Trinitroresorcinol elutes at 2.76 minutes. The sample showed peaks at 1.57 and 3.15 minutes.

(2) Gas chromatography: The sample was studied with gas chromatography.

Instrument: Bendix 2500 equipped with flame ionization detector.
Column: Glass, 6 ft x 1/4 in.
Flow rate: 30 cc N_2/min.
Column temperature: Start at 100°C and heat at 5°/min to 250°C.
Injection port: 200°C.
Detector temperature: 200°C.

No peaks were observed. Trinitroresorcinol did not give a peak using this system.

(3) Thin-layer chromatography: The sample was examined by thin-layer chromatography.

Plate: Brinkmann Silica Gel F-254.
Solvent system: Acetone saturated with ammonium acetate.
Material spotted: 5, 0.5 µg trinitroresorcinol
20 µl of sample having a dissolved solids content of 170 µg/µl.
Detection: (a) Visual
(b) UV-254 nm
(c) Spray with 2% diphenylamine in 95% ethanol followed by IV irradiation.

Trinitroresorcinol has an R_f of 0.42 and a detection limit of 0.5 µg. The sample showed no spots above R_f 0.0.

g. Spectral characteristics: The sample was studied with infrared, ultraviolet and visible spectroscopy.

(1) Infrared spectrum: A vacuum-dried sample in a Fluoro-Mac mull showed bands at 3400 (broad, s) 2150 (w), 1665 (s), 1475 (s), 1340 (w), 1330 (w), 1160 (w), 1075 (m) and 875 (w). Trinitroresorcinol shows bands at 3200 (m), 1660 (s), 1620 (s), 1600 (s), 1550 (s), 1490 (s), 1466 (s), 1380 (s), 1310 (s), 1210 (w), 1190 (w), 1085 (s), 935 (m), 920 (m), 785 (m), 763 (m) and 732 (w) cm^{-1}.

(2) Ultraviolet spectrum: No peaks were observed. A 20-fold dilution showed an absorbance of 0.28 at 216 nm and 0.12 at 300 nm.

(3) Visible spectrum: No peaks were observed from 400 to 800 nm.

5. Acute Toxicity

Animal tests were performed on the neutralized concentrated desensitized trinitroresorcinol, prepared as described above. The material contained 170 mg solids/ml.

a. Skin and eye irritation: The primary skin and eye irritations were determined by the Draize procedure[6] using six New Zealand white rabbits. Desensitized trinitroresorcinol gave negative results in the eye irritation test and a score of 0.00 on the skin irritation test.

b. Oral toxicity: Desensitized trinitroresorcinol given orally at 5 g/kg killed none of the three male albino mice (Charles River CD-1 strain) and none of the three male and six female albino rats (Charles River CD strain) dosed.

6. Conclusions

The sample as received was basic (pH 11.7) and contained the equivalent of 21 mg NaOH/ml. A homogenous aliquot contained 0.8% aluminum and 17.3% sodium. The initial total reducible NO_2 content was 0.39 mg NO_2/ml. The inorganic nitrite content was 0.033 mg/ml and the nitrite content was 0.17 mg/ml. Thus the covalently bound NO_2 content is similar to the inorganic NO_3 plus NO_2 content. High pressure liquid chromatography (HPLC) was particularly useful for monitoring nonvolatile ultraviolet absorbing compounds. No trinitroresorcinol (< 0.01%) was observed in the waste sample. There are at least four uncharacterized ultraviolet absorbing components by HPLC. Thin-layer chromatography also indicated the absence

of trinitroresorcinol, but did not show as many minor components as seen by HPLC. Gas chromatography on an OV-1 column did not indicate the presence of any volatile components.

The desensitized trinitroresorcinol was concentrated and neutralized and found to be nonirritating to rabbits and nontoxic to rats and mice after oral administration.

7. Recommendations

Because of the lack of irritancy and toxicity, no further toxicological research is warranted. Samples should be taken periodically and characterized by high pressure liquid chromatography to insure no significant variations in composition.

F. Desensitization "Blank"

1. Introduction

The sample is a mixture of water, sodium hydroxide and aluminum.

2. Sample Preparation

The following procedure was used by the Army for preparation of the blank:

a. To 4 liters of water containing 258 g of NaOH was added 129 g of atomized aluminum during a 40-minute period.

b. The solution was heated at 82° to 85° for 4 hours by injecting steam.

c. The final volume was 6.8 liters.

d. The pH of the solution was 13.1.

3. Sample Concentration for Acute Toxicity Studies

a. Sample description: The sample was a translucent white suspension. The sample was shaken vigorously before removing an aliquot. The pH was 11.6.

b. Concentration: The approximate concentration of dissolved solids per ml was determined prior to concentration. A 10 ml aliquot was reduced to dryness under 20 mm Hg vacuum at 50° to 60°. As received the concentration of dissolved solids was 90 mg/ml.

The concentration of dissolved solids was now adjusted by solvent removal under 20 mm Hg vacuum at 50 to 60°. After concentration the pH was adjusted to 7.2 by addition of acetic acid. Addition of acetic acid caused the solution to become viscous. The final concentration of dissolved solids was 205 mg/ml. The concentration factor was 2.28.

4. Chemical Assays and "Fingerprinting"

a. Alkalinity: The sample supernatant as received required 0.925 meq of HCL/ml of sample to neutralize it. This alkalinity corresponds to 37 mg NaOH/ml.

b. Metal content: The sample as received contained the following concentrations of metals.

Metal	% in Supernatant	% in Precipitate[a/]
Aluminum	0.65	65.7
Antimony	< 0.0005	0.025
Barium	< 0.0002	0.025
Calcium	0.0010	0.062
Lead	< 0.0005	0.016
Magnesium	< 0.0001	0.032
Sodium	2.06	1.1

a/ The sample was removed by spatula and dried 2 hours at 100°C.

c. Reducible NO_2 content: The concentration of reducible NO_2 groups was determined by the method of Selig.[3/] This procedure will determine the presence of aliphatic nitrates, aliphatic nitrites, aromatic nitro groups and inorganic nitrite and nitrate ions.

The reducible NO_2 content of the supernatant when assayed on 20 May 1975, and again 41 days later was also negative.

d. Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method described by the Environmental Protection Agency.[4/] The sulfate content of the supernatant as received was 0.00 mg SO_4/ml of sample.

e. Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite contents were determined by the cadmium reduction method described by the Environmental Protection Agency.[5/] The nitrate and nitrite contents of the supernatant as received were 0.00 mg/ml.

f. Chromatographic characterization: The sample was studied by gas, liquid and thin-layer chromatography. The primary purpose of this work was to provide chromatographic "fingerprints" of the sample.

(1) Liquid chromatography: The sample supernatant was studied using the following conditions:

Instrument: Waters Associates 301 equipped with 254 nm UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 30 minutes using solvent program No. 6.

Flow rate: 1 ml/min.

No peaks were observed.

(2) Gas chromatography: The sample was studied by gas chromatography as described below:

Column: Glass, 6 ft x 1/4 in., 3% OV-1 on gas chromatography.
Instrument: Bendix 2500 equipped with flame ionization detector.
Flow rate: 30 cc N_2/min.
Column temperature: Start at 100°C and heat at 5°/min to 250°C.
Injection port: 200°C
Detector temperature: 200°C

No peaks were observed.

(3) Thin-layer chromatography: The sample was studied by thin-layer chromatography.

Plate: Brinkmann Silica Gel F-254.
Solvent system: acetone saturated with ammonium acetate.
Material spotted: 20 µl of sample having a dissolved content of 205 µg/µl.
Detection: (a) Visual
(b) UV-254 nm
(c) Spray with 2% diphenylamine in 95% ethanol followed by UV irradiation.

The sample showed no spots.

g. Spectral characterization: The sample was studied by infrared, ultraviolet and visible spectroscopy.

(1) Infrared spectrum: A vacuum-dried sample in KBr showed broad bands at 3300 and 1020 cm^{-1}.

(2) Ultraviolet spectrum: An undiluted sample showed a maximum at 265 nm (absorbance 0.11).

(3) Visible spectrum: No peaks were observed in the range 400 to 800 nm.

5. Acute Toxicity

Animal tests were performed on the neutralized concentrated desensitization blank. The material contained 205 mg solids/ml.

a. Skin and eye irritation: The primary skin and eye irritations were determined by the Draize procedure[6] using six New Zealand white rabbits. The desensitization blank gave negative results on the eye irritation test and a score of 0.08 on the skin irritation test. This is well below the 0.20 score required for a "mild irritant" rating, so the blank is nonirritating.

b. Oral toxicity: The desensitization blank given orally in water at 5 g/kg killed none of three male albino mice (Charles River CD-1 strain) and none of three male albino rats (Charles River CD strain). When given to a first group of three female rats, one died during the first night. None of a second group of three females died. The death is not treatment related.

6. Conclusions

The sample as received was basic (pH 11.6) and contained the equivalent of 37 mg NaOH/ml. The supernatant contained 0.65% aluminum and 2% sodium. The precipitate contained 66% aluminum and 1.1% sodium. Chromatographic techniques showed no organic impurities.

The concentrated, neutralized desensitization "blank" was nonirritating and nontoxic in animals. Adverse effects observed in testing desensitized compounds can be assumed to be due to the compound itself, not the desensitization materials.

G. Wastewater from Tetrazene Production

1. Introduction

a. Sample origin: The sample is wastewater produced during the manufacture of tetrazene at Lake City Army Ammunition Plant, Building 85. Three samples were studied. The first shipment was sampled by the Army. Two later lots were sampled and shipped by MRI.

b. Chemical and toxicological literature: See section A on tetrazene.

2. Production Methods

a. Production of wastewaters: The production and generation of wastewaters have been summarized by Small and Rosenblatt[1] and are given below:

> "To produce tetrazene, a 65 liter solution of 26.2 lb aminoguanidine bicarbonate in dilute sulfuric acid is mixed with 15 liters of solution containing 22.5 lb sodium nitrite. Tetrazene precipitates, and its further processing is similar to that used for lead styphnate. However, the collected mother liquors (previously neutralized with sodium carbonate) and washwaters are simply boiled after collection. The wastes are then routed to the lead styphnate lagoons. The tetrazene yield is about 16 lb/batch, which indicates 87% conversion. It is assumed that the remainder is lost product, or about 2.4 lb tetrazene/batch."

b. Fractionation of wastewaters: Toxicological study indicated that the concentrated supernatant of Lot 3 wastewater from tetrazene production was more toxic than the precipitate (see Section 5b(2) below). Therefore the supernatant was further fractionated.

A quantity of Lot 3 supernatant was centrifuged, decanted, and dried in vacuo. Four grams of supernatant solids were chromatographed on 225 g of silica gel G (Brinkmann 70 to 230 mesh) in 10% $CH_3OH/CHCl_3$ using a 4 x 40 cm column. The column was eluted progressively with 1,000 ml 10% $CH_3OH/CHCl_3$, 500 ml 20% $CH_3OH/CHCl_3$, 500 ml 30% $CH_3OH/CHCl_3$, 500 ml 40% $CH_3OH/CHCl_3$ and 2,000 ml 50% $CH_3OH/CHCl_3$. The first 1,000 ml left no residue on evaporation. Forty ml fractions were collected and monitored using thin-layer chromatography on silica gel 60 F-254 using 50% $CH_3OH/CHCl_3$ with UV and ninhydrin spray for detection. The results were:

Fraction	UV (R_f)	Ninhydrin (R_f)	Mg, Approximate
1 to 14	0.8	no spot	20
15 to 20	0.8 and 0.67	no spot	10
21 to 32	0.8 and 0.67	white spot 0.67 yellow spot 0.62 red spot origin	20
33 to 35	0.8 and 0.67	white spot 0.67 yellow spot 0.62 red spot origin	20
36 to 40	0.67	red spot origin	20[a/]
41 to 55 - (Fractions were combined and the solvent removed in vacuo. CH_3OH was added to just dissolve the residue. CH_3Cl was added dropwise until the precipitate stopped forming. The precipitate was removed by filtration (41-55 S) and the filtrate (41-55 L) dried in vacuo.)			
41-55 S	0.73 and 0.64	no spot	20
41-55 L	0.47	no spot	300
56	0.71, 0.57 and 0.21	red spot 0.73 white spot 0.57	10
57 to 63 - Treated same as 41 to 55 to give 57-63 S and 57-63 L.			
57-63 S	0.46	no spot	10
57-63 L	0.32	yellow spot 0.32	100
64	0.32 and origin	2 orange spots, 0.32 and origin	10
65-end of column	0.32 and origin	2 orange spots, 0.32 and origin	20

a/ During attempted weighing this sample exploded causing minor operator injury.

The combined 41-46 L fraction gave about 300 mg of material and the combined 57-63 L fractions gave about 100 mg of material; these two fractions were used for chemical characterization and toxicological studies as described below. The other fractions totaled about 160 mg. Thus about 85% of the total sample was not eluted from the column with the strongest solvent.

3. Sample Concentration Procedure for Acute Toxicity Studies

a. Sample description: The three samples studied were light brown and contained no precipitate. The samples were used as received. Their pH was in the range of 7 to 8.

b. Concentration: The approximate concentration of dissolved solids/ml was determined prior to concentration. A 10-ml aliquot was reduced to dryness under 20 mm Hg vacuum at 50° to 60°. As received, the concentration of dissolved solids in Lot 1 was 0.8 mg/ml. The concentration of dissolved solids in this first lot of tetrazene waste (Lot 1) was too low to be useful. Two more lots of wastewater (Lots 2 and 3) were obtained. Only Lots 2 and 3 were used in animal studies. The initial concentration in Lot 2 was 6 mg dissolved solids/ml. The initial concentration in Lot 3 was 12 mg/ml.

The concentration of dissolved solids was adjusted by solvent removal under 20 mm Hg vacuum at 50° to 60°. On solvent removal, a light brown turbid solution resulted. The final concentration of dissolved plus suspended solids in Lot 2 and Lot 3 was 100 mg/ml. The pH of Lot 2 and 3 as concentrated was 7.3 and 7.6, respectively.

One percent carboxymethyl cellulose ether sodium salt was added to the concentrated Lots 2 and 3 samples to hold the precipitate in solution.

c. Distillate examination: The distillate resulting from sample concentration was studied by gas chromatography, thin-layer chromatography and ultraviolet spectroscopy for the presence of components other than water. The techniques used are described below under Section 4, Chemical Assays. No other components were detected in the distillate.

4. Chemical Assays and "Fingerprinting"

a. Unfractionated wastewaters: The unfractionated wastewaters from tetrazene production were assayed and "fingerprinted" as follows:

(1) Alkalinity: The samples as received were in the pH range of 7 to 8 and therefore were used unchanged.

(2) Metal content: Only Lot 1 was assayed for metal content. As received, it contained < 0.004% of aluminum, antimony, barium, calcium, lead, or magnesium.

(3) Reducible NO_2 content: The concentration of reducible NO_2 groups was determined by the method of Selig.[3] This procedure will determine the presence of aliphatic nitrates, aliphatic nitrites, aromatic nitroso and nitro groups, as well as inorganic nitrite and nitrate ions. Although a number of functionalities are detected simultaneously, the results are expressed in terms of mg NO_2 for comparison purposes. The nonspecificity of the assay can be considered an advantage since when combined with the inorganic NO_2 and NO_3 assays, the covalently bound NO_2 content can be estimated.

After concentrating to 100 mg/ml, Lot 2 supernatant had a reducible NO_2 content of 4.26 mg/ml. Lot 2 precipitate had a reducible NO_2 content of 47 mg/g of precipitate. The precipitate was obtained by centrifugation and vacuum drying.

After concentrating to 100 mg/ml, Lot 3 supernatant had a reducible NO_2 content of 5.23 mg/ml. Lot 3 precipitate also had a reducible NO_2 content of 47 mg/g precipitate.

(4) Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method described by the Environmental Protection Agency.[4] The sulfate content of Lot 2 after concentration to 100 mg total solids/ml was 4.4 mg SO_4/ml and that of Lot 3 was 43 mg/ml.

(5) Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite content was determined by the cadmium reduction method described by the Environmental Protection Agency.[5] After concentrating the sample to 100 mg total solids/ml, the nitrate and nitrite contents of the supernatant of Lot 2 were 2.7 mg/ml and 1.0 mg/ml, respectively. Similarly, the nitrate and nitrite contents of the supernatant of Lot 3 were 6.7 mg/ml and 18.9 mg/ml, respectively.

(6) Chromatographic characterization: The samples were studied by gas, liquid and thin-layer chromatography. The primary purpose of this work was to provide chromatographic "fingerprints" of the samples.

(a) Liquid chromatography: The samples were studied as described below:

Instrument: Waters Associates 301 equipped with 235 nm Schoeffel 770 UV detector.
Column: Porasil AX, 2 ft x 1/3 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 30 minutes using linear solvent program No. 6.

Flow rate: 1 ml/min.

Lot 1 showed peaks at 1.6 and 2.76 minutes. Tetrazene had a retention time of 1.6 minutes and tetrazole had a retention time of 2.76 minutes. Lot 2 supernatant showed peaks with retention times of 1.6, 2.76, 5.51, 23.6, 29.5, 31.5 and 32.7 minutes. Lot 2 precipitate chromatogram was similar to the supernatant. A sample of 5-aminotetrazole had a retention time of 5.51 minutes. Lot 3 supernatant showed peaks with retention times of 2.76, 5.51, 18.1, 22.0, 25.2 and 37.4 minutes.

(b) Gas chromatography: The samples were studied by gas chromatography as described below:

Instrument: Bendix 2500 equipped with flame ionization detector.
Column: glass, 6 ft x 1/4 in.
(i) 3% OV-1 on Gas Chrom Q.
(ii) 1.5% DCLSX-3-0295 and 1.5% GE XE-60 on Gas Chrom Q.
Flow rate: 30 cc N_2/min.
Column temperature: Start at 100°C and heat to 5°/min to 250°C.
Injection port: 200°C
Detector temperature: 200°C

Only Lot 1 was studied. Using Column (i), no peaks were observed. Using Column (ii), a single broad peak was observed at 8 minutes. A reference sample of 1-H-tetrazole had a retention time of 7 minutes on Column (ii). No peak was observed for 5-aminotetrazole or tetrazene on either column.

(c) Thin-layer chromatography: Lot 2 sample was studied as described below:

Plate: Brinkmann Silica Gel F-254.
Solvent system: 95% ethanol/conc. NH_4OH (4:1).
Material spotted: 5, 1, 0.5 µg 5-aminotetrazole (10% HCl).
10 µg cyanamide (acetone).
500 µg of dissolved solids. (H_2O)
Detection: (i) UV-254 nm.
(ii) Spray with ninhydrin and heat.

UV light showed spots at R_f 0.54, 0.56 and 0.67. The references showed no spot for cyanamide and a faint spot for 5-aminotetrazole (R_f 0.0). With ninhydrin, the homogenous sample showed spots at R_f 0.0 to 0.15 (pink), 0.2 to 0.4 (purple), 0.48 (white), 0.54 (white), 0.60 (pink), 0.63 (pink) and 0.66 (yellow). Cyanamide gave a white spot at 0.77 and 5-aminotetrazole remained at the origin (pink).

(d) Lots 2 and 3 samples were studied as described below:

Plate: Brinkmann Silica Gel F-254
Solvent system: $CHCl_3$:CH_3OH (1:1).
Material spotted: 10 µg 5-aminotetrazole.
10 µg 1-H-tetrazole.
10 µg cyanamide.
4 µl concentrated Lot 2 (100 µg/µl).
1 µl concentrated Lot 3 (332 µg/µl).
Detection: UV-254 nm.
ninhydrin spray.

On detection with UV (254 nm) no spots were observed for the 5-aminotetrazole, 1-H-tetrazole or cyanamide references. Lot 2 showed spots at R_f 0.08, 0.36, 0.42 and 0.58. Lot 3 showed spots at R_f 0.05, 0.21 and 0.47.

On detection with ninhydrin, 5-aminotetrazole gave a light pink spot at R_f 0.37, 1-H-tetrazole gave a pink spot at R_f 0.03 and cyanamide gave a white spot at R_f 0.58. Lot 2 showed a white streak from the origin to R_f 0.36 and an orange spot at R_f 0.42. Lot 3 showed a white streak from the origin to R_f 0.15, a yellow spot at R_f 0.21 and a white streak from R_f 0.27 to 0.44.

(7) Spectral characterization: Lots 2 and 3 were studied by infrared, ultraviolet and visible spectroscopy.

(a) Infrared spectrum: A vacuum-dried sample of Lot 2 in KBr showed bands at 3450 (s), 2160 (s), 1685 (s), 1630 (m), 1480 (m), 1420 (w), 1140 (s), 810 (w), 680 (m), 510 (m) and 470 (m) cm^{-1}.

A vacuum-dried sample of Lot 3 in KBr showed bands at 3440 (broad, s), 2160 (m), 1690 (w), 1560 (m), 1470 (m), 1260 (s), 1150 (s), 850 (m), 830 (m) and 620 (m) cm^{-1}. The band at 2160 cm^{-1} was less intense in Lot 3 than in Lot 2.

(b) Ultraviolet spectrum: Lot 2 as received showed no peaks above 230 nm. With a 20-fold dilution in water the absorbance at 230 nm was 0.91. Lot 3 as received with a dilution of 333 showed a peak at 266 nm with a broad shoulder extending to about 320 nm. The absorbance at 230 nm was 2.7 as it neared end absorption.

(c) Visible spectrum: For Lot 2 as received, no peaks above 0.1 absorbance were observed in the range of 400 to 800 nm. For Lot 3 as received, no peaks were observed in the range of 400 to 800 nm. A shoulder leading to the UV region was observed. With a dilution of 200, the absorbance at 400 nm was 0.23.

(8) Similarities between Lots 2 and 3:

(a) Alkalinity: Both lots are in the pH range 7 to 8.

(b) Reducible NO_2 content: The reducible NO_2 contents of the lots were similar after concentration.

(c) Inorganic nitrate and nitrite content: The nitrate and nitrite content in Lot 3 was slightly higher.

(d) Infrared spectrum: A band at 2160 cm^{-1}, usually associated with the isonitrile group[20] or azide group,[21] was present in both lots.

(e) Visible spectrum: Lots 2 and 3 showed no significant peaks in the visible spectrum.

(9) Differences between Lots 2 and 3:

(a) Initial concentration: Lot 3 as received had twice the dissolved solids content of Lot 2.

(b) Sulfate content: After concentration the Lot 3 sulfate content was about 10 times that of Lot 2.

(c) Liquid chromatograph pattern: The HPLC traces show major qualitative and quantitative differences. In Lot 2 by peak area comparison, the compounds eluting at 2.76 and 23.6 minutes are the major components with the peak at 23.6 minutes about 5 times the area of the 2.76 minutes peak. In Lot 3 by peak area comparison the compounds at 2.76 and 27.4 minutes are the major components with about an equal distribution of peak area. By comparison, 1-H-tetrazole has a retention time of 2.76 minutes; however, this should not be considered a positive identification.

(d) Thin-layer pattern: Qualitative differences were noted; however, the HPLC pattern (see c.) gives better resolution of the components.

(e) Infrared spectrum: A band at 2160 cm^{-1}, usually associated with the isonitrile group[20] or azide group[21], was more intense in Lot 2 than in Lot 3. There were many differences in the spectra below 1500 cm^{-1}.

(f) Ultraviolet spectrum: Lot 2 showed no significant absorption above 230 nm. Lot 3 showed a peak at 266 nm with a broad shoulder extending to about 320 nm.

b. Fractions from wastewaters: The isolated fractions 41-55 L and 57-63 L were characterized by methods similar to those used above.

(1) Fraction 41-55 L:

(a) Description: The sample is dark red, semicrystalline and hydroscopic.

(b) Capillary melting point: Decomposed at about 146°.

(c) Infrared spectrum: Absorptions were observed at 3500 (broad, s), 2160 (m), 1665 (broad, m), 1480 (w), 1270 (s), and 840 (w) cm^{-1}.

(d) Ultraviolet: No absorption maximum was observed above 230 nm.

(e) Mass spectrum: The sample was studied using an Atlas CH4 operating at 70 eV. Spectra were taken at 20° intervals while raising the temperature from 30° to 400°C. The spectrum changed drastically for each 20° interval, making the data of minimal use for structural identification.

(f) Thin-layer chromatography:

Plate: Silica Gel F-254.
Solvent system: $CH_3OH/CHCl_3$ (1:1).
Detection: (i) UV 254 nm.
(ii) ninhydrin
Material spotted: 100 ug fraction 41-55 L.

The UV light showed one spot at R_f 0.47. The ninhydrin spray showed no spots.

(g) Elemental analysis: The sample contained 3.71% C, 2.67% H and 28.50% N by combustion analysis.

(h) Discussion of characterization data: Fraction 41-55 L has not been identified. Thin-layer chromatography indicates the sample to be reasonably pure, however verification is needed before one can interpret the elemental analysis. The IR band at 2160 cm^{-1} (4.63 μ) is probably due to an azide group.[21]

(2) Fraction 57-63 L:

(a) Description: Light brown powder.

(b) Capillary melting point: > 350°

(c) Infrared spectrum: Absorptions were observed at 3500 (broad, s), 1600 (m), 1470 (m), 1420 (w) and 1270 (s) cm^{-1}.

(d) Ultraviolet: An absorption maximum was observed at 303 nm with E_1^1 = 204.

(e) Mass spectrum: The sample was studied using an Atlas CH4 operating at 70 eV. Spectra were taken at 20° intervals while raising the temperature from 30° to 400°C. The spectrum changed drastically for each 20° interval, making the data of minimal use for structural identification.

(f) Thin-layer chromatography:

Plate: Silica Gel F-254.
Solvent system: $CH_3OH/CHCl_3$ (1:1).
Detection: (i) UV-254 nm.
(ii) ninhydrin
Material spotted: 100 μg Fraction 57-63 L.

The UV light showed one spot at R_f 0.32. The ninhydrin spray showed a yellow spot at R_f 0.32.

(g) Elemental analysis: The sample contained 7.36% C, 2.45% H and 36.44% N by combustion analysis.

(h) Discussion of characterization data: Fraction 57-63 L has not been identified. Thin-layer chromatography indicates the sample to be reasonably pure, however verification is needed before one can interpret the elemental analysis. The UV data indicate considerable conjugation (λ max 303 nm). This absorption maximum is probably not due to the tetrazole ring since tetrazene, 5-aminotetrazole and 1-H-tetrazole do not have a significant extention coefficient above 230 nm.

5. Acute Toxicity

Various lots were tested for acute toxicity. The samples tested contained 100 mg solids/ml, and were diluted as necessary for the specific study.

a. Skin and eye irritation: The primary skin and eye irritations were determined by the Draize procedure[6] using six New Zealand white rabbits. Lot 3 of the tetrazene wastewater was nonirritating in the eye test and scored 0.00 in the skin test. Other samples were not tested, because of these negative results and because of insufficient quantity.

b. Oral toxicity: As described below, various lots and fractions were tested for acute oral toxicity in albino rats (Charles River CD strain) and/or albino mice (Charles River CD-1 strain). LD_{50}'s were calculated by the probit method of Finney[7] adapted for computer.

(1) Toxicity of Lot 2: Because insufficient quantity of Lot 2, this lot was tested only in female albino rats. Eight of nine rats given 5 g solids/kg body weight orally in water died (see Table 3). Both rats given 4 g/kg died, but both given 3 g/kg survived. Therefore, the LD_{50} is estimated to be 3.5 g/kg.

(2) Toxicity of Lot 3: Lot 3 was much more toxic than Lot 2. As shown in Tables 3 and 4, the LD_{50}'s (with 95% confidence limits) of Lot 3 were 0.622 (0.568 to 0.664) g solids/kg body weight in male rats and 0.311 (0.262 to 0.371) g/kg in female rats. This sex difference is significant and probably results from sex differences in the metabolism of the toxic compounds. The tetrazene wastewater was somewhat less toxic to mice, with LD_{50}'s of 0.711 (0.589 to 0.845) g/kg in males and 0.805 (0.705 to 0.875) g/kg in females. This sex difference in mice is not significant. All animals died on the first day with no apparent symptoms except depression or survived the entire 14 days of observation.

TABLE 3

ACUTE ORAL LD_{50}'S OF EXTRACTS OF TETRAZENE WASTEWATERS IN RATS

Dose (g solids/kg body weight)	Mortality	
	Male	Female
(Lot 2)		
5.0	ND	8/9
4.0	ND	2/2
3.0	ND	0/2
(Lot 3)		
0.75	5/5	ND
0.70	5/6	ND
0.65	3/5	5/5
0.60	3/5	5/5
0.55	0/5	5/5
0.50	0/5	ND
0.40	0/5	4/5
0.35	ND	4/5
0.30	ND	2/5
0.25	ND	1/5
0.20	ND	0/5
LD_{50} (g/kg)	0.622	0.311
95% Confidence Limits (g/kg)	0.568-0.664	0.262-0.371
Slope ± Standard Error	10.4 ± 3.4	4.5 ± 1.5

ND = Not Determined.

TABLE 4

ACUTE ORAL LD_{50}'S OF EXTRACTS OF TETRAZENE WASTEWATERS IN MICE

Dose (g solids/kg body weight)	Mortality	
	Male	Female
1.2	5/5	ND
1.0	4/5	5/5
0.9	ND	3/5
0.85	ND	4/5
0.8	4/5	2/5
0.75	ND	2/5
0.7	2/5	ND
0.6	2/5	0/10
0.5	0/5	0/5
LD_{50} (g/kg)	0.711	0.805
95% Confidence Limits (g/kg)	0.589-0.845	0.705-0.875
Slope ± Standard Error	3.9 ± 1.2	7.4 ± 2.6

ND = Not Determined.
All data using Lot 3.

Since this lot, as received contained 12 mg/ml total solids, the required volume to produce LD_{50} in female rats was about 25 ml/kg. Extrapolated to a 70 kg man, this is about 2 qt. The required volumes to produce LD_{50} in male rats and male and female mice were even greater. While unfit for drinking, the wastewater is not a severe acute hazard to the workers.

(3) Toxicity of Lot 3 fractions: Since the tetrazene wastewater samples are very complex mixtures, an attempt was made to identify the more toxic components. First, a portion of Lot 3 was separated into supernatant and precipitate by centrifugation, decantation of the supernatant, and vacuum-drying of the precipitate. Two groups of four albino male mice were given a single oral dose of 0.5 g total solids/kg body weight in water of either mixture. The mice given the precipitate were unaffected. Those given the supernatant were listless and unresponsive for several hours but did not die within 14 days.

As described in Section 2b above, the Lot 3 supernatant solids were fractionated by chromatography. Two fractions, (41-55 L and 57-63 L), were tested in male mice. A single oral dose of 0.5 g of total solids/kg of body weight of each fraction in water was given to four mice. Two given fraction 41-55 L and one given 57-63 L died within an hour of dosing; none died thereafter. From these data and their abundance in the wastewater it is concluded that these two fractions have LD_{50}'s near 0.5 g/kg and represent much of the toxicity of the wastewater.

6. Conclusions

The wastewater samples received were significantly different. High pressure liquid chromatography and thin-layer chromatography are particularly useful in characterizing the samples. High pressure liquid chromatography indicated the presence of at least seven components. Lot 2 was moderately toxic after oral dosing; Lot 3 was nonirritating to rabbits but more toxic to rats. The toxicity appears to be due to two characterized but unidentified components, which can be readily monitored by high pressure liquid chromatography. These components are present in the wastewater as received in concentrations of 0.9 and 0.3 mg/ml. The more abundant component probably contains an azide linkage. By IR spectroscopy, neither of these compounds are among those listed in the attached appendix.[21]

7. Recommendations

Further work is warranted due to the toxicity of the unknown compounds in the wastewater. This should include:

a. Devising a better fractionation scheme to give a better mass balance.

b. Identifying the toxic compounds.

c. Locating the source of the toxic compounds, whether in the synthesis or in later steps. High pressure liquid chromatography is the most useful technique for detecting the compounds.

d. Developing a practical method of cleaning up tetrazene wastewater. This may eliminate the toxic compounds and make further toxicological testing unnecessary.

e. Devising a practical synthesis of the toxic compounds so enough can be made for long-term toxicity studies.

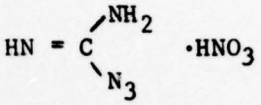
f. Carrying out appropriate long-term toxicity studies on the appropriate compounds.

APPENDIX

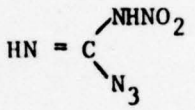
The infrared spectra of toxic fractions 41-55 L and 57-63 L (see attached spectra) are different from the spectra of the compounds listed below:[21]

1. Sodium azide

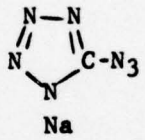
2. Guanyl azide nitrate



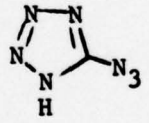
3. Nitroguanyl azide



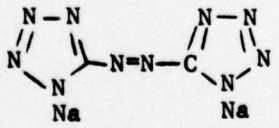
4. Sodium tetrazoyl azide



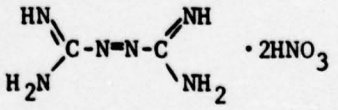
5. Tetrazolyl azide



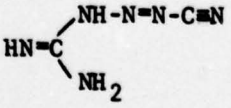
6. Disodium azotetrazole pentahydrate



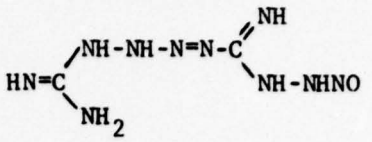
7. Azidicarbamidine dinitrate



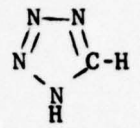
8. Diazoguanidine cyanide



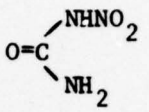
9. 1-Guanyl-4-nitrosoaminoguanyl-iso-tetrazene



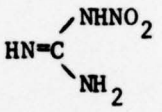
10. Tetrazole



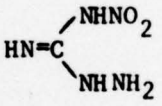
11. Nitrourea



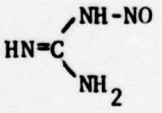
12. Nitroguanidine



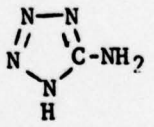
13. Nitroaminoguanidine



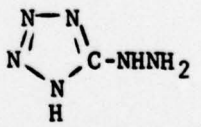
14. Nitrosoguanidine



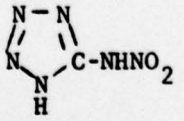
15. 5-Aminotetrazole



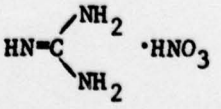
16. Tetrazoyl hydrazine



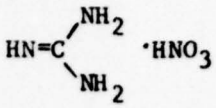
17. 5-Nitroaminotetrazole



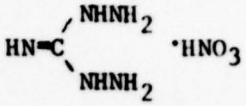
18. Guanidine nitrate



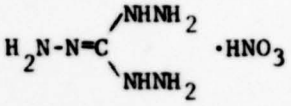
19. Aminoguanidine nitrate



20. Diaminoguanidine nitrate



21. Triaminoguanidine nitrate



22. Hydrazine monohydrochloride

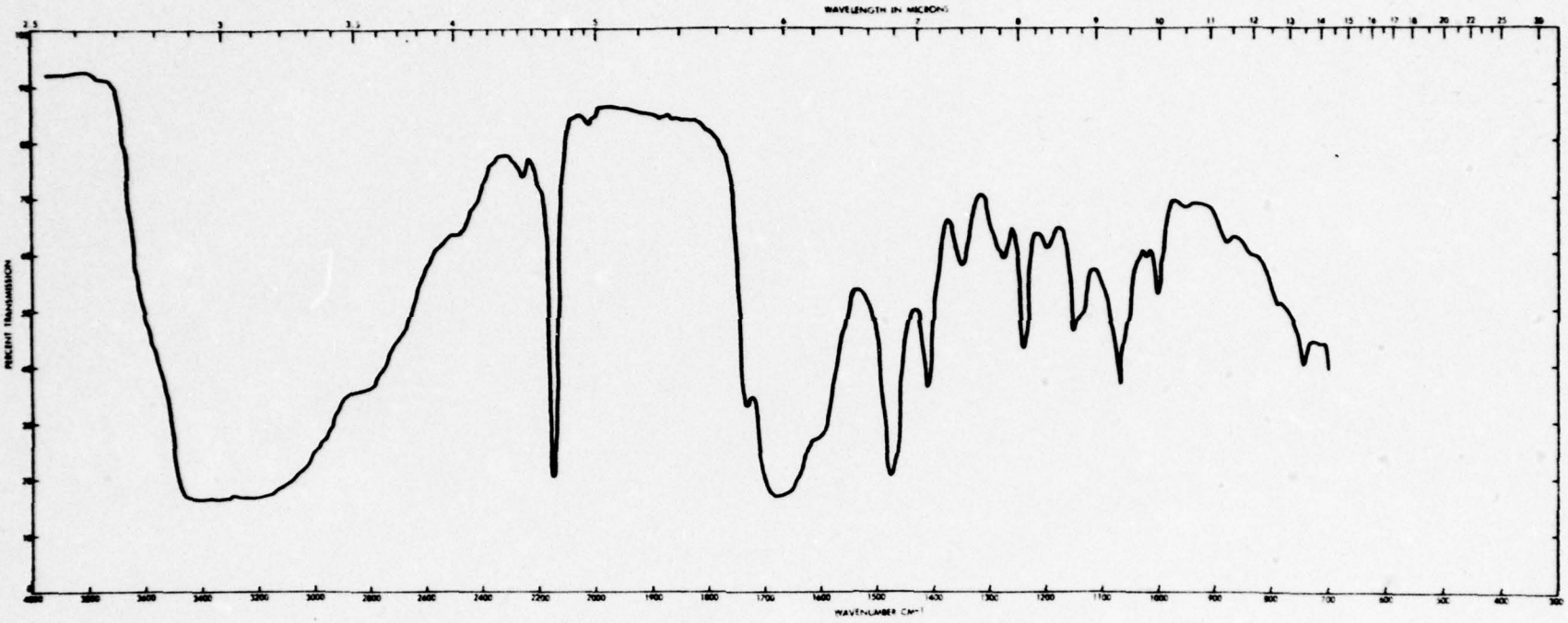


Figure 1 - Sample 1: Desensitized Pure Tetrazene.

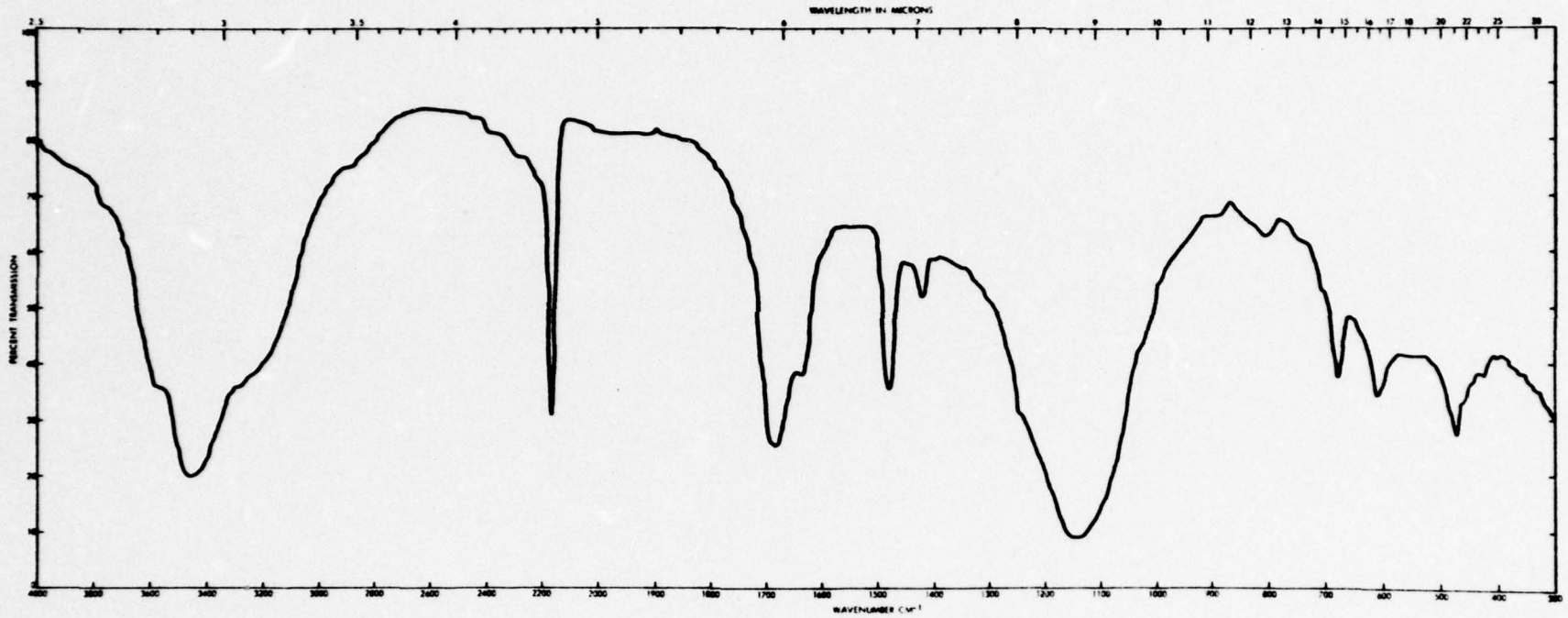


Figure 2 - Sample 7, Lot 2: Tetrazene Wastewater.

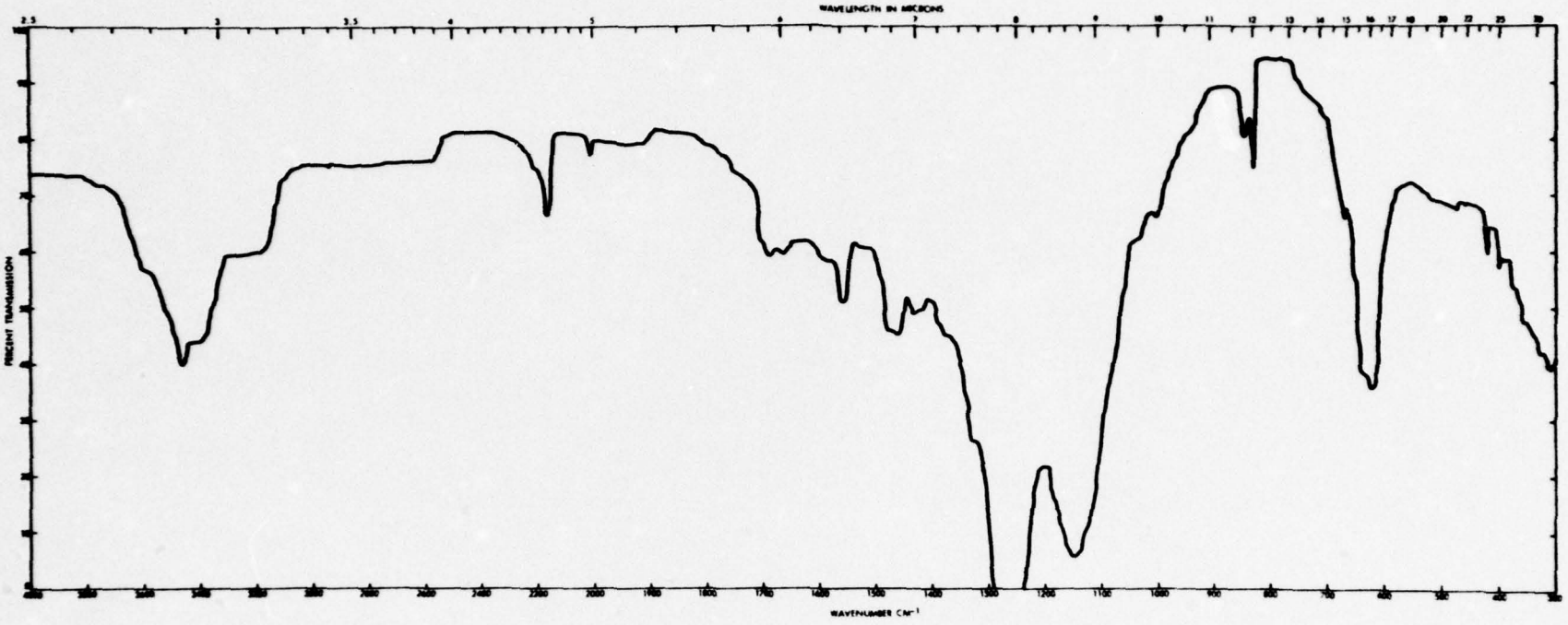


Figure 3 - Sample 7, Lot 3: Tetrazene Wastewater.

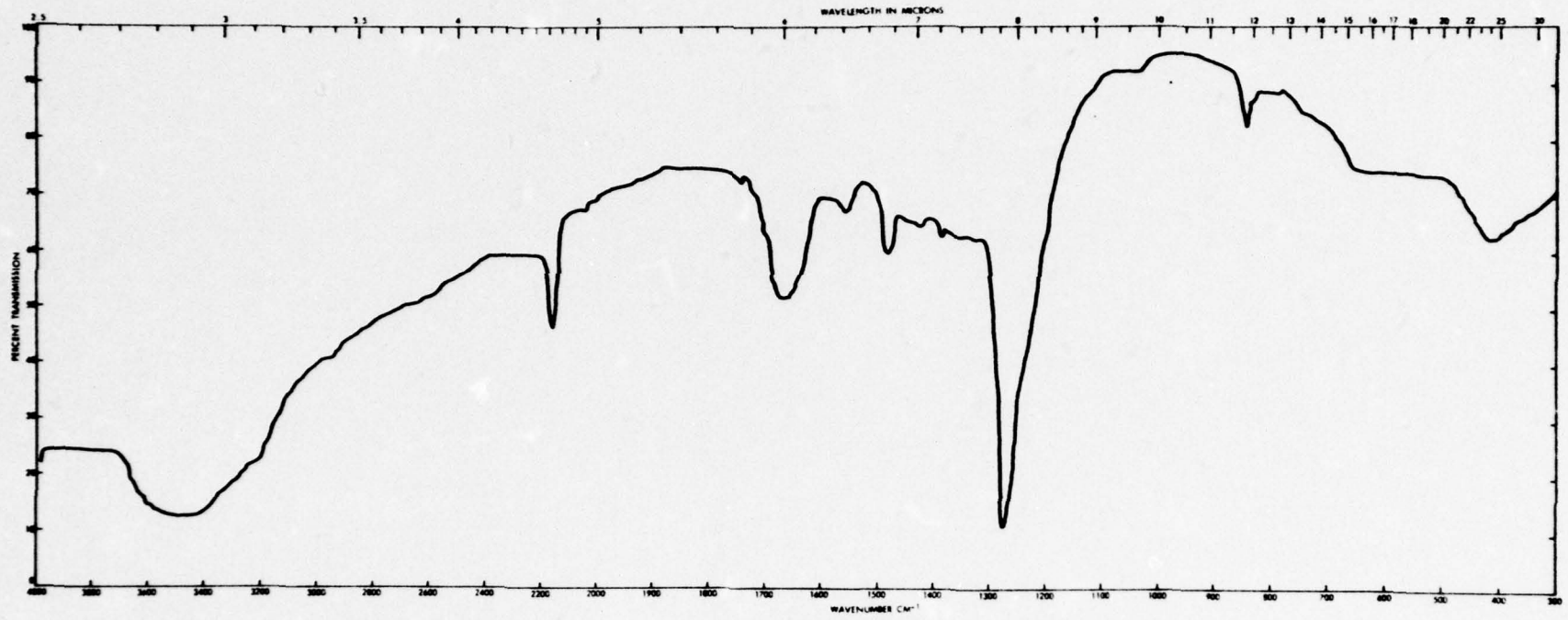


Figure 4 - Sample 7, Lot 3, Fraction 47-55 L: Tetrazene Wastewater.

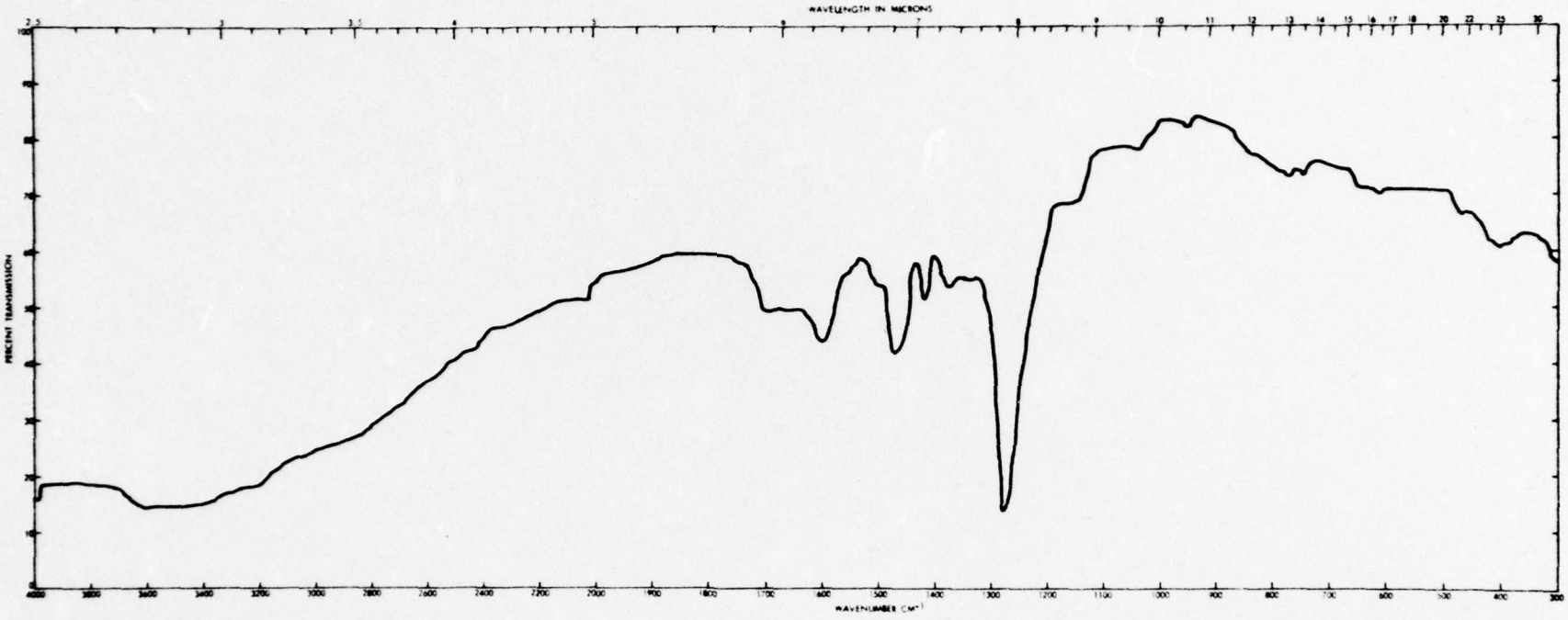


Figure 5 - Sample 7, Lot 3, Fraction 57-63 L: Tetrazene Wastewater.

H. Wastewater from Primer Mixture FA 956 Production

1. Introduction

The sample is wastewater produced during the manufacture of primer mixture FA 956 at Lake City Army Ammunition Plant, Building 90C. The sample was sampled and shipped by the Army.

2. Production Methods

The production methods and wastewater generation procedures have been summarized by Small and Rosenblatt[1/] and are given below:

Because of the high explosive hazard, primer mixtures are prepared in small quantities and used rapidly after preparation. To prepare the primers, screened ingredients are mixed in a blender. There is no definitive breakdown of primer losses in formulation or use. Lake City AAP supervisory personnel estimate that about 12% of the amount of primer used in small arms ammunition is wasted. This percentage represents primer material cleaned out from screens and blenders, spills, unused or dried-out mixtures and washdowns. The wastewaters with primer ingredients are dosed with sodium hydroxide and aluminum powder and then heated. The primer-desensitized wastewaters are then routed to the LCAAP industrial wastewater treatment plant (IWTP) where they contact other wastes from LCAAP activities. The IWTP treatment at LCAAP consists of aeration, with subsequent skimming to remove oils and greases, dosing with alum and lime to adjust pH and promote flocculation, and settling of solids from effluent prior to discharge to surface flow.

3. Sample Concentration for Acute Toxicity Studies

a. Sample description: The sample was a transparent brown liquid with a small amount of light brown finely divided precipitate. The sample was shaken vigorously before removing an aliquot. The pH was 12.3.

b. Concentration: The approximate concentration of dissolved solids/ml was determined prior to concentration. A 10-ml aliquot was reduced to dryness under 20 mm Hg vacuum at 50° to 60°. As received the concentration of dissolved solids was 7.5 mg/ml.

The concentration of dissolved solids was adjusted by solvent removal under 20 mm HG vacuum at 50 to 60°. After concentration the pH was adjusted to 7.2 by addition of acetic acid. On addition of acetic acid the solution became very turbid. The final concentration of dissolved plus suspended solids was 153 mg/ml. The concentration factor was 20.4.

c. Distillate examination: The distillate resulting from sample concentration was studied by gas chromatography, thin-layer chromatography, and ultraviolet spectroscopy for the presence of components other than water. The techniques used are described under Section 4, Chemical Assays. No other components were detected in the distillate.

4. Chemical Assays and "Fingerprinting"

a. Alkalinity: The sample supernatant as received required 0.0775 meq of HCl/ml of sample to neutralize it. This alkalinity corresponds to 3.1 mg NaOH/ml.

b. Metal content: The sample as received contained the following concentrations of metals:

Metal	% in Supernatant	% in Precipitate
Aluminum	0.080	51.2
Antimony	0.0030	0.48
Barium	0.0007	1.55
Calcium	0.0015	< 0.24
Lead	0.0025	4.4
Magnesium	< 0.0001	< 0.12
Sodium	0.170	1.9

c. Reducible NO_2 content: The concentration of reducible NO_2 group is determined by the method of Selig.[3] This procedure determines the presence of aliphatic nitrates, aliphatic nitrites, aromatic nitro groups and inorganic nitrite and nitrate ions. Although a number of functionalities are detected simultaneously, the results are expressed in terms of mg NO_2 for comparison purposes. The nonspecificity of the assay can be considered an advantage since, when combined with the inorganic NO_2 and NO_3 assays, the covalently bound NO_2 content can be estimated.

The reducible NO_2 content of the supernatant when assayed on 21 May 1975 and again 36 days later was 0.1 mg NO_2/ml. The reducible NO_2 content of the precipitate when assayed on 29 May 1975 and again 34 days later was 0.00 g NO_2/g precipitate.

d. Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method described by the Environmental Protection Agency.[4] The sulfate content of the supernatant as received was 0.28 mg SO_4/ml of sample.

e. Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite contents were determined by the cadmium reduction method described by the Environmental Protection Agency.[5] The nitrate content of the supernatant as received was 0.0044 mg/ml. The nitrite content of the supernatant as received was 0.0197 mg/ml.

f. Chromatographic characterization: The sample was studied by gas, liquid, and thin-layer chromatography. The primary purpose of this work was to provide chromatographic "fingerprints" of the sample.

(1) Liquid chromatography: The sample supernatant was studied using the following conditions:

System 1

Instrument: Waters Associates 301 equipped with 254 nm UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 30 minutes using solvent program No. 6.
Flow rate: 1 ml/min.

A single broad peak was observed at 20.5 minutes. Trinitroresorcinol and lead styphnate do not give peaks using this system.

System 2

Instrument: Waters Associates 301 equipped with 235 nm UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 3% H_2O in CH_3CN.
Flow rate: 1 ml/min.

Trinitroresorcinol and lead styphnate elute at 2.76 minutes. The sample showed peaks at 1.18, 3.15, 6.69 and 8.66 minutes. The peaks at 6.69 and 8.66 minutes were ill-defined, suggesting the presence of unresolved components.

(2) Gas chromatography: The concentrated sample supernatant and precipitate were studied by gas chromatography as described below. The supernatant was injected "as is". A chloroform extract of the precipitate was injected.

Instrument: Bendix 2500 equipped with flame ionization detector.
Column: Glass, 6 ft x 1/4 in.
(a) 3% OV-1 on Gas Chrom Q
(b) 5% OV-17 on Anakrom ABS
Flow rate: 50 cc N_2/min.
Column temperature: (a) Start at 100°C and heat at 5°/min. to 250°C.
(b) Isothermal at 130°C.
Injection port: (a) 200°C.
(b) 130°C.
Detector temperature: 200°C.

No peaks were observed using column (a) or (b), column temperature (a) or (b), or injection temperature (a) or (b). Using the (b) conditions, reference PETN had a retention time of 5.7 minutes.

(3) Thin-layer chromatography: The sample was studied in two thin-layer chromatography systems.

System 1 (for TNR and lead styphnate)

Plate: Brinkmann Silica Gel F-254
Solvent system: Acetone saturated with ammonium acetate.
Material spotted: 5 µg lead styphnate
5 µg trinitroresorcinol
20 µl of sample having a dissolved solids content of 153 mg/ml (i.e., 3,060 µg of waste solids).
Detection: (a) visual
(b) UV-254 nm
(c) Spray with 2% diphenylamine in 95% ethanol followed by UV irradiation.

Lead styphnate has an R_f of 0.42 and a detection limit of 0.5 µg. Trinitroresorcinol has an R_f of 0.42 and a detection limit of 0.5 µg. The sample showed one spot at R_f 0.125.

System 2 (for PETN)

The precipitate (33 mg) was extracted twice with 5 ml chloroform using sonication. The chloroform was concentrated and used for spotting in the following TLC system:

Plate: Brinkmann Silica Gel F-254
Solvent system: Cyclohexane/acetone (1:1)
Material spotted: 5, 1, 0.5 µg, chloroform extract of 250 mg of sample precipitate.
Detection: (a) UV-254 nm
(b) Spray with 2% diphenylamine dissolved in 95% ethanol, radiate with UV light.

UV light indicated no spots. Diphenylamine spray indicated the presence of PETN at R_f 0.59 in the reference. PETN can be detected at the 0.5 µg level. The sample showed no spots.

g. Spectral characterization: The sample was studied by infrared, ultraviolet and visible spectroscopy.

(1) Infrared spectrum: A vacuum dried sample of the supernatant showed (KBr wafer) broad, ill-defined bands at 3300, 1620 and 1075 cm^{-1}.

(2) Ultraviolet spectrum: The supernatant showed no peaks in the range 230 to 400 nm. With a 10-fold dilution the sample indicated an absorbance of 0.55 at 230 nm.

(3) Visible spectrum: The supernatant showed no peaks in the range 400 to 800 nm. An undiluted sample had an absorbance of 0.12 at 400 nm.

5. Acute Toxicity

Animal tests were performed on the neutralized, concentrated primer mixture FA 956 wastewater, prepared as described above. This material contained 153 mg solids/ml.

a. Skin and eye irritation: The primary skin and eye irritations were determined by the Draize procedure[6] using six New Zealand white rabbits. Primer mixture FA 956 wastewater was nonirritating in the eye test and scored 0.00 in the skin test. It is less irritating than the desensitized primer itself.

b. Oral toxicity: Primer mixture FA 956 wastewater given orally at 5 g/kg killed none of three male albino mice (Charles River CD-1 strain) and none of three male and six female albino rats (Charles River CD strain). Presumably the antimony and lead are sufficiently diluted to be nontoxic in these acute tests. However, these metals are notoriously toxic in repeated doses because of their accumulation in the body.

6. Conclusions

The sample as received was basic (pH 12.3) and contained the equivalent of 3.1 mg NaOH/ml. The sample supernatant as received contained 0.08% aluminum and 0.17% sodium. The precipitate contained 51% aluminum, 1.6% barium, 0.5% antimony, 4.4% lead and 1.9% sodium. The sulfate content was 0.28 mg/ml. The initial total reducible NO_2 content of the supernatant was 0.10 mg/ml. The inorganic nitrate content was 0.0044 mg/ml and the nitrite content was 0.02 mg/ml. Thus, the covalently bound NO_2 content is slightly higher than the inorganic NO_3 plus NO_2 content. High pressure liquid chromatography (HPLC) was particularly useful for monitoring non-volatile ultraviolet absorbing compounds. No trinitroresorcinol (less than 0.01%) was observed in the waste sample. There are at least five uncharacterized ultraviolet absorbing components present by HPLC. Thin-layer chromatography (TLC) also indicated the absence of trinitroresorcinol, but did not show as many minor components as seen by HPLC. TLC did not indicate the presence of pentaerythritol tetranitrate in the precipitate at the 0.02% concentration level. Gas chromatography on OV-1 and OV-17 columns did not indicate the presence of any volatile components. PETN would have been readily detectable in the precipitate at a concentration of $< 0.01\%$.

The concentrated, neutralized sample was nonirritating to rabbits and nontoxic to rats and mice after oral administration.

7. Recommendations

Because of the lack of irritancy and toxicity, no further toxicological research is warranted. Antimony and lead content should be kept within accepted standards for industrial effluents. Samples should be taken periodically and characterized by high pressure liquid chromatography (for organic constituents) and by elemental analysis (for antimony and lead) to insure no significant variations in composition.

I. Wastewater from Lead Styphnate Production

1. Introduction

a. Sample origin: The sample is wastewater produced during the manufacture of lead styphnate at Lake City Army Ammunition Plant, Building 85. The material was sampled and shipped by the Army.

b. Chemical and toxicological literature: See Section B on Lead Styphnate.

2. Production Methods

The production methods and wastewaters generation procedures have been summarized by Small and Rosenblatt[1/] and are given below:

Two "master solutions" are prepared for lead styphnate synthesis, the first 720-liter solution of 130 lb TNR and 21 lb magnesium oxide, the other a 150-liter solution containing 100 lb of lead nitrate. Seventy liters of the first solution are mixed with 26 liters of the second, whereupon lead styphnate precipitates. The spent solution with product is drained through 50 mesh screen on which the product is collected. The spent solution is neutralized (the solution becomes acid as the reaction progresses) with sodium carbonate. The lead styphnate is washed several times, the washwater from each wash being removed by vacuum. These washwaters and spent solution are routed to storage tanks. Once daily, sodium hydroxide and aluminum powder are added to these tanks, and the contents steam boiled. The contents of the tanks are then discharged to a series of evaporation lagoons, different lagoons from the ones used for TNR disposal.

About 19 lb of lead styphnate are produced per batch which indicates a 79% yield. The remaining 21% of yield or about 5 lb of lead styphnate is lost per batch.

3. Sample Concentration for Acute Toxicity Studies

a. Sample description: The solution was greenish-black and did not contain a precipitate. The sample was used as received for sampling purposes. The pH was 11.7.

b. Concentration: The approximate concentration of dissolved solids/ml was determined prior to concentration. A 10-ml aliquot was reduced to dryness under 20 mm Hg at 50° to 60°C. As received, the concentration of dissolved solids was 20 mg/ml.

The concentration of dissolved solids was now adjusted by solvent removal under 20 mm Hg at 50° to 60°C. After concentration, the pH was adjusted to 7.4 using acetic acid. On addition of acetic acid, the solution became very turbid. The final concentration of dissolved plus suspended solids was 154 mg/ml. The concentration factor was 7.7.

c. Distillate examination: The distillate resulting from sample concentration was studied by gas chromatography, thin-layer chromatography, and ultraviolet spectroscopy for the presence of components other than water. The techniques used are described under Section 4, Chemical Assays. No other components were detected in the distillate.

4. Chemical Assays and "Fingerprinting"

a. Alkalinity: The sample as received required 0.11 meq HCl/ml of sample to neutralize it. This alkalinity corresponds to 4.4 mg NaOH/ml.

b. Metal content: The sample as received contained the following concentrations of metals.

Metal	% in Homogenous Aliquot
Aluminum	0.090
Antimony	0.0005
Barium	0.0002
Calcium	0.0010
Lead	0.0075
Magnesium	0.0001
Sodium	0.440

c. Reducible NO_2 content: The solutions were assayed as received. The concentration of reducible NO_2 groups was determined by the method of Selig.[3] This procedure will determine the presence of aliphatic nitrates, aliphatic nitrites, aromatic nitro group and inorganic nitrite and nitrate ions. Although a number of functionalities are detected simultaneously, the results are expressed in terms of mg NO_2 for comparison purposes. The nonspecificity of the assay can be considered an advantage since when combined with the inorganic NO_2 and NO_3 assays, the covalently bound NO_2 content can be estimated.

The reducible NO_2 content of the homogenous solution assayed on 21 May 1975 was 3.0 mg NO_2/ml. Thirty-four days later the assay indicated 3.5 mg NO_2/ml.

d. Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method used by the Environmental Protection Agency.[4] The sulfate content of the supernatant as received was 0.44 mg SO_4/ml of sample.

e. Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite contents were determined by the cadmium reduction method described by the Environmental Protection Agency.[5] The nitrate content of the supernatant as received was 2.69 mg/ml. The nitrite content of the supernatant as received was 0.222 mg/ml.

f. Chromatographic characterization: The sample was studied by gas, liquid and thin-layer chromatography. The primary purpose of this work was to provide chromatographic "fingerprints" of the sample.

(1) Liquid chromatography: The sample supernatant was studied using the following conditions:

System 1

Instrument: Waters Associates 301 equipped with 254 nm UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 30 min using solvent program No. 6.
Flow rate: 1 ml/min.

Peaks were observed at 3.1 and 3.5 min. Trinitroresorcinol and lead styphnate do not give peaks using this system.

System 2

Instrument: Waters Associates 301 equipped with 235 nm UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 3% H_2O in CH_3CN.
Flow rate: 1 ml/min.

Trinitroresorcinol and lead styphnate elute at 2.76 min. The sample showed peaks at 3.15, 5.12 and 9.06 min.

(2) Gas chromatography: The sample was studied by gas chromatography as described below:

Instrument: Bendix 2500 equipped with flame ionization detector.
Column: Glass, 6 ft x 1/8 in., 3% OV-1 on Gas Chrom Q.
Flow rate: 30 cc N_2/min.
Column temperature: Start at 100°C and heat at 5°C/min to 250°C.
Injection port: 200°C.
Detector temperature: 200°C.

No peaks were observed.

(3) Thin-layer chromatography: The sample was studied by thin-layer chromatography as described below:

Plate: Brinkmann Silica Gel F-254.
Solvent system: Acetone saturated with ammonium acetate.
Material spotted: 5 µg lead styphnate.
5 µg trinitroresorcinol.
20 µl of sample having a dissolved solids content of 54 mg/ml.
Detection: Visual.
UV - 254 nm.
Spray with 2% diphenylamine in 95% ethanol followed by UV irradiation.

Lead styphnate has an R_f of 0.42 and detection limit of 0.5 µg. Trinitroresorcinol has an R_f of 0.42 and a detection limit of 0.5 µg. The sample showed one spot of R_f 0.13.

g. Spectral characterization: The sample was studied by infrared, ultraviolet and visible spectroscopy.

(1) Infrared spectrum: Reducing an aliquot to dryness gave a residue whose IR spectrum in KBr showed absorptions at 3300 (Broad, s), 1660 (s), 1470 (Broad, s), 1410 (Broad, s), 1280 (s), 1160 (s), 870 (m), 840 (w), 740 (s), and 640 (m) cm^{-1}. The $C-NO_2$ group normally has bands near 1560 and 1350 cm^{-1}.12/ Lead styphnate in KBr shows bands at 3570 (m), 1590 (s), 1490 (m), 1410 (m), 1315 (s), 1230 (s), 1100 (m), 790 (m), and 740 (w) cm^{-1}.

(2) Ultraviolet spectrum: A 1,000-fold dilution of the sample showed a maximum at 309 nm (absorbance 0.38).

(3) Visible spectrum: No peaks were observed in the region 400 to 800 nm. With a 10-fold dilution the absorbance at 400 nm was 0.05.

5. Acute Toxicity

Animal tests were performed on the neutralized concentrated lead styphnate wastewater prepared as described above. This material contained 154 mg solids/ml.

a. Skin and eye irritation: The primary skin and eye irritations were determined by the Draize procedure[6] using six New Zealand white rabbits. Lead styphnate wastewater was negative on the eye irritation test and scored 0.12 on the skin irritation test, less than the 0.20 required for a rating of "mild irritant." Therefore, the wastewater is nonirritating.

b. Oral toxicity: Lead styphnate wastewater given orally at 5 solids/kg body weight killed none of three male albino mice (Charles River CD-1 strain) and none of three male and six female albino rats (Charles River CD strain).

6. Conclusions

The sample as received was basic (pH 11.7) and contained the equivalent of 4.4 mg NaOH/ml. As received, a homogenous sample contained 0.90% aluminum, 0.0075% lead and 0.44% sodium. The initial total reducible NO_2 content was 13 mg NO_2/ml. After 34 days storage at 28°C, the reducible NO_2 content was 3.5 NO_2/ml. When assayed at the same time as the second assay for total reducible NO_2 content, the inorganic nitrate content was 2.7 mg/ml and the nitrite content was 0.22 mg/ml. High pressure liquid chromatography (HPLC) was useful for monitoring nonvolatile ultraviolet absorbing compounds. No trinitroresorcinol or lead styphnate (< 0.01%) was observed in the sample. There are at least three uncharacterized ultraviolet absorbing components present by HPLC. Thin-layer chromatography also indicated the absence of trinitroresorcinol, but did not show as many minor components as seen by HPLC. Gas chromatography on an OV-1 column did not indicate the presence of any volatile components.

The neutralized concentrated lead styphnate wastewater was nonirritating to rabbits and nontoxic to rats and mice after oral administration.

7. Recommendations

Because of the lack of irritancy and toxicity, no further toxicological research is warranted. Lead content should be kept within recognized limits for industrial effluents. Samples should be taken periodically and characterized by high pressure liquid chromatography (for organic constituents) and by elemental analysis (for lead) to insure no significant variations in composition.

J. Trinitroresorcinol Wastewater from Lake City Army Ammunition Plant

1. Introduction

a. Sample origin: The sample is wastewater produced during the manufacture of trinitroresorcinol at Lake City Army Ammunition Plant, Building 83. The material was sampled and shipped by the Army.

b. Chemical and toxicological literature: See Section E on trinitroresorcinol.

2. Production Method

The production methods and wastewater generation procedures have been summarized by Small and Rosenblatt[1/] and are given below:

> "To produce TNR, a 30 lb batch of resorcinol is first mixed with 240 lb of 98% sulfuric acid. Then 72 lb of 95% nitric acid is added to the resulting mixture of resorcinol-4,6-disulfonate in sulfuric acid. The crude TNR is removed from the spent acid and pooled with two other TNR batches. The batches are washed with about 100 gal. of 1 N nitric acid to remove traces of sulfate and filtered. The acids from these processes are collected in a sump and treated with sodium carbonate solution. Once a week, the collected TNR sludge is treated with a mixture of 30 lb sodium hydroxide and 20 lb powdered aluminum. The resultant degraded sludge is a black, possibly polymeric material. The neutralized acids and the treated sludge are routed to a series of wastewater evaporation lagoons."

Lake City AAP personnel indicate that about 60 lb TNR is produced per 30 lb of resorcinol charge. This is about a 90% yield; smaller scale TNR production (a slightly different process) at Frankford Arsenal, Philadelphia, Pennsylvania has resulted in a 76% yield. Their product has been analyzed and found to be 93.5% TNR. The identified impurities include a 1:1 TNR-dinitroresorcinol addition product (0.8%), dinitroresorcinol (1%), 6-nitroso-2-resorcinol (1%), 2-nitroresorcinol (0.5%) and mesoxalic acid ($HON{=}C(COOH)_2$). On the basis of 90% conversion at LCAAP, about 6 lb of TNR is lost per batch produced. With a solubility of the order of 7 g/liter, some of this TNR could be washed out with the 1 N nitric acid washes and be discharged to the evaporation pond as the sodium salt of TNR.

3. Sample Concentration for Acute Toxicity Studies

a. Sample description: The solution as received was light brown and contained numerous large (about 1 in.) colorless monoclinic crystals. These crystals are characterized as sodium sulfate decahydrate. Only the supernatant was used for toxicology sample preparation. The pH was 11.4.

b. Concentration: The approximate concentration of dissolved solids/ml was determined prior to concentration. A 10-ml aliquot was reduced to dryness under 20 mm Hg vacuum at 50° to 60°C. As received, the concentration of dissolved solids was approximately 180 mg/ml.

This concentration of dissolved solids is useful without further concentration. The pH was adjusted to 7.3 by addition of acetic acid. The solution becomes very turbid and dark brown on addition of acetic acid. The final concentration of dissolved plus suspended solids was 180 mg/ml.

4. Chemical Assays and "Fingerprinting"

a. Alkalinity: The sample supernatant as received required 0.30 meq of HCl/ml of sample for neutralization. This alkalinity corresponds to 12 mg NaOH/ml.

b. Metal content: The sample as received contained the following concentrations of metals.

Metal	% in Supernatant	% in Precipitate[a/]
Aluminum	0.20	0.052
Antimony	< 0.0005	0.0090
Barium	< 0.0002	< 0.0035
Calcium	0.0010	< 0.035
Lead	0.0005	< 0.0090
Magnesium	< 0.0001	< 0.0001
Sodium	7.12	26.2

a/ The sample was air dried at 27° for 24 hours. A white powder resulted which by sodium assay was $Na_2SO_4.2H_2O$.

When a crystal was water washed and dried at 100° for 24 hours under 0.1 mm vacuum the sodium content was 32%. This sodium content corresponds to that of sodium sulfate. The weight loss on drying data indicated the crystal was sodium sulfate decahydrate. The infrared spectrum of the dried material was identical to that of sodium sulfate.

c. Reducible NO_2 content: The concentration of reducible NO_2 groups was determined by the method of Selig.[3] This procedure determines the presence of aliphatic nitrates and nitrites, aromatic nitroso and nitro groups, and inorganic nitrite and nitrate ions. Although a number of functionalities are detected simultaneously, the results are expressed in terms of mg NO_2 for comparison purposes. The nonspecificity of the assay can be considered an advantage since, when combined with the inorganic NO_2 and NO_3 assays, the covalently bound NO_2 content can be estimated.

The reducible NO_2 content of the supernatant when assayed on 21 May 1975 and again 36 days later was 14 mg NO_2/ml. The reducible NO_2 content of the precipitate when assayed on 28 May 1975 and again 34 days later, was 0.00 mg NO_2/g of precipitate.

d. Sulfate content: The concentration of ionic sulfate was determined by an automated methylthymol blue method described by the Environmental Protection Agency.[4] The sulfate content of the supernatant was 125 mg SO_4/ml of sample. The sulfate content of the precipitate without drying was 28.4 ± 0.4%. This value is slightly lower than the 29.8% theoretical value for $Na_2SO_4.10H_2O$. The discrepancy is probably due to occluded water.

e. Inorganic nitrate and nitrite content: The inorganic nitrate and nitrite contents were determined by the cadmium reduction method described by the Environmental Protection Agency.[5] The nitrate content of the supernatant as received was 19.2 mg/ml. The nitrite content of the supernatant as received was 0.89 mg/ml.

f. Chromatographic characterization: The sample was studied by gas, liquid and thin-layer chromatography. The primary purpose of this work was to provide chromatographic "fingerprints" of the sample.

(1) Liquid chromatography: The sample supernatant was studied as described below:

System 1

Instrument: Waters Associates 301 equipped with 254 nm UV detector.

Column: Porasil AX, 2 ft x 1/3 in.
Solvent: 100% CH_3CN programmed to 60% H_2O in 30 minutes using linear solvent program No. 6.
Flow rate: 1 ml/min.

Peaks were observed at 3.1, 3.5 and 4.3 minutes. Ill-defined peaks were observed at 9.5 to 12.6 minutes and 13.8 to 19.7 minutes. Trinitroresorcinol does not give a peak using this system.

System 2

Instrument: Waters Associates 301 equipped with 235 nm Schoeffel UV detector.
Column: Porasil AX, 2 ft x 1/8 in.
Solvent: 3% H_2O in CH_3CN.
Flow rate: 1 ml/min.

Trinitroresorcinol elutes at 2.76 minutes. The sample showed peaks at 3.15, 8.26 and 10.6 minutes.

(2) Gas chromatography: The sample was studied by gas chromatography as described below:

Instrument: Bendix 2500 equipped with flame ionization detector.
Column: Glass 6 ft x 1/8 in., 3% OV-1 on Gas Chrom Q.
Flow rate: 30 cc N_2/min.
Column temperature: Start at 100°C and heat at 5°C/min to 250°C.
Injection port: 200°C
Detector temperature: 200°C

No peaks were observed.

(3) Thin-layer chromatography: The supernatant was studied by thin-layer chromatography.

Plate: Brinkmann Silica Gel F-254.
Solvent system: Acetone saturated with ammonium acetate.
Material spotted: 5, 2, 1, 0.5 mg trinitroresorcinol. 3,600, 360, 36 mg of residue from dried supernatant.

Detection: (a) Visual
(b) UV (254 nm)
(c) Spray with 2% diphenylamine in 95% ethanol followed by UV irradiation.

Trinitroresorcinol has an R_f of 0.42 and a detection limit of 0.5 mg. The sample shows one spot at R_f 0.13.

g. Spectral characterization: The sample was studied by infrared, ultraviolet and visible spectroscopy.

(1) Infrared spectrum:

Precipitate: The spectrum of the vacuum dried precipitate in KBr is identical to that of sodium sulfate. A single absorption at 1120 cm^{-1} was observed.

Supernatant: The spectrum of the vacuum dried sample in KBr showed absorptions at 3500 (m), 1660 (m), 1450 (m), 1390 (s), 1280 (w), 1130 (s), 880 (w), 840 (w), 640 (s) and 620 (s) cm^{-1}. Trinitroresorcinol showed bands at 3200 (m), 1660 (s), 1620 (s), 1600 (s), 1550 (s), 1490 (s), 1466 (s), 1380 (s), 1310 (s), 1210 (w), 1190 (m), 1085 (s), 935 (m), 920 (m), 785 (m), 763 (m), and 732 (w) cm^{-1}.

(2) Ultraviolet spectrum: With 200-fold dilution, the supernatant showed a maximum at 329 nm (absorbance 0.55). Trinitroresorcinol in 95% ethanol absorbs at 258 nm (ϵ = 8,579), 345 nm (ϵ = 9,246) and 398 nm (ϵ = 15,570).

(3) Visible spectrum: The supernatant showed no peaks in the range 400 to 800 nm, using a 100-fold dilution. The absorbance at 400 nm was 0.02.

5. Acute Toxicity

Animal tests were performed on the neutralized supernatant of trinitroresorcinol (TNR) wastewater, prepared as described above. This material had 180 mg solids/ml.

a. Skin and eye irritation: The primary skin and eye irritations were determined by the Draize procedure[6] using six New Zealand white rabbits. TNR wastewater gave negative results on the eye irritation test and a score of 0.08 on the skin irritation test. This is well below the 0.20 score required for a "mild irritant" rating.

b. Oral toxicity: TNR wastewater given orally in water at 5 g solids/kg body weight killed none of three male albino mice (Charles River CD-1 strain) and none of three male albino rats (Charles River CD strain). When given to a first group of three female rats, one died on the first day. When given to a second group of three females, none died. Similar results were observed with the desensitization blank, so the death is not due to the compound.

6. Conclusions

The sample as received was basic (pH 11.4) and contained the equivalent of 12 mg NaOH/ml of sample. The sulfate content of the supernatant was 125 mg/ml.

The supernatant contained 0.2% aluminum and 7.1% sodium. The precipitate contained varying amounts of sodium depending on the precipitate drying time and temperature. The crystalline precipitate was identified as sodium sulfate decahydrate. The initial total reducible NO_2 content of the supernatant was 14 mg NO_2/ml. The inorganic nitrate content was 19 mg/ml and the nitrite content was 0.9 mg/ml. Thus, the covalently bound NO_2 content was low relative to the inorganic NO_3 plus NO_2 content. High pressure liquid chromatography (HPLC) was particularly useful for monitoring nonvolatile ultraviolet absorbing compounds. No trinitroresorcinol (less than 0.01%) was observed in the waste sample. There are at least five identified ultraviolet absorbing components present by HPLC. Thin-layer chromatography also indicated the absence of trinitroresorcinol, but did not show as many minor components as seen by HPLC. Gas chromatography on an OV-1 column did not indicate the presence of any volatile components.

The neutralized supernatant was nonirritating and nontoxic in the animal tests performed.

7. Recommendations

Because of the lack of irritancy and toxicity, no further toxicological research is warranted. Samples should be taken periodically and charaterized by high pressure liquid chromatography to insure no significant variations in composition.

III. RECOMMENDATIONS

A. Introduction

The ultimate objective of this research is to determine the effects of munitions wastewaters on mammalian life to provide a basis for the development of environmental criteria. The results of these toxicity studies are summarized in Tables 5 and 6. Only desensitized tetrazene, its wastewater and desensitized primer FA 956 shows significant toxicity. The toxicity of primer mixture FA 956 is probably due to its antimony and lead content. Antimony is well-known as an arsenic-like toxic metal. It is used as an antiparasitic agent in compounds such as tartar emetic (antimony potassium tartrate) and stibophen. Lead is very well known as an accumulating poison. These metals can be readily precipitated. The supernatant should be monitored to insure that release of the metals is within accepted industrial practice. The precipitate should be reclaimed or appropriately disposed of.

Desensitized pure tetrazene and tetrazene wastewaters require more work. The toxicity of desensitized pure tetrazene is of less importance. However, components found in the wastewaters were much more toxic.

B. Tasks

The following tasks, in order of priority, are recommended to follow up this study:

1. Continue the study of the toxic components of tetrazene wastewater to ascertain:

   a. Better fractionation procedures.

   b. Their identity.

   c. Their source, whether by-products of the synthesis or products formed later during the desensitization and disposal processes.

   d. A practical synthesis. The two toxic components totalled 1.2 mg solids/ml wastewater. Extraction of kilogram quantities of these materials required for chronic toxicity testing is not practical.

2. Develop a practical method of cleaning-up tetrazene wastewater. This will include checking the toxicity of the products formed.

TABLE 5

PRIMARY SKIN AND EYE IRRITATION IN NEW ZEALAND WHITE RABBITS[a/] TREATED WITH DESENSITIZED PRIMER COMPOUNDS OR EXTRACTS OF PRIMER WASTEWATERS

Compound		Irritation Scores	
Number	Name	Skin[b/]	Eye
1	Desensitized Pure Tetrazene	0.25	negative
2	Desensitized Pure Lead Styphnate	0.16	negative
3	Desensitized Primer Mixture FA 956	0.16	negative
4	Desensitized Pure PETN	0.00	negative
5	Desensitized Pure Trinitro-resorcinol (TNR)	0.00	negative
6	Desensitization Blank	0.08	negative
7	Tetrazene Wastewater	0.00	negative
8	Primer Mixture FA 956 Wastewater	0.00	negative
9	Lead Styphnate Wastewater	0.12	negative
10	TNR Wastewater	0.08	negative

a/ Six rabbits per compound.
b/ > 0.2, mild; > 2.5, moderate; > 5.0, severe.

TABLE 6

ACUTE ORAL LD_{50}'S IN RATS[a] AND MICE[b] OF DESENSITIZED PRIMER COMPOUNDS OR EXTRACTS OF PRIMER WASTEWATERS

Compound Number	Compound Name	Rat (g solids/kg body weight) Male	Rat (g solids/kg body weight) Female	Mice (g solids/kg body weight) Male	Mice (g solids/kg body weight) Female
1	Desensitized Pure Tetrazene	3.40 (3.28-3.55)[c]	3.30 (3.12-3.44)	> 5.0	ND[d]
2	Desensitized Pure Lead Styphnate	> 5.0	> 5.0	> 5.0	ND
3	Desensitized Primer Mixture FA 956	4.84 (4.62-5.19)	3.95 (3.85-4.04)	> 5.0	ND
4	Desensitized Pure PETN	> 5.0	> 5.0	> 5.0	ND
5	Desensitized Pure Trinitro-resorcinol (TNR)	> 5.0	> 5.0	> 5.0	ND
6	Desensitization Blank	> 5.0	> 5.0	> 5.0	ND
7	Tetrazene Wastewater, Lot 2	ND	est. 3.5	ND	ND
	Lot 3	0.622 (0.568-0.664)	0.311 (0.262-0.371)	0.711 (0.589-0.845)	0.805 (0.705-0.875)
8	Primer Mixture FA 956 Wastewater	> 5.0	> 5.0	> 5.0	ND
9	Lead Styphnate Wastewater	> 5.0	> 5.0	> 5.0	ND
10	TNR Wastewater	> 5.0	> 5.0	> 5.0	ND

[a] Albino Rats, CD Strain, Charles River Breeding Laboratories
[b] Albino Swiss Mice, CD-1 Strain, Charles River Breeding Laboratories
[c] 95% Confidence Limits
[d] Not determined

3. Carry out appropriate long-term toxicity studies on:

a. Compounds released into the environment after treatment of tetrazene wastewater.

b. Compounds to which workers are exposed.

4. Test a number of samples of all the wastewaters for variations in toxicity. This is necessary because two lots of tetrazene wastewater differ greatly in chemical characteristics and toxicity. High pressure liquid chromatography (HPLC) provides a quick, convenient method for testing. If the HPLC shows significant variation in chemical composition, the wastewaters should be checked for variations in acute toxicity.

C. Time Requirements

We estimate that Task 1 will require 4 to 6 months, if suitable synthetic methods (Sub-task c) are available. Task 2 can be carried out concurrently and would also require 4 to 6 months. It may be extended by longer stability tests of the treated wastewaters.

After sufficient quantities of the substances to be tested are at hand, Task 3 will require 3 years. If only subchronic studies are performed, 1 year will suffice.

The time required by Task 4 will depend on the sampling scheme, which should include all phases of production. Sampling intermittently for a period of 6 months should be adequate if there are no major variations. The study can be completed 2 months after receipt of the last sample.

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