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PROBLEM DEFINITION STUDY ON 1,3-DINITROBENZENE, 1,3,5-TRINITROBENZENE AND DI-N-PROPYL ADIPATE

Final Report

by:

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November 1979

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U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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1,3,5-Trinitrobenzene	Mammals	Biodegradation	
Trinitrobenzene	Fish	Pollution	

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This document presents the results of a problem definition study on munitions-related chemicals. The purpose of this study was to review the literature and make preliminary assessments of the environmental hazards associated with discharges of 1,3-dinitrobenzene, 1,3,5-trinitrobenzene and di-n-propyl adipate..

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EXECUTIVE SUMMARY

The goal of this problem definition study was to evaluate the literature relating to the toxicological and environmental hazards associated with Army use or pollution of 1,3-dinitrobenzene, 1,3,5-trinitrobenzene and di-n-propyl adipate.

1,3-Dinitrobenzene and 1,3,5-trinitrobenzene are not intentionally produced by the Army. However, during the production of 2,4,6-trinitrotoluene (TNT), small but significant quantities of these compounds are produced as by-products. 1,3-Dinitrobenzene and 1,3,5-trinitrobenzene enter the environment in the red water evaporator condensate. 1,3-Dinitrobenzene is also present in minor amounts in the TNT final product. Thus, 1,3-dinitrobenzene can also enter the environment from TNT blending and loading operations. In addition to actual discharges of these nitrobenzenes, 1,3-dinitrobenzene and 1,3,5-trinitrobenzene can be formed in the environment by photolysis of 2,4-dinitrotoluene and TNT, respectively.

Both nitrobenzenes are toxic to humans and mammals. The main biochemical activity of these compounds is the production of methemoglobin. The rate and degree of methemoglobin formation differs. 1,3,5-Trinitrobenzene produces methemoglobin with peak levels 1 to 2 hours after exposure. In contract, methemoglobin levels do not peak until about 4 hours after exposure to 1,3-dinitrobenzene and are significantly higher than the levels observed for 1,3,5-trinitrobenzene. Both compounds have been shown to exhibit a dose-response mutagenicity in the Ames test.

In the aquatic environment, 1,3-dinitrobenzene and 1,3,5-trinitrobenzene were moderately toxic to fish in acute exposure. They were less toxic to invertebrates. Both compounds had low bioconcentration factors, ≤ 10.

Microbial growth is inhibited by low concentrations of these nitrobenzenes with the trinitro compound slightly more toxic than the dinitrobenzene. Degradation of these compounds by microorganisms is slow and appears to be a reductive process yielding amino groups in place of the nitro groups.

The nitrobenzenes are also toxic to plants. The toxicity appears to be associated with interference with the photosynthetic process.

Di-n-propyl adipate is used exclusively by the Army in the production of propellants. There are no other uses of this compound in the United States. Although this compound does not appear to be a major pollution problem under current Army usage, very little information exists on its toxicological and environmental hazards.

Only one study on the effects of di-n-propyl adipate to mammals was found in the literature. This compound has a low acute toxicity, however, it was mildly teratogenic in acute doses. No subacute or chronic studies on this compound were uncovered.

No aquatic toxicity data were available on di-n-propyl adipate. Similar adipates produced no toxic effects to fish when exposed to low ppm concentrations. However, the calculated bioconcentration factor for di-n-propyl adipate is high. Thus, the compound could present a problem in the aquatic environment. The magnitude of the aquatic toxicity problem with di-n-propyl adipate is dependent on several interesting factors - the ability of aquatic organisms to degrade this compound; any biomagnification through the food chain and potential mutagenic or teratogenic effects on the organisms.

FOREWORD

The purpose of this study was to gather, collate and evaluate the available information on the toxicological and environmental hazards of 1,3-dinitrobenzene, 1,3,5-trinitrobenzene and di-n-propyl adipate. The results of this study will aid the Army in determining future research needs for defining the hazards of these chemicals.

In the preparation of this report, several reference sources have been directly quoted. Permission has been obtained from the appropriate sources for reprint of the quoted information.

TABLE OF CONTENTS

Table of Contents (cont.) Page

Table of Contents (cont.) Page

LIST OF TABLES

List of Tables (cont.)

LIST OF FIGURES

List of Figures (cont.)

PROBLEM DEFINITION STUDY ON
1,3-DINITROBENZENE

SUMMARY

The U.S. Army does not intentionally produce 1,3-dinitrobenzene. However, during TNT production, benzene impurities in the toluene are nitrated to dinitrobenzene. Of the three isomers, 1,3-dinitrobenzene is 93% of the product.

The predicted environmental discharge of 1,3-dinitrobenzene from TNT manufacturing should range from 0.2 - 2.0 lb/line/day. 1,3-Dinitrobenzene is discharged into the environment in the red water evaporator condensate wastewater. The amount discharged is highly dependent on whether batch or continuous process is used. 1,3-Dinitrobenzene may also enter the environment through photoconversion of discharged 2,4-dinitrotoluene from the propellant manufacturing. Photoconversion should only result in the equivalent of 0.005 lb/line/day of 1,3-dinitrobenzene entering the environment.

There is only one civilian manufacturer of 1,3-dinitrobenzene. All of the product produced in the civilian sector is used as a synthetic intermediate mainly in the dye industry. The pollution from civilian manufacture and use is not known.

1,3-Dinitrobenzene is highly toxic to humans. The symptoms observed are mainly the result of oxygen deprivation to the tissues due to the presence of methemoglobin in the blood. These symptoms vary from general weakness, fatigue, and headaches in the cases of limited exposure to extreme cyanosis, nervous system disorders, gastrointestinal upsets, enlarged spleens, coma and possibly death from circulatory collapse in cases of severe poisoning. The symotoms vary widely with the state of health, national origin, and food and drink. habits of the individual exposed. Normally, methemoglobin and/or Heinz bodies can be detected in the blood. However, if the tests are performed several hours after a slight exposure, no blood abnormalities may be found. Most occupational exposure to this compound occurred before 1940. In general, the exposure was traced to skin absorption or inhalation of dusts, but ingestion due to poor hygiene practices can not be discounted. The lowest published toxic dose in a controlled experiment is 4 mg/kg placed on the skin for 30 hours.

The effects of 1,3-dinitrobenzene on experimental animals include methemoglobinemia, Heinz body formation, cyanosis, gastrointestinal upset, nervous system disturbances, coma. The sensitivity of 1,3-dinitrobenzene poisoning varies widely among the species and from individual to individual within the species.

The metabolism of 1,3-dinitrobenzene has been studied to some extent. It is generally believed that a reduced product is the agent responsible for methemoglobin formation, however, evidence as to the nature of this metabolite is not conclusive.

In the aquatic environment, 1,3-dinitrobenzene is moderately toxic to fish in acute exposure. It is less toxic to aquatic invertebrates. This compound has a low bioconcentration factor of about 10, indicating that aquatic organisms

should not concentrate 1,3-dinitrobenzene to a great extent. The mutagenic properties of 1,3-dinitrobenzene have been evaluated by the Ames test and yeast recombinogenic system. These *in vitro* tests have yielded positive results for mutagenicity in most cases, although further verification is needed.

Microbial growth is inhibited by concentration from 5 ppm to greater than 100 ppm, depending upon the orgarisms. 1,3-Dinitrobenzene is highly resistant to microbial degradation. Degradation that occurs appears to be a reduction process yielding amino groups from nitro groups.

1,3-Dinitrobenzene may adversely affect plants by interfering with their photosynthetic processes. This compound has been reported to be toxic to tomatoes and corn. Algae had a toxicity threshold of from 170 to 700 ppb.

The data available on 1,3-dinitrobenzene indicate that it is a potential hazard to all life forms if released into the environment. Several additional studies are recommended to verify old existing work and to further determine the potential hazards of this compound. These studies should include:

- Evaluation of the wastewater treatment facilities to effectively remove this compound and its precursors from the wastewaters

- Chronic aquatic toxicity and reproductive tests with fish and invertebrates to determine the effects of long term exposure to this compound and its potential effects on reproduction

- Chronic mammalian toxicity and reproductive tests to determine long term exposure effects including carcinogenicity, mutagenicity and teratogenicity

- Metabolic studies with ^{14}C-1,3-dinitrobenzene using cats, dogs or rats to determine the metabolite responsible for methemoglobin formation and if significant quantities of 2,4-dinitrophenol are formed *in vivo* to lead to uncoupling of oxidative phosphorylation

- Confirmation of *in vitro* mutagenicity tests results.

FOREWORD

A. Study Goals

This report presents the results of an evaluation of the available information on the toxicological and environmental hazards of 1,3-dinitrobenzene. 1,3-Dinitrobenzene is a by-product of TNT (2,4,6-trinitrotoluene) manufactured at various Army Ammunition Plants. This compound is also present in small quantities in the final TNT product. It can thus enter the environment during TNT blending operations at Holston AAP and loading operations at Army load, assembly and pack plants. 1,3-Dinitrobenzene can also be found in the environment from photochemical reactions of 2,4-dinitrotoluene, a major pollutant from the manufacture of TNT. This evaluation of the toxicological and environmental hazards of 1,3-dinitrobenzene was undertaken in order to aid the Army in identification of research needs.

B. Study Methodology

The methodology utilized to gather information for this report included a detailed search of the literature and numerous personal contacts. During the literature search, the following sources were reviewed for pertinent information on 1,3-dinitrobenzene:

- Chemical Abstracts	1940 - present
- Biological Abstracts	1950 - present
- Excerpta Medica	1950 - present
- TOXLINE	1965 - present
- National Technical Information Service	1964 - present
- Defense Documentation Center	1958 - present
- COMPENDEX	1970 - present

Personal contacts were made with Army Ammunition Plant personnel and Army and civilian researchers The specific contacts made and results are presented below

1. Contacts with U.S. Manufacturers

Mr. Leo Zeftel of E.I. duPont deNemours was contacted on September 27, 1979. Mr. Zeftel provided a Material Safety Data Sheet which included a TLV of 1 mg/m^3.

Mr. Weitzer of the American Hoechst Company was contacted on October 1, 1979. He could provide no information.

2. Foreign Contacts

Five foreign companies listed in the 1979 Directory of Chemical Producers in Western Europe were contacted by Telex in October 1979:

England

Hopkin and Williams
Koch-Light Laboratories Ltd.
BDH Chemicals Ltd.

Germany

Bayer AG
Hoechst

One company, BDH, responded on October 23, 1979. E.P. Long provided data on the acute oral toxicity to cats, wild birds and humans. Dr. Meyer-Dullheuer of Hoechst responded on November 28, 1979, providing the same information on toxicity to humans, wild birds and cats.

Koch-Light Laboratories Ltd. responded on December 3, 1979. They also provided the same information on toxicity to cats and humans.

Bayer AG responded on November 19, 1979. They no longer make 1,3-dinitrobenzene and have no information concerning this compound.

TABLE OF CONTENTS

Table of Contents (cont.)

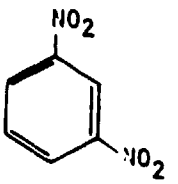
LIST OF FIGURES

LIST OF TABLES

I. 1,3-Dinitrobenzene

A. Alternate Names

1,3-Dinitrobenzene is one of three possible dinitrobenzene isomers. This isomer has the following structure:



The molecular formula of 1,3-dinitrobenzene is $C_6H_4N_2O_4$ equivalent to a molecular weight of 168.11 g/mole. Pertinent alternate names for 1,3-dinitrobenzene are listed below:

CAS Registry No.: 99-65-0
CA Name (9CI): Benzene, 1,3-dinitro-
CA Name (8CI): Benzene, m-dinitro-
Wiswesser Line Notation: WNR CNW
Synonyms: m-dinitrobenzene; 1,3-dinitrobenzene; oil of mirbane; oil of myrbane; 1,3-dinitrobenzol

B. Physical Properties

The physical properties of 1,3-dinitrobenzene are listed in Table I-1. The infrared, ultraviolet, NMR, and mass spectra are shown in Figures I-1 through I-4.

The complete characterization of the vibrational spectra of the dinitrobenzenes is reported by Shukla *et al.* (1968) and also by Green and La wers (1971). The Raman spectra were also reported by Shukla and Upadhya (1969). The data of Shukla are shown in Tables I-2 and I-3. Shukla and Upadhya (1969) note a difference in the low frequency Raman (lattice) vibrations which correspond to the difference in point group for the para-isomer (D_{2h}) compared to the meta and ortho isomers (C_{2v}).

According to Maksimov (1972), there are differences in the melting points and rates of decomposition of the isomers. p-Dinitrobenzene melts at 175.0°C while the ortho-and meta-isomers melt at 118°C and 90°C, respectively. Though the data are not given, Maksimov states that the order of the rate constants for the decomposition of the dinitrobenzenes is o>p>m.

Leiga and Sarmousakis (1966) reported the change in solubility of 1,3-dinitrobenzene in NaCl and other salt solutions according to Table I-4. These authors also note differences in the behavior of the dinitrobenzene isomers which are also dependent on the salt and its concentration. For example, in p-toluenesulfonate solutions, the solubility of the o- and m-isomers increases with the salt concentration up to about 1M, then decreases sharply. The p-isomer does not exhibit this behavior but its solubility increases monotonically with the concentration of the salt. At low concentrations, the m- and p-isomers exhibit essentially identical behavior with respect to change in solubility with salt concentration and differ from the o-isomer. Leiga and Sarmousakis (1966) discount the effects of the dipole moment of the nonelectrolyte on its solubility in this case and instead postulate a complex of ion and nonelectrolyte paired with "cages" of water molecules.

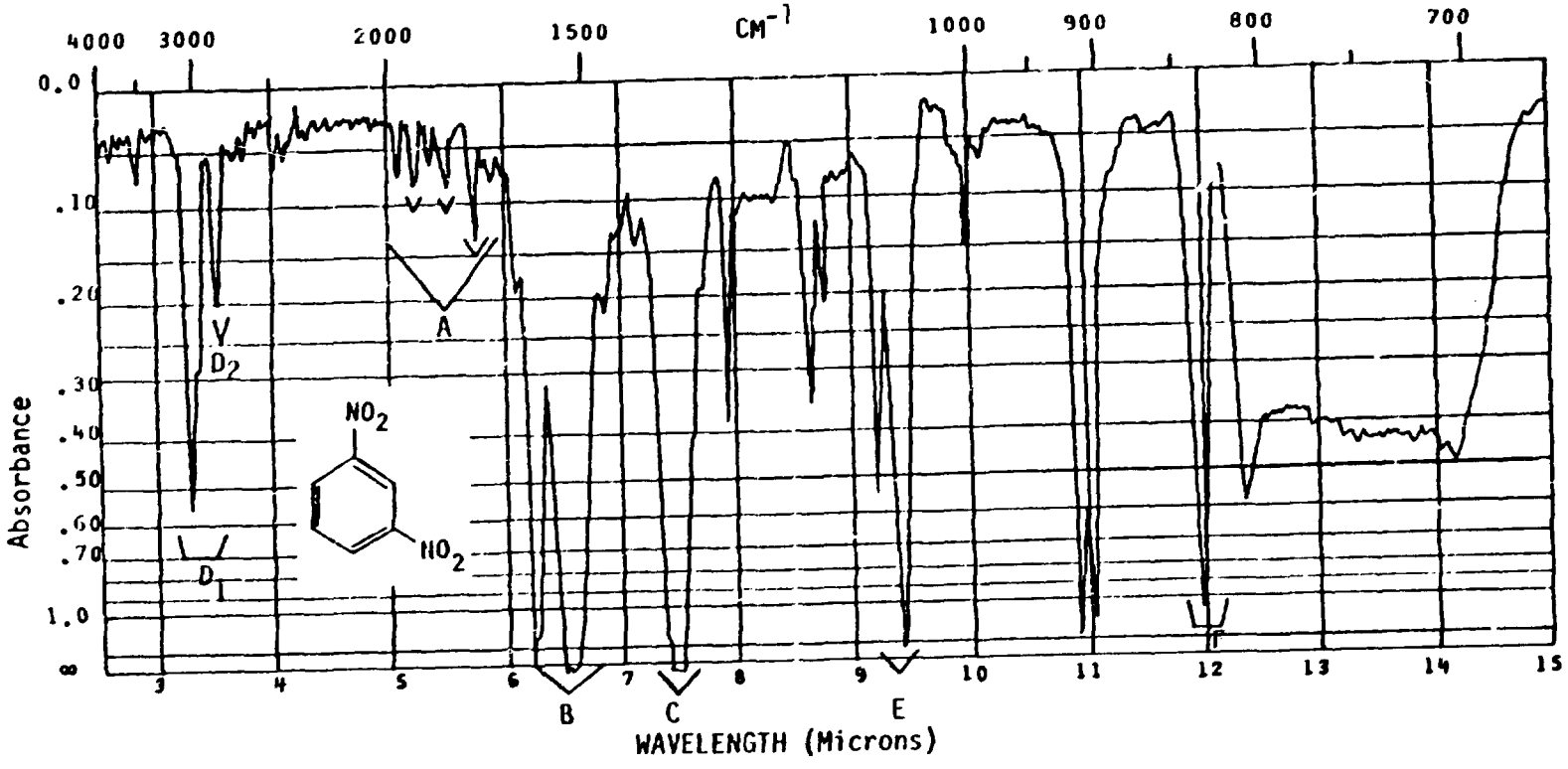
The NMR spectra of 1,3-dinitrobenzene in a number of solvents have been reported by Shapiro and Mohrmann (1977). In CCl_4, for example, chemical shifts (δ) of 9.018, 8.542 and 7.779 ppm, respectively, are listed for the protons at the 2, 4 and 5 positions. Computer analysis of the AB_2X system (Martin and Dailey, 1962) has yielded values of 8.3, 0.5 and 2.2, respectively, for $J_{4,5}$, $J_{2,5}$ and $J_{2,4}$.

Table I-1. Physical Properties of 1,3-Dinitrobenzene*

Physical form @ 20°C:	monoclinic needles, rhombic plates
Color:	colorless to yellow
M.P.:	89.57°C
B.P.:	302.8°C @ 770 mm Hg; 300-303°C @ 760 mm Hg; 291°C @ 14 mm Hg
Vapor Pressure:	1.0 mm Hg @ 234°C
Vapor density:	5.8 (air=1)
Crystal density:	d_{δ}^{4} 1.571
	d_{4}^{30} 1.5656
Symmetry group:	C_{2v}
Flash point:	150°C (closed cup)
Heat of combustion (ΔH_p):	4.145 cal/g
Solubility:	water: 0.046 g/100 g @ 15°C 0.32 g/100 g @ 100°C ethanol: 2.60 g/100 g @ 20°C ethyl ether: 6.7 g/100 g @ 15°C benzene: 34.7 g/100 g @ 18°C also soluble in acetone, pyridine, ethyl acetate and toluene
Dipole moment:	3.8 D
Electron affinity:	3.47 eV
Partition coefficient:	octanol-water log 1.49 (calculated)
Ultraviolet Spectral data:	λ_{max} = 233 nm (methanol) ε_{max} = 18,500 l/mole-cm (Weast. 1973) λ_{max} = 228 nm (n-heptane) ε_{max} = 18,800 l/mole-cm (Howard *et al*,1976). λ_{max} = 233.8 nm (methanol) ε_{max} = 17,700 l/mole-cm (Spanggord *et al*, 1978)
Liquid density:	1.511 g/ml @ 0°C 1.575 g/ml @ 18°C
Specific gravity:	1.546
Infrared absorption bands:	3030, 1610, 1520, 1450, 1080, 930, 730 cm^{-1}
Proton NMR chemical shifts (δ)	7.85, 8.55, 9.05 ($CHCl_3$)
Mass spectral data[m/e(% intensity)]:	168(100), 75(60), 76(55), 30(50), 18(41), 50(39), 122(30), 92(21)
m/e:	168.02

*Reference: Kirk and Othmer (1966, 1967); Hawley (1977); Windholz (1976); Howard *et al.* (1976); Dupont deNemours & Company (1979); Spanggord *et al.* (1978).

Figure I-1. The Infrared Spectrum of 1,3-Dinitrobenzene
(Spanggord *et al.*, 1978)



Sample: 1,3-Dinitrobenzene in $CHCl_3$

Ref. Cell: $CHCl_3$

Legend:
A. overtones - possibly not all meta
B. N=O asymmetric and C=C ring stretch
C. N=O symmetric
D_1. aromatic C-H stretch
D_2. methyl C-H stretch - $CHCl_3$
E. in Plane C-H bend
F. possibly Ar-NO_2 C-N stretch

Figure I-2. Ultraviolet Spectrum of 1,3-Dinitrobenzene (Spanggord *et al.*, 1978)

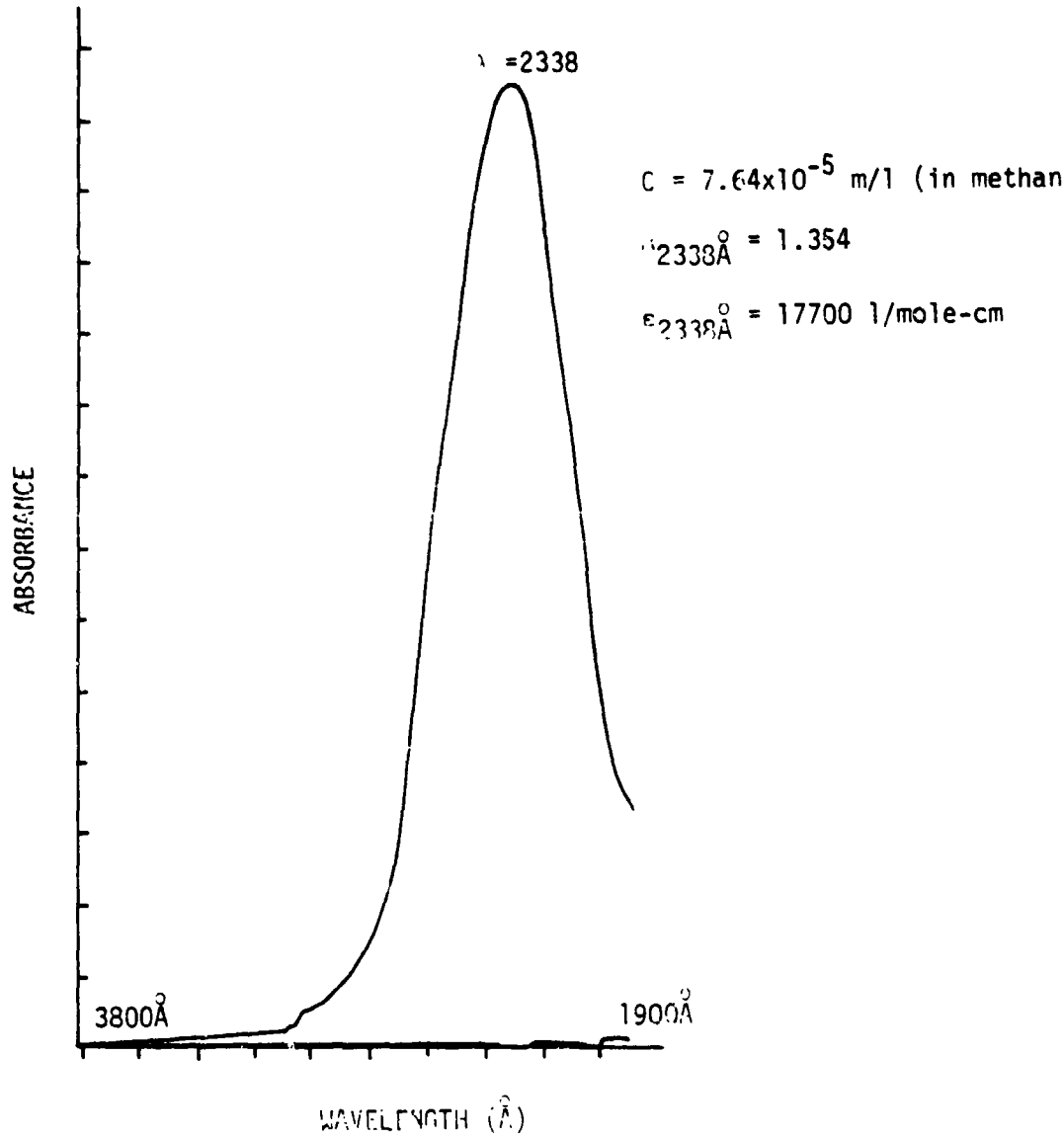
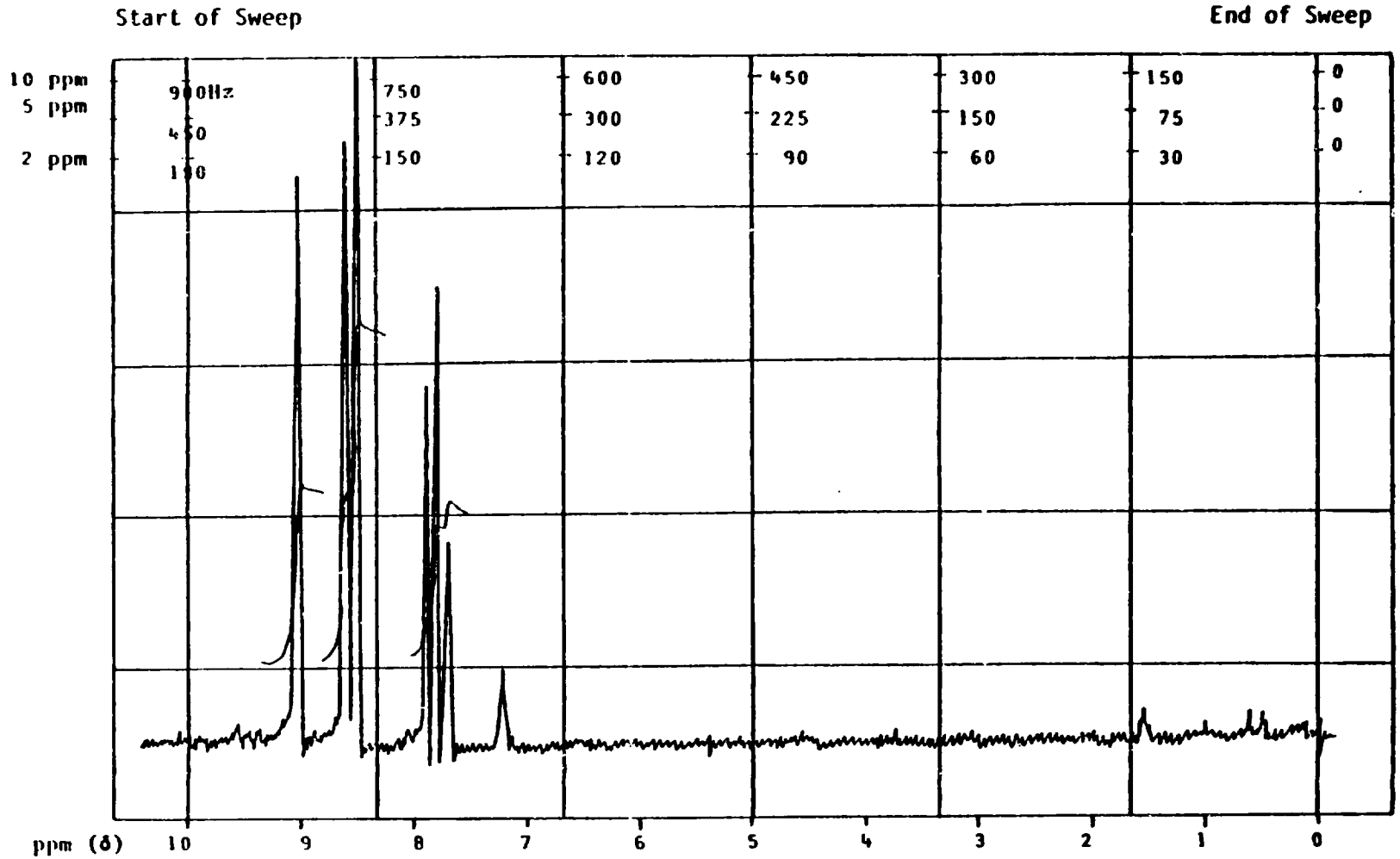


Figure I-3. NMR Spectrum of 1,3-Dinitrobenzene
(Spanggord *et al.*, 1978)



Lock Pos._____ppm
Lock Power .004 mG
Decouple Pos.____ppm
Decoupling Power_____mG
Solvent: $CDCl_3$

Spectrum Ampl. 5
Filter .05 sec
RF Power .04 mG
EM-390 90 MHz NMR Spectrometer

Sweep Time 5 min
Sweep Width 10 ppm
End of Sweep____ppm

Nucleus____
Zero Ref____
Sample Temp.___°C
Sample_____

Figure I-4. Mass Spectrum of 1,3-Dinitrobenzene
(Spanggord *et al.*, 1978)

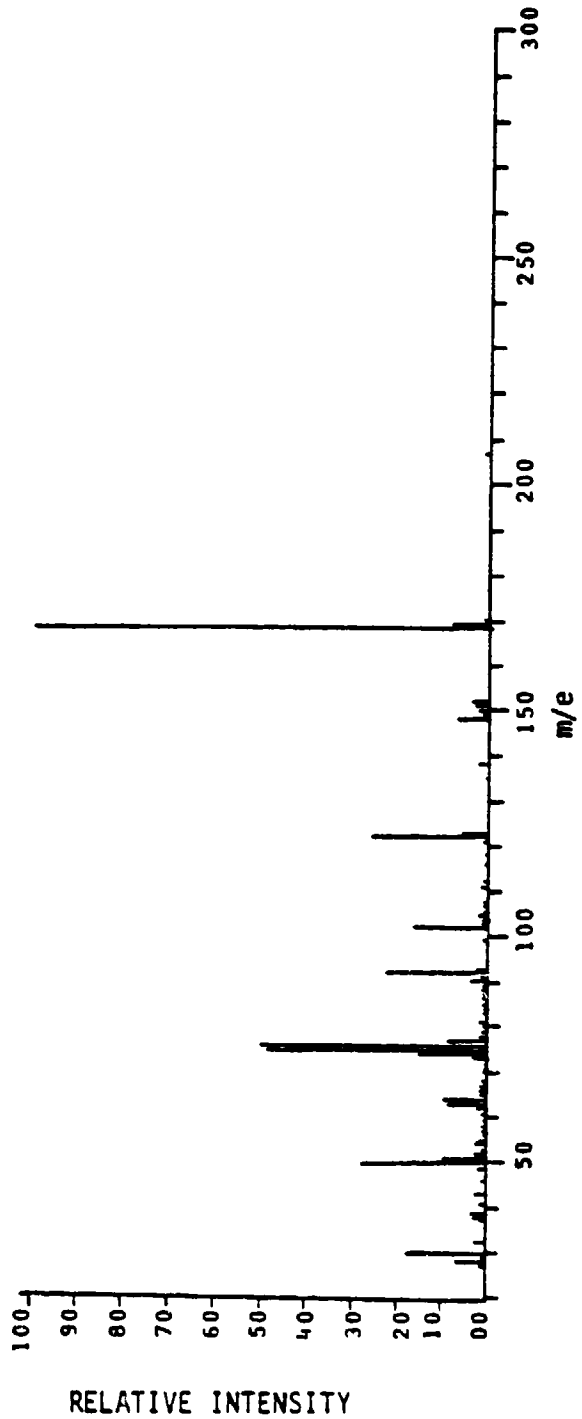


Table I-2. Vibrational Frequencies and Their Assignments for 1,3-Dinitrobenzene (Shukla *et al.*, 1968)

Raman (solution)		Infrared (KBr pellet)		Assignment
cm^{-1}	Relative Intensities	cm^{-1}	Relative Intensities	
702	(0)	683	(10)	a_1 C-C-C i.p. bending
		724	(9)	b_1 C-C-C o.p. bending
		760	(2)	
		791	(2)	b_1 C-H o.p. bending
		817	(7)	b_1 C-H o.p. bending
840	(3)	837	(6)	a_1 NO_2 i.p. bending
909	(0)	915	(7)	b_1 C-H o.p. bending
		944	(2)	b_1 C-H o.p. bending
1005	(4)	1003	(5)	a_1 C-C stretching (ring breathing)
		1027	(4)	a_1 C-C-C i.p. bending
		1067	(8)	b_2 C-H i.p. bending
		1092	(6)	
		1128	(4)	
1149	(9)	1145	(5)	a_1 C-H i.p. bending
		1172	(5)	a_1 C-H i.p. bending
1210	(1)	1212	(5)	
		1273	(7)	b_2 C-H i.p. bending
1353	(0)	1347	(8)	a_1 C-N stretching
1365	(3)	1357	(10)	a_1 N=O sym. stretching
1440	(0)	1441	(5)	b_2 C=C stretching
		1476	(7)	a_1 C=C stretching
		1510	(9)	b_2 C=C stretching
1538	(4)	1530	(10)	b_2 N=O asym. stretching

Table I-2. (cont.)

1600	(3)	1603	(7)	b_1 C=C stretching
		1694	(1)	A_1 791+915 = 1706
		1758	(1)	B_1 724+1027 = 1751
		1820	(1)	A_1 2X915 = 1830
		1904	(2)	B_1 724+1172 = 1896
		1996	(2)	A_1 2X1003 = 2006
		2252	(3)	A_1 915+1347 = 2262
		2359	(3)	A_1 1003+1357 = 2360
		2383	(3)	A_1 1027+1357 = 2384
		2431	(3)	B_2 1067+1357 = 2424
		2492	(3)	A_1 1146+1347 = 2493
		2888	(5)	B_2 1357+1530 = 2887
		3009	(6)	b_2 C-H stretching
		3461	(5)	B_2 1027+1357+1067 = 3451
		3574	(2)	B_2 1067+1357+1146 = 3570
		3028	(4)	B_2 837+3009 = 3936

i.p. = in-plane; o.p. = out-of-plane; sym. = symmetric; and asym. = asymmetric.

Table I-3. Fundamental Raman Frequencies (in cm^{-1}) in Dinitrobenzenes (Shukla and Upadhya, 1969)

Isomer			Assignment
ortho	meta	para	
25	23	26	lattice vibration
39	37	30	do
57	58	60	do
79	76	68	do
112 (a_2)	113 (a_2)	113	torsional vibration
138 (a_2)	162 (a_2)	138	C-NO_2 twisting
186 (b_2)	194 (b_1)		o.p. ring bending
367 (a_2)	349 (a_2)	297 (a_u)	do
422 (b_2)	400 (b_2)		C-NO_3 rocking
		436 (b_{3g})	i.p. ring bending
638 (b_2)	645 (b_2)	613 (a_{1g})	do
794 (b_1)	813 (b_1)	763 (b_{1g})	C-H o.p. bending
842 (a_1)	831 (a_1)	858 (b_{2g})	do
1032 (a_1)	994 (a_1)	1096 (a_{1g})	C-C stretching
1148 (b_2)	1141 (b_2)		C-H i.p. bending
1194 (a_1)	1166 (a_1)		do
1357 (a_1)	1345 (a_1)	1341 (a_{1g})	N=O symmetrical stretching
1434 (b_1)			
1450 (a_1)	1504 (a_1)		C-C stretching
1530 (b_2)	1530 (b_2)	1528 (b_{3g})	N=O asymmetrical stretching
1600 (a_1)	1595 (a_1)	1575 (a_{1g})	C-C stretching

o.p., out-of-plane; and i.p., in-plane.

Table I-4. Solubility of 1,3-Dinitrobenzene in Several Salt Solutions (Leiga and Sarmousakis, 1966)

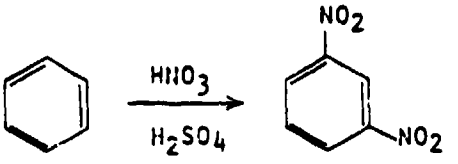
NaCl		$(C_2H_5)_4NCl$		$p\text{-}CH_3C_6H_4SO_3Na$	
moles/l	g/l	moles/l	g/l	moles/l	g/l
0.1735	0.511	0.0998	0.550	0.1162	0.629
0.4876	0.466	0.2096	0.560	0.2192	0.730
1.017	0.415	0.2995	0.572	0.2986	0.813
1.510	0.361	0.3981	0.581	0.3991	0.929
2.058	0.320	0.5988	0.586	1.001	1.975
2.497	0.287	0.993	0.586	1.507	0.784
3.006	0.258	1.407	0.567	2.004	0.320
3.502	0.233	1.895	0.515		
4.002	0.207				
4.597	0.164				
5.000	0.166				

C. Chemical Properties

1. General Chemistry

a. Synthesis

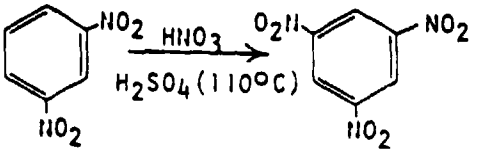
The synthesis of 1,3-dinitrobenzene is generally accomplished by the mixed acid nitration of benzene (Richter and Anschutz, 1946).



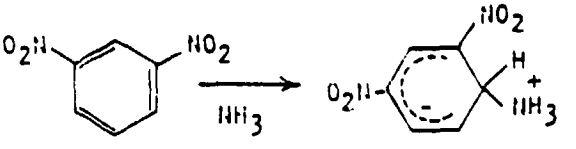
The introduction of the second nitro group requires rather severe conditions (95°C) as the ring is strongly deactivated. Only traces of the ortho and para isomers are encountered as these positions receive the majority of the deactivation (Fieser and Fieser, 1961).

b. Aromatic Ring Reactions

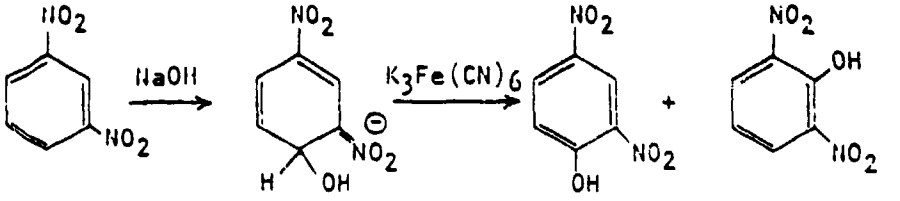
The aromatic ring, although quite deactivated by the influence of the two nitro groups, will undergo electrophilic substitution under forced conditions predominantly at carbon #5. For example, the mixed acid nitration gives 1,3,5-trinitrobenzene (March, 1968).



The electron withdrawing nitro groups enhance attack by nucleophiles. In liquid ammonia, 1,3-dinitrobenzene forms a stable Zwitterion (Foster and Mackie, 1962).

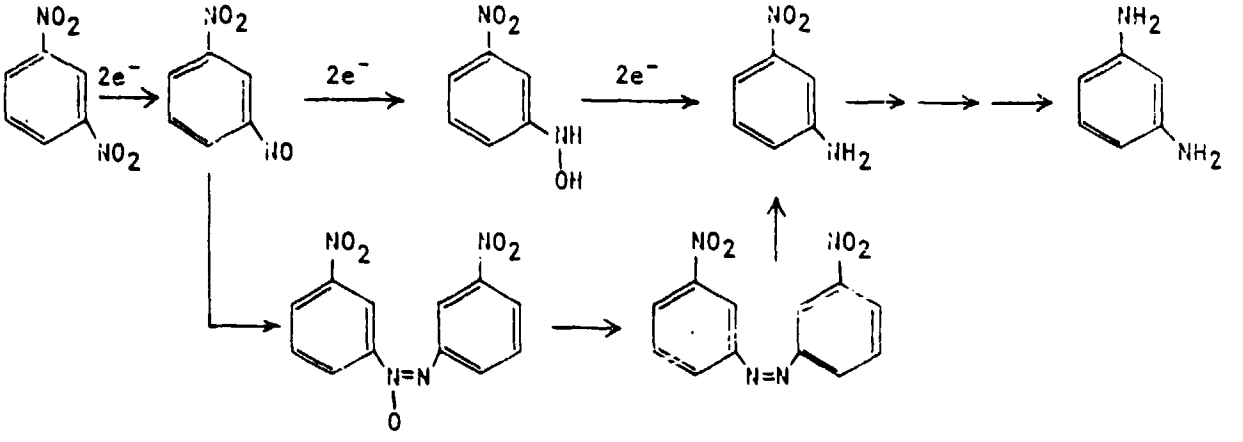


In the absence of a leaving group, a neutral species may be generated by oxidation. For example, 2,4-dinitrophenol is formed by treating 1,3-dinitrobenzene with hydroxide followed by oxidation with ferricyanide (Fieser and Fieser, 1961).



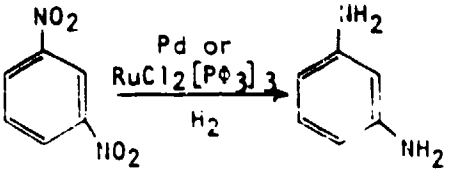
c. Reduction

Reduction of the nitro groups of 1,3-dinitrobenzene have been reported by numerous authors. Electrolytic reductions involve the initial formation of a radical anion (Freed *et al.*, 1962; Geske *et al.*, 1964) eventually leading to 1,3-phenylenediamene through a series of intermediates including nitroso-, hydroxylamino-, azo-, and azoxy- substituted species (Walbrick, 1970; Chandra *et al.*, 1976; Endrey and Reilly, 1968; Glicksman and Morehouse, 1958).

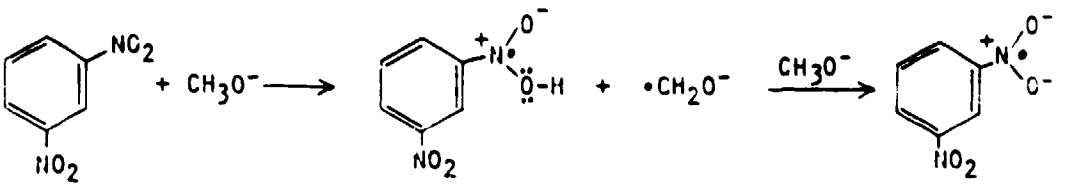


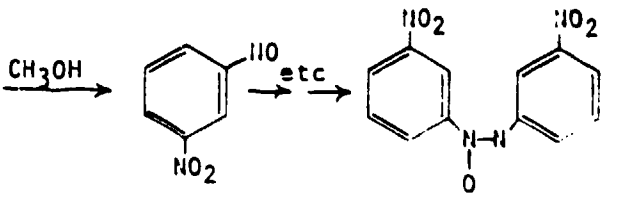
Nitrobenzenes in general are reduced by metals such as zinc, iron or tin in protic solvents. For example, nitrobenzene is reduced to aniline by Zn/HCl; to N-hydroxylaniline by Zn/H_2O and to hydrazobenzene by Zn/NaOH (March, 1968). A similar set of reductions is expected for 1,3-dinitrobenzene.

1,3-Dinitrobenzene is reduced by H_2 with a $RuCl_2$ $[P\phi_3]_3$ catalyst at 1200 psi (Knifton and Suggitt, 1974) or with a Pd catalyst at 300 psig (Dovell *et al.*, 1970) to form 1,3-diaminobenzene.

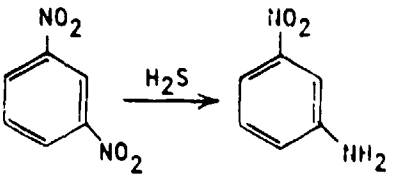


Treatment of 1,3-dinitrobenzene with sodium methoxide gives 3,3'-dinitroazoxybenzene (Bellobono *et al.*, 1972). The proposed mechanism involves the formation of radical species by hydrogen abstraction from the alkoxide.



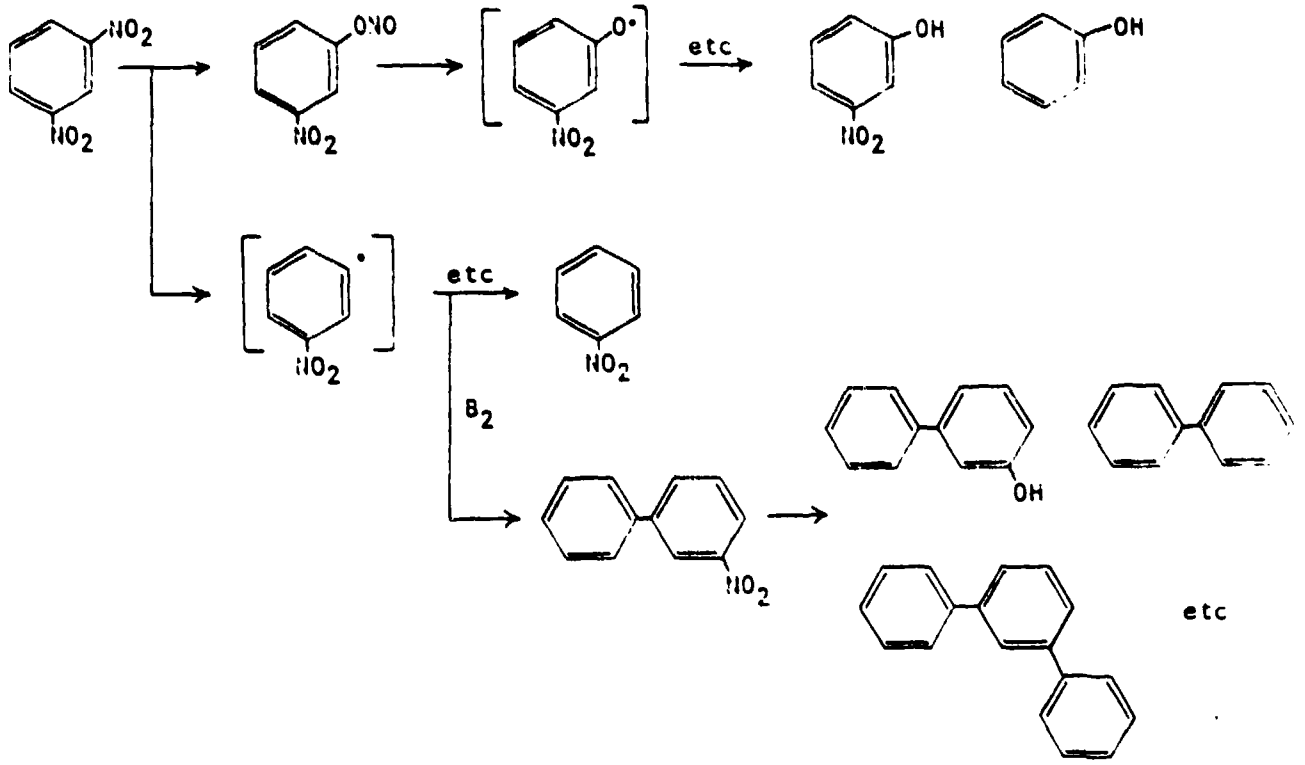


Selective reduction of one nitro group can be accomplished by the action of hydrogen sulfide (Roberts and Caserio, 1964).



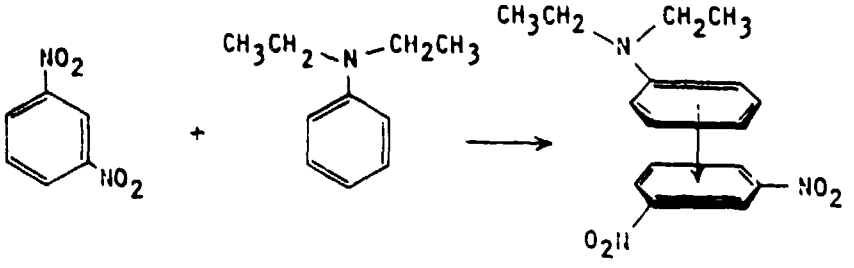
d. Thermal Decomposition

The thermal decomposition of 1,3-dinitrobenzene in benzene has been reported by Fields and Meyerson (1972a, 1972b). The initial processes are assumed to be (1) a rearrangement leading to m-nitrophenyl nitrite which looses NO to form m-nitrophenoxy radicals and (2) the direct loss of NO_2 leading to m-nitrophenyl radicals. Subsequent reactions lead to the formation of a variety of substituted benzenes, phenols, biphenyls and terphenyls.



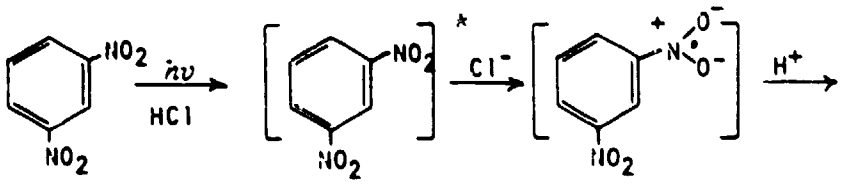
e. Charge Transfer Reactions

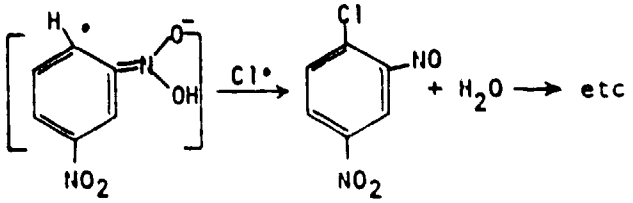
The electron attracting effect of the nitro groups leaves the aromatic system of 1,3-dinitrobenzene electron deficient. This makes the formation of π-π charge transfer complexes with electron rich aromatics very favorable. For example, π complexes have been reported with diphenylamine, N-N-diethylaniline and p-anisidine (Sharma and Tewari, 1974; Pariher *et al.*, 1968a; 1968b).



f. Photochemical Reactions

Wubbels and Letsinger (1974) have studied the photoreduction of 1,3-dinitrobenzene in concentrated HCl. A mechanism involving an electron transfer from chloride to the photoexcited dinitrobenzene is postulated.





2. Environmental Reactions

Chemical and photochemical reactions of 1,3-dinitrobenzene are expected to play only a minor role in the environmental fate of this chemical. Newell *et al.* (1976) reported that 1,3-dinitrobenzene in simulated waste streams was not photochemically reduced when irradiated with a 1200 watt medium pressure Hanover lamp .

3. Monitoring and Analysis

a. Analytical Methods

Several Techniques have been described for the estimation of 1,3-dinitrobenzene. Gas chromatography has been used by several authors. Chromatographic conditions employed by these investigators are listed in Table I-5.

Rawat and Singh (1978) have reported the spectrophotometric determination of 1,3-dinitrobenzene by reduction to the analogous hydroxylamine with Zn/NH_4Cl and formation of a colored complex by treatment with Fe^{+++}/acetylchloride. A similar study by Tiwari and Pande (1972) involved the $LiAlH_4$ reduction to the analogous azo compounds with spectrophotometric analysis at 355 nm. Samples in the range of 100-600 µg were studied.

High performance liquid chromatography has been employed by Meier *et al.* (1978). Zorbux-ODS and Partisil 10-ODS reverse phase columns were used with a 40% acetonitrile mobile phase. A detection limit of less than 0.1 ppm was possible with UV detection at 230 nm. Spanggord *et al.* (1978) employed reverse phase HPLC on µ Bondapak C_{18} for the analysis of 1,3-dinitrobenzene with 50% methanol/water as the solvent and UV detection at 254 nm. Detection limits were not given. HPLC was also employed by Newell *et al.* (1976) for the analysis of 1,3-dinitrobenzene in simulated pink water. A normal phase µ Porasil column was used with 35% hexane/65% methylene chloride as a

Table I-5. Gas Chromatographic Analysis of 1,3-Dinitrobenzene

Reference	Column	Detector	Conditions	Sensitivity
Fields and Meyerson, 1972a, 1972b	Polyethylene-glycolsebacate on Chromosorb W	Mass Spectroscopy	Not Specified	Not Specified
Habboush and Norman, 1962	SGR-glass beads	Flame Ionization	100°C	Not Specified
Camera *et al.*, 1965	10% Silicon grease E301 on Celite	Thermal Conductivity	Not Specified	Not Specified
Chang, 1971	15% SE 30	FID/Mass Spectroscopy	150-200°C	Not Specified
Kaplan and Zitrin, 1977	3% OV-17	FID/E.C.	160°C	1 ng
Spanggord *et al.*, 1978	3.5% Dexsel 300 GC on Chromosorb WAWDMCS	E.C.	Temperature Programmed between 40°C and 220°C	1 µg
Sullivan *et al.*, 1978	2% OV-101/3% QF1 on 100/120 mesh Gas Chrom Q or 8% UCW 98 on Gas Chromatograph Q 80/100 mesh	EC/Alkali flame ionization detector	175°C	Not Specified

solvent and UV detection at 254 nm. A detection limit was not specified, however, values were reported as low as 0.8 ppm. A method for analysis of air samples has been described (NIOSH, 1978). The method involves trapping by bubbling into ethylene glycol and analysis by high performance liquid chromatography. A Sperisorb ODS column was employed with a 20% methanol mobile phase and UV detection at 254 nm. The detection limit was 0.42 mg/m^3.

A number of qualitative methods for analysis of 1,3-dinitrobenzene have been described in the literature. Sharma and Tewari (1974) have used $\pi-\pi$ charge transfer complex of 1,3-dinitrobenzene with N-N-diethylaniline for spot tests and spectrophotometric analysis. A λ_{max} of 412 nm and a yellow spot test allowed detection of 100 µg of 1,3-dinitrobenzene. However, interferences from other nitroaromatic compounds limit the utility of this method.

Thin layer chromatography of 1,3-dinitrobenzene has been employed by several researchers. Conditions used for the analyses are presented in Table I-6. Paper partition chromatography has also been utilized. Prey and Kabil (1956) studied the chromatography of 1,3-dinitrobenzene on acylated paper using a butanol, methanol, formic acid (83:15:2) solvent system. Concentrations with the 0.1-1.0% range were used. Franc and Stránský (1959) obtained a chromatographic spectrum by plotting the rf values in 12 different stationary and mobile phase combinations. The results are shown in Figure I-5.

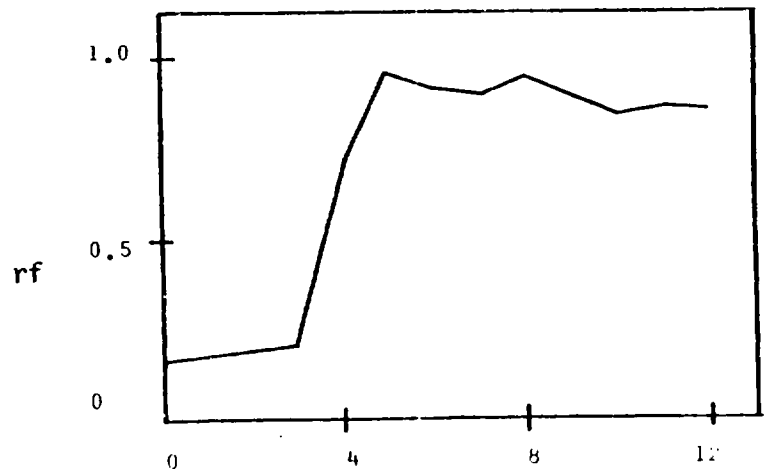
Snyder (1963) used column chromatography on Florisil for analysis of 1,3-dinitrobenzene. A methylene chloride/pentane mobile phase was used. Chmielowiec and Kemula (1974) chromatographed 1,3-dinitrobenzene on a Wojatit KPS (H+)/divinylbenzene cation exchange resin using a 10% acetone/.005 M H_2SO_4 solvent system.

b. Monitoring

A monitoring study for 1,3-dinitrobenzene was recently sponsored by the Army. During this study, 79 samples of condensate water discharge at Volunteer AAP were collected. Average levels of 1,3-dinitrobenzene in the samples were 2.05 mg/l. The 90th percentile concentration was 4.000 mg/l (Spanggord *et al.*, 1978). Monitoring for 1,3-dinitrobenzene at Volunteer AAP has also been reported by Sullivan *et al.* (1978). Values at nine sampling sites ranged from below the detection limit of 1 ppb to a high of 6 ppb during December 1976 and from below a detection limit of 0.25 ppb to a high of 4.1 ppb during March of 1977.

Table I-6. Thin Layer Chromatography of 1,3-Dinitrobenzene

Reference	Stationary Phase(s)	Mobile Phase(s)	Sensitivity	Visualization
Franck-Neumann and Jossang, 1964	Silica gel G	cyclohexane/chloroform anthracene	Not Specified	Ultraviolet
Pejkovic-Tadic *et al.*, 1966	Silica gel G	Benzene/ethyl acetate	26 µg	Not specified
Parihar *et al.*, 1968a	Silica gel G or Silica gel G+Magnesium Silicate	CCl_4/chlorobenzene xylene xylene/petroleum ether xylene/ethylenedichloride (2% complexing agent *i.e.*, solvent)	1-2 µg	Analyzed as m-chloroaniline, dimethylaniline, or toluidine complexes
Parihar *et al.*, 1968b	Silica gel G Magnesium Silicate or Alumina	monochlorobenzene/ethylene dichloride or petroleum ether/ ethyl acetate or monochloro-benzene/ethylene dichloride or carbon tetrachloride	Not Specified	Analyzed as δ-Complexes with diphenylamine, diethylaniline or p-anisidine
Klemm *et al.*, 1978	Silica gel G or Alumina	benzene	7 µg	Rhodamine B and UV
Boehm, 1967	Silica gel G	ethyl acetate/petroleum ether	Not Specified	Diphenylamine
Boehm, 1966	Silica gel G	benzene/petroleum ether/ methanol	10 µg	Not Specified



System Number	Stationary Phase	Mobile Phase
1	formamide	cyclohexane
2	dimethyl formamide	cyclohexane
3	formamide	cyclohexane/pyridine (25:1)
4	---	chlorocyclohexane
5	---	ethyl acetate/formic acid/water (10:2:5)
6	---	isoamyl alcohol/ethanol/pyridine/water (1:1:1:1)
7	---	n-butanol/10% NH_3 (85:15)
8	---	n-butanol/pyridine/water (5:3:3)
9	---	n-butanol/3 NHCl (86.5:13.5)
10	---	ethanol/10% NH_3 (80:20)
11	paraffin oil	80% ethanol
12	paraffin oil	ethanol/acetic acid/water (20:2:13)

--- = no stationary phase

Figure I-5. Chromatographic Spectrum of 1,3-Dinitrobenzene (Franc and Stransky, 1959)

D. Health Effects

1,3-Dinitrobenzene was widely employed in the munitions industry in the late nineteenth and early twentieth centuries. However, as the toxicological properties of this compound were recognized, it was gradually replaced by the less toxic, trinitrotoluene (TNT). Because of the decline in the use of 1,3-dinitrobenzene, the basic toxicological studies on this compound were conducted in the period from 1860 to 1930. The importance of these earlier studies was recognized by their inclusion in this evaluation.

1. Biology

a. Absorption

1,3-Dinitrobenzene can enter the mammalian body by ingestion, inhalation of dust or vapor, and by absorption through the skin (Monks, 1902; White and Hay, 1901; Steiner, 1918; Ishihara *et al.*, 1976). Skin absorption of 1,3-dinitrobenzene was a controversial issue in the late 1800's. In 1901 White and Hay showed that skin absorption of 1,3-dinitrobenzene does occur in both man and animals. These authors applied an ointment of 25% 1,3-dinitrobenzene in anhydrous wool fat to three square inches of the freshly shaved back of a cat. The area was covered with a piece of lint, then a gauze and collodion dressing. Within 24 hours, the cat was dying. Symptoms were classical for 1,3-dinitrobenzene poisoning, *i.e.*, methemoglobinemia, blue tongue and mucous membranes, stupor, gasping for breath. No blood or 1,3-dinitrobenzene were found in the urine of this cat. A second cat was dosed in the same manner, except the back was shaved 3 days before dosing to prevent entry of the 1,3-dinitrobenzene through any cuts. The cat was cyanotic within 24 hours after dosing and died within 4 days. The urine of this cat contained blood. To determine the effects of prolonged skin exposure, a third cat was dosed with the 1,3-dinitrobenzene ointment. After the shaven back had been allowed to heal for 3 days, the ointment was applied to 3 square inches of parchment. The parchment was placed on the shaven area and covered as before to prevent any licking of the material. The cat showed slight cyanosis in two days. On the second day, the parchment was replaced with a freshly treated piece. The cat was more cyanotic by the third day and was dying on the sixth day after the initial dose. The urine was highly discolored but contained no bile. The blood was chocolate brown and contained irregular shaped corpuscles. The cat was killed on the sixth day and necropsied. Both the urine and blood taken after death contained methemoglobin and oxyhemoglobin but no 1,3-dinitrobenzene. The kidneys were inflammed.

To further clarify the skin absorption of 1,3-dinitrobenzene, a second set of experiments were performed by White and Hay (1901). In these experiments, 0.1 g of chemically pure 1,3-dinitrobenzene crystals was applied to 2 square inches of the shaven back of a cat and covered as previously described. At the same time, 0.1 g of chemically pure 1,3-dinitrobenzene dissolved in mononitrobenzene (a greasy compound which the authors consider relatively non-toxic) was applied to the same skin area of a second cat. The

cat which was dosed with the 1,3-dinitrobenzene dissolved in mononitrobenzene showed cyanosis. No cyanosis was evident in the cat dosed with the chemically pure crystals. The indication from this experiment is that an oily solvent is necessary for skin absorption of 1,3-dinitrobenzene.

Although these studies were not performed on a statistically significant number of animals, these physicians (White and Hay, 1901) took the proper precautions to insure that the toxic symptoms could only be due to skin absorption of 1,3-dinitrobenzene. The results of their experiments on cats indicate that 1,3-dinitrobenzene is absorbed through the skin of cats. However, an oily solvent appears to be necessary to aid skin absorption.

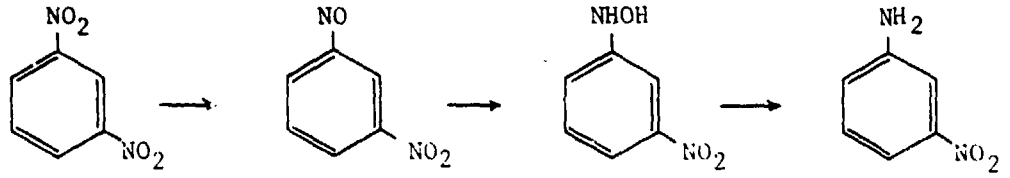
In a classic human experiment, a physician, Dr. Hay (White and Hay, 1901), annointed his groin with 400 mg of anhydrous wool fat containing 100 mg of 1,3-dinitrobenzene. The procedure was repeated approximately 12 hours later. In 24 hours, his tongue, lips and fingernails were blue. The dosing was repeated for the third time at 24 hours. At approximately 30 hours after the first dose, the lips were livid blue and the nails and skin had a deadly blue appearance. The pulse rate was elevated (100-120). He had a throbbing headache. The blood was brown color. The urine was not discolored and contained no 1,3-dinitrobenzene. Due to the cyanosis, all the ointment was removed at approximately 30 hours. At 35 hours, the pulse was full and bounding, *i.e.*, 96-98. He had a metallic taste in his mouth and an orbital headache. The veins in his hands and ears were full and engorged. There was a tremer in the hand. These subsisted within two days with a return to normal health. This experiment proved conclusively that 1,3-dinitrobenzene can be absorbed into the human body through the skin.

Since 1901, there have been many reported cased of 1,3-dinitrobenzene poisoning as a result of skin absorption of this compound. For example Monks (1902) described a case in which a family suffered from 1,3-dinitrobenzene poisoning. This family had slept in beds in which the sheets had been sprinkled with a home concocted mixture of the explosive, roburite (7% 1,3-dinitrobenzene) and an insecticide. Steiner (1918) also described a case of 1,3-dinitrobenzene poisoning through skin contact with clothing contaminated with this compound. More recently, Ishihara *et al.* (1976) reported a case of skin absorption of 1,3-dinitrobenzene through supposed protective gloves. (See Occupational Exposure Section). In general, it is believed that most occupational poisonings due to 1,3-dinitrobenzene were the result of skin absorption (von Oettingen, 1941; Am. Conf. Gov. Ind. Hyg., 1971.)

b. Metabolism and Transport

The earliest reported study on the metabolism of 1,3-dinitrobenzene was conducted by Lipschitz (1920). He found that 1,3-dinitrobenzene was transformed into nitrophenylhydroxylamine in various animal tissues and in cell suspensions. Lipschitz believed that the nitrophenylhydroxylamine metabolite was the chemical responsible for methemoglobin production (see pharmocological section) in cases of 1,3-dinitrobenzene poisoning. He assumed

that the 1,3-dinitrobenzene was partially excreted as m-nitroaniline. This assumption was based on the ability of the urine contents to undergo a diazotization reaction with naphthol. Lipschitz postulated the following reductive pathway for *in vivo* metabolism of 1,3-dinitrobenzene (Lipschitz, 1920; Lipschitz and Osteroth, 1924):



1,3-dinitrobenzene — 1,3-nitroso-nitrobenzene — 1,3-nitrophenyl-hydroxylamine — m-nitroaniline

Heubner and Lo-Sing (1938) disagree with Lipschitz on the 1,3-dinitrobenzene metabolite that is responsible for methemoglobin formation. They believe that aminophenols are responsible for this reaction not nitrophenylhydroxylamine.

Sufficient data is not presented in these papers for a detailed analysis of the conclusions. In addition, the age of these works (before modern instrumental analysis and identification techniques were available) render the conclusions suspect. Both of the intermediates predicted by these authors, aminophenols and N-hydroxylarylamines, have been implicated in methemoglobin production. N-hydroxylarylamines are very active methemoglobin formers. They react with hemoglobin to yield methemoglobin and nitrosobenzenes *e.g.* 1,3-nitrosobenzene. Normal blood cells supplied with glucose can reduce the nitrosobenzenes to regenerate the N-hydroxylarylamine. This recycling accounts for a methemoglobin to poison ratio of greater than one (Smith, 1975). Methemoglobin ratios of greater than one have been found for 1,3-dinitrobenzene (see pharmacological section).

The only detailed study of 1,3-dinitrobenzene metabolism was conducted by Parke (1961) This author used oral doses of 50-100 mg/kg of ^{14}C labelled 1,3-dinitrobenzene to study the metabolism of this compound in the rabbit. Urine and feces were collected and analyzed for radioactivity and metabolites. The respired air was not collected. Metabolites were separated by paper chromatagraphy and identified by chemical reactivity and spectral properties. Radioactivity was measured by an end window counter tube.

In two days, 65-93% of the radioactive dose was excreted in the urine and 1-5% in the feces. The remaining amount of radioactivity was presumed to be retained in the tissues and then slowly excreted in the urine. However, this assumption cannot be assured since respired $^{14}CO_2$ was not measured. The major metabolites identified in the urine were: m-nitroaniline and m-phenylenediamine which together comprised an average of 35% of the dose; 2,5-diaminophenol, 31% of the dose; and 2-amino-4-nitrophenol, 14% of the dose.

Thirty percent of the dose was excreted as glucoronides and 6% as ethereal sulphates in the urine.

Although this study of the metabolism of 1,3-dinitrobenzene is the most believable reported in the literature, it is lacking in many points. First rabbits are not a particularly good species from which to extrapolate metabolism to man. Indeed rabbits are reported to have different reactions to methemoglobin formers than man (Smith, 1975) (see pharmacology section). Second, $^{14}CO_2$ was not measured. The decision not to measure $^{14}CO_2$ may have been due to the small amount of $^{14}CO_2$ the author found in a similar study with nitrobenzene (Parke, 1956). However, data on $^{14}CO_2$ production would have placed additional credence on the results. Third, the methods of metabolite identification and quantification are crude by today's standards. Fourth, the end-window counter is notorously inefficient and irreproducible for ^{14}C counting. Liquid scintillation counting is now the accepted method. Thus, it is not known if the low radioactivity recovery was due to poor techniques or actual tissue retention of dinitrobenzene or some metabolite(s).

Essentially the metabolic fate of 1,3-dinitrobenzene and the metabolite(s) responsible for methemoglobinemia are unknown. Several compounds have been assumed to be responsible. These compounds are the result of logical reduction and/or hydroxylation mechanisms *in vivo*. However, their presence and actions in mammalian and human cases of 1,3-dinitrobenzene poisoning have not been conclusively proven. Another question which needs to be answered is the potential for *in vivo* 2,4-dinitrophenol formation. Parke (1961) found that this metabolite accounted for 0.1% of the dose. It is unknown if direct hydroxylation of 1,3-dinitrobenzene can occur in man or other mammalian systems. If it does occur, uncoupling of oxidative phosphorylation due to the presence of this metabolite may be in part responsible for some of the symptoms observed in some cases of 1,3-dinitrobenzene poisoning.

c. Elimination

As early as 1902, White *et al* recognized dark-colored acidic urine as symptomatic of chronic 1,3-dinitrobenzene poisoning. They found traces of 1,3-dinitrobenzene in some of the urine samples. The presence of m-nitroaniline and other diazotizable products in the urine has also been reported (Lipschitz, 1920; Parke, 1961; Watanabe *et al.*, 1976). According to Parke (1961) (described in the previous section), 3-4-diaminophenol, 2-amino-4-nitrophenol and m-phenylenediamine are also excreted by rabbits poisoned with 1,3-dinitrobenzene.

d. Pharmacology

The main physiological effect of 1,3-dinitrobenzene poisoning is the conversion of normal oxyhemoglobin to methemoglobin (Fairhall, 1969; Bodansky, 1951). Production of methemoglobin diminishes the oxygen-carrying capacity of the blood by binding irreversibly with oxygen. In addition, the presence of methemoglobin interferes with the release of oxygen from normal hemoglobin (Hamblin, 1962). Cyanosis (and tissue hypoxia) become evident

when the oxygenated hemoglobin levels fall below critical cellular oxygen demand. Another characteristic of methemoglobin is its ability to initiate the dissociation and exchange of complete hemo groups as units.

Different species have been shown to vary in their susceptibility to chemically induced methemoglobinemia by 1,3-dinitrobenzene (Smith, 1975; Kunz, 1942; Kiese, 1949a). This species sensitivity difference is due to three factors: 1) the ability to reduce 1,3-dinitrobenzene to its active form; 2) differences in susceptibility of the various hemoglobins to oxidation; and 3) differences in the methemoglobin reductase activity in the erythrocytes of the species (Smith, 1975). Of these factors, the ability to reduce methemoglobin is the most important. In mammalian erythrocytes, methemoglobin reduction is normally carried out by the enzyme, diaphorase. This enzyme utilizes NADPH as a cofactor. In most erythrocytes, a second reductive system which utilizes NADPH as a cofactor is also available. Smith (1975) shows a comparison of data on the ability of different species to reduce methemoglobin. The pig and the horse red cells have reduction rates much lower than man. Cats, cows, goats and dogs have methemoglobin reductase activity approximately equal to that of man while rats, guinea pigs, mice and rabbits have a higher rate of methemoglobin reductase activity than man.

Methemoglobin is normally present in low concentrations in the blood of man and other mammals with an equilibrium existing between hemoglobin and methemoglobin (Hamblin, 1962). With massive chemical exposure, the reduction mechanisms are inadequate to handle the extra amount of abnormal pigment and cyanosis becomes evident (Hamblin, 1962). The onset of cyanosis may be delayed for up to several hours, with the time required depending on the amount absorbed and the individual involved (Linch, 1974). Cyanosis is usually detectable when the amount of methemoglobin reaches fifteen percent (Hamblin, 1962). Exposure to aromatic mitro compounds, *e.g.* 1,3-dinitrobenzene, may be either acute or chronic. Each of these situations has its own particular manifestations. Acute exposure usually produces cyanosis with or without a significant loss of hemoglobin, whereas chronic exposure due to prolonged absorption may produce reversible anemia (Linch, 1974). This overall effect has been referred to as the cyanosis-anemia syndrome (Linch, 1974).

Another hematological effect associated with exposure to 1,3-dinitrobenzene in the presence of Heinz bodies in the blood. These bodies are protein inclusion granules in red blood cells, thought to consist of denatured hemoglobin (Smith, 1975). Dissociation of heme (as can occur in methemoglobinemia) results in its decreased solubility, leading to precipitation of the pigment as a Heinz body (Smith, 1975). Thus, the formation of Heinz bodies can be regarded as a continum of oxidative stress to the red blood cell (Smith, 1975). Premature capture of these distorted cells occurs, which may explain the many observations of temporary spleen enlargement.

As with methemoglobinemia, individual species (and individuals within species) vary in their susceptibility to agents producing Heinz bodies. The erythrocytes of cats, mice, dogs and humans are highly susceptible to Heinz bodies formation. Least responsive to Heinz body formation are the erythrocytes of monkeys, guinea pigs, rabbits and chickens.

Many studies have been conducted in both animals and man to follow the course of methemoglobin formation due to 1,3-dinitrobenzene. The results of these studies are summarized and discussed in the following paragraphs.

A controlled study of human 1,3-dinitrobenzene intoxication was reported by Ishihara *et al.*, (1976). The volunteer was a Japanese male aged 30. Blood samples for methemoglobin analysis were drawn prior to exposure and several times after exposure. Methemoglobin was detected immediately after exposure. The methemoglobin concentration reached the maximum level (11% of total hemoglobin) one hour after exposure and remained at this level for approximately 4 hours. The level of methemoglobin in the blood then decreased rapidly. No methemoglobin was detected in the man's blood 21 hours after exposure.

The *in vivo* and *in vitro* methemoglobin-forming capacity of various benzene derivatives was studied by Watanabe *et al.* (1976). For the *in vivo* study, the authors used Wistar rats of both sexes as test animals. The rats weighed between 150 and 220 g.

A preliminary experiment was conducted to determine the maximum non-lethal dose and the time after dosing during which maximum methemoglobin levels occur. This preliminary experiment was conducted using 1,3-diamino-2,4,6-trinitrobenzene, aniline and nitrobenzene. Male rats were injected intraperitoneally at dose levels of 25, 50, 100 and 200 μ moles/kg. For these 4 compounds, methemoglobin concentration in the blood was linearly related to the logarithm of the dose. The concentration of methemoglobin increased within 1 hour after dosing. Maximum methemoglobin concentrations in the blood were observed from 3 to 8 hours after dosing. From this preliminary experiment, the authors chose a dose of 100 μM/g for all compounds. They also set the time for sacrifice and measurement of methemoglobin levels in the blood at 5 hours after dosing.

The procedure used for the *in vivo* experiment was as follows. 1,3-Dinitrobenzene was dissolved in or dispersed in propylene glycol to yield 2 ml of solution containing the dose of 100 μM/kg. Five rats received 2 ml of the solution by intraperitoneal injection. The five control animals received 2 ml of the vehicle alone. The urine of each rat was collected and analyzed for diazopositive metabolites. For this determination, the urine was hydrolyzed with HCl and reduced by addition of Zn powder. The reduced product was diazotized with $NaNO_2$, ammonium sulfamate and N-(1-naphthyl)-ethylenediamine dihydrochloride. The resulting color was measured at 560 nm on a spectrometer. Five hours after dosing, blood was collected from the rats by decapitation. The blood was analyzed for methemoblobin, alanine transaminase and aspartate transaminase levels.

For the *in vitro* study, 0.5 μmole of each compound was dissolved in 1 ml of propylene glycol. This solution was mixed with 3.5 ml of 0.14M potassium phosphate buffer (pH = 6.6) and 1 ml of hemolyzate containing 0.1

μ mole of hemoglobin from the control rats. The mixture was incubated at 37°C for 5 hours. After 5 hours, the methemoglobin levels were measured.

The results of the *in vivo* and *in vitro* studies for 1,3-dinitrobenzene are presented below:

Diazopositive metabolites in urine (based on 1.80±0.58 mg/kg as p-aminophenol equivalent is equal to 1)	5.2 rat #1 6.0 rat #2
Methemoglobin Concentration of the blood *in vivo*	25.2±5.5 for 5 animals
Methemoglobin Concentration *in vitro*	8.2±0.9 for 5 trials ($p<0.01$)
In vitro control	4.2±1.0 for 5 trials
Asparate aminotransferase (Karmen units)	227.2±48.3 for 5 animals
Control	195.0±22.6 for 5 animals
Alanine aminotransferase (Karmen units)	483.3±13.5 for 5 animals
Control	33.7±6.3 for 5 animals

The *in vivo* data reported in this study agree with the reports that 1,3-dinitrobenzene is a potent methemoglobin former. However, the methemoglobin levels (reported to be maximum) are in doubt. These measurements were taken 5 hours after dosing based on results of other compounds. Pankow *et al.* (1975), in essentially the same type of poisoning, (.15 μ mole/kg) found the methemoglobin levels in the blood at 4 hours after dosing were approximately 2/3 the maximum levels observed at 1 to 2 hours after dosing. In contrast Seńczuk *et al.* (1976) found the maximum methemoglobin levels occurred at 4 hours after dosing (.4 μ mole/kg). Thus, the methemoglobin levels reported by Watanabe *et al.* (1976) are probably not real maximum levels.

In vitro formation of methemoglobin occurred, however, the percentage of methemoglobin found was small compared to the *in vivo* formation. These results indicate that the high levels of methemoglobin formed *in vivo* by 1,3-dinitrobenzene poisoning are due to a metabolite(s) of the 1,3-dinitrobenzene and not the compound itself.

Diazopositive urinary metabolites increased to 5 to 6 times that of the controls within 5 hours after dosing with 1,3-dinitrobenzene. The authors suggest that monitoring for diazopositive urinary metabolites is applicable for detecting a wide range of nitro and amino benzene poisonings.

The increase in the alanine transferase activity was significant ($0.025<p<0.05$) for administration of 1,3-dinitrobenzene. This increase in the level of this enzyme may be an early indication of possible hepatotoxicity. However, further investigation is necessary to conform or deny this possibility (Watanabe *et al.*, 1976).

Seńczuk *et al.* (1976) also studied the formation of methemoglobin in white rats (Wistar) after a single dose of 1,3-dinitrobenzene. These rats weighed between 200 and 300 g. These animals were given a dose of 0.4 mM of 1,3-dinitrobenzene per kg of body weight. Methemoglobin levels in the blood were measured 1,2,4,6,8,10 and 12 hours after dosing. Methemoglobin levels were 52% of total hemoglobin one hour after dosing. The maximum methemoglobin level was 78% of total hemoglobin four hours after dosing. A gradual decline in methemoglobin level was observed for the next 8 hours.

In a study with guinea pigs, Kunz (1942) followed the time course of methemoglobin, hemoglobin and reticulocyte concentrations in the blood after intraperitoneal injections of 1,3-dinitrobenzene. Single doses of 10, 20, 30, 40 and 50 mg/kg in poppy seed oil were given to the guinea pigs. One to four animals weighing between 0.19 and 0.55 kg were used at each dosage level. Blood was taken from the heart, ear or juglar vein periodically after dosing. Monitoring of the blood was continued until the animal's death or the blood chemistry returned to essentially that observed before the poisoning. Total hemoglobin, methemoglobin, and reticulocyte concentrations in the blood were measured.

In general, the higher doses of 1,3-dinitrobenzene produced higher levels of methemoglobin. Otherwise the time course of production and disappearance of methemoglobin varied among animals within a specific dosage. These variations may have been due to the variation in blood taking methods or to difference among individuals of the same species. Peak methemoglobin concentrations were observed .5 to 1.5 hours after dosing after which the methemoglobin concentration in the blood slowly declined. Generally a fall in the total hemoglobin concentration was observed within 1-24 hours after dosing. The reticulocyte concentrations varied erradically with a general increase observed within a few hours after dosing.

Kunz (1942) also calculated the molar ratio of methemoglobin to 1,3-dinitrobenzene for his experiments. He found an average ratio of 1.5 for the guinea pig. Kunz (1942) also compared the methemoglobin levels at a given dose of 1,3-dinitrobenzene for his guinea pig experiments to those observed by his collegues with other animals. He found the following order of sensitivity: rabbit < guinea pig < dog < cat.

In a second set of experiments, Kunz (1942) administered daily subcutaneous doses of 10, 15, 20 and 30 mg/kg 1,3-dinitrobenzene to guinea pigs. The guinea pigs weighed between .21 and .3 kg. Blood samples were taken periodically from the heart or ear. Only total hemoglobin and reticulocytes were determined on the blood samples. In general the hemoglobin concentrations decreased with time (52% after 7 days of poisoning) and the

reticulocyte concentrations increased with time. The experiments lasted from 4 to 10 days and were terminated due to death of the animals. Unfortunately methemoglobin levels and Heinz body concentrations were not measured during this study. Therefore a complete picture of the blood chemistry was not obtained.

Kiese (1949a) studied the pharmacological effects of acute 1,3-dinitrobenzene on dogs. These dogs received 10 or 20 mg/kg subcutaneous injections of 1,3-dinitrobenzene as a 1 to 2% solution in olive or poppy seed oil. The blood was examined for number of red and white blood cells, reticulocytes, cells with Heinz bodies; whole blood color; hemiglobin; (this author uses the word hemiglobin for the oxidized form of hemoglobin instead of the more common methemoglobin); verdoglobin (not translatable, presumably sulfhemoglobin or a similar compound); bile color and osmotic pressure of the red blood cells. The urine was examined for protein, bile color, and bilirubin. For those animals which died as a result of the poisoning, a histological examination of the organs was undertaken.

The effects of 1,3-dinitrobenzene on the blood of dogs receiving 10 and 20 mg/kg are shown in Figure I-6a and b and discussed below. For the 10 mg/kg dose, the maximum concentration of methemoglobin in the blood of the 10 test animals ranged from 3.0 to 11.0 g/100 ml of blood. The median was 6.6±0.9 g/100 ml blood or 53±3% of total blood color. This maximum in methemoglobin concentration occurred at approximately 12 hours after dosing. For the 20 mg/kg dose, the maximum methemoglobin levels ranged from 4.0 to 13.8 g/100 ml blood with a median of 10.2±0.4 g/100 ml of blood or 71.0±1.7% of total blood color. The maximum methemoglobin concentration occurred ~ 13 hours after dosing. At 24 hours after dosing, the mean methemoglobin concentrations were 27.7±2.8% and 48.2±3.1% of total blood color for the 10 mg/kg and 20 mg/kg dose, respectively. Methemoglobin levels in surviving animals did not return to normal for 58±4 hours for the 10 mg/kg doses and a mean of 80 hours for the 20 mg/kg dose.

Increases in verdoglobin concentrations in the blood were not always observed. When they were observed, they somewhat paralleled the methemoglobin variations.

A decrease in the number of red blood cells was observed. Heinz bodies were present in many of the red blood cells with as many as 100% of the cells having these inclusions within 24 hours after the 20 mg/kg dose. Maximum concentrations of Heinz bodies were observed at ~36 hours after the 10 mg/kg dose and 24 hours after the 20 mg/kg dose. The number of reticulocytes increased while the number of white blood cells showed an increase after a rapid decline.

No increases in the amount of protein, sugar or bilirubin were observed in the urine.

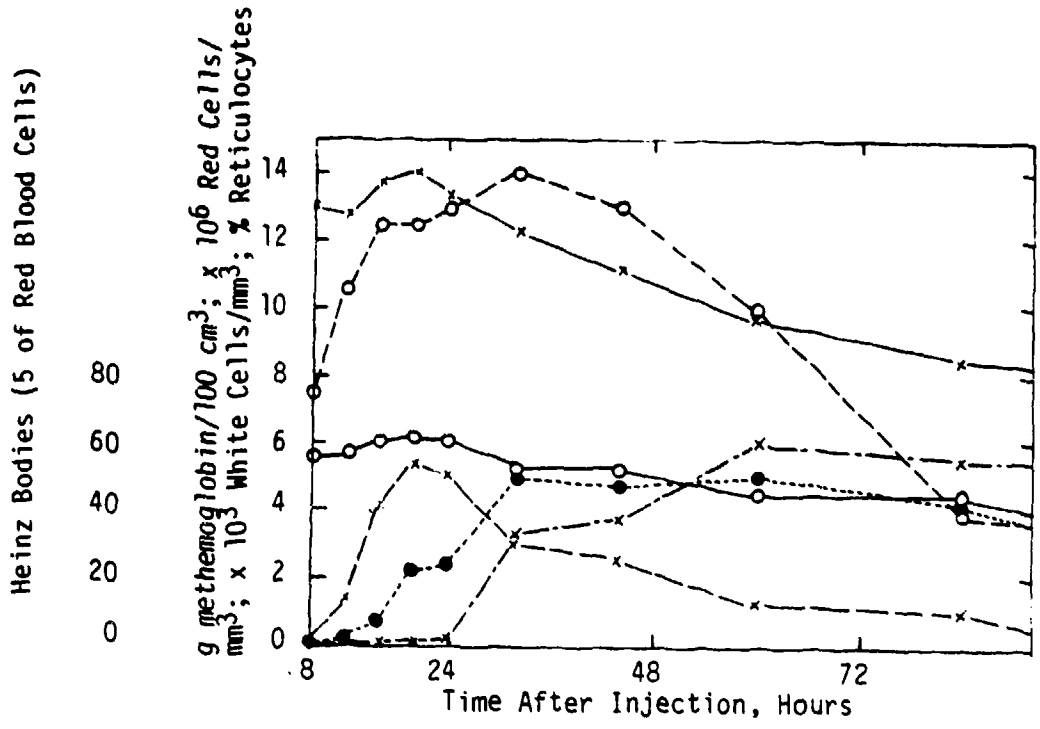


Figure 6a. Blood Profile of a 21 kg Dog After Subcutaneous Injection of 10 mg/kg 1,3-Dinitrobenzene as a 2% Olive Oil Solution

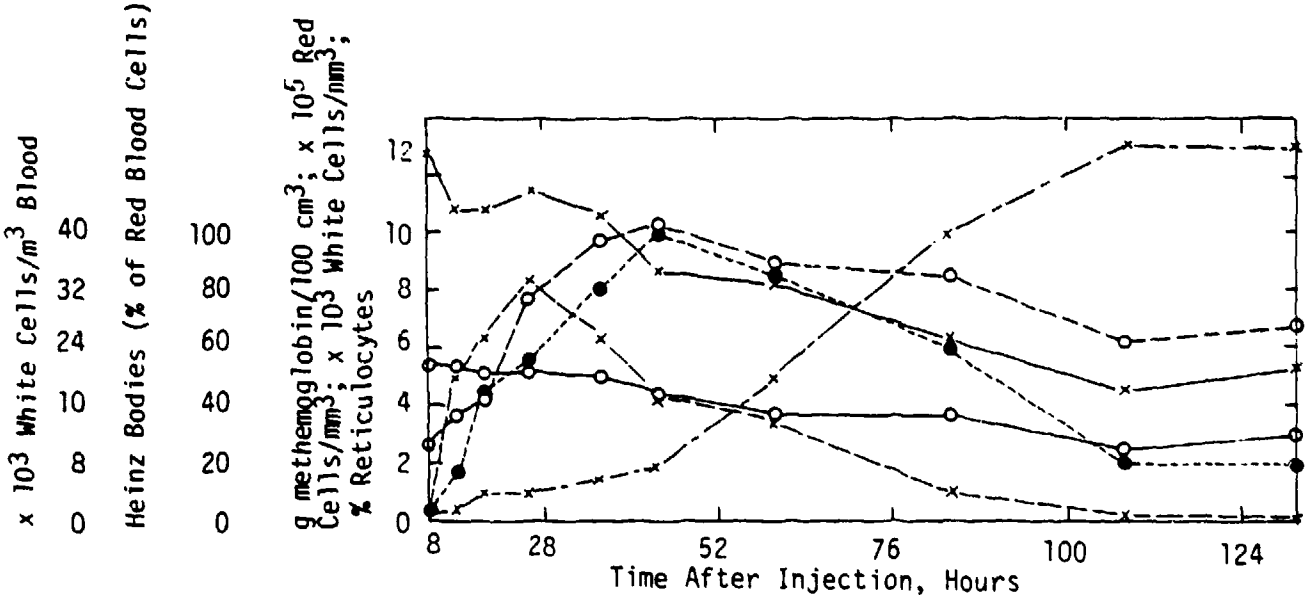
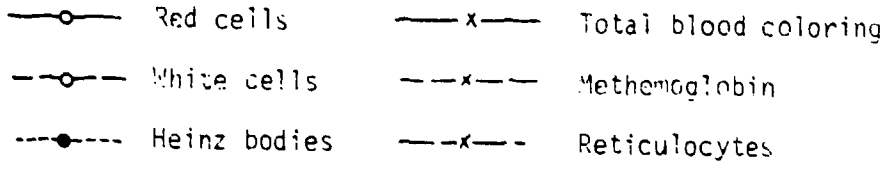


Figure 6b. Blood Profile of a 18 kg Dog After Subcutaneous Injection of 20 mg/kg 1,3-Dinitrobenzene as a 2% Olive Oil Solution



Kiese (1949b) also studied the effect of long term exposure to 1,3-dinitrobenzene on dogs. These dogs were given daily subcutaneous injections of 1 to 2% 1,3-dinitrobenzene in olive oil. Daily doses were 6, 2, 1.5, 1.0, 0.5, 0.2 and 0.1 mg/kg. Animals receiving the higher doses developed anemia and convulsions. Death followed shortly after the onset of convulsions. (See chronic toxicity) As shown in Figure I-7, the blood of animals receiving 1.0 to 1.5 mg/kg/day showed low levels of methemoglobin and reticulocytes. The red blood cell count and the total blood color dropped and the number of Heinz bodies in the red blood cells increased. At lower doses(0.1 and 0.2 mg/kg), the methemoglobin concentrations in the blood were too low to measure. As shown in Figure I-8a and b, there is a gradual decrease in blood color material, body weight and red blood cells. The number of Heinz-bodies in the red blood cells gradually increase. Death due to poisoning occurred in these animals on the last day shown in the figures. No increase in protein, sugar or bilirubin was found in the urine of these dogs.

The cat has been a favorite animal for studying the methemoglobin forming capacity of the arylamine and nitro compounds. Researchers who have used this animal to study the pharmacology of 1,3-dinitrobenzene include Heubner and Lo-Sing (1938), Issekutz (1939) and Bredow and Jung (1942).

Heubner and Lo-Sing (1938) studied the ability of the ortho, meta and para isomers of dinitrobenzene to form methemoglobin *in vivo* in cats. Dosing was accomplished by subcutaneous injection of an olive oil solution containing between 0.1 and 5% of the compound to be tested. Doses of 1.0, 2.0, 10, 17 and 37 mg/kg were administered. Loosely bound oxygen in the blood was measured before and after dosing. From the difference in manometer readings, the authors calculated the percentage of hemoglobin that was oxidized to methemoglobin. They then calculated the molar ratio of methemoglobin formed to 1,3-dinitrobenzene administered. This ratio varied from cat to cat and depended on dosage and time after dosage at which the readings were taken. For 1,3-dinitrobenzene, the following data were reported:

Wt. of Cats, kg	Dosage, mg/kg	Molar Ratio of Methemoglobin to 1,3-Dinitrobenzene Administered
2.7	1.0	0
2.5	2.0	19.6
4.6	10.0	1.7
2.5	17.0	3.0
2.0	37.0	1.4
2.5	2.0	7.0

The ratio for 1,2-dinitrobenzene was less then 1,3-dinitrobenzene while that of the para isomer was much higher.

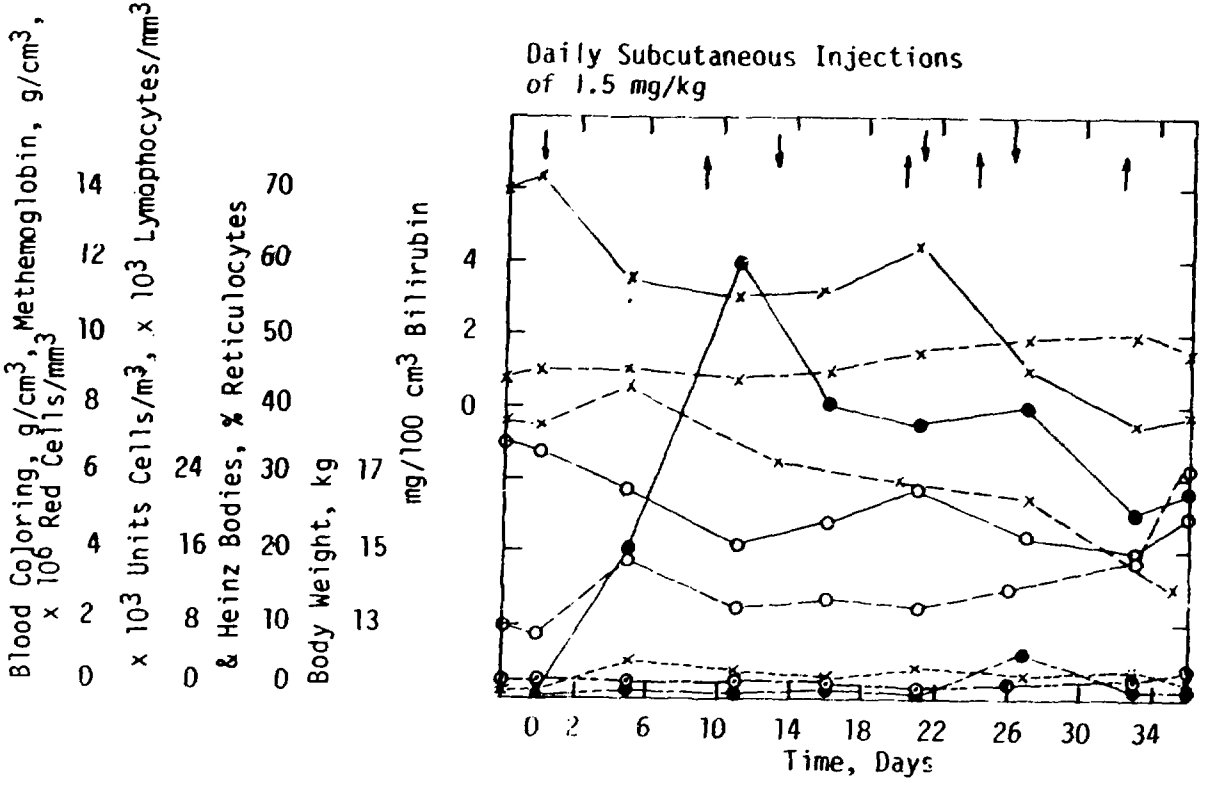


Figure I-7. Blood Profiles and Body Weight of Dog Receiving Daily Subcutaneous Injections of 1.5 mg/kg of 1,3-Dinitrobenzene as a 2% Poppy Seed Oil Solution (Kiese, 1949b)

Heinz Bodies
Reticulocytes
Lymphocytes
Red Cells
White cells
Total Blood Color
Bilirubin
Body Weight
Methemoglobin
Injections Interrupted
Injections Restarted

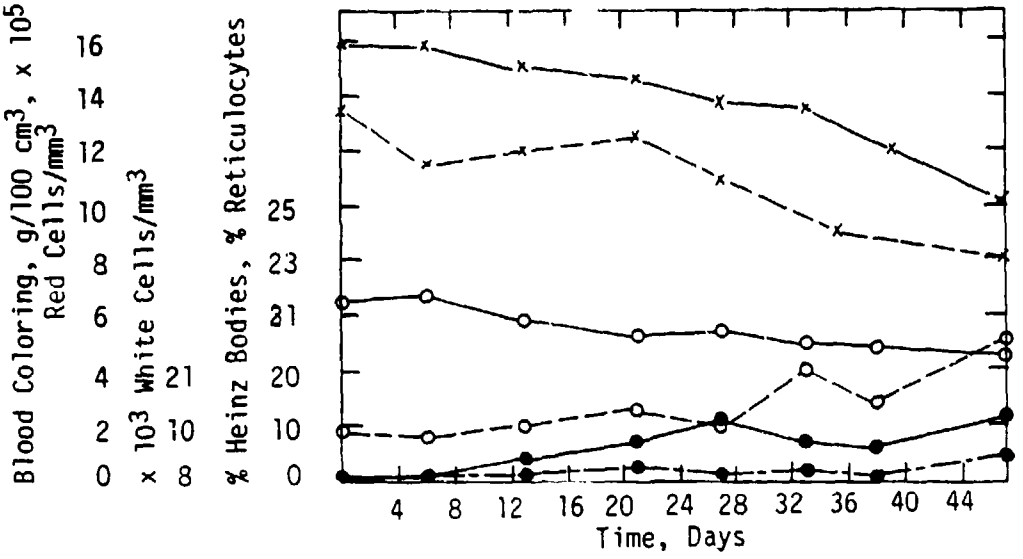


Figure I-8a. Blood Profile and Body Weight of a Dog Receiving Daily Subcutaneous Injections of 0.2 mg/kg of 1,3-Dinitrobenzene as a 1% Poppy Seed Oil Solution (Kiese, 1949b)

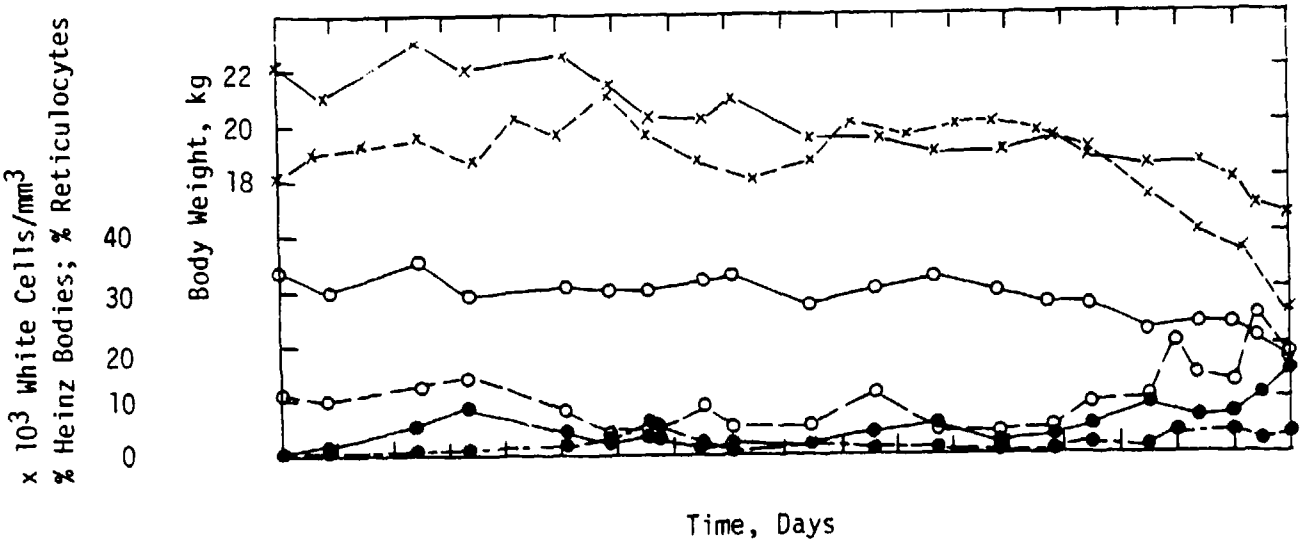


Figure I-8b. Blood Profile and Body Weight of a Dog Receiving Daily Subcutaneous Injections of 0.1 mg/kg of 1,3-Dinitrobenzene as a 1% Poppy Seed Oil Solution (Kiese, 1949b)

Issekutz (1939) studied the formation of methemoglobin in cats (1700-3000 g in weight) after subcutaneous dosage of 1,3-dinitrobenzene as a 1% olive oil solution. He used the spectrophotometric method for methemoglobin determination after showing that this method yielded almost identical results with the manometric method. Doses of 1, 5, 6.5, 8 and 10 mg/kg were administered. Insufficient data were presented to determine the time rate of methemoglobin formation and decline. However, the author did show that alcohol increased the amount of methemoglobin formed from exposure to the same doses of 1,3-dinitrobenzene. For example, the molar ratio of methemoglobin to 1,3-dinitrobenzene is approximately 5.3. In the presence of alcohol, this ratio rises to 8.0.

Bredow and Jung (1942) followed the course of methemoglobin and Heinz bodies formation in the blood of cats after single and multiple intraperitoneal injections of oil solutions of 1,3-dinitrobenzene. Doses ranged from 6.7 to 8.3 mg/kg. The time course of methemoglobin and Heinz bodies in the blood after a dosage of 6.7 mg/kg is shown in Figure I-9. Upon repeated injections of the same dose, 2-9 days later, the maximum methemoglobin levels occurred sooner. However, maximum levels were approximately the same as observed in the first dose.

Bodansky (1951) summarized the molecular ratio of methemoglobin to molar dosage for ortho, meta and para dinitrobenzene as follows:

-	o-dinitrobenzene	0.86 Bredow and Jung, 1942 3.7 Issekutz, 1939
-	m-dinitrobenzene	7.1 Issekutz, 1939 7.8 Bredow and Jung, 1942 6.4 Heubner and Lo-Sing, 1938
-	p-dinitrobenzene	55 Heubner and Lo-Sing, 1938 198 Issekutz, 1939

The data for this summary are from the papers discussed above. These summary numbers do not quantitatively agree with the data presented in the papers. However, qualitatively, there is agreement that para-dinitrobenzene is the most potent *in vivo* methemoglobin former of the three compounds. The meta-isomer is second in activity and the ortho-isomer third.

Another pharmacological effect of the nitro-aryl compounds has been reported - uncoupling of oxidative phosphorylation resulting in metabolic disruption and neurologic symptoms. Uncoupling of oxidative phosphorylation by dinitrophenol is well known. Whether 1,3-dinitrobenzene or any of its metabolites produce this effect is unclear. However, the presence of dinitrophenol as a metabolite of 1,3-dinitrobenzene could explain some of the effects on the nervous system which have been observed as a result of 1,3-dinitrobenzene poisoning. These effects could also be a direct result of methemoglobinemia.

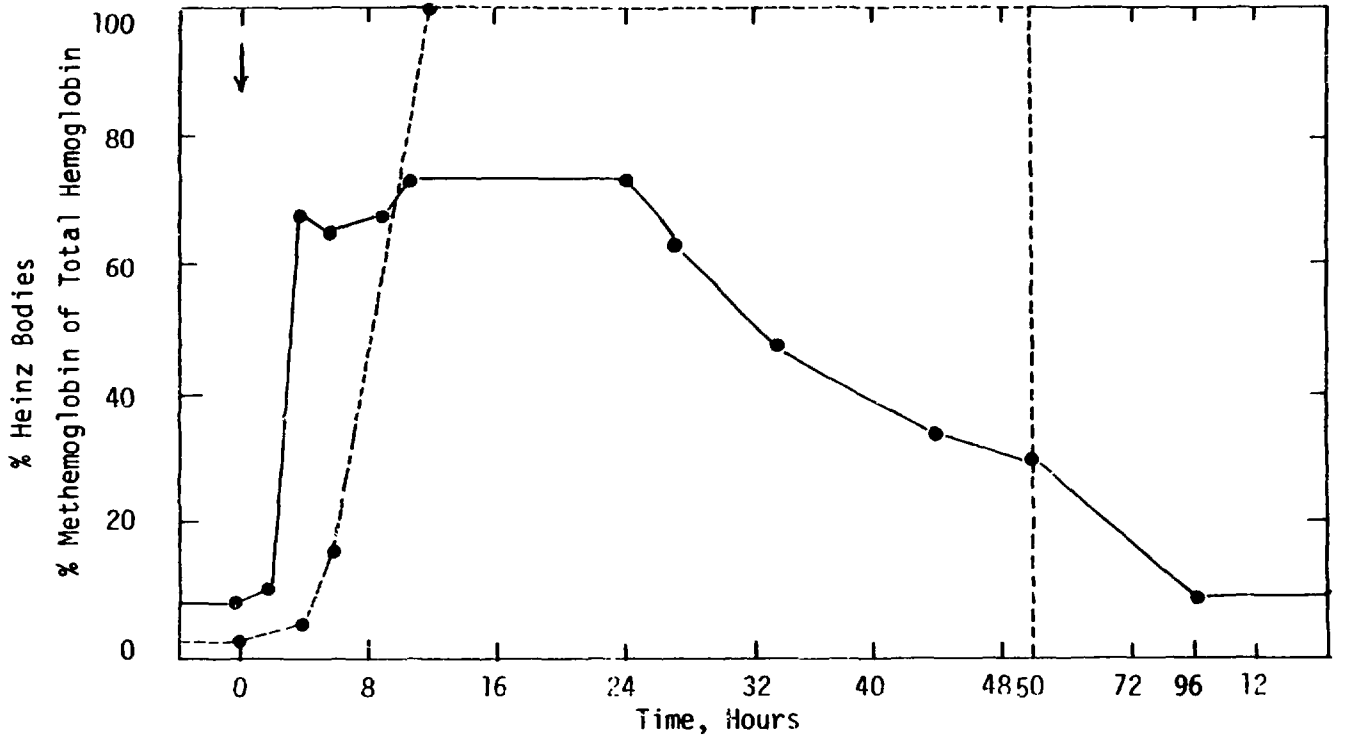


Figure I-9. Blood Profile of a 3 kg Cat After Intraperitoneal Injection of Injection of 1,3-Dinitrobenzene in Poppy Seed Oil (Bredow and Jung, 1942)

——— Methemoglobin

-------- Heinz Bodies

The effects of 1,3-dinitrobenzene induced methemoglobinemia on the peripheral motor nerve conduction velocity in rats were studied by Pankow *et al.* (1974) and Pankow *et al.* (1975). Male outbred albino rats, about 70 days old and weighing approximately 230 g, were used in the test. Each rat received a single intraperitoneal injection of 0.15 m mole/kg of 1,3-dinitrobenzene dissolved in polyethylene glycol M600. Blood samples were taken from the retro-orbital plexus at 0.5, 1, 3, 4 and 24 hours after dosing and then periodically for 28 days for methemoglobin determination. Determination of the sciatic nerve conduction velocity was accomplished at room temperature after hexobarbital anaesthetization. All reported values were based on the mean value obtained from 8 to 10 rats.

The methemoglobin content of the blood increased quickly to 60% of total hemoglobin level. The methemoglobin level in the 4-hour blood sample had dropped to 40% of total hemoglobin. The methemoglobin level continued to decline and had reached normal levels (0.7±0.5% of total hemoglobin) by 24 hours after dosing. The leucone aminopeptidase activity at 24 hours had increased 60% over that observed for the controls (260 U/l compared to 185 U/l for the controls). The sciatic motor nerve conduction velocity at 24 hours after dosing showed a decrease of 16% from that observed in the controls (57.2±2.9 m/sec for the controls and 48.0±6.2 for the poisoned rats). This decrease in sciatic motor conduction velocity was significant at the $P<0.001$ level. Motor nerve conduction velocity was believed to be due the impaired unloading of oxygen in the tissues following methemoglobinemia.

2. Effects of Human Exposure

a. Epidemiology

The very old literature contains some brief accounts of 1,3-dinitrobenzene poisoning to babies by label inks containing this compound. No information was uncovered relating to human poisoning through drinking water containing 1,3-dinitrobenzene. However, a large body of literature on human poisoning by this compound does not exist. These poisonings were mainly as a result of roburite production or loading operations, laboratory nitration reactions in schools and workers taking roburite home.

b. Occupational Exposure Studies

The use of 1,3-dinitrobenzene in the explosive, roburite, resulted in a high frequency of industrial poisonings from this compound. The major occupational exposure to 1,3-dinitrobenzene occurred prior to 1940. Poor hygienic conditions in the workplace were the main cause of industrial poisonings due to this compound. The number of pre-1940 cases of industrial poisonings from 1,3-dinitrobenzene is too large to describe each case in detail in this report. Therefore, the findings of these earlier investigators will be summarized and a few of the more recent cases of occupational exposure to this compound will be discussed in detail.

(1) Summary of Pre-1940 Occupational Exposure to 1,3-Dinitrobenzene

In general, the occupational exposure to 1,3-dinitrobenzene in the early 1900's was traced to inhalation of the dust or vapors and absorption of the compound through the skin (Müller, 1923; Koelsch, 1917). However, it must be remembered that proper hygienic conditions were not employed during this period. Thus, ingestion of 1,3-dinitrobenzene, due to contamination on the hands and under fingernails, can not be ruled out.

The main effect of 1,3-dinitrobenzene poisoning in humans is the formation of methemoglobin with the concurrent reduction in the oxygen carrying capacity of the blood (Rabe, 1919; Clark and Paul, 1935; Ishihara *et al.*, 1976; Beritić, 1956 and others). The presence of methemoglobin has not been demonstrated by all investigators. This failure to find methemoglobin may be due to insensitive chemical procedures; decomposition of the methemoglobin before detectable amounts are accumulated in the blood due to slow poisoning; or the length of time between poisoning and the tests. In general, a reduction in red blood cells (Müller, 1923; Steiner, 1918) and hemoglobin is reported. The presence of hematin (Olson, 1918; Koelsch, 1917) and Heinz bodies (Beritić, 1956) have also been reported. Other reported blood abnormalities include polychromasia (Walker, 1908) aniso- and poikilocytosis (Walker, 1908).

The majority of outward symptoms and pathological findings can be related to the alteration or destruction of the hemoglobin and resulting oxygen deprivation. The most obvious symptoms of 1,3-dinitrobenzene poisoning is cyanosis. Other symptoms include changes in the nervous system and respiratory and circulatory systems, dark (chocolate) colored blood, enlarged liver and spleen and highly colored urine. The degree of the symptoms vary depending on the type (acute, subacute, chronic) and degree of exposure (Steiner, 1918), as well as the health of the individual exposed (debilitated persons and alcoholics have lower resistance [Steiner, 1918]). Often the symptoms do not appear until several hours after exposure (Steiner, 1918; Koelsch, 1917; Clark and Paul, 1935 and others). In slight cases of exposure, symptoms disappear within 2-3 days (Steiner, 1918; Hüber, 1891). In contrast, symptoms can remain for 10 days to several months for severe cases of exposure.

The most obvious outward signs of oxygen deprivation is cyanosis. The cyanosis may be observed as a blue-grey coloring of the mucous membranes in slight exposure cases (Steiner, 1918). In severe cases, the skin is colored blue-black or a dark brown. The effects on the central nervous system are also characteristic of oxygen deprivation. These symptoms include headache, sleeplessness, fatigue in mild cases (Koelsch, 1917; Hüber, 1891). Other central nervous symptoms appear depending on the severity of the poisoning and include flickering before the eyes, numbness in the extremeties, tremors, staggering gait, vertigo, convulsions (Steiner, 1918; Capellini and Zanotti, 1946). In more severe cases, excitement, psychosis (Hübner, 1919 and Müller, 1923), unconsciousness and coma (Steiner, 1918; Clark and Paul, 1935; Hübner, 1919; Stukowski, 1922) can occur. Usually the patient recovers but deaths have occurred as a result of circulatory and respiratory failure (Koelsch, 1917).

Effects on the respiratory and circulatory systems are observed in moderate to severe cases of 1,3-dinitrobenzene poisoning. The symptoms include dyspnea and air hunger (Steiner, 1918), weak and rapid pulse (White and Hay, 1901; Steiner, 1918; Hüber, 1891). In more severe cases, chest pain, palpitation and oppression may be present (Clark and Paul, 1935) and very low blood pressure (Müller, 1923). As mentioned previously, circulatory and respiratory failure have been reported (Koelsch, 1917).

Gastrointestinal tract upsets vary from a metallic or bitter almond taste and loss of appetite (White and Hay, 1901; Koelsch, 1918) to nausea, vomiting and constipation in the more severe cases (Steiner, 1918).

1,3-Dinitrobenzene is a potent *in vivo* methemoglobin former. The clinical picture of poisoning by this compound is mainly a result of the destruction of the oxygen carrier pigments in the blood. Due to the destructive effects of this compound on the blood and blood pigments, enlarged liver and spleen and icterus are often observed in severe cases of poisoning (Walker, 1908; Olsen, 1918; Koelsch, 1918; Müller, 1923; Beritić, 1956). Pathological findings include yellow atropy of the liver (Walker, 1908) and fatty degeneration of the liver (Koelsch, 1917).

The urine of patients with subacute or chronic poisoning by 1,3-dinitrobenzene is usually dark in color (Müller, 1923; Steiner, 1918; White *et al.*, 1902) and highly acid (White *et al.*, 1902). The presence of urobilin has also been reported in severe cases of poisoning (White *et al*, 1902). The presence of trace amounts of 1,3-dinitrobenzene has also been found on occassion (Koelsch, 1918; White *et al.*, 1902).

(2) Recent cases of Occupational Poisoning Due to 1,3-Dinitrobenzene

Rejsek (1947) described several interesting case studies of 1,3-dinitrobenzene poisoning. All of his patients filled prepared bombs with molten 1,3-dinitrobenzene. Frequently the melt spilled over and stuck to the walls and floor. The solidified compound was scrubbed from the walls, but not from the floor. The first case described was a 20-year old male, who had been working with 1,3-dinitrobenzene for six months. He became blue at work, had a fever, felt weak, vomited and had shortness of breath upon climbing stairs. After being declared unfit for work, and not feeling well even two weeks after the cyanosis was over, he was sent home to recover. Four weeks after the cyanosis had subsided, he drank a glass of beer. About three hours later, he had nausea and headache. Soon afterward, he vomited and became blue. After vomiting all night, he was sent to Rejsek in the morning. Upon admission, the cyanosis was markedly reduced, being more pronounced on the mucous membrane of the palate. However, his palms were yellow and the skin beneath his fingernails and toe nails was orange colored.

The day after admission, the worker was no longer cyanotic, and it was not possible to determine how much poisoning still persisted. Therefore, an alcohol mobilization was attempted, based upon the patient's statements given and on previous work on TNT poisoning (Rejsek, 1947). The

man was then given a pint of beer. Half an hour later, he had a headache, and an hour later, he began to turn blue. Two hours after drinking the beer, he was ash-blue all over. His cyanosis did not recede until the next day. During this time, blood was drawn several times. It was chocolate brown and showed only the spectrum of (normal) oxyhemoglobin. Microscopic examination revealed 2-4% Heinz bodies. Blood serum analyzed polarographically prior to the experiment was normal. At the height of cyanosis, the serum showed a typical double-wave of dinitro compounds.

In addition to these findings, it was found that exposure to sunlight produced a similar response. A week after the beer experiment, the worker took a sunbath for an hour, undressed from the waist up. Immediately afterward, his lips were cyanotic, and he had a severe headache. Both symptoms had disappeared by the next day. Subacute poisoning thus became acute under the influence of alcohol or sunlight. These effects were already known, and employees working with nitroaromatics were forbidden to drink alcoholic beverages. They were also advised to avoid resting in the sun (Rejsek, 1947; Fairhall, 1969).

Two other detailed case studies were included in the Rejsek report, both with essentially identical circumstances. The only difference for these two cases was that no Heinz bodies were present. No methemoglobin was ever observed spectrophotometrically. However, with the spectrophotometer used, the detection limit for methemoglobin was 30%. By the time this level of methemoglobinemia is reached, cyanosis had been long present, and the blood had become chocolate-brown.

The number of Heinz bodies present was not directly dependent upon the intensity of cyanosis. In the first case, only about 2-3% Heinz bodies were present with strong cyanosis. For the other two workers, even in the presence of intense cyanosis no Heinz bodies were found. In one other case in which cyanosis was hardly marked, there were 60% Heinz bodies. This worker was not a hospital patient, and the alcohol mobilization experiment was not conducted on him. From these two observations, Rejsek (1947) noted that the maximum of Heinz bodies seemed to be present sometime after the most intense cyanosis.

In all cases described, no nitrocompounds were present polarographically prior to alcohol consumption (Rejsek, 1947). It was therefore possible to detect poisoning by dinitrobenzene even after a considerable length of time. For example, the first worker underwent the alcohol mobilization study more than six weeks after his cyanosis had ceased. After drinking the beer, there was subsequent cyanosis and appearance of nitrobodies in the polarograph. Rejsek (1947) suggested that this method could be an important contribution to the diagnosis of poisoning by dinitrobenzene.

The data supplied by Rejsek suggests accumulation of 1,3-dinitrobenzene or some metabolite in the human body for several weeks and confirms earlier data on alcohol and sunlight mobilization. However, no

evidence is available on how or where the 1,3-dinitrobenzene or its metabolite is stored in the body or the mechanisms leading to alcohol or sunlight mobilization of the symptoms.

An important factor to remember in the diagnosis of 1,3-dinitrobenzene poisoning is that each individual responds differently. The toxic effects of aromatic nitrocompounds are known to vary with intensity and length of exposure, and with individual susceptibility (Beritić, 1956; Monks, 1902; Steiner, 1918). An account of two patients who worked under identical conditions but developed different clinical pictures was given by Beritić (1956). The two patients were female process workers in a chemical firm manufacturing 1,3-dinitrobenzene in small quantities on an occasional basis. Occupational exposure during this synthesis occurred in the handling of the product before and after recrystallization. Although the workers were provided with protective gloves, and warned against the hazard of handling the substance with bare hands, they did not always wear the gloves. Thus exposure could have been due to skin absorption or ingestion as a result of poor hygienic procedures. For both of these workers, the length and period of exposure ($\sim$ one week) and the kind of work performed were equal. Thus, although it is difficult to assess the degree of exposure for each individual, all other parameters of the exposure were equal.

The first case was a woman, aged 22 years. She had abdominal pain, nausea, dizziness and cyanosis of the lips, oral mucosa, conjunctivae, ears and finger tips when admitted to the hospital. Examination revealed a slightly elevated temperature 99.3°F, high pulse rate (98) and a blood pressure of 105/55. The liver was very tender and palpable. Laboratory analyses performed on the patient's blood showed that 28% of the total hemoglobin was methomoglobin. Reticulocytes were 1.8% of the blood cells. No Heinz bodies were present. Serum bibirubin was slightly elevated (1.66 mg/100 ml), however, the blood urea, serum iron and fasting blood sugar were all within normal levels.

The second case was a 25-year-old woman from the same place. She had had the following symptoms for three days: fatigue, weakness, loss of appetite, and nervousness. Her lips were slightly blue. No nausea, abnominal pain, headaches or elevated temperature were experienced. Upon examination she was found to be slightly cyanotic, pulse rate of 76 per minute with a blood pressure of 120/65. No tenderness was found in the liver area. Methemoglobin levels were 60% of the total hemoglobin. Reticulocytes and Heinz bodies were found to be 4.7 and 38% of the blood cells, respectively. No abnormal coloration was observed in the urine.

No evidence of retention of 1,3-dinitrobenzene or any metabolite was shown in these case histories. However, no mobilization tests (alcohol or sunlight) were attempted.

These cases confirm the multitude of earlier literature reports in that the poisoning due to 1,3-dinitrobenzene is highly dependent on the individual exposed. The state of health of the individual, his diet,

and even his nationality have an effect on the symptoms which will be observed (Beretić, 1956; Rejsek, 1947; Steiner, 1918; Monks, 1902; Koelsch, 1917). These observations have been made too frequently to be attributed to artifacts.

A case study of an industrial exposure to 1,3-dinitrobenzene was reported by Ishihara *et al.* (1976). A 37-year old female worker in an electronics factory was engaged in the arrangement of chemically-immersed electronic parts for approximately 30-45 minutes per day. In performing this task, the worker wore protective latex gloves. A new chemical mixture containing 0.5% w/w 1,3-dinitrobenzene was introduced for cleaning the parts. Three days following the initial exposure, her lips and fingernails were cyanotic. After spending a day at home, all of her symptoms disappeared and she returned to work. However, cyanosis, general malaise and anorexia appeared within two days after returning to work and she was admitted to the hospital. Upon admission, physical examination revealed that her face and sclerae were icteric and the liver was palpable. Liver function tests and hematology were negative except for increased bile pigments due to an industrial chemical. No determination of methemoglobin was made of this worker.

In order to determine the details of this intoxication, a simulation study was initiated. A male volunteer, aged 30 years, was asked to follow exactly what had been done by the patient. The volunteer performed the same work as the patient for the same amount of time (45 minutes). During this period, ambient air was monitored in the volunteer's breathing zone. Midget impingers containing a 1:1 ethanol:water mixture were used to sample the air. 1,3-Dinitrobenzene analysis was accomplished by reduction with Zn powder followed by diazotization with $NaNO_2$, ammonium sulfamate and N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting color was measured at 560 nm on a spectrometer. Detection limit was 250 $\mu g/m^3$ of air. Venous blood samples were taken immediately before and after exposure as well as 3, 5 and 21 hours afterwards for determination of methemoglobin. Urine samples were obtained as excreted for 21 hours. These samples were analyzed for metabolites spectrophotometrically after hydrolysis, reduction with Zn and diazotization. Rates of excretion of diazo-positive urinary metabolites were reported as mg of 2,4-dinitrophenol/hr.

The concentration of methemoglobin in the volunteer's blood increased from 3% before exposure to 11% immediately after exposure. The methemoglobin concentration remained at this level for 3 hours after which time it fell rapidly. No methemoglobin was detected in the blood sample taken 21 hours after the 1,3-dinitrobenzene exposure. Urinary metabolites (measured as 2,4-dinitrophenol) of 1,3-dinitrobenzene increase from the 0.2 mg/hr preexposure levels to a maximum of 0.6 mg/hr. The rate of excretion of diazo-positive urinary metabolites returned to the preexposure levels 10 hours after exposure. During the period of the volunteer's exposure, no 1,3-dinitrobenzene was detected in the air.

From the volunteer experiment, Ishihara *et al.* (1976) suspected skin exposure. To confirm this suspicion, new latex gloves of the same brand utilized by the patient and volunteer were studied to determine whether

they were permeable to 1,3-dinitrobenzene. The finger portions of the gloves were removed and water was added to each. The fingers were dipped into the same solution as was used by the patient, for a similar amount of time. Thus, the finger portions were dialysis bags with water inside and sample solution outside. The water was then analyzed for 1,3-dinitrobenzene. Results of five such experiments revealed an average of 181 ppm of 1,3-dinitrobenzene in the internal water. Absence of 1,3-dinitrobenzene in the ambient air excluded inhalation as a route of entry. This portion of the study, clearly determined that the exposure was due to skin absorption of 1,3-dinitrobenzene through the gloves. The authors even suggested that repeated use of the same gloves might have aggravated the exposure situation.

Linch (1974) monitored the urine of chemical workers exposed to nitro and amino aromatic compounds as an early warning of excessive exposure. In addition, he monitored blood to determine levels of methemoglobin. The reason for this study was that air analysis could not provide an adequate surveillance of compounds which entered the body through the skin. In addition, chronic or prolonged subacute exposure to these compounds usually produced reversible anemia without cyanosis. Therefore, the monitoring of over-exposure by appearance of cyanosis was not valid for these cases. Linch undertook a clinical and laboratory evaluation of 187 cyanosis cases during the ten years following 1956. The relationship of frequency of abnormal specimens to the probability of occurrence of cyanosis followed an S-shaped probability curve. If less than 12% of the blood specimens from a given work crew were abnormal, then cyanosis would not be expected to develop. A 20% control limit would predict more than 70% of potential cyanosis cases. It was also determined that changes in the ambient temperatures in working areas changed the incidence of cyanosis. An increase or decrease of $5^{o}F$ caused an increase or decrease of 7% in the occurrence of cyanosis (Linch, 1974). Of all the amino and nitro aromatic compounds tested, 1,3-dinitrobenzene had the highest potential for causing cyanosis-anemia (Linch, 1974). Urinary excretion data collected during cyanosis episodes were used to establish biological threshold limit values for 1,3-dinitrobenzene. The data from this study led Linch to recommend a revision of the threshold limit value (TLV) for 1,3-dinitrobenzene. He suggested that the new limit be set at 1 ppm, a value 1000 times more sensitive than that recommended by the 1971 American Conference of Governmental Industrial Hygienists. When urinary metabolites exceeded 10 mg/liter, it was recommended that corrective action be taken to reduce further exposure. Routine regular examination of urine and blood for each employee exposed to cyanogenic chemical provided a control of exposure to these chemicals. An interesting additional finding of this study was that certain workers were found to be predisposed to cyanosis. Five percent of the workers in the study were classified as chronic repeaters, and were permanently removed from areas of potential exposure to cyanogenic compounds. Pre-employment screening was recommended to identify these individuals.

In summary, 1,3-dinitrobenzene has been shown to produce toxic symptoms in man as a result of acute, subacute and chronic exposure. Absorption in occupational situations can occur through the skin, by inhalation

of dusts and vapor and by ingestion as a result of inadequate hygienic procedures. Symptoms are generally those associated with oxygen deprivation and initially closely resemble those of carbon monoxide poisoning. These symptoms vary with the degree of poisoning and with individuals. Deaths are rare and usually result from respiratory or circulatory collapse.

There is evidence that 1,3-dinitrobenzene or some metabolite is retained in the body for at least several weeks. Mobilization by alcohol or sunlight several weeks after all symptoms have subsided results in a return of severe cyanosis, nausea, etc. However, no studies have been conducted to determine what the retained agent is, how and where it is retained, or to illucidate the mechanism of mobilization.

3. Effects on Experimental Animals

a. Acute Toxicity

The earliest reported study on the acute toxicity to laboratory animals appears to be that reported by Hüber (1891). Earlier reviews (von Oettingen, 1941) report the work of Ollivier and Bergeron (1863) to be the first experimental toxic studies on dinitrobenzene. However, these studies were on nitrobenzene. Hüber (1891) performed multiple poisonings by 1,3-dinitrobenzene on rabbits, frogs and dogs. Doses were usually administered orally as a powder or an oil solution. The minimal fatal doses of 1,3-dinitrobenzene were:

frogs	:	0.005 - 0.01g
rabbits	:	0.4 - 0.5g
dogs	:	0.6g

Dosages based on weight can not be calculated since the weight of the animals was not reported. Hüber (1891) reported the main symptoms of 1,3-dinitrobenzene poisoning to be blood changes, dyspnea and paralysis of the central nervous system. He did not identify methemoglobin in the blood.

In 1901, White and Hay reported the results of acute toxicity tests with 1,3-dinitrobenzene to cats. The smallest lethal oral dose to a 6 pound cat was 0.08g (33 mg/kg). A dose of 0.06 g (22 mg/kg) was almost fatal and a dose of 1.2 g (44 mg/kg) was fatal within 3 hours. Hypodermically (subcutaneously) a dose of 0.04g was not fatal to a cat. These authors estimated that 1.82 g would be fatal to a 265 kg man. These authors observed cyanosis and effects on the central nervous system including loss of vaso-motor tone and symptoms of multiple neuritis.

Kiese (1949a) investigated the acute toxicity of 1,3-dinitrobenzene to dogs. The 1,3-dinitrobenzene was administered subcutaneously as a 1-2% solution in olive or poppy seed oil. A single 1,3-dinitrobenzene dose of 1 or 2 mg/kg had no observable effect on the dogs. A dose of 10 mg/kg was fatal to 9 of 19 dogs. Doses of 20 mg/kg killed 9 of the 12 dogs. From these results the LD50 was taken to be ~10 mg/kg.

Changes were noted in the dogs receiving the 10 and 20 mg/kg within one hour. After 3-5 hours, the dogs were quiet and appeared tired. A few animals receiving the 20 mg/kg had difficulty in standing at 3-5 hours. Most animals laid on their sides and the dogs on the higher dose exhibited spasms. Two dogs receiving the 20 mg/kg does died within 24 hours; one at 7 hours and the other at 13 hours after dosing. Other dogs died from 2 to 17 days after dosing. Blood chemistry of these dogs showed the presence of increased amounts of methemoglobin, Heinz bodies, decreased red blood cells, and an increase in reticulocyles (see Pharmacological Section). On autopsy, fluid was observed in the body cavities. Tissue degeneration was noted in the liver, spleen and kidneys.

Christian *et al.* (1976) used male and female rats aged 8 to 10 weeks, to determine the acute toxicity of 1,3-dinitrobenzene. These animals received the compound by oral gavage as a one percent suspension in corn oil. Signs of illness and fatalities were noted daily for three weeks after dosing. Doses used in this study were 36, 55, 66, 80, 120 and 180 mg/kg. For each of these six doses, 12 animals, six males and six females, were used. Ingestion of 1,3-dinitrobenzene caused illness characterized by reduction in ambulatory motions, ataxia, weakness, dyspnea and rapid heart beat. Paws, ears, lips and nostrils appeared cyanotic. As the illness progressed, there was eventual respiratory collapse and terminal coma. Deaths occurred in a period ranging from one to twenty-six hours. At the two lowest doses, there was no incidence of mortality. However, at the highest dose, all of the animals died. Both mortality and severity of illness were almost evenly distributed between sexes and were related to the magnitude of the dose. The LD50 for this experiment was 83.1 mg/kg (confidence limits 56-124) for both sexes. The males alone had an LD50 of 91 mg/kg (confidence limits 54.8-151 mg/kg) and the females had an LD50 of 81.4 mg/kg (confidence limits 50-133 mg/kg). Rats that received sublethal doses and became ill recovered promptly. After one week, they had all returned to normal.

b. Subacute Toxicity

The subacute toxicity of 1,3-dinitrobenzene to rats was studied by Christian *et al.* (1976). These rats were 8 to 10 weeks old at the beginning of the 6 week study. Four groups of rats, with 6 males and 6 females in each group, received various concentrations of 1,3-dinitrobenzene in their drinking water. The concentrations of 1,3-dinitrobenzene used were 0 (control), 50, 100 and 200 ppm. The rats were allowed water *ad libitum*. The weekly average dosage was calculated from the amount of water consumed. However, this calculation did not take into account loses of 1,3-dinitrobenzene through volatilization on adherence to the container walls. Weekly calculated dosages varied considerably.

The effects observed during this study included mortality, signs of illness, changes in growth rate, changes in food consumption, hematological changes, changes in metabolic enzyme activity, changes in specific organ weights and gross and microscopic pathology.

Three males died during the third week and one in the fourth week at the 200 ppm level. All the remaining animals survived the study. Other than mortality, the predominant effect of ingested 1,3-dinitrobenzene was a loss in body weight. The amount of weight loss was generally related to the concentrations of 1,3-dinitrobenzene in the drinking water. The loss in body weight also paralleled food consumption. This relationship was not further examined. In general the 1,3-dinitrobenzene was more toxic to males than females, causing mortality and a greater loss in body weight in the males than in the females. The effect is opposite of that observed in the acute studies.

Hematological examination of these rats was conducted by obtaining peripheral blood after 5 weeks of ingesting the specific concentrations of 1,3-dinitrobenzene. Parameters examined were hematocrit, hemoglobin content, number of leukocytes and percentage distribution of differential leukocytes. Neither the total number of leukocytes nor their differential distribution showed any marked change compared to controls. However, there was a consistent mild reduction in hemoglobin content and hematocrit. At the 200 ppm level, the hematocrit was reduced from 51% to 48% for males and from 49% to 42% for females. Hemoglobin contents at this same level decreased from 15.5 g/100 ml to 14.3 g/100 ml for males, and from 14.8 g/100 ml to 12.8 g/100 ml for females. Decreases for the two lower levels of 1,3-dinitrobenzene were also noted with the highest dosage level producing the largest decrease.

Changes in the metabolic activity of liver microsomal enzymes were measured indirectly by the length of the period of sleep induced by a certain dose of sodium hexobarbital. For females, there was a significant ($p<0.01$) decrease in sleeptime at all levels of 1,3-dinitrobenzene. Males on the 100 ppm level and an occasional male on the 50 ppm level showed some reduction in sleeptime, while males at the highest level showed no reduction at all.

Animals were terminated by exsanguination after the sixth week and the average terminal weights of the body and various organs were determined. For both males and females, there was a reduction in final body weight proportional to the concentration of 1,3-dinitrobenzene in the water. Spleen enlargement was evident in males at the 50 and 100 ppm levels and in females at all levels. In addition, atrophy of the testes was evident in males at all three levels. There was no weight reduction of ovaries in any of the females at any level. Other organs weighed included liver, heart, lungs, kidneys, brain, pituitary and adrenals. For these organs, weights were essentially the same as controls for both sexes and all dose levels.

Pathological examination was also conducted on the following organs: heart, aorta, lungs, liver, spleen, kidney, stomach, duodenum-pyloris, small intestine, large intestine, urinary bladder, prostate and testes, ovary and uterus, pancreas, thyroid, thymus, adrenals, pituitary, brain, spinal cord, eye, skeletal muscle and skin. In all animals of both sexes, the liver contained a deposition of a brownish-yellow pigment in the Kupffer's cells. This

deposition was less pronounced at the 50 ppm level and severe at the 200 ppm level. The testes of all males showed atrophy, while the ovaries of the females were not affected. In the 200 ppm group, both males and females showed atrophy and fibrosis of the spleen, with marked deposition of hemosiderin. No other significant pathological changes were found in the other tissues when compared to control animals.

In a 16 week study, Christian *et al.* (1976) provided four groups of 40 rats with drinking water containing 0 (control), 3, 8, and 20 ppm 1,3-dinitrobenzene. The experimental methodology and parameters observed were essentially the same as in the 6 week study. All the animals survived the 16-week study. For both sexes, the variations in water consumption between the four 1,3-dinitrobenzene levels was no greater than among the cases within levels.

The average weekly weights of both males and females on the 3 and 8 ppm levels were similar to controls. However, while females on the 20 ppm level showed a weight reduction after 8 weeks, males on this level grew as well as controls. A statistical relationship between body weight and food intake was obtained by covariance analysis (Christian *et al.*, 1976). With this analysis, final body weights were adjusted to the same food intake as controls. It was determined that the low final weight of females on the 20 ppm level was only slightly dependent upon reduced food intake. No explanation for this extra weight loss was given. Adjusted weights were comparable at other dosage levels.

Samples of peripheral blood were analyzed for hematocrit, hemohemoglobin content, number of leukocytes and percentage distribution of differentiated leukocytes. The same six male rats were used in each group after periods of 5, 10, and 14 weeks. Hemoglobin levels at the 20 ppm dose were decreased by 6% after 5 weeks and by 9% after 10 weeks. These values appeared to be returning to control levels by the 14th week, since there was only a 3% decrease of hemoglobin. After 5 weeks at the 20 ppm level, the hematocrit had decreased by 7%. At 10 and 14 weeks, the hematocrit was similar to the control. At all other levels of 1,3-dinitrobenzene, there were no effects attributable to the compound.

The average length of sleep resulting from a standard dose of sodium hexobarbital was determined after rats had been receiving 1,3-dinitrobenzene for 14 weeks. Generally, there was no significant difference in the length of period of sleep for any of the animals at any level. However, males at the 8 ppm level had significantly ($p<0.01$) prolonged sleep periods. The author noted that in the absence of similar changes in males on greater intake levels, and with females exhibiting no effects, the increase represents an improbable sampling error, or may be due to some extraneous factor (Christian *et al.*, 1976).

At the end of 16 weeks, animals were exsanguinated and weights of the body and various organs were determined. In both sexes, spleens were increased in weight in proportion to the 1,3-dinitrobenzene content of the water. The increases were statistically significant (level of significance not given) at the 8 and 20 ppm doses (Christian *et al.*, 1976). The testes were sharply (60%) reduced in weight only at the 20 ppm level. Other organs examined included liver, heart, lungs, kidneys, brain, ovaries, pituitary and adrenals. These weights, along with body weights, showed no remarkable change attributable to 1,3-dinitrobenzene.

Pathological examination of the spleens of all of these animals revealed deposition of hemosiderin. This effect was most pronounced at 20 ppm of 1,3-dinitrobenzene in the water. The testes showed no abnormalities in animals exposed to 3 or 8 ppm of the compound. However, a slight to moderate atrophy of the testes was evident in the 20 ppm group. There were no pathological alterations that could be attributed to 1,3-dinitrobenzene exposure in the other organs examined. These organs included heart, aorta, lungs, liver, kidney, stomach, duodenum-pyloris, small intestine, large intestine, urinary bladder, prostate, ovary and uterus, pancreas, thyroid, thymus, adrenals, pituitary, brain, spinal cord, eye, skeletal muscle and skin.

The methodology used in these subacute studies is questionable. The use of drinking water *ad libitum* to supply a toxic chemical to an animal leads to variation in daily dosage. In addition the actual intake of water or chemical can never be known with any certainty. The parameters observed in this study lead one to believe that the authors were not familiar with the literature on 1,3-dinitrobenzene intoxication. For example, the blood was not monitored for methemoglobin or the presence of Heinz body. Behavioral effects which indicate nervous system toxicity were also not monitored.

c. Chronic Toxicity

K se (1949b) studied the chronic toxicity of 1,3-dinitrobenzene to dogs. The dogs received daily subcutaneous injections of 2.0, 1.5, 1.0, 0.5, 0.2 and 0.1 mg/kg. One dog received a 6 mg/kg dose on days 1, 2, 6, 7 and 8. This animal died on the eighth day after beginning to convulse on the seventh day. Other animals were dosed for various lengths of time. Dosing was sometimes interrupted and restarted a number of times due to the appearance of convulsions and nervousness. At doses of 2.0, 1.5, 1.0 and 0.5 mg/kg, the animals died shortly after convulsions started and dosing stopped. Of the 5 animals receiving the daily 2 mg/kg dose, death occurred between 8 and 10 days in 3 animals. The dosage was stopped on the 9th day in the fourth dog and the fifth dog lived for 24 days. A general loss in weight was observed as well as a decline in blood pigments material. Heinz bodies were observed in 24 to 54% of the red blood cells. Nervous disturbances and spasms were observed usually around day 5 to 8.

Twelve dogs received daily doses of 1.5 mg/kg. As with the 2.0 mg/kg dose, losses in weight were observed. Blood pigment concentrations also declined. Heinz bodies were observed in 15 to 43% of the red blood cells. Nervousness and spasms were observed as early as day 7. The time of death varied from 17 to 113 days, however, the dosing was irregular making it difficult to draw any conclusions from the data.

Three animals each received daily doses of 1.0 and 0.5 mg/kg. As in higher doses, a general loss in weight and decline in percentage of blood pigment were observed. Heinz bodies were observed in 21-30% and 15 to 20% of the red blood cells at the 1.0 and 0.5 mg/kg doses, respectively. The on-set of nervousness and spasms varied widely with death following closely behind the on-set of spasms.

Doses of 0.2 mg/kg were given daily to 7 days. The dosings were for 20, 25, 30, 40, 48, 68 and 70 days. Only two animals died (48 and 68 days of dosing). Slight weight losses and a decline in blood pigment were observed. Heinz bodies were in 6 to 15% of the red blood cells.

At the 0.1 mg/kg doses, one of the two dogs died at 144 days (14.4 mg/kg total dosage). The other dog was dosed daily for 8 months (23.4 mg/kg total dose) without any sign of nervous disorders.

This study is the only long term poisoning with 1,3-dinitrobenzene found in the literature. In general the doses used were too high and death occurred prematurely. Even at the lowest dosage tested (0.1 mg/kg), 1,3-dinitrobenzene killed one of the two animals. This dosage is 1/100 of the reported LD50 of 10 mg/kg for dogs. Usually chronic doses of 1/25 LD50 are tolerated. The toxicity of 1,3-dinitrobenzene at 1/100 LD50 appears to indicate a degree of accumulation. However, the accumulation potential of 1,3-dinitrobenzene has never been studied.

3. Teratogenicity, Mutagenicity and Carcinogenicity

Teratogenic evaluations for 1,3-dinitrobenzene have not been reported. The compound has been evaluated for mutagenicity by the Ames Salmonella Mutagenicity Assay and for DNA damage by the *E. coli* toxicity test and the yeast recombinogenic assay.

Simmon *et al.* (1977) subjected 1,3-dinitrobenzene to the Ames screening procedure before and after chlorination and ozonation. Over a concentration range of 0.92-46 µg/plate, no mutagenicity was demonstrated to any of the tester strains used (TA1535, TA1537, TA1538, TA98, TA100) with or without activation. Chiu *et al.* (1978) subjected 1,3-dinitrobenzene to this procedure and demonstrated mutagenicity of the 1,3-dinitrobenzene to strain TA98 over a concentration range of 10 µmoles (1680 µg) to 0.1 mole (16.8 µg) per plate without activation. McGregor (1979) subjected 1,3-dinitrobenzene to testing in the bacterial system and reported a positive, dose-related response in TA98 over a 3 log range.

Methodology for this mutagenic screening procedure is that specified by Ames (Ames *et al.*, 1975). In this procedure, *S. typhimurium* strains auxotrophic for histidine (his^-) are incubated on histidine deficient media with the sample in the presence or absence of an enzyme system capable of metabolizing many chemicals. Only those cells reverting to histidine independence (his^+) will grow and form colonies on the histidine deficient media. These colonies are scored for evaluation of the sample when compared to positive and negative control levels. Two criteria important to the evaluation of the completed tests are: 1) positive tests must exhibit a dose response effect over at least three concentrations separated by at least half-log steps. 2) response to dose must equal at least two times the control level to be considered positive.

Simmon *et al.* (1977) did not demonstrate mutagenicity of 1,3-dinitrobenzene at the low levels they tested, however, Chiu *et al.* (1978) and McGregor (1979) demonstrated a mutagenic response to TA98 at higher levels over a concentration range spanning two logs. In the Chui paper, actual data were not provided for evaluation of dose response or actual numbers of revertants scored. These studies indicate that 1,3-dinitrobenzene is genetically active in the Ames test, however, further studies should be performed to confirm thise results.

Simmon *et al.* (1977) reported mutagenicity testing of 1,3-dinitrobenzene in the yeast *Saccharomyces cervisiae*. This yeast is a diploid microorganism heterozygous for a mutation resulting in a defective enzyme in the adenine metabolic pathway. Homozygous mutant cells produce a red pigment when grown on medium containing adenine. Such homozygous mutants can be generated by mitotic recombination. The frequency of this recombinational event may be increased by incubation of the organism with potential mutagens and the degree of mutagenicity determined from the number of red pigmented colonies appearing on the plates. The munitions wastewater chemicals tested in this system gave no evidence of mutagenic activity. McGregor (1979) also tested 1,3-dinitrobenzene in this system. His results indicate recombinogenic activity with metabolic activation.

McGregor (1979) subjected 1,3-dinitrobenzene to the *E. coli* toxicity test. This test is a DNA repair test in which an *E. coli* strain with normal repair function and an *E. coli* strain with defective repair function are incubated with the test chemical. Plates are evaluated for preferential killing of the repair deficient strain, indicating DNA damage. No DNA damage to *E. coli* as measured by this test was observed.

Purchase *et al.* (1976) reported on evaluation of six short term tests for detecting organic chemical carcinogens (Ames test, cell transformation assay, Rabin's test, subcutaneous implants, sebaceous gland suppression, tetrazoleum reduction). One of the chemicals subjected to these six short-term tests was 1,3-dinitrobenzene. Results of those tests demonstrated a positive response only in the sebaceous gland suppression test. Purchase *et al.* (1976) indicate that only the Ames test and the "cell transformation" assay were suitable tests for detection of potential carcinogens. This study was primarily for evaluation of the short term tests and did not provide data for evaluation of test results.

Evaluation of mutagenic studies must be approached with caution. No single test system is adequate for a submammalian mutagen screen. False responses may occur with any one test. Microbial assays are useful in identification of those samples and/or their breakdown products which are genetically active and provide an indication of their relative potency. Extrapolation from these systems to assessment of human risk is beyond the capability of these test systems.

E. Environmental Effects

1. Entry into the Environment

The U.S. Army unintentionally produces 1,3-dinitrobenzene during the manufacture of TNT. Chandler *et al.* (1972) identified 1,3-dinitrobenzene using thin layer chromatography in the final product of TNT. The 1,3-dinitrobenzene is produced from the nitration of benzene impurities in the toluene. Of the possible dinitrobenzene isomers, 1,3-dinitrobenzene is approximately 93% of the product. Typical benzene impurity concentrations in toluene are 250 mg/l (Kohlbeck and Chandler, 1973). If all the benzene present is nitrated, then 0.018% of the TNT crude product could be 1,3-dinitrobenzene. Thus, the maximum amount of 1,3-dinitrobenzene produced by each TNT line would be approximately 17 lb/day.

Discharges of 1,3-dinitrobenzene in the process effluent are considerably less than 17 lb/line/day due to:

- presence of 1,3-dinitrobenzene in the TNT final product (Chandler *et al.*, 1972)
- incomplete nitration of the benzene to 1,3-dinitrobenzene
- degradation of 1,3-dinitrobenzene during the processing.

Kitchens *et al.* (1978) predicted environmental discharge of 1,3-dinitrobenzene from TNT manufacture to range from 0.2-2.0 lb/line/day. However, losses are highly dependent on the process used, batch or continuous, and the purity of the toluene. Spanggord *et al.* (1978) determined the average 1,3-dinitrobenzene levels of 79 samples in the evaporator condensate water at Volunteer AAP to be 2.05 ppm. One continuous line was in operation when these samples were collected.

The Holston AAP blends TNT with RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine). The levels of 1,3-dinitrobenzene in Holston AAP effluents have not been measured. At full mobilization, an estimated 650 lb/day of TNT would be released into the Holston River. Using the 0.017% 1,3-dinitrobenzene in TNT ratio, 1.1-2.2 lb/day of 1,3-dinitrobenzene could be released into the Holston River.

2. Behavior in Soil and Water

No information was available on the adsorption or leachability of 1,3-dinitrobenzene in soils and sediments.

Spanggord *et al.* (1978) exposed 1,3-dinitrobenzene to visible light in a photodegradation reactor. They found 1,3-dinitrobenzene to be photochemically stable. In another experiment, Spanggord *et al.* (1978) exposed 1,3-dinitrobenzene solutions in water to sunlight or shade for 17 days. In the experiment, 2200 ml of aqueous solution were used for each condition. Glass containers were used and lined with paper, so photolysis could only occur near the surface of the solution. Water loss from the solutions was measured daily and water was added to the original level before sampling. The results were:

Time (hrs)	1,3-dinitrobenzene levels in ppm	
	Sunlight	Shade
10	11.4	11.4
24	11.0	10.1
48	10.4	10.2
72	9.7	10.0
168	7.8	8.4
240	6.4	7.2
312	5.1	6.2
408	3.2	4.6

The photodegradation experiments conducted by Spanggord *et al.* (1978) supported each other and indicated that 1,3-dinitrobenzene is photochemically stable. However, no experimental check was performed on the amount of adsorption of 1,3-dinitrobenzene onto the glass.

Spanggord *et al.* (1978) also conducted an experiment on the losses of 1,3-dinitrobenzene due to volatilization. An aqueous solution was left in a darkened hood for 8 days. They found that 41% of the 1,3-dinitrobenzene was lost over the 8-day period. From this experiment, Spanggord *et al.* (1978) determined the rate loss in water of 1,3-dinitrobenzene to be 1.88×10^{-2} ppm/hr. Again, no measurement of glass adsorption was conducted.

Sullivan *et al.* (1978) measured 1,3-dinitrobenzene in Volunteer AAP effluents and in the receiving water, Waconda Bay. They found no detectable levels of 1,3-dinitrobenzene (< 0.25 ppb) in Volunteer AAP effluents during March, 1977. However, 1,3-dinitrobenzene was present at the first sampling site in Waconda Bay, with mean levels of 4.1 ppb. Analyses of sediments for 1,3-dinitrobenzene showed levels ranging from less than 6.3 to 14 ppb.

3. Biodegradation and Bioconcentration

a. Biodegradation

Alexander and Lustigman (1966) found that 1,3-dinitrobenzene was resistant to degradation by a mixed population of soil microorganisms. A 5 ppm concentration of 1,3-dinitrobenzene was not degraded after 64 days. The presence of the chemical in the soil was determined by ultraviolet absorbance at 250 nm. If the benzene ring was cleaved, the absorbance would be lost. The test soil inoculum was compared to a control soil, where the microorganism population was inhibited by $HgCl_2$, to determine if biodegradation had occurred. The analytical procedures used on this study were not specific for 1,3-dinitrobenzene. Thus, the conclusion drawn by the authors may not be valid since partial degradation or biotransformation can occur without cleavage of the benzene ring.

Chambers *et al.* (1963) evaluated the degradation of 1,3-dinitrobenzene by a mixed bacteria culture with *Pseudomonas* the dominant group. They also found 1,3-dinitrobenzene to be resistant to degradation. A Warburg test, with a mixed culture, used less than 40 µl of oxygen over a 150 minute period in a 100 ppm solution of 1,3-dinitrobenzene.

McCormick *et al.* (1976) conducted tests on the rate of hydrogen consumption by cell-free extracts of *Veillonella alkalescens* incubated with several nitroaromatic compounds. The data indicate that 1,3-dinitrobenzene is the most resistant to degradation of the dinitrobenzene compounds. McCormick *et al.* (1976) speculated that the 1,4-dinitrobenzene is rapidly reduced because both nitro groups undergo reduction to amino groups simultaneously. This reaction does not occur in 1,3-dinitrobenzene as the first formed amino group inhibits the reduction of the second nitro group. The reduction of nitro groups to amino groups occurs by the following steps (McCormick *et al.*, 1976):

$$R - NO_2 \xrightarrow{H_2} R - NO + H_2O$$

$$R - NO \xrightarrow{H_2} RNHOH$$

$$RNHOH \xrightarrow{H_2} RNH_2 + H_2O$$

Bringmann and Kuehn (1971) employed a two-stage aerated reactor for the degradation of 1,3-dinitrobenzene. The first stage contained *Azobacter agilis* and the second stage, activated sludge. After 36 hours of treatment, 95-97% of the 1,3-dinitrobenzene was transformed.

Villanueva (1961) used *Nocardia* species to degrade 1,3-dinitrobenzene. Relative amounts of arylamine formed were used to assess degradation. Arylamine was determined with a spectrophotometer at a wavelength of 540 nm. The relative degradation of 1,3-dinitrobenzene by *Nocardia* were:

Substrate	Relative Amounts of Arylamine Formed After 1 hr at 37°C when incubating with (*)	
	Whole Organism	Cell-Free Extract
o-Dinitrobenzene	72	75
m-Dinitrobenzene	58	56
p-Dinitrobenzene	100	100

* Referred to p-dinitrobenzene reduction as 100

No percent removal data were presented by Villaneuva. The lack of detail and the apparent absence of quality control measures make the data from this study of questionable value.

b. Bioconcentration

The calculated octanol-water partition coefficient and bioconcentration values for 1,3-dinitrobenzene are presented in Table I-7 The values indicate that 1,3-dinitrobenzene should be concentrated to a low degree by fish.

4. Effects on Animals

a. Mammals

The only available information on the effects of 1,3-dinitrobenzene on mammals is on experimental animals. These effects were discussed in Section D.

b. Birds

Schafer (1972) exposed wild starlings and red-winged blackbirds to 1,3-dinitrobenzene. The 1,3-dinitrobenzene in propylene glycol was given to the birds by oral gavage. The LD50 for the red-winged blackbird was found to be 42 mg/kg. The LD50 for the starling was greater than 100 mg/kg.

Table I-7. Octanol-Water Partition Coefficients and Bioconcentration Calculations for 1,3-Dinitrobenzene

log P	BCF[3]
1.49[1]	8
1.62[2]	10

[1] Fujita *et al.*, 1964; Leo *et al.*, 1971

[2] Spanggord *et al.*, 1978

[3] log BCF = .76 log P - .23
(Federal Register, 1979)

c. Fish

The acute toxicity of 1,3-dinitrobenzene to fish is presented in Table I-8. The two 96-hour LC50 values that are reported are in good agreement. Juhnke and Leudemann (1978) and LeClerc (1960) present very little information on their methods. Therefore, the results are suspect. Liu and Bailey (1977) used a standard static acute toxicity test, so their values are probably reproducible. No lethal acute effects were observed at 3 ppm. From these data, it appears that 1,3-dinitrobenzene is moderately toxic to fish during a short term exposure. The estimated levels of 1,3-dinitrobenzene in rivers receiving AAP effluents should not produce toxic effects on fish. However, no sublethal or chronic effects studies on 1,3-dinitrobenzene on fish have been performed.

The low bioconcentration factor estimates of 10 or less, indicate that fish will not concentrate 1,3-dinitrobenzene from the water to any great extent.

d. Amphibians

No information was available on the toxicity of 1,3-dinitrobenzene to amphibians.

e. Invertebrates

Liu and Bailey (1977) conducted the only acute toxicity test of 1,3-dinitrobenzene on aquatic invertebrates. They determined a 48-hr EC50 for the *daphnia magna* to be 53.0 ppm in soft water at 20°C. These data indicate that fish are more susceptible to 1,3-dinitrobenzene than invertebrates during short term exposure.

f. Microorganisms

The toxic effects of 1,3-dinitrobenzene are presented in Table I-9. 1,3-Dinitrobenzene was found to be inhibitory to microorganisms at levels of 4-5 ppm. Chiu *et al.* (1978) tested the effects of 1,3-dinitrobenzene on *Salmonella typhimurium* (TA98 and TA100). In this study, the growth was partially inhibited at 1,3-dinitrobenzene concentrations of 1 μ mole and TA100 was completely inhibited at 10 μ mole.

Higgins (1960) found that 1,3-dinitrobenzene inhibits growth of *A. niger* by indirectly depressing amino acid synthesis during the early growth phase. The specific enzyme type inhibited by 1,3-dinitrobenzene in *A. niger*, was found to be glucose oxidase (Higgins and Chambers, 1963). Higgins (1960) also observed that the inhibitory effects of 1,3-dinitrobenzene could be reversed by the addition of intermediates of carbohydrate metabolism such as leucine, proline, citric acid or succinic acid. The author also found that mature mycelia of *A. niger* could detoxify 1,3-dinitrobenzene by the production of an enzyme, nitroaryl reductase, which reduces the compound.

Table I-8. Acute Toxicity of 1,3-Dinitrobenzene to Fish

Organism	Level ppm	Water	Temperature °C	Effect	Reference
Golden Orfe (*Leuciscus idus melanotus*)	3	-	-	no deaths	Juhnke and Luedemann, 1978
" " " "	10	-	-	LC50	" " " "
" " " "	20	-	-	LC100	" " " "
Redbelly dace (*Chrosomus sp*)	10-12	distilled	23	6-hr lethal threshold	LeClerc, 1960
" "	8-10	hard 150	23	6-hr lethal threshold	" "
Fathead minnow (*Pimephales promelas*)	7.4	soft	20	96-hr LC50	Liu and Bailey, 1977

Table I-9. Growth Inhibition of Bacteria and Fungi by 1,3-Dinitrobenzene

Species	Conc. mg/l	Medium	Effect	Reference
Bacteria				
Streptococcus sp.	33	-	inhibition	Lecoq and Landrin (1951)
Human tuberculin bacillus	5	-	inhibition	" " " "
Pseudomonas putida	5-14	-	toxicity threshold	Bringmann and Kuehn (1976)
Fungi				
Aspergillus niger	4	glucose	complete inhibition	Higgins (1958)
" "	0.4	"	no inhibition	" "
" "	40	serum-mash	complete inhibition	Zsolnai (1961)
Penicillium simplicissimum	40	" "	" "	" "
Tricothecium roseum	100	" "	" "	" "
Candida albicans	200	" "	partial inhibition	" "
Acherion quincheamun	100	" "	complete inhibition	" "
Trichophyton gypseum	100	" "	" "	" "
Epidermophyton Kaufman-Wolf	100	" "	" "	" "

5. Effects on Plants

a. Phytotoxicity

The phytotoxicity of nitro-substituted benzenes to higher plants has received very little attention. Aromatic nitro compounds have been reported to interfere with seed germination in rape and wheat plants at concentrations of 50 ppm (Jones *et al.*, 1954). The authors attributed this interference to a correlation with the water solubility of the compound and not with the chemical structure. Simon and Blackman (1953) reported that the frond multiplication rate of *Lemna minor* at pH 5.1-5.4 was reduced by 1,3-dinitrobenzene solution. The respiration of infiltrated leaf disks of *Brassica alba* at pH 3.0 was also reduced by 1,3-dinitrobenzene solution. An application of 2 lbs/acre of 1,3-dinitrobenzene had almost total herbicidal effects with no phytotoxicity to crop plants excepting tomato (McRae, 1970). However, Stom and Khutorianskii (1972) reported that 1,3-dinitrobenzene derivatives showed growth inhibitory action on corn at a concentration of 0.17 g/l. This inhibitory action was directly related to the electron acceptor ability of the molecule.

The effect of 1,3-dinitrobenzene on photosynthesis in *Chlorella* and spinach chloroplasts was examined by Howard *et al.* (1976). Among the two phases of fluorescence induction (photochemical and thermal), the thermal phase was inhibited at concentrations of less than 10^{-4}M. The authors attributed the inhibition to an irreversible binding of the toxicant to the reaction centers. At higher 1,3-dinitrobenzene concentrations, the photochemical phase appeared to be inhibited. The data suggest that 1,3-dinitrobenzene may adversely affect plants by interfering with their photosynthetic processes.

Bringman and Kuehn (1978) determined the onset of inhibitory action of 1,3-dinitrobenzene upon cell multiplication of *Microcystis aeroginosa* and *Scendesmus quaricauds*, a blue-green and green algae, respectively. In double distilled water at pH 7.0, the toxicity threshold (chemical concentration causing the onset of all multiplication inhibition) for *Microcystis* was 0.17 mg/l and 0.7 mg/l for *Scenedesmus*.

No other specific 1,3-dinitrobenzene phytotoxicity information has been encountered

b. Bioaccumulation

No information was found on the bioaccumulation of 1,3-dinitrobenzene by plants.

c. Degradation

No information was found on the degradation of 1,3-dinitrobenzene by plants.

F. Regulations and Standards

1. Air and Water Regulations

Dinitrobenzene (mixed isomers) is on the list of hazardous substances published by the Environmental Protection Agency (EPA) (Federal Register, 1978). This material is placed in category "C" by the EPA indicating that a discharge of greater than 1000 lb of dinitrobenzene within a 24-hour period would constitute a harmful quantity. Discharges of greater than 1000 lb of dinitrobenzene with 24 hours would be in violation of Section 311 of the Federal Water Pollution Control Act and would subject the violators to penalties as spelled out in the Federal Register, March 13, 1978.

2. Human Exposure Standards

The American Conference of Governmental Industrial Hygienists (1977) recommends a maximum skin exposure TLV of 1 mg/m^3. This value has been adopted by OSHA (Federal Register, 1974a). The USSR standard for drinking water is 0.5 mg/l dinitrobenzene (Stofen, 1973). 1,3-Dinitrobenzene is not on the current list of chemicals to be evaluated for carcinogenic potential by NCI in their bioassay program.

3. Department of Tranpsortation

The Department of Transportation classified dinitrobenzene as a hazardous chemical (Federal Register, 1974b). Containers must bear a "Poison" label (Federal Register, 1976).

G. Conclusions and Recommendatons

1,3-Dinitrobenzene is currently manufactured in the United States by one company: E.I. duPont deNemours. Environmental contamination due to this manufacturing process is not known.

Effects of exposure of mammals and humans to 1,3-dinitrobenzene are well documented in the literature. The main route of occupational exposure of 1,3-dinitrobenzene is through skin absorption, although inhalation of the dust and possible ingestion are also efficient absorption routes. Symptoms of exposure to this compound range from headache, vertigo, nausea, vomiting, diarrhea, fever, exhaustion to cyanosis, dark chocolate colored blood, lower blood pressure and circulatory collapse. Subacute and chronic exposures produce secondary anemia, anorexia, weight loss, insomnia, moderate cyanosis and yellow-brown discoloration of the skin. After removal from 1,3-dinitrobenzene exposure and dissipation of the symptoms, alcohol and sun exposure can quickly regenerate the symptoms of acute poisoning.

1,3-Dinitrobenzene is a powerful methemoglobin former. The presence of methemoglobin and Heinz bodies in the blood are valuable diagnostic tools of 1,3-dinitrobenzene poisoning. However, individuals vary significantly in their response to this compound and the presence or absence of methemoglobin or Heinz bodies can not be utilized as the sole diagnostic tool.

The mutagenicity of 1,3-dinitrobenzene has been evaluated by several *in vitro* tests. This compound has been shown to be mutagenic by the Ames test and the yeast recombinogenic system. However, further work is needed to verify the results since the data presented in the published papers is not overwhelmingly conclusive.

In the environment, 1,3-dinitrobenzene is toxic to fish in the low ppm concentrations in acute exposure. From the limited data available, it is less toxic to invertebrates such as *Daphnia magna*. No data on chronic exposure effects of 1,3-dinitrobenzene to aquatic organisms were found in the literature. The growth of microorganisms is inhibited by concentrations of 1,3-dinitrobenzene varying from 5 mg/l to greater than 100 mg/l. Microbial degradation proceeds with difficulty and appears to be a reduction process yielding amino groups from the nitro groups.

1,3-Dinitrobenzene is not expected to bioconcentrate to a high degree in aquatic organisms. However, it is relatively stable in the environment.

After evaluation of the available data and taking into consideration the decline in civilian usage of 1,3-dinitrobenzene, it is recommended that the Army undertake additional studies on this compound. This work should include:

- studies to determine the ability of proposed biological and carbon adsorption treatment facilities at TNT manufacturing, blending and loading plants to remove 1,3-dinitrobenzene and its precursors from the effluent streams

- chronic and reproductive effects of 1,3-dinitrobenzene and its potential degradation products on aquatic organisms

- confirmation of *in vitro* mutagenicity test results

- chronic mammalian toxicity and reproductive studies to determine effects of long term exposures including carcinogenicity, mutagenicity and teratogenicity

- metabolic studies with cats, dogs or rats to determine the metabolite responsible for methemoglobin formation and if significant 2,4-dinitrophenol is formed *in vivo* leading to uncoupling or oxidative phosphorylation.

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PROBLEM DEFINITION STUDY ON 1,3,5-TRINITROBENZENE

SUMMARY

1,3,5-Trinitrobenzene is not manufactured by either the civilian or military community. However, it is a by-product of TNT manufacture. TNT is manufactured at Radford, Volunteer and Joliet Army Ammunition Plants. During the nitration of toluene, competitive oxidation of TNT forms 2,4,6-trinitrobenzoic acid. The acid is decarboxylated in either the acid or Sellite wash to form 1,3,5-trinitrobenzene. Neither of these two washes is released directly into the environment. However, the red water evaporator condensate is released untreated into water bodies. The amount of 1,3,5-trinitrobenzene released into the environment is highly dependent upon whether the batch or continuous process is used for TNT manufacture. Estimates of discharges are 0.39 lb/line/day for continuous process and 0.07 lb/line/day for batch process. Photoconversion of TNT to 1,3,5-trinitrobenzene by sunlight could add additional amounts of 1,3,5-trinitrobenzene to the wastewater and receiving waters.

1,3,5-Trinitrobenzene is also present in the TNT final product in concentrations ranging from 0.1% to 0.7%. Thus, 1,3,5-trinitrobenzene is also expected to be present in the effluent from the blending operations at Holston AAP and the loading operations at various Army Ammunition Plants. Again photoconversion of TNT to 1,3,5-trinitrobenzene will increase the concentration of this compound in the receiving waters.

1,3,5-Trinitrobenzene is weakly toxic to experimental animals in acute doses. However, in subacute and chronic doses, it causes central nervous system and blood chemistry disorders. Methemoglobin formation is the most prevalent blood disorder. Heinz bodies are also present.

In the limited studies that have been performed, no evidence has been uncovered to hint at any carcinogenic or mutagenic effects of long-term exposure to 1,3,5-trinitrobenzene. However, 1,3,5-trinitrobenzene has been shown to have a dose response mutagenic effect in the Ames assay.

In the aquatic environment, 1,3,5-trinitrobenzene is moderately toxic to fish in acute doses. It is less toxic to aquatic invertebrates. No chronic aquatic toxicity data are available.

Microbial growth inhibition is observed with low concentrations (ppm levels) of 1,3,5-trinitrobenzene. Microbial degradation of 1,3,5-trinitrobenzene proceeds slowly. This mechanism of degradation is a reductive process yielding amino groups from the nitro groups.

The following studies are recommended in order to fill in the information gaps on 1,3,5-trinitrobenzene:

- chronic mammalian toxicology studies
- environmental degradation studies
- chronic aquatic toxicity studies
- repeat of the Ames and yeast assays

FOREWORD

A. Study Goals

This report presents the results of an evaluation of the available information on the toxicological and environmental hazards of 1,3,5-trinitrobenzene. 1,3,5-Trinitrobenzene is a by-product of TNT (2,4,6-trinitrotoluene) production at various Army ammunition plants.

During the nitration process some of the TNT formed is oxidized to 2,4,6-trinitrobenzoic acid. This acid can then be decarboxylated under process conditions to form 1,3,5-trinitrobenzene. This compound is present in the final TNT product and in the wastewaters generated during TNT production compounding and loading. Since 1,3,5-trinitrobenzene is not manufactured in the United States, the wastewaters from Army Ammunition Plants handling TNT are the only sources of entry of this compound into the environment. This evaluation of the toxicological and environment hazards of 1,3,5-trinitrobenzene was undertaken in order to aid the Army in identification of research needs and in recommendation of effluent criteria for this compound.

B. Study Methodology

The methodology utilized to gather information for this report included a detailed search of the literature and numerous personal contacts. During the literature search, the following sources were reviewed for pertinent information on 1,3,5-trinitrobenzene.

- Chemical Abstracts	1940 - present
- Biological Abstracts	1950 - present
- Excerpta Medica	1950 - present
- TOXLINE	1965 - present
- National Technical Information Service	1964 - present
- Defense Documentation Center	1958 - present
- Compendex	1970 - present

Personal contacts were made with Army ammunition plant personnel and Army and civilian researchers. The specific contacts made and results are presented below:

1. Contacts with U.S. Manufacturers

Mr. Bill Lyman of Eastman Organic Chemicals was contacted on September 27, 1979. Eastman does not supply 1,3,5-trinitrobenzene, and has no information on that compound.

Mr. David Griffiths of Aldrich Chemicals was contacted on September 27, 1979. Aldrich does not have the chemical in their catalog. They had no information on the compound. Mr. Griffiths suggested calling Polysciences.

Dr. Jack Radelle of Polysciences was contacted on September 28, 1978. Polysciences does not supply the compound, and has no information concerning it.

Mr. Schulthis of Chem Tronics was contacted on October 1, 1979. He said that the company once made small amounts of the compound; however, the chemical has not been made there in the last three years.

Other companies were contacted, but had no information on 1,3,5-trinitrobenzene. These companies included:

Fisher Scientific
Hercules
Mason Honger
VIOR Scientific

2. Foreign Contacts

No manufacturers of 1,3,5-trinitrobenzene were noted in the 1979 Directory of Chemical Producers of Western Europe.

3. Other Sources

In September 1979, Mr. J. Gareth Pearson of USAMBRDL, Fort Detrick, Md. provided a reprint of the paper "An Approach to the Toxicological Evaluation of a Compax Industrial Wastewater," by J.G. Pearson, J.P. Glennon, J.J. Barkley and J.W. Highfill. Data on the mutagenicity of this compound were provided.

TABLE OF CONTENTS

Table of Contents (cont.)

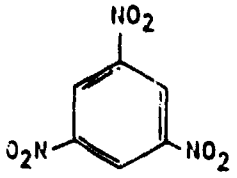
LIST OF TABLES

LIST OF FIGURES

II. 1,3,5-Trinitrobenzene

A. Alternate Names

1,3,5-Trinitrobenzene is an explosive having the following chemical structure:



It has a molecular formula of $C_6H_3N_3O_6$ and a molecular weight of 213.11 g/mole. Pertinent alternate names for 1,3,5-trinitrobenzene are listed below:

CAS Registry No.:	94-35-4
CA Name (8CI)	benzene, 1,3,5-trinitro-
Wiswesser Line Notation:	WNR CNW ENW
Synonyms:	Benzite; s-trinitrobenzene; sym-trinitrobenzene; TNB; symmetric trinitrobenzene; trinitrobenzene; 1,3,5-trinitrobenzene

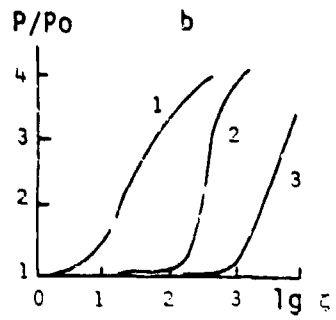
B. Physical Properties

The physical properties of 1,3,5-trinitrobenzene are presented in Table II-1. The infrared, ultraviolet, NMR, and mass spectra are shown in Figures II-1 through II-4.

The Raman spectra are reproduced in Figures II-5 and II-6. The spectral assignments, based on the assumption of a planar molecule with D_{3h} symmetry, are given in Table II-2 (Shurvell *et al.*, 1969). This work also reports two fundamental absorption bands in the far infrared at wavenumbers 156 cm^{-1} and 130 cm^{-1}. An earlier study by Shurvell *et al.* (1967) gives a more complete explanation of the I.R. spectral assignments which are included in Table II-2.

1,3,5-Trinitrobenzene is reported to be polymorphic with melting points (I) 123°C, (II) 110°C, (III) 106°C by Ravich and Bogush (1962). Ravich further states that in studies involving microthermal analysis and differential recording a solid state phase transformation occurs at 101 - 102°C. Ordinarily, 1,3,5-trinitrobenzene melts at 123°C; however, it is capable of super cooling and forming metastable forms at 110°C and 106°C. If the cooling is continued, the phase transformation occurs at 101°C and on subsequent reheating the substance melts at 123°C. However, if the substance is reheated immediately, an endothermal effect is seen at 110°C. The 106°C form is reportedly stable and can be crystallized, melted, and stored. When melting is carried out on a heated stage beneath a microscope, the phase transformation at 101°C appears as a bright color.

Maksimov (1972) reports differences in the rate of decomposition of the isomers trinitrobenzene and states that the amount of gas generated during decomposition amounts to 4 - 4.5 moles per mole of 1,3,5-trinitrobenzene. The differences in the acceleration of decomposition are shown in the graph below:

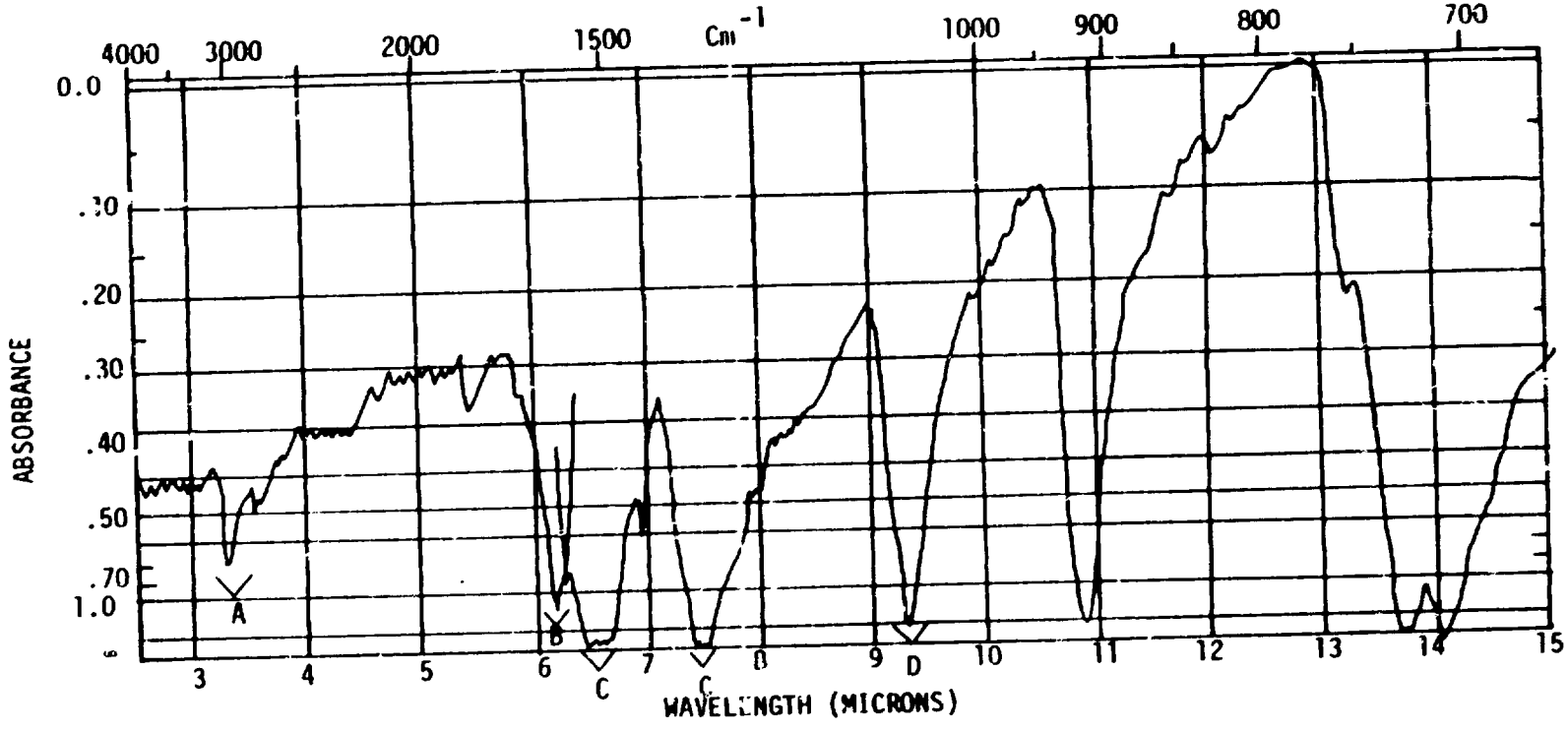


Effect of Isomerism on the Thermal Decomposition of the Vapors of Trinitrobenzenes at 300°C and an Initial Pressure of about 100 mm Hg; 1) 1,2,3-trinitrobenzene; 2) 1,2,4-trinitrobenzene; 3) 1,3,5-trinitrobenzene

Table II-1. Physical Properties of 1,3,5-Trinitrobenzene*

Physical form @ 20°C:	dimorphous solid: Form I (most common) orthorhombic bipyramidal plates; Form II-plates
Color:	pale yellow
M.P.:	122.5°C (Form I) 61°C (Form II)
B.P.:	315°C @ 760 mm Hg 250°C @ 50 mm Hg 175°C @ 2 mm Hg sublimes with careful heating; explodes on rapid heating
Crystal density:	d_2^{20} 1.76 d_4^{152} 1.4775
Solubility:	water: 0.0278 g/100 g @ 15°C 0.102 g/100 g @ 50°C 0.498 g/100 g @ 100°C acetone: 59.105 g/100 g @ 17°C 160.67 g/100 g @ 50°C methanol: 3.759 g/100 g @ 17°C 7.62 g/100 g @ 50°C 95% ethanol: 1.392 g/100 g @ 17°C 3.52 g/100 g @ 50°C ethyl ether: 1.703 g/100 g @ 17°C benzene: 6.176 g/100 g @ 17°C 25.70 g/100 g @ 50°C ethyl acetate: 29.826 g/100 g @ 17°C 52.50 g/100 g @ 50°C pyridine: 112.605 g/100 g @ 17°C 194.23 g/100 g @ 50°C
Specific gravity:	1.688
Vapor pressure:	2 mm Hg @ 175°C 50 mm Hg @ 250°C
Heat of combustion:	3113 cal/gm
Absorption of water:	0.04%

*References: Desvergnes, 1931; Windholz, 1976; Sax, 1976; Hawley, 1977; Spanggord *et al.*, 1978.



Spectrum No._____

Thickness_____

Sample 1,3,5-trinitrobenzene

Legend A: Aromatic C-H stretch
B: C=C ring stretch
C: N=O stretch
D: C-H bend (in plane)

Figure II-1. Infrared Spectrum of 1,3,5-Trinitrobenzene (Spanggord *et al.*, 1978)

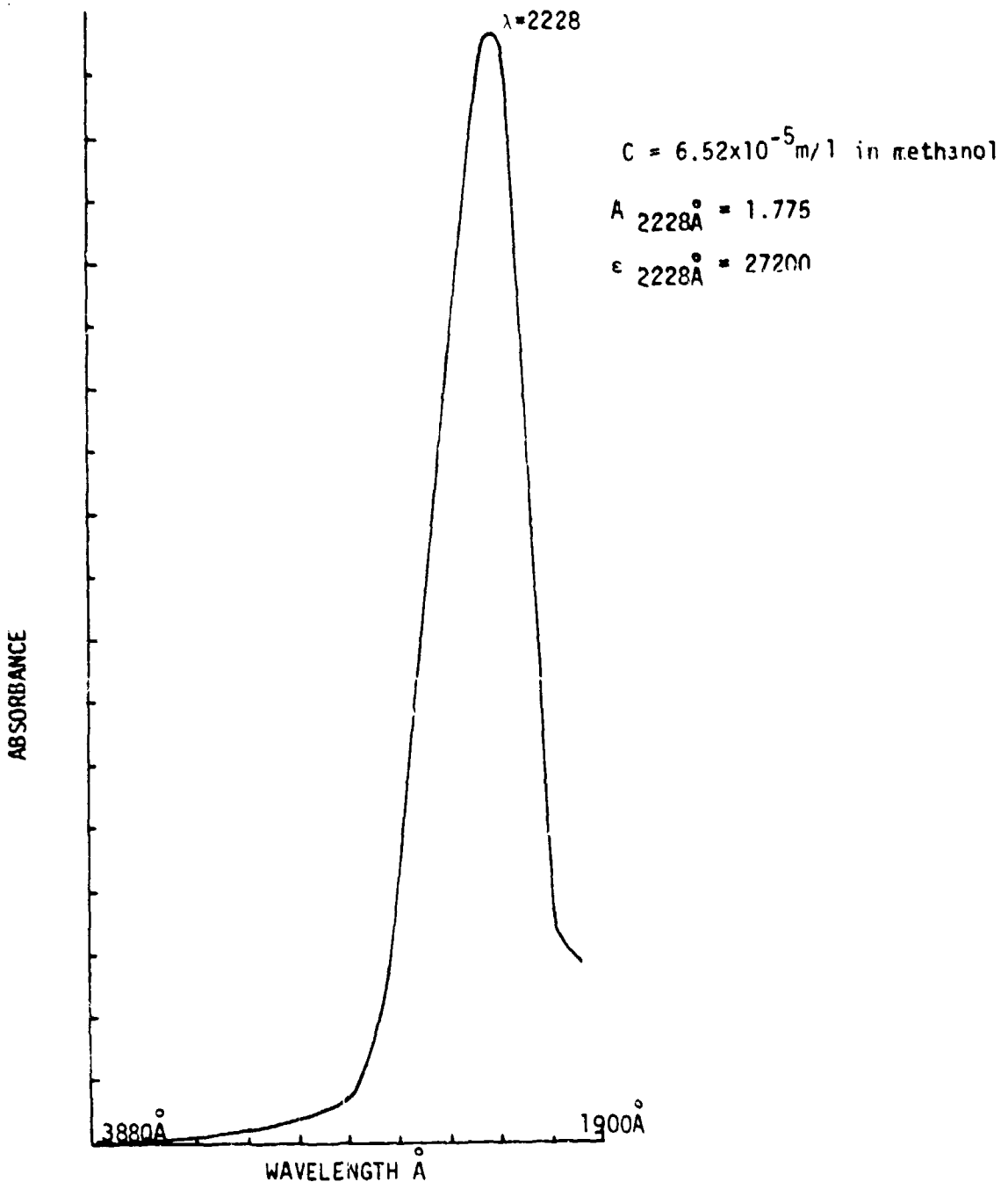


Figure II-2. Ultraviolet Spectrum of 1,3,5-Trinitrobenzene (Spanggord *et al.*, 1978)

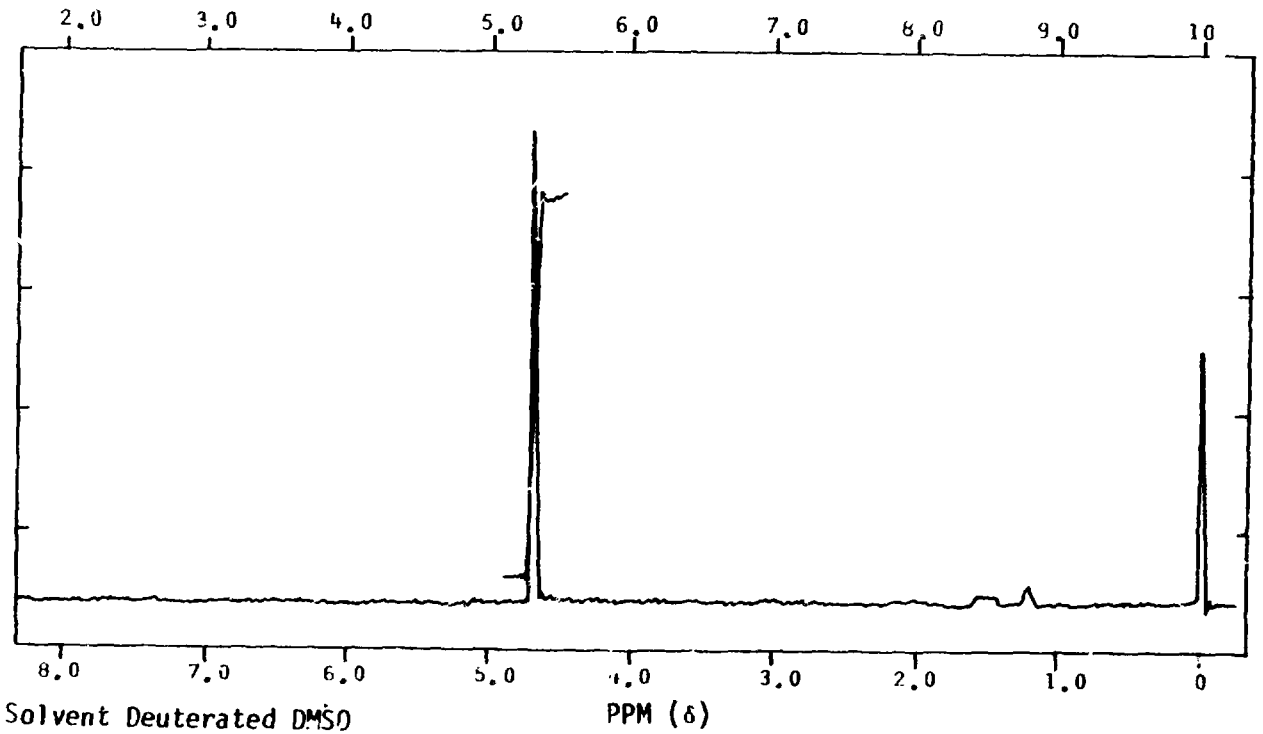


Figure II-3. NMR Spectrum of 1,3,5-Trinitrobenzene (Spanggord *et al.*, 1978)

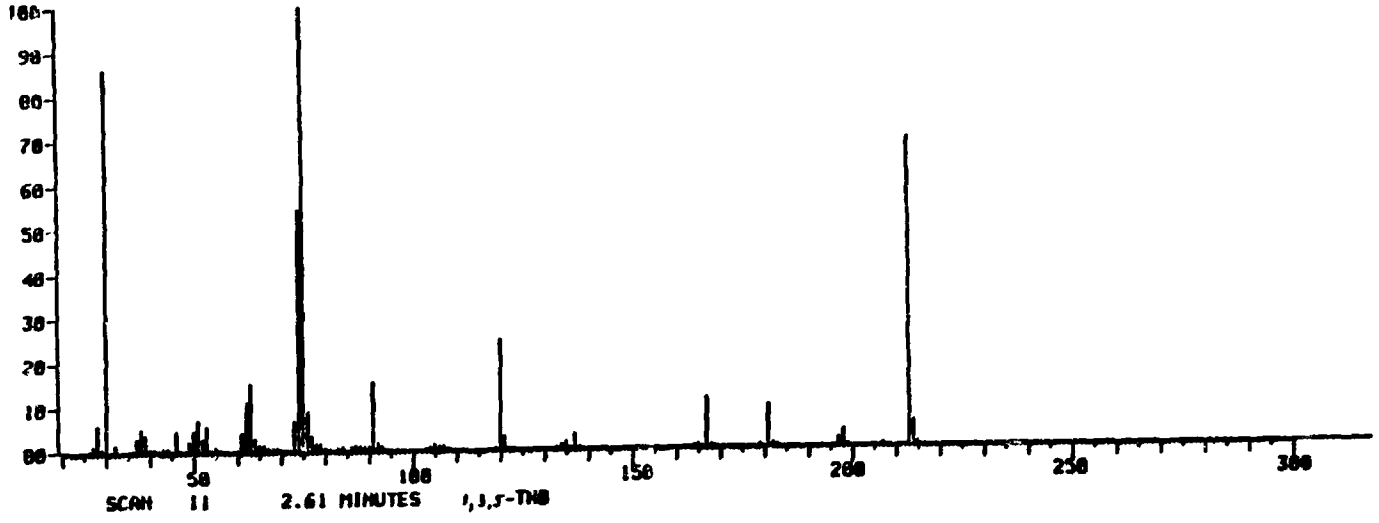


Figure II-4. Mass Spectrum of 1,3,5-Trinitrobenzene
(Spanggord *et al.*, 1978)

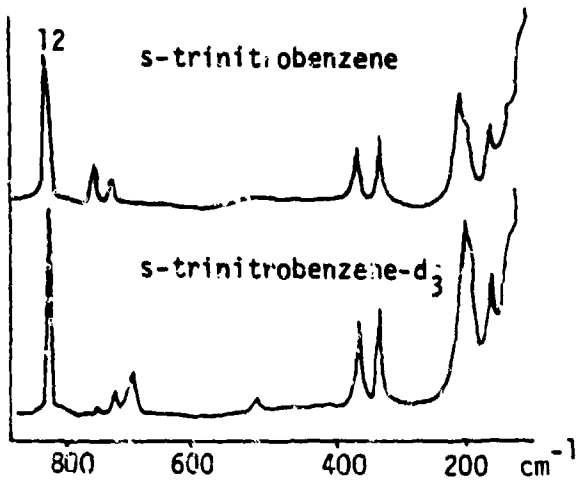


Figure II-5. Raman Spectra of Solid 1,3,5-Trinitrobenzene and 1,3,5-Trinitrobenzene-d_3 in the Region 100-800 cm^{-1}, excited by the 4880Å line of an Argon Ion Laser (Shurvell *et al.*, 1969)

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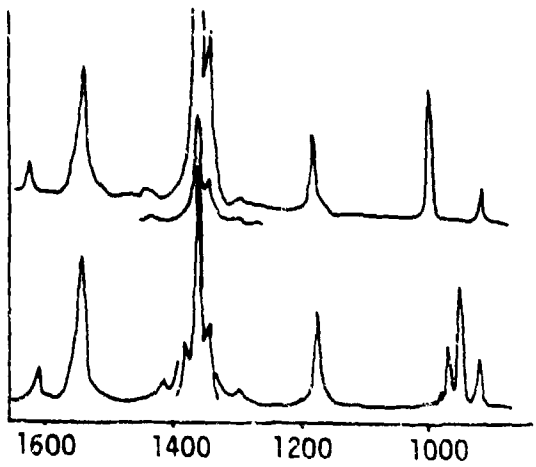

Figure II-6. Raman Spectra of Solid 1,3,5-Trinitrobenzene and 1,3,5-Trinitrobenzene-d_3 in the Region 800-1600 cm^{-1}, excited by the 5145Å line of an Argon Ion Laser (Shurvell *et al.*, 1969)

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Table II-2. Raman and Infrared Frequencies (in cm^{-1}) for 1,3,5-Trinitrobenzene (Shurvell *et al.*, 1969)

Identification	Raman*	Infrared	Assignment	
1	3100 w^	3100	vCH (vCD)	E'
2	3100 W^	-	vCH (vCD)	A'
3	1623 m. dp	1625	vCC	E'
4	1547 vs. dp	1545	NO_2 asym. stretching	E'
-	1520 vw, sh	-	c	-
5	1440 w	1443	vCC	E'
6	1365vvs. p	-	NO_2 sym stretching	A'
7	1345 s, dp	1345	NO_2 stretching	E'
-	1296 vw	-	c	-
8	1184 s. p	-	vCN	A'
9	-	1075	βCH (βCD)	E'
-	-	-	c	-
10	1002 s. p	-	ring mode	A_1'
11	918 m. dp	918	vCN	E'
12	825 s. p	-	NO_2 sym. def	A_1'
-	-	-	c	-
13	754 w	-	γCH (γCD)	E"
14	725 vw	728	NO_2 in-plane def.	E'
15	-	620	δCCC	E'
16	370 m	360	NO_2 rock	E'
17	355 m. p	-	Ring def.	A_1'
18	212 s	-	φCC	E_u''
19	200 sh	-	NO_2 out-of-plane def.	E"
20	160 w	156	βCN	E'
21	133 sh	-	NO_2 out-of-plane def.	E"

v = very; s = strong, m = medium, w = weak, p = polarized, dp = depolarized, c = combination or overtone frequency, sh = shoulder.

^ = Frequency used twice.

Prominent infrared absorption bands are at 3100, 1620, 1540, 1340, 1080, 920, 730 and 710 cm^{-1}. 1,3,5-Trinitrobenzene has a UV maximum at 226 nm and molar absorptivity of 26,925 l/mol cm. The mass spectral distribution is 75 (100), 30(92), 74(65), 213(32), 120(14), 62(12), 91(11), 167(5). The mass ratio is 213.00. ^{13}C NMR coupling constants are reported as:

$Jc^{13}H$ = 179.5±1.5 (acetone)

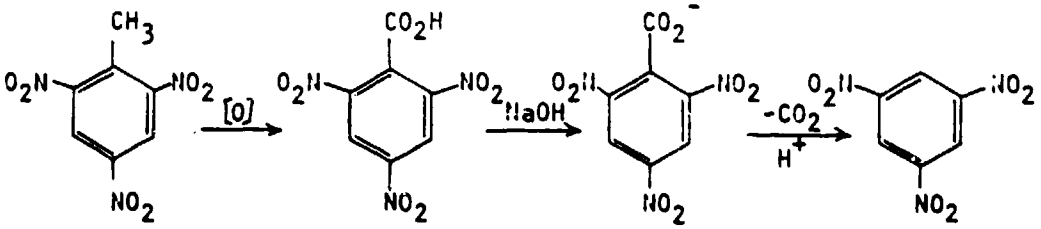
Jm^{HH} = 1.9±0.2 (acetone)

C. Chemical Properties

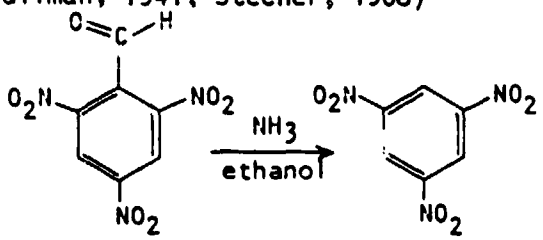
1. General Chemistry

a. Synthesis

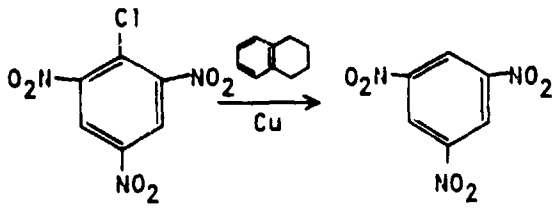
1,3,5-Trinitrobenzene can be prepared by the nitration of 1,3-dinitrobenzene. The deactivating effect of the two nitro groups requires that extreme conditions be used for the addition of the third nitro group. The normal mixed acid nitration conditions proceed slowly and give low yields (Radcliffe and Pollitt, 1921; Gilman, 1941). Improved yields can be obtained by the reaction with nitronium tetrafluoroborate in fluorosulfuric acid (Olah and Lin, 1974 a,b). Other precursors also result in better yields of 1,3,5-trinitrobenzene. For example, 2,4,6-trinitrotoluene can be converted to 1,3,5-trinitrobenzene by oxidation to 2,4,6-trinitrobenzenoic acid followed by decarboxylation in base (Gilman, 1941; Stecher, 1968).



1,3,5-trinitrobenzene can also be formed by the treatment of 2,4,6-trinitrobenzaldehyde with base (Gilman, 1941; Stecher, 1968)



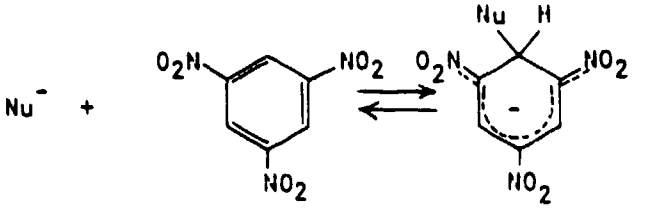
or by the reduction of picryl chloride with tetralin and copper bronze (Lesslie and Turner, 1932).



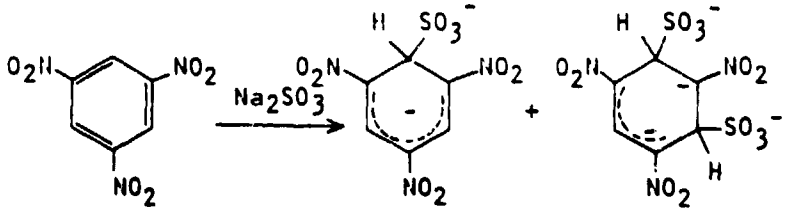
b. Substitution and Addition Reactions

Unlike other aromatic molecules, 1,3,5-trinitrobenzene does not undergo electrophilic substitution due to the deactivating effect of the

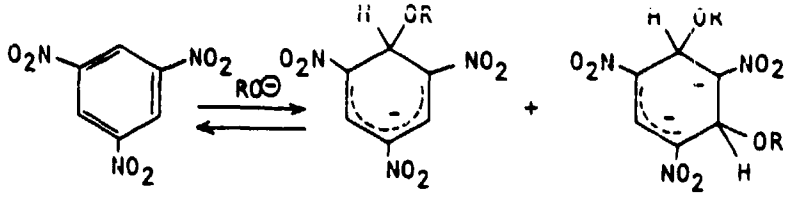
nitro groups. On the other hand, the withdrawing effect of the nitro groups has a stabilizing effect on negative charges. Thus, the aromatic hydrogens are sufficiently active to exchange with barium (Radulescu and Popa, 1935). This effect is also responsible for the susceptibility of the molecule toward nucleophilic attack. Undoubtedly, the most studied reaction of 1,3,5-trinitrobenzene is the formation of Meisenheimer complexes with nucleophiles:



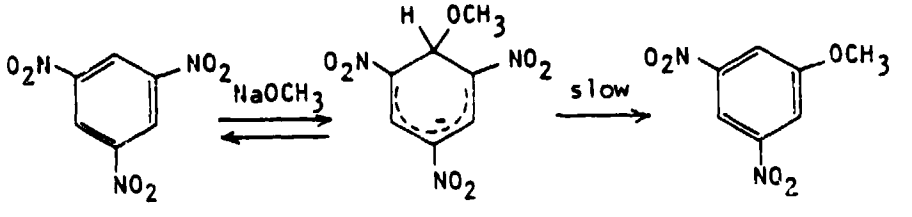
For example, attack by sulfite ions results in a pair of complexes (Bernasconi and Bergstrom, 1973; Sasaki, 1973).



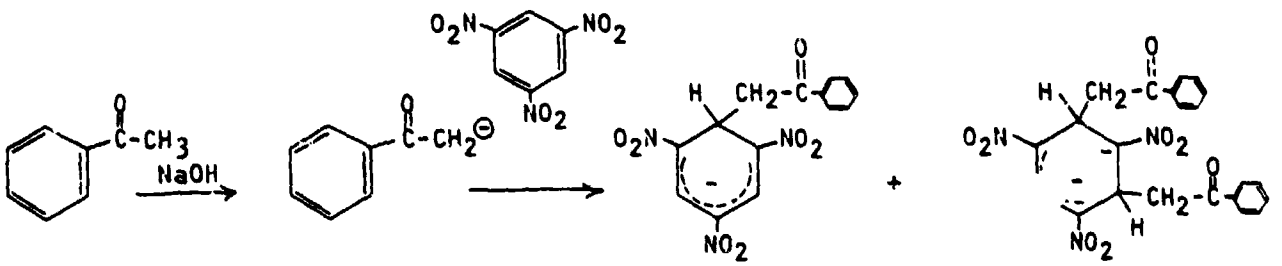
Alkoxides yield a similar set of products (Bernasconi and Bergstrom, 1974; Gan and Norris, 1971),



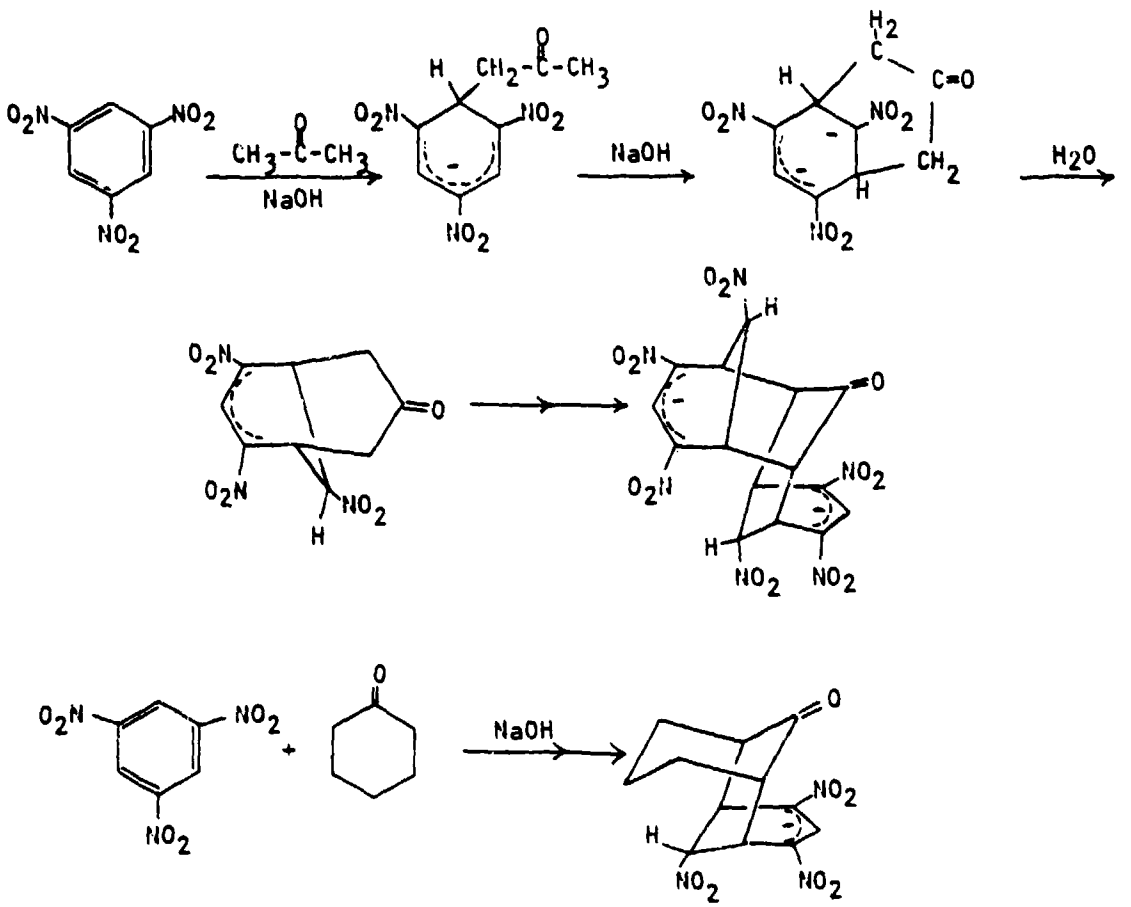
however, a second slower process involving displacement of NO_2 has also been described (Bellobono, 1969).



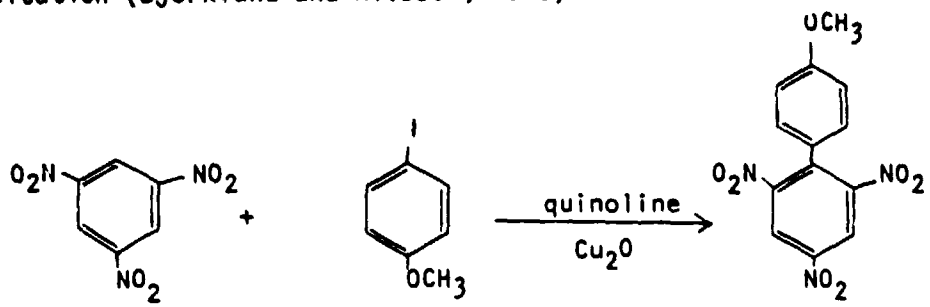
Some of the more interesting Meisenheimer complexes are those resulting from the attack of carbanions. For example, acetylbenzene forms a carbanion in sodium hydroxide which will attack trinitrobenzene (Kohashi *et al.*, 1977).



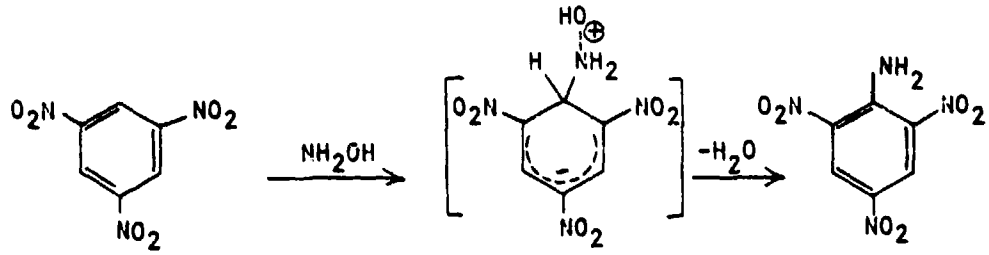
Molecules capable of forming carbanions at more than one site can yield bicyclic and polycyclic complexes (Kohashi *et al.*, 1973; Kolb *et al.*, 1976; Strauss and Schran, 1969).



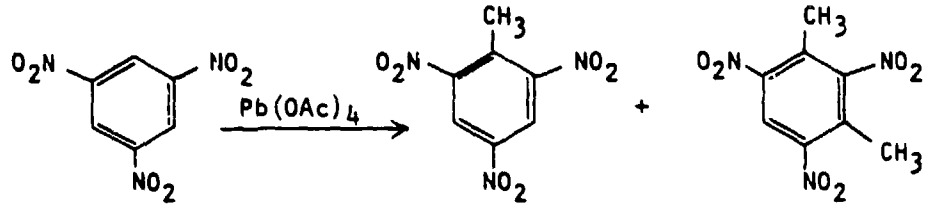
If the nucleophilic attack is accompanied by oxidation, substitution results. For example, aryl halides, in the presence of base and cuprous oxide yield aryl substitution (Bjorklund and Nilsson, 1968).



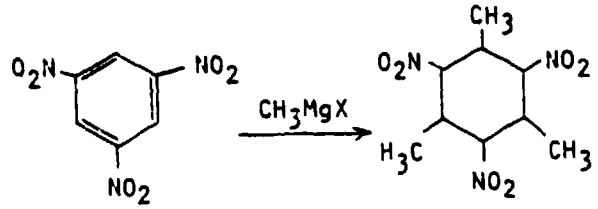
If a suitable leaving group is convenient, neutral species can be generated (Fieser and Fieser, 1961).



Substitution by the action of radical species has also been reported (Fieser *et al.*, 1942). By treatment with lead tetraacetate, mono- and dimethyl substitution results.

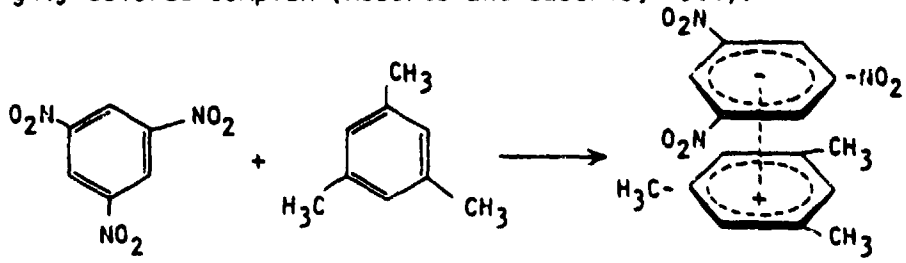


1,3,5-Trinitrobenzene is also subject to nucelophilic addition reactions. On treatment with Grignard reagents, substituted cyclohexanes are obtained (March, 1968).



c. π Complexation

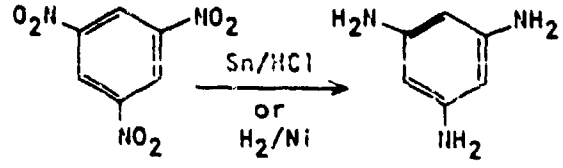
Another reaction related to the electron deficiency of the aromatic ring in 1,3,5-trinitrobenzene is the formation of π complexes. For example, 1,3,5-trinitrobenzene reacts with 1,3,5-trimethylbenzene to form a highly colored complex (Roberts and Caserio, 1964).



Other π complexes have been reported for 1,3,5-trinitrobenzene with anthracene (Liebler *et al.*, 1972; Hertel and Romer, 1930) and about forty aromatic amines and alkylbenzenes (Cooper *et al.*, 1967).

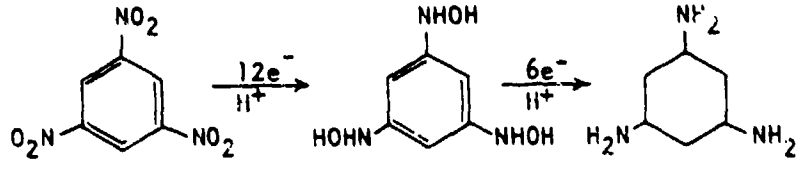
d. Reduction

The nitro groups of 1,3,5-trinitrobenzene are subject to reduction and are expected to undergo reductions typical of nitroaromatics. For example, treatment with metal acid solutions such as Sn/HCl or catalytic hydrogenation should yield 1,3,5-triaminobenzene.

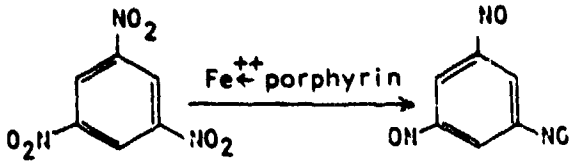


Reduction to hydroxylamine substituents should occur on treatment with Zn/H_2O (March, 1968). Nitrobenzenes are reduced to hydrazobenzene by Zn/NaOH, to azobenzene by Sn/NaOH, and to azoxybenzene by methanol and sodium hydroxide These reagents will certainly reduce trinitrobenzene, however, complex mixtures of dimeric and polymeric products are likely.

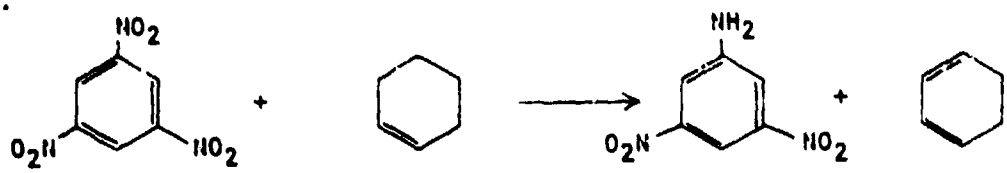
The electrolytic reduction of 1,3,5-trinitrobenzene has been reported by Holleck and Perret (1955).



Reduction to trinitrosobenzene by iron porphyrins has been described by Ong and Castro (1977).

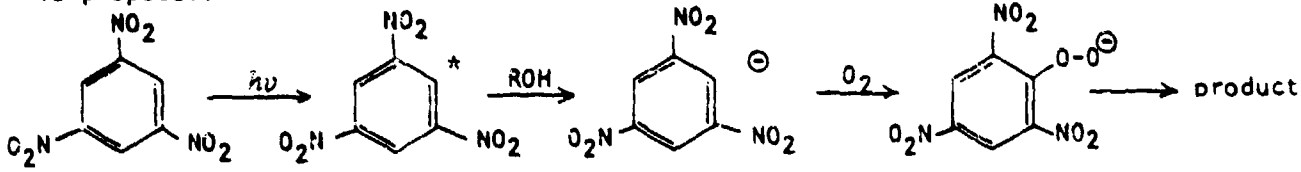


Hydrogen transfer from cyclohexene has also been reported (Braude *et al.*, 1954).

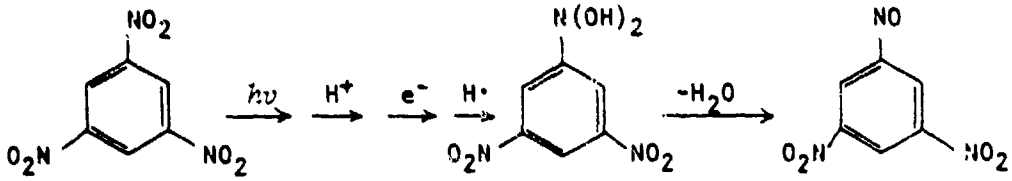


e. Photochemistry

The photolysis of 1,3,5-trinitrobenzene in alconol has been reported by Capellos and Iyer (1978). Proton abstraction and oxygen coupling is proposed.



In water, a similar mechanism was proposed with flash photolysis, but steady irradiation gives reduction to a nitroso intermediate (Capellos and Suryanarayanan, 1972).

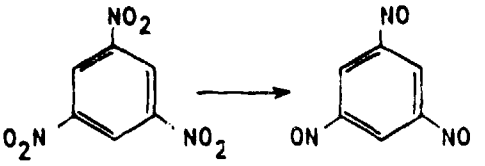


f. Thermal Decomposition

The thermal decomposition of 1,3,5-trinitrobenzene in the gaseous state has been investigated (Maksimov and Egorycheva, 1971). The gaseous products were N_2, CO, CO_2, H_2O and small amounts of NO. No nitrogen dioxide was found. A multi-component liquid residue product was formed. Possible constituents were benzene, phenol, aniline, biphenyl, and their nitro and nitroso derivatives. A brown film of nonuniform make-up formed on the vessel wall during decomposition. Spectroscopic investigation indicated the presence of unsaturated aromatic bonds and the $C-NO_2$ functionality.

2. Environmental Reactions

The environmental chemistry of 1,3,5-trinitrobenzene has not been described. Photoreduction to nitroso analogs is expected.



Reduction by metals to hydroxylamino, azo, hydrazo, amino and azoxy-species or a combination, thereof, is also possible. Hydroxylation and/or rearrangement could also lead to phenolic species.

3. Monitoring and Analysis

a. Analytical Methods

Numerous techniques have been reported for the determination of 1,3,5-trinitrobenzene. Most of the procedures have been designed to enable analysis in the presence of chemically similar compounds and compounds expected to be found in explosive mixtures.

Gas chromatography has been employed by numerous authors. A few examples are listed in Table II-3.

Meier *et al.* (1978) have reported the analysis of 1,3,5-trinitrobenzene by high performance liquid chromatography (HPLC). A Partisil 10-ODS column was employed with a 40% aqueous acetonitrile mobile phase. A 0.1 ppm detection limit was possible with UV detection at 230 nm. Analysis by HPLC was also reported by Inveresk Research International (1979). A silica column was employed with a 1.0 - 1.5% acetonitrile in hexane mobile phase. Ultraviolet detection at 225 nm was used.

Thin layer chromatography has been used extensively. Several examples are listed in Table II-4. No direct comparison of the sensitivity of the individual TLC methods for 1,3,5-trinitrobenzene has been made. Therefore, it is not possible to select an optimum method.

Several other techniques have also been employed for analysis of 1,3,5-trinitrobenzene. Colman (1963) investigated the application of paper chromatography. Both polar (25% formamide) and reverse phase (mineral oil) treated paper were used with cyclohexane/benzene (1:1) and water as mobile phases, respectively. A colorimetric monitoring technique has been

Table II-3. Gas Chromatographic Analyses of 1,3,5-Trinitrobenzene

Reference	Column	Detector	Sensitivity	Conditions
Dalton *et al.*, 1970	10% UC-W 98 on Diatoport-S	FID	not specified	150°- 240°C
Chang, 1971	15% SE30	M.S./FID	not specified	150°- 200°C
Olah and Lin, 1974a	steel capillary with butanediol succinate	FID	not specified	170°C
Kaplan and Zitrin, 1977	3% OV-17	FID/EC	1 ng EC 1 µg FID	160°C
Spanggord *et al.*, 1978	3.5% Dexsel 300 GC on Chromosorb WAWDMCS	E C	not specified	temperature programmed between 40°C and 220°C
Sullivan *et al.*, 1978	2% OV-101/3% QFI gas chrom Q and 8% vc-w 98 on gas chrom Q	EC/AFID	not specified	175°C

Table II-4. Thin Layer Chromatography of 1,3,5-Trinitrobenzene

Reference	Stationary Phase	Mobil Phase	Visualization	Sensitivity
Parihar *et al.*, 1971	silica gel, silica gel/aniline silica gel/phenylene diamine	$C_6H_5Cl/C_2H_4Cl_2$ 9:1 xylene/$C_2H_4Cl_2$ 9:1 chloroform/cyclohexane 3:1	UV analysis of extracted spots	1.5 µg
Boehm, 1967	silica gel G	ethyl acetate/petroleum ether	diphenylamine	not specified
Hansson, 1963	silica gel G	petroleum ether/acetone	UV, and diphenyl amine/UV	1 µg
Kaplan and Zitrin, 1977	silica gel G	trichloroethylene/acetone	KOH; Griess Reagent or o-toluidine	1 µg
Fisco, 1975	silica gel	toluene/benzene/hexane/ pentane/acetone 5:5:4:1:1 nitromethane/benzene 1:3	UV	not specified
Yasuda, 1964	silica gel/An	ethyl acetate/petroleum ether 15:85 dichloroethane/petroleum ether 25:75	benzaldehyde	not specified
Hoffsommer and McCullough, 1968	silica gel	toluene/benzene/pentane/ acetone 40:40:20:5	DMSO/ethylenediamine	not specified

described by Hess *et al.* (1975). The aqueous samples were simultaneously treated with (a) air/KOH and (b) air/KOH/Sultzman reagent prior to dual channel colorimetric analysis at 440 and 550 nm, respectively. Detection limits were in the low ppm range. Selig (1961) has developed a method involving reduction of 1,3,5-trinitrobenzene with $CrCl_2$ and back titration with Fe^{+++}. Detection limits were in the mg range. Kaplan and Zitrin (1977) have used ultraviolet spectroscopy for the analysis of 1,3,5-trinitrobenzene. The λ_{max} at 222-224 nm was employed. Concentrations of 10 ppm were detected. Other techniques which have been used in polarography (Marple and Rogers, 1953) and infrared spectroscopy (Pristera *et al.*, 1960).

b. Monitoring

A recently completed monitoring study for 1,3,5-trinitrobenzene was sponsored by the Army (Spanggord *et al.*, 1978). During this study, 79 samples of condensate water effluent were collected at Volunteer AAP. 1,3,5-trinitrobenzene was not present in all the samples. The 90th percentile concentration of this compound (mean of all non-zero values) was 0.390 mg/l. Monitoring for 1,3,5-trinitrobenzene has also been reported by Sullivan *et al.* (1978). Values at nine sampling sites ranged from <1 to 2 ppb during December 1976 and from <0.75 to 20.0 ppb during March 1977.

D. Health Effects

1. Biology

a. Absorption, Transport, Metabolism and Elimination

Studies have shown 1,3,5-trinitrobenzene to be toxic by the oral and subcutaneous routes of administration (Fogleman *et al.*, 1955; Watanabe *et al.*, 1976; Timofievskaya and Rodionova, 1973. Toxicity for the inhalation and dermal absorption routes is implied by studies with the 1,3,5-trinitrobenzene-aniline complex (Fogleman *et al.*, 1955); by the ability of the lower nitrated benzenes to be absorbed through these routes and by studies conducted by Timofievskaya and Rodionova (1973).

The only reported studies on inhalation and cutaneous absorption of 1,3,5-trinitrobenzene were conducted by Timofievskaya and Rodionova (1973). For the inhalation exposure studies, Timofievskaya and Rodionova (1973 used 15 liter chambers. Petri dishes containing 1,3,5-trinitrobenzene were placed in the chambers and allowed to remain there for 24 hours before the animals were admitted. This method was reported to result in saturated atmospheres inside the chamber. However, no analyses were conducted to determine the actual concentration of 1,3,5-trinitrobenzene in the air. After the 24 hour period, six white mice were placed in two chambers (3 in each chamber) containing the 1,3,5-trinitrobenzene vapors for 2 hours. Six control white mice were placed in chambers without the 1,3,5-trinitrobenzene for 2 hours. The animals were observed for 2 weeks after the exposure. No toxic effects were observed. However, the negative results from this study should not eliminate the potential toxic effects from inhalation of 1,3,5-trinitrobenzene dust.

The dermal absorption of 1,3,5-trinitrobenzene was studied by Timofievskaya and Rodionova (1973). 1,3,5-Trinitrobenzene was applied to the shaved skin of mice as a 33% ointment in fat. After repeated applications of the ointment (10 times in 3 hours), signs of intoxication were noted, e.g. respiratory disturbances and a change in the color of the urine. None of the animals died. Thus, the result of this study appears to indicate that 1,3,5-trinitrobenzene is capable of penetrating the skin in a manner similar to 1,3-dinitrobenzene. However, the experimental details in this study are sketchy and further experimental work is needed to determine the hazards associated with dermal contact with 1,3,5-trinitrobenzene.

No specific studies on the transport, metabolism or elimination of 1,3,5-trinitrobenzene were found in the literature.

b. Pharmacology

The main pharmacological action of the nitrobenzene family is the production of methemoglobin (Bodansky, 1951). Several studies have been conducted to define the percentage of *in vivo* and *in vitro* methemoglobin formation associated with absorption of these compounds.

The *in vivo* and *in vitro* methemoglobin-forming capacities of various amino and nitrobenzene derivatives, including 1,3,5-trinitrobenzene, were studied by Watanabe *et al.* (1976). For the *in vivo* study, the authors used Wistar rats of both sexes as test animals. The rats weighed between 150 and 200 g. A preliminary experiment was conducted to determine the maximum non-lethal dose and the time after dosing during which maximum methemoglobin levels occurred. This preliminary experiment was conducted using 1,3-diamino-2,4,6-trinitrobenzene, aniline and nitrobenzene as test compounds. Male rats were injected intraperitoneally at dose levels of 25, 50, 100 and 200 μM/kg. For these 3 compounds, methemoglobin concentration in the blood was linearly related to the logarithm of the dose. The concentration of methemoglobin in the blood increased within 1 hour after dosing. Methemoglobin concentrations in the blood were maximum from 3 to 8 hours after dosing. From this preliminary experiment, the authors chose a dose of 100 μM/kg for all compounds. They also set the time for sacrifice and measurement of methemoglobin levels in the blood at 5 hours after dosing.

The procedure used for the *in vivo* experiment was as follows. 1,3,5-Trinitrobenzene was dissolved in or dispersed in propylene glycol to yield 2 ml of solution containing the dose of 100 μM/kg. This solution was injected intraperitoneally into five rats. Five control animals received 2 ml of the vehicle alone. The urine of each rat was collected and analyzed for diazo-positive metabolites. For this determination, the urine was hydrolyzed with HCl and reduced by addition of Zn powder. The reduced product was diazotized with $NaNO_2$, ammonium sulfamate and N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting color was measured at 560 nm on a spectrometer. Blood was collected from the rats by decapitation five hours after dosing. The blood was analyzed for methemoglobin, alanine transaminase and aspartate transaminase levels.

For the *in vitro* study, 0.5 μM of each compound was dissolved in 1 ml of propylene glycol. This solution was mixed with 3.5 ml of 0.15 M potassium phosphate buffer (pH = 6.6) and 1 ml of hemolyzate containing 0.1 μM of hemoglobin from the control rats. The mixture was incubated at 37°C for 5 hours. After 5 hours, the methemoglobin levels were measured.

The results of the *in vivo* and *in vitro* studies on 1,3,5-trinitrobenzene are presented below (Watanabe *et al.*, 1976):

Diazopositive metabolites in urine (based on 1.80 ± 0.58 mg/kg as p-aminophenol equivalent is equal to 1)	1.9 rat #1 1.8 rat #2
Methemoglobin concentration in the blood in *in vivo*	18.9 ± 2.1 for 5 animals
Methemoglobin concentration in blood of controls	no value reported
Methemoglobin concentration *in vitro*	13.9 ± 0.7 for 5 trials ($p<0.01$)
In vitro control	4.2 ± 1.0 for 5 trials

Asparate aminotransferase (Karmen units)	200.2 ± 25.9 for 5 animals
Control	195.0 ± 22.6 for 5 animals
Alanine aminotransferase (Karmen units)	38.7 ± 9.4 for 5 animals
Control	33.7 ± 6.3 for 5 animals

The *in vivo* study showed that 1,3,5-trinitrobenzene promotes the formation of methemoglobin; however, it is not as potent as a methemoglobin former as 1,3-dinitrobenzene. The data would be more convincing if variations of the methemoglobin levels in the blood of the controls had been reported. In contrast to the 1,3-dinitrobenzene, 1,3,5-trinitrobenzene is a potent *in vitro* methemoglobin former (Watanabe *et al.*, 1976).

Senczuk *et al.* (1976) monitored the production of methemoglobin in rats after administration of various nitroaromatic compounds. Wistar rats weighing between 200 and 300 grams received single doses of 0.5 mM/kg of 1,3-dinitrobenzene (67.2 mg/kg) and 1,3,5-trinitrobenzene (85.2 mg/kg). A comparison of the time course and level of methemoglobin formation after administration of these two compounds is presented in Figure II-7. From this figure, it is apparent that 1,3-dinitrobenzene produced the higher level of methemoglobin (78% of total hemoglobin). The peak methemoglobin level occurred ∿4 hours after dosing. For 1,3,5-trinitrobenzene, the peak methemoglobin level was ∿50% of total hemoglobin and occurred at 3 hours after dosing. Thus, for equal molar doses, 1,3,5-trinitrobenzene produced methemoglobin faster than 1,3-dinitrobenzene but at a lower level. This study indicates that Watanabe *et al.* (1976) may not have measured the peak methemoglobin concentration for 1,3,5-trinitrobenzene since all their measurements were made at 5 hours.

In general, substances which lead to the formation of methemoglobin, also lead to the formation of Heinz bodies (Buckell and Richardson, 1950; Smith, 1975). Heinz bodies are refractile inclusions in the erythrocytes. The connection between methemoglobin and Heinz bodies has long been discussed, but has not yet been satisfactorily explained (Buckell and Richardson, 1950). The bodies are thought to possibly consist of denatured hemoglobin, since their formation is always accompanied by a drop in the hemoglobin level (Buckell and Richardson, 1950; Smith, 1975). It has been found that not all methemoglobin producing chemicals produce Heinz bodies (Buckell and Richardson, 1950).

Bredow and Jung (1942) studied the effects of 1,3,5-trinitrobenzene injected intraperitoneally in cats. These authors monitored the formation of both methemoglobin and Heinz bodies as a function of time after dosing. In this study, 4 animals each received a 10 mg/kg dose of 1,3,5-trinitrobenzene. Blood samples were taken and monitored for methemoglobin and Heinz body concentrations periodically for 24 hours. The animals were redosed with 5 mg/kg at a later time (not specified). One animal died on the day following the second injection. Two other cats were injected with a single dose of 15 mg/kg. One of these animals died. All the other animals recovered without any apparent permanent effects.

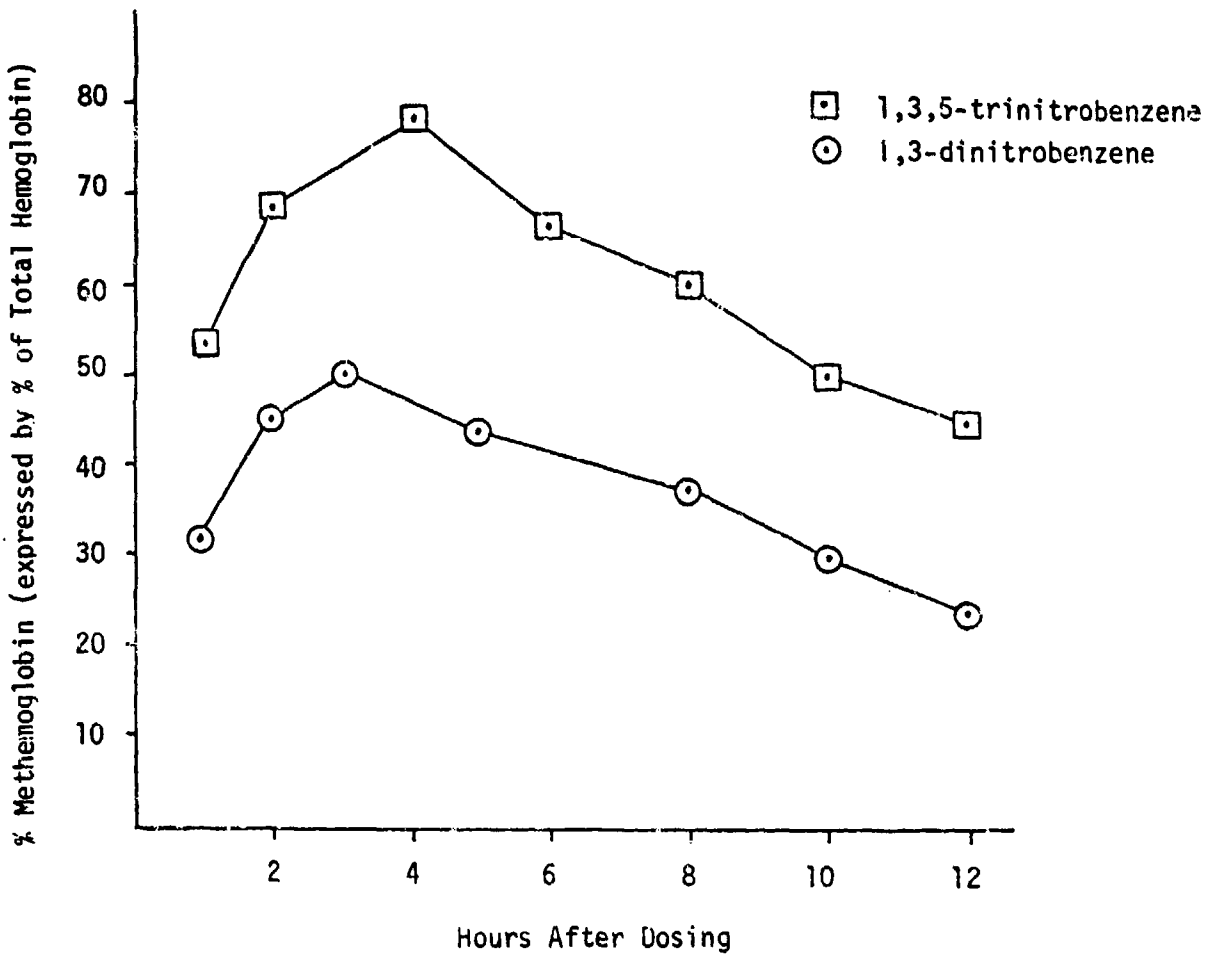


Figure II-7. Concentrations of Methemoglobin Following Administration of 1,3-Dinitrobenzene and 1,3,5-Trinitrobenzene (Senczuk *et al.*, 1976)

Maximum methemoglobin concentrations in the blood were found at ~1 hour after dosing. As shown in Figure II-8, the methemoglobin levels gradually declined, returning to normal levels after about 24 hours after dosing. The percentage of Heinz bodies in the blood rose steadily for the 24 hour monitoring period. Thus, it appears that in the cat, 1,3,5-trinitrobenzene poisoning results in the initial formation of methemoglobin. Heinz body formation closely follows the decline in methemoglobin concentration. The time required before the peak methemoglobin concentrations are observed is also shorter in cats (1 hour) compared to that observed in rats (3-5 hours).

2. Effects of Human Exposure

No literature was found dealing with human exposure to 1,3,5-trinitrobenzene. From a comparison of the animal studies of 1,3-dinitrobenzene and 1,3,5-trinitrobenzene, the effects of 1,3,5-trinitrobenzene on humans should be similar to those described for 1,3-dinitrobenzene. However, the effects of 1,3,5-trinitrobenzene intoxication should be less severe than 1,3-dinitrobenzene and evident sooner after exposure.

3. Effects on Experimental Animals

a. Acute Oral Toxicity

Korolev *et al.* (1977) determined the acute toxicity of 1,3,5-trinitrobenzene to white mice, white rats and guinea pigs. The 1,3,5-trinitrobenzene was dissolved in vegetable oil before oral administration. The LD50 (calculated by Litchfield and Wilcoxin and Deichman methods) were:

white mice	600 mg/kg
white rats	450 mg/kg
guinea pigs	730 mg/kg

Chemical signs of the 1,3,5-trinitrobenzene toxicity were central nervous system disturbances, respiratory disturbances and cyanosis. No other information on the number of animals, doses used or any autopsy results were given.

Fogleman *et al.* (1955) investigated the toxicity of 1,3,5-trinitrobenzene to rats. For the study, male albino rats, weighing between 100 and 170 grams, received 5% w/v 1,3,5-trinitrobenzene in 0.5% methylcellulose by gavage. They were observed for seven days, with signs of toxicity and mortality noted daily. All survivors were sacrificed and gross autopsies were performed. The acute oral toxicity of 1,3,5-trinitrobenzene was found to be about 505 mg/kg. (The authors stated that the data were not suited for calculation of confidence limits). Gross signs of intoxication were depression, hypernea, gasping, salivation, cyanosis, loss of reflexes, tachycardia, coma and death. Necropsy revealed hemorrhagic lungs and rusty colored blood. Autopsies of the survivors were essentially normal, except that the kidneys and blood were darkened.

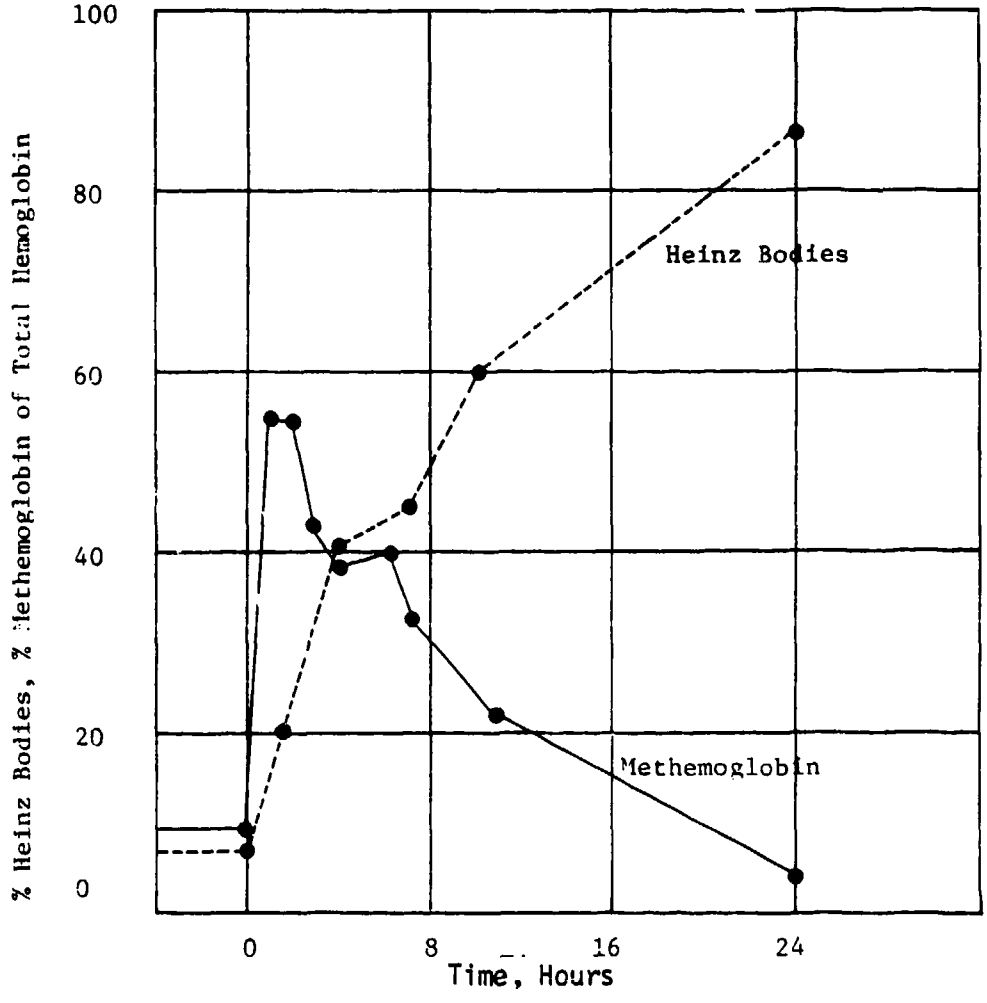


Figure II-8. Methemoglobin and Heinz Body Concentrations in the Blood of Cats Following Oral Administration of 10 mg 1,3,5-Trinitrobenzene (Bredow and Jung, 1942)

Timofievskaya and Rodionova (1973) also investigated the oral acute toxicity of 1,3,5-trinitrobenzene to white mice and rats. The 1,3,5-trinitrobenzene was administered as a suspension in a 3% starch solution. The animals were monitored for 14 days after dosing. The calculated LD16, LD50 and LD84 doses were:

LD16	mouse	374 mg/kg
LD50	mouse	572 mg/kg
	female rat	280 mg/kg
	male rat	440 mg/kg
LD84	mouse	770 mg/kg

Following the dosing, a transitory period of stimulation was observed. This behavior was followed by a period of inhibition, narcosis, tonic-clonic convulsions and motor coordination disturbances. As the poisoning proceeded, decreased sensitivity to pain and auditory stimulation was observed. Respiration grew shallow and labored. Cyanosis was also present. Methemoglobin was present in the blood of the animals. Methemoglobin levels reached 57% of the total hemoglobin in animals administered the LD50 dose within 1 to 1 1/2 hours after dosing.

In general, the experimental details presented in the reports of these acute toxicity studies are too sketchy to thoroughly evaluate the results. However, the LD50 values for rats and mice from these studies are in good agreement and thus, give credence to the numbers. The oral acute LD50 results indicate that 1,3,5-trinitrobenzene is weakly toxic to experimental animals. It appears that the female of the species is more susceptible to the toxic effects of 1,3,5-trinitrobenzene than the male (Timofievskaya and Rodionova, 1973). However, additional studies are needed to confirm this observation and extend it to other species.

b. Acute Effects on the Eye

The effects of a single application of 1,3,5-trinitrobenzene on the mucous membrane of the rabbit eye were determined by introduction of 50 mg into the congunctival sac (Timofievskaya and Rodionova, 1973). A control received 50 mg of powdered sugar to account for mechanical irritation. Immediately after application, epiphora, blepharitis and hyperemia were observed in the 1,3,5-trinitrobenzene treated eye. No other details of the experiment were given.

c. Subacute Oral Toxicity

Korolev *et al.* (1977) also conducted subacute studies with white rats (numbers unspecified, possibly 10 total) using a dose equal to 1/5 to 1/25 of the LD50. The studies lasted for 30 days (direct translation). The exact methodology is unclear. Presumably, the authors dosed the rats at levels ranging from 90 mg/kg (1/5 LD50) to 1.8 mg/kg (1/25 LD50) for 30 days by oral gavage. According to the authors, the following parameters were evaluated: dynamic body weight; numbers of erythrocytes, leucocytes, and reticulocytes; hemoglobin and methemoglobin levels; cholinesterase, peroxidase, alanine and aspartic transaminase and aldolase activities; ceruloplasmin and SPP (not further identified) to characterize the state of the central nervous system; and stained leucocytes to evaluate allergenic action. At autopsy, the weights of the internal organs and the activity of the liver cholinasterase were determined.

Two of 10 animals died during the study (doses not specified). No details of the changes of these parameters during the study were given, however, the authors state that statistically significant changes ($p<0.05$-0.01) were found in these parameters at 1/10 and 1/25 LD50 dose levels. The threshold level was determined to be 1/25 LD50 since even at this level, the peroxidase activity, ceruloplasm, number of erythrocytes, and SPP varied significantly when compared to the controls. The authors found 1,3,5-trinitrobenzene to be "very highly cumulative."

The subacute toxicity of 1,3,5-trinitrobenzene to mice was studied by Timofievskaya and Rodionova (1973). Two separate experiments were carried out to determine the coefficient of accumulation of 1,3,5-trinitrobenzene. In the first experiment, mice (probably 30, although numbers were unclear) were dosed with 1/10 LD50 dose daily for 30 days. Dosing was accomplished by oral gavage of a 3% starch suspension. Controls received only the starch solution. Only one animal died after administration of 3 LD50. No coefficient of accumulation was calculable from this experiment. During the experiment, clinical signs of intoxication increased. The treated animals weighed more than the controls at the end of 30 days.

In a second experiment, Timofievskaya and Rodionova (1973 administered 1,3,5-trinitrobenzene to mice. The initial dose was 0.09 LD50. The remaining doses were administered every 4 days and increased in a geometrical progression with a 1.5 multiplication factor. The experiment was 24 $\pm$ 4 days in length. Seventy percent of the animals died. The coefficient of accumulation was >10 (low, unexpressed accumulation of the substance).

The subacute toxicity of 1,3,5-trinitrobenzene to dogs was studied by Fogleman *et al.* (1955). For this study, two male and two female mongrel dogs were used, with two additional dogs serving as controls. Each animal was individually housed and fed a basic laboratory diet. Complete blood counts, urinalysis, bromosulfalein liver function tests, determination of blood urea nitrogen and methemoglobin were made at intervals throughout the study. The dogs were observed daily for signs of intoxication, and their body weights were recorded weekly. At the termination of the study, all of the animals were sacrificed by exsanguination, and autopsies were performed. Various tissues were sectioned and examined for histological changes.

The two dogs received four doses of 100 mg/kg (~1/5 LD50) over a four day period. The compound was administered orally in a gelatin capsule. The female developed convulsions, anorexia and soft feces. The erythrocytes had an abnormal morphology, and methemoglobin levels in the female and male were 11.4% and 21.8% of the total hemoglobin, respectively. Hemoglobin values and other clinical laboratory tests were within normal units. The dogs were allowed to recover for 30 days and were then returned to a daily dosage of 10 mg/kg. They received 13 doses in a 19-day period, during which time they appeared clinically normal. When the study was terminated, the methemoglobin levels were 1.0% of the total hemoglobin for the female and 5.9% for the male. All other laboratory tests were within normal limits.

In the second part of the study, two dogs received 37 doses of 25 mg/kg (1/20 LD50) over a 54-day period. The female exhibited anorexia, depression and nausea during the first 30 days of the study, but returned to normal during the rest of the period. The male became ataxic on the 39th day after receiving 29 doses. After 34 doses, the dog had developed generalized stiffness and ataxia. This condition persisted until the end of the study. Methemoglobin levels in the animals reached a maximum of 24.4% of the total hemoglobin for the female and 4.9% for the male. The male developed mild anemia, but all other tests were normal.

At autopsy, the spleens and livers of all dogs receiving 1,3,5-trinitrobenzene were heavier than controls (no numbers given). No gross pathological changes were noted. Microscopic examination of the spleens showed thickening of the trabeculae. Since the spleens were otherwise normal, the authors concluded that gross enlargement was due to passive congestion (Fogleman *et al.*, 1955). Kidney sections showed indications of toxic reactions with both endothelial and epithelial cells of the glomerular tufts undergoing proliferation and cloudy swelling (Fogleman *et al.*, 1955). In the dogs receiving the 100 mg/kg followed by 10 mg/kg dose, the cells in the loops of Henle were undergoing fatty metamorphosis. The other group of dogs receiving 25 mg/kg had more severe changes, with the loops of Henle undergoing degeneration. In addition, there was involvement of the distal convulted tubules, where the cells appeared swollen and granular. Sections of the liver, gonads and bone marrow from animals ingesting 1,3,5-trinitrobenzene were comparable to controls.

Korolev *et al.* (1977) and Timofievskaya and Rodionova (1973) appear to disagree on the accumulation properties of 1,3,5-trinitrobenzene. Neither paper presented sufficient experimental details or results upon which to evaluate the reported subacute studies. Fogleman *et al.* (1955) showed that repeated subacute doses as low as 1/20 LD50 in dogs had definite physiological effects on the animals. These results would tend to agree with those of Korolev *et al.* (1977). However, insufficient details of the experimental results, *e.g.*, daily blood chemistry, urinalysis, etc. were presented by Fogleman *et al.* (1955) to make an evaluation of the reliability of the final data and conclusions.

d. Subacute Effects on the Skin

The local effects of repeated application of 1,3,5-trinitrobenzene to the shaven skin of rabbits were studied by Timofievskaya and Rodioncva (1973). 1,3,5-Trinitrobenzene was applied as a 33% ointment in fat 5 times daily for 3 hours (presumably for only one day). Local effects of the application included hyperemia, edema and tiny hemorrhages. The skin had bright yellow-orange discoloration. Following desquamation, complete regeneration of the skin in the treated area occurred within 1 to 2 weeks. No intoxication effects were reported. However, the authors did show that 1,3,5-trinitrobenzene penetrated the intact skin of mice to cause intoxication. This study was discussed in Section I, D, 1, a.

e. Chronic Toxicity Studies

Chronic toxicity studies of the effects of 1,3,5-trinitrobenzene on male white rats were conducted by Korolev *et al.* (1977). Three dose levels were tested, 2, 0.2 and 0.02 mg/kg. These doses were administered presumably daily for at least 6 months, although the study may have been conducted for close to the lifetime of the animals. Dosing was presumably by oral gavage, however, methodology was not specified. Parameters monitored during the study were dynamic body weight, numbers of erythrocytes, leucocytes and reticulocytes; hemoglobin and methemoglobin; cholinestinase, peroxidase, alanine and aspartic transaminase and aldolase activities; ceruloplasmin; SPP (not further identified in text) to characterize state of central nervous system; measurement of leucocytes to determine allergenic activity; activity of conditioned reflexes; activity of alkaline phosphatase; and quantity of β-lipoproteins in the blood serum. At the conclusion of the study, the animals were autopsied. The internal organs were weighed; cholinesterase activities of the organs were measured and pathomorphological experiments (not specified) were conducted.

Reportedly, doses of 0.2 and 0.02 mg/kg[1] of 1,3,5-trinitrobenzene produced statistically significant ($p<0.05$) changes in the peroxidase and alkaline phosphatase activities, ceruloplasmin and the amount of SH groups in the blood. Conditioned reflex activity was also disturbed. At a dose of 3 mg/kg, increases in blood histamine levels were observed by the fifth and sixth months, indicating weakly allergenic properties of 1,3,5-trinitrobenzene.

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[1] These numbers are probably supposed to be 2.0 and 0.2 mg/kg since the authors state later in the paper that the 1,3,5-trinitrobenzene dose of 0.02 mg/kg does not produce any changes in the test animals when compared to the controls.

f. Carcinogenicity

The carcinogenic activity of 1,3,5-trinitrobenzene was investigated by Gorski (1969). In his study, Gorski used 20 male BALB/c strain mice aged 2-4 months. The mice received 1,3,5-trinitrobenzene injected subcutaneously. The 1,3,5-trinitrobenzene may have been disolved in 0.4 ml of paraffin oil. The methodology section of this paper is ambiguous. The amount of 1,3,5-trinitrobenzene injected per dose and the number of doses is not given. In this experiment, three animals died (no reasons given), however, no tumors were found during the lifetime of the remaining animals.

g. Teratogenicity

No information was found in the literature relating to any teratogenic studies on 1,3,5-trinitrobenzene.

h. Mutagenicity

Korolev *et al.* (1977) evaluated the effects of 1,3,5-trinitrobenzene on the reproductive function of animals during a chronic toxicity study (described earlier). Data from this study are presented in Table II-5. 1,3,5-Trinitrobenzene did not produce any disturbances in the functional state of the spermatozoa or morphological changes in the animal epithelium.

1,3,5-Trinitrobenzene has been subjected to microbial testing for mutagenesis and toxicity in the Ames assay, the *E. coli* toxicity test and the yeast recombinogenic assay (see Section I for description of procedures).

McGregor (1979a) reports mutagenic activity of 1,3,5-trinitrobenzene not requiring metabolic activation in the Ames assay (strains TA1535 and TA98). A dose-related response was observed over a concentration range of 1-33 µg/plate. Simmon *et al.* (1977) also reported mutagenic activity of 1,3,5-trinitrobenzene in strains TA98 and TA100 with and without activation, before and after chlorination and ozonation over a range of 18-71 µg/plate.

McGregor (1979b) subjected 1,3,5-trinitrobenzene to testing in the yeast recombinogenic assay. Toxicity of 1,3,5-trinitrobenzene at the levels tested prevented evaluation of mitotic recombinational events. Simmon *et al.* (1977) reported no genetic activity by 1,3,5-trinitrobenzene in the yeast assay.

Further studies are needed to confirm the dose related response in the Ames assay over a concentration range of one and one-half logs. The yeast assay should be re-evaluated utilizing lower levels of 1,3,5-trinitrobenzene to allow for increased cell survival and scoring of recombinational events.

Table II-5. Changes in the Function of the Gonads During Chronic Intoxication by Trinitrobenzene (TNB) (Kcrolev *et al.*, 1977)

Doses of TNB (mg/kg)	Index of Spermatogenesis	Quantity of Spermatogonia	No. of Tubules with Desquamated Epithelium	No. of Tubules with 12 stages of meiosis	No. of Spermatozoa (thousands)	Time of Motility of Spermatozoa (minutes)	Acid Resistance of Spermatozoa pH	Osmotic Resistance of Spermatoza (% Physiological) Solution	Weight Coefficient of the Gonads
Control	3.68 ± 0.027	23.6 ± 2.42	4.4 ± 0.80	3.2 ± 0.27	216 ± 13.75	410 ± 13.0	3.10 ± 0.09	2.60 ± 0.15	3.4% ± 0.2%
0.02	3.72 ± 0.031	22.0 ± 2.14	5.3 ± 1.23	4.0 ± 0.38	195 ± 12.42	398 ± 11.3	3.06 ± 0.083	2.77 ± 0.16	3.3% ± 0.15
P	0.2	>0.5	>0.5	>0.1	>0.2	>0.1	>0.5	0.5	0.5
0.2	3.66 ± 0.041	26.7 ± 3.05	3.8 ± 0.77	2.75 ± 0.35	238 ± 18.3	400 ± 12.7	3.14 ± 0.10	2.82 ± 0.18	3.51 ± 0.42
P	>0.5	>0.1	>0.3	>0.1	>0.2	>0.3	>0.3	>0.1	0.5
2.0	3.65 ± 0.032	24.3 ± 2.52	4.3 ± 1.12	3.5 ± 0.37	175 ± 9.3	368 ± 11.8	3.19 ± 0.075	2.52 ± 0.15	3.60 ± 0.37
P	>0.5	>0.3	>0.5	>0.5	>0.1	>0.1	>0.5	>0.1	0.2

E. Environmental Effects

1. Entry into the Environment

The release of 1,3,5-trinitrobenzene into the environment is primarily as a by-product in the manufacture of TNT (2,4,6-trinitrotoluene). 1,3,5-trinitrobenzene is formed during the nitration of TNT. During this process, competitive oxidation of the methyl group to a carboxyl group can occur forming 2,4,6-trinitrobenzoic acid. The acid is then decarboxylated to form 1,3.5-trinitrobenzene. The benzene impurities in toluene (Kohlbeck *et al.*, 1973) do not form 1,3,5-trinitrobenzene upon direct nitration because the third nitration is not kinetically favored. The losses of 1,3,5-trinitrobenzene in the manufacture of TNT are from the acid wash (yellow) water and the sellite wash (red) water. Although the yellow and red water are not released directly into the environment, the evaporator condensate, from the red water condensation process, is released untreated into the water bodies at Volunteer AAP and Joliet AAP (Patterson *et al.*, 1976).

The 1,3,5-trinitrobenzene concentrations in munition plants effluents are not routinely monitored, however, some data are available to assess the levels in condensate wastewater. Patterson *et al.* (1976) found nitrobody (nitro containing organics) levels of approximately 15 mg/l in the evaporator condensate at Joliet AAP. Epstein *et al.* (1975) analyzed Radford AAP condensate wastewater and found 0.6% of the nitrogen containing organics were 1,3,5-trinitrobenzene and 8% were TNT (a TNT/1,3,5-trinitrobenzene ratio of 145/1). Combining the data of Patterson *et al.* (1976) and Epstein *et al.* (1975), the concentrations of 1,3,5-trinitrobenzene and TNT in the condensate wastewater can be calculated as 0.009 mg/l 1,3,5-trinitrobenzene and 1.2 mg/l of TNT. Of the 23 combined Volunteer AAP condensate water samples collected from 9/8-10/29, 1976, only three samples had detectable 1,3,5-trinitrobenzene levels (Spanggord *et al.*, 1978):

Date	Levels (ppm) of 1,3,5-trinitrobenzene
9/16	0.06
9/17	0.20
10/20	0.20

TNT was identified in only one of the 23 samples at a level of 0.2 ppm on 9/17/76 (Spanggord *et al.*, 1978).

Volunteer AAP composite effluent samples were collected in March, 1977 by Sullivan *et al.* (1978). These samples were collected when one continuous line was in operation. Of the 20 samples collected, only 2 had measurable 1,3,5-trinitrobenzene levels:

Date	1,3,5-trinitrobenzene Levels in ppb	Effluent Volume MGD	Pounds per day Released
3/9	8.2	4.2	.29
3/18	9.8	5.2	.43

The release of 1,3,5-trinitrobenzene at infrequent times could be related to the time of release of the evaporator condensate waste water from the red water condensation process into the condensate effluent.

Using a TNT/1,3,5-trinitrobenzene ratio of 145/1 for levels in the condensate wastewater (Epstein *et al.*, 1975), estimates for the release of 1,3,5-trinitrobenzene in continuous batch lines were calculated to be 0.39 lb/line/day and 0.07 lb/line/day, respectively (Kitchens *et al.*, 1978). The estimate of 0.39 lb/line/day for continuous line operation is very close to the effluent levels measured by Sullivan *et al.* (1978) when 1,3,5-trinitrobenzene was above detection limits. Photoconversion of TNT to 1,3,5-trinitrobenzene could add additional levels of 1,3,5-trinitrobenzene to the wastewater. Burlinson *et al.* (1973) found that approximately 1% of the TNT was photoconverted to 1,3,5-trinitrobenzene by sunlight. Estimated discharges of 1,3,5-trinitrobenzene at full TNT production are presented in Table II-6. These levels take into account release in wastewater and photoconversion of 1,3,5-trinitrobenzene.

1,3,5-Trinitrobenzene is also lost to the environment when TNT is blended with RDX or HMX at Holston AAP. Approximately 0.1 to 0.7% of the final TNT product is 1,3,5-trinitrobenzene (Kitchens *et al.*, 1978). At full production, 110 mils/lb of TNT would be used at Holston AAP each year. Of this TNT, 110,000 to 770,000 lb is 1,3,5-trinitrobenzene. Estimated discharges of TNT at full production run as high as 650 lb/day. If a comparable percentage of 1,3,5-trinitrobenzene were lost, the discharge would be 0.65 to 4.6 lb/day. However, 1,3,5-trinitrobenzene is approximately twice as soluble as TNT. Therefore, as much as 1.3 to 9.1 lb/day of 1,3,5-trinitrobenzene could be discharged per day from Holston AAP. Photolysis of TNT could lead to an additional 6.5 lb/day of 1,3,5-trinitrobenzene in the Holston River. The resulting Holston River concentration (assuming full mixing) could be as high as 0.9 ppb.

2. Behavior in Soil and Water

Sullivan *et al.* (1978) measured 1,3,5-trinitrobenzene, TNT and other munition compounds from the effluent from Volunteer AAP into the receiving water, Waconda Bay, in March, 1977. The data on 1,3,5-trinitrobenzene and TNT levels in the water and sediment are presented in Table II-7. The locations of the sampling sites are presented in Figure II-9.

TNT levels in the water samples were initially in the 40 ppb range. The 1,3,5-trinitrobenzene levels were below the detection limit (.75 µg/l) in the effluent and three of the first four sampling stations. Stations 5-7 have roughly the same 1,3,5-trinitrobenzene levels and TNT levels. However, at stations 8 and 9, the 1,3,5-trinitrobenzene levels were 10.6 and 20.6 µg/l.

Table II-6. Estimated Discharges and River Concentrations of 1,3,5-Trinitrobenzene at Full TNT Production

Plant	No. of Lines	Total Discharge Rates lb/day	River	River Flow MGD	River Concentration ppb
RAAP	2 continuous	1.9	New	2,380	0.1
VAAP	6 continuous 6 batch	6.7	Tennessee	23,750	0.03
JAAP	6 continous 3 batch	6.2	Illinois	5,390	0.14

Table II-7. Water and Sediment Analyses of 1,3,5-Trinitrobenzene and 2,4,6-Trinitrotoluene, March, 1977 (Sullivan *et al.*, 1978)

	1,3,5-Trinitrobenzene*			TNT*		
	Water			Water		
Station No.	Average	Range	Sediment Average	Average	Range	Sediment Average
1	2.9	<0.75-11.5	73	48.0	<0.10-146	91
2	<0.75	<0.75	≤179	40.3	<0.10-148	119
3	<0.75	<0.75	76	43.7	<0.10-139	119
4	<0.75	<0.75	135	13.3	<0.10-49.4	86
5	17.1	<0.75-66.	80	26.2	<0.10-95.5	70
6	1.2	<0.75-2.0	221	1.8	<0.10-4.8	138
7	1.6	0.9 -2.2	250	0.2	<0.10-0.3	105
8	10.6	0.75-39.	304	0.1	<0.10-0.2	142
9	20.6	0.75-38.4	263	0.3	<0.10-0.4	148
units	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l

* 5 samples collected

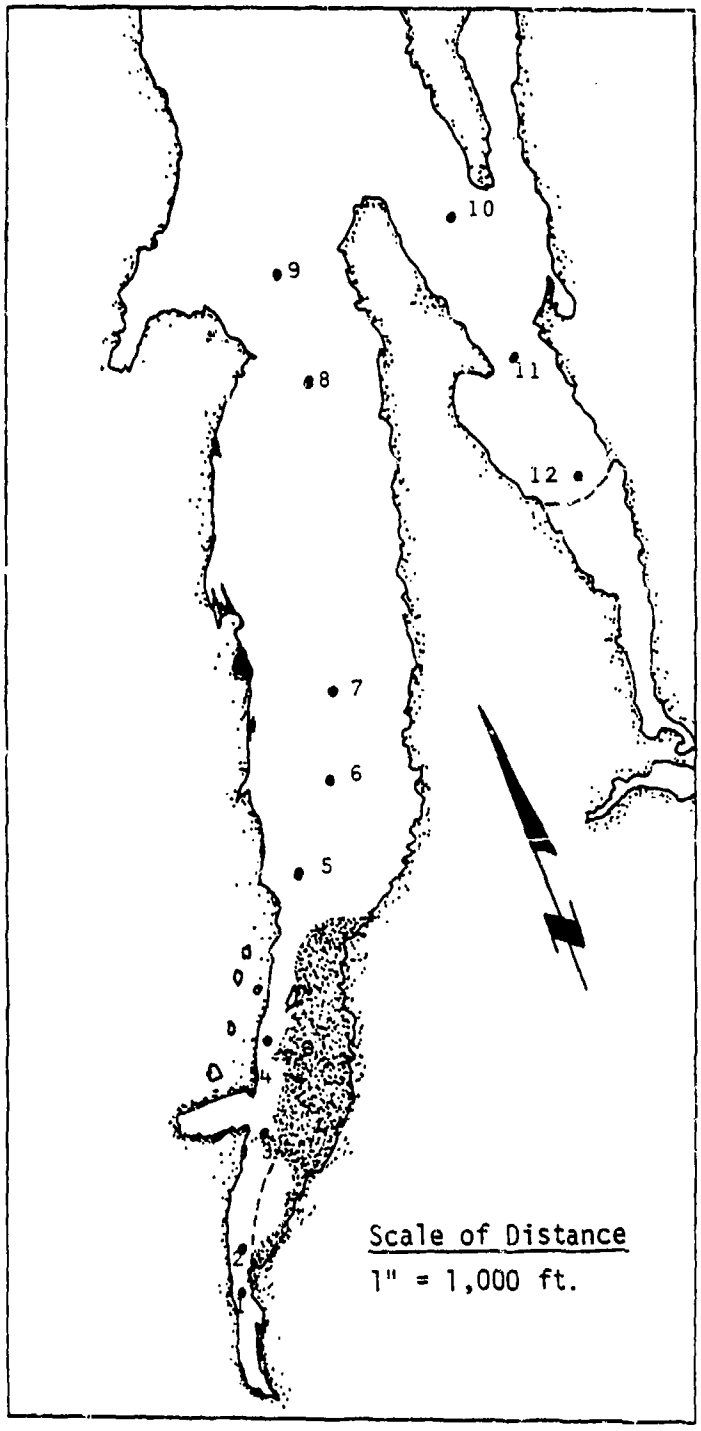

Figure II-9. Sampling Sites for Volunteer AAP (Sullivan *et al.*, 1978)

and the TNT levels were less than 1 ppb. The same trend for the increasing 1,3,5-trinitrobenzene levels in the sediment was also observed. 1,3,5-Trinitrobenzene levels increase from 73 µg/kg at station 1 to 200-300 µg/kg at stations 6-9.

These data seem to indicate that 1,3,5-trinitrobenzene is being formed in Waconda Bay, probably as a degradation product of from the photolysis of TNT. However, the 200-300 µg/kg levels found in the sediment do not indicate a long term build up of 1,3,5-trinitrobenzene.

Spanggord *et al.* (1978) found that 1,3,5-trinitrobenzene will not be substantially removed from the environment by photolysis. No information is available on any complexation of 1,3,5-trinitrobenzene in the environment. If complexation occurs, degradation of the resulting compounds will be more difficult.

3. Biodegradation and Bioconcentration

a. Biodegradation

The results of several studies on the biodegradation of 1,3,5-trinitrobenzene are presented in Table II-8. The data indicate that 1,3,5-trinitrobenzene can be degraded with rigorous treatment as shown by Bringmann and Keuhn (1971). However, under more natural conditions, 1,3,5-trinitrobenzene is only slightly degraded. McCormick *et al.* (1976) found that cell-free extracts of *Veillonella alkalescens* had the greater rate of hydrogen consumption with 1,3,5-trinitrobenzene than the other nitroaromatic compounds tested, thus, indicating that 1,3,5-trinitrobenzene was more readily reduced than other nitroaromatic compounds. Chambers *et al.* (1963), using a mixed culture with *Pseudomonads* predominating, and Villanueva (1960 and 1961), using *Nocardia sp.*, also found low levels of biodegradation of 1,3,5-trinitrobenzene.

The high levels of hydrogen used in McCormick's test indicates that the nitro groups are reduced to amino groups. McCormick *et al.* (1976) proposed the following mechanism:

$$R\text{-}NO_2 + H_2 \rightarrow R\text{-}NO + H_2O$$

$$R\text{-}NO + H_2 \rightarrow RNHOH$$

$$RNHOH + H_2 \rightarrow RNH_2 + H_2$$

Oxidative deamination then could take place. However, for ring cleavage to occur, the two hydroxyl groups should be ortho or para to each other. Since this is not the case for 1,3,5-trinitrobenzene, further degradation of 1,3,5-triaminobenzene or 1,3,5-trihydroxybenzene should be low.

b. Bioconcentration

The calculated octanol-water partition coefficient and bioconcentration factor for 1,3,5-trinitrobenzene and degradation products are presented in Table II-9. The values indicate that the chemical will not be highly concentrated by fish.

Table II-8. Summary of the Biodegradation Studies on 1,3,5-Trinitrobenzene

Reference	Test Chemicals	Concentration Used	Source of Microorganisms	Duration of Test	Criteria for Test Chemical Alteration	Results
Bringmann and Kuehn, 1971	1,3,5-trinitro-benzene	118-146 mg/l	Combined action of *Azotobacter agilis*, and microorganisms in activated sludge	36 hours	Estimation of nitro-reduced metabolites	95-97% reduction after second sludge
Chambers *et al.*, 1963	1,3,5-trinitro-benzene	100 mg/l	Microorganisms in soil, compost, or mud from a catalytic cracking plant waste Lagoon, adapted to degrade phenol	150 minutes	Oxygen Consumption in Warburg	Low oxygen consumption
Villanueva, 1960	1,3,5-trinitro-benzene	250 mg/l	Pure culture of *Nocardia* sp.	16 days	Growth	No growth as carbon source
Villanueva, 1961	1,3,5-trinitro-benzene	-	Pure culture of *Nocardia* sp.	1 hours	Arylamine levels	Arylamine formation

- not specified

Table II-9. Octanol-Water Partition Coeffecients and Bioconcentration Factor for Fish Exposed to 1,3,5-Trinitrobenzene and likely Degradation Products

Chemical	log P	BCF[2]
1,3,5-Trinitrobenzene	1.36[1]	6.4
1,3,5-Trihydroxybenzene	.19[3]	1.8
1,3,5-Triaminobenzene	.19[3]	1.8

[1] Spanggord *et al.*, 1978

[2] log BCF = .76 log P - .23 (Federal Register, 1979)

[3] Leo *et al.*, 1971

4. Effects on Animals

a. Mammals

The available information on the toxic effects of 1,3,5-trinitrobenzene to mammals is presented in Section D.

b. Birds

No information was found on the environmental effects of 1,3,5-trinitrobenzene on birds.

c. Fish

The toxicity of 1,3,5-trinitrobenzene to fish is presented in Table II-10. The data indicate that 1,3,5-trinitrobenzene is moderately toxic to fish during acute exposures.

Spanggord *et al.* (1978) reported that 1,3,5-trinitrobenzene was present in less than 10% of 79 condensate wastewater samples collected from Volunteer AAP. However, when 1,3,5-trinitrobenzene was present, the average levels were 0.15 ppm. Also, 1,3,5-trinitrobenzene levels were found to increase in the environment, due to photo or biodegradation of other munitions compounds in the effluent. Therefore, levels of 1,3,5-trinitrobenzene released in effluents could stress fish in the receiving waters near the effluent.

. Amphibians

No studies have been conducted on the effects of 1,3,5-trinitrobenzene on amphibians.

e. Invertebrates

The only study to determine the toxicity of 1,3,5-trinitrobenzene to invertebrates was conducted by Liu and Bailey (1977). They found that 1,3,5-trinitrobenzene levels of 2.7 ppm produced a 48-hr EC50 on the water flea (*Daphnia magna*). Thus, 1,3,5-trinitrobenzene should not be acutely toxic to aquatic invertebrates.

f. Microorganisms

The toxicity of 1,3,5-trinitrobenzene to microorganisms was determined by inhibition of growth. These data, presented in Table II-11 indicate that 1,3,5-trinitrobenzene is toxic to microorganisms at concentration in the low ppm range. The bacteria, *Streptococcus* sp. was the most sensitive organism tested, with growth inhibition at 1 mg/l.

Table II-10. Toxic Effects of 1,3,5-Trinitrobenzene to Fish

Species	Water Type	Hardness (ppm as $CaCO_3$)	Temperature °C	pH	Concentration (ppm)	Effect	Reference
Fathead Minnow *(Pimephales promelas)*	fresh	26	20	7.2 - 8.6	1.03	96-hr LC50	Liu and Bailey (1977)
Kuhlia sandvicensis	salt	--	--	--	10	violent irritant in 2 minutes	Hiatt *et al.*, (1953)
Kuhlia sandvicensis	salt	--	--	--	.1	slight irritant in 2 minutes	"

-- not specified

Table II-11. Growth Inhibition of Bacteria and Fungi by 1,3,5-Trinitrobenzene

Species	Concentration mg/l	Effect	Reference
Bacteria			
Streptococcus sp.	1	inhibition	Lecoq and Landrin (1951)
Human tuberculin bacillus	2	"	"
Staphylococcus aureus pyogenes	21	inhibition threshold	Zsolnai (1970)
Staphylococcus albus	8.5	" "	"
Escherichia coli communis	8.5	" "	"
Aerobacter aerogenes	21	" "	"
Proteus vulgaris	8.5	" "	"
Staphylococcus oxford	35	complete inhibition	Secareanu *et al.* (1965)
Escherichi coli	35	" "	"
Klebsiella pneumoniae	100	" "	"
Fungi			
Trichoderma viride	21	50% inhibition	Simon and Blackman (1953)
Trichophyton gypseum	43	inhibition threshold	Zsolnai (1970)
Epidermophyton	43	" "	"

Simon and Blackman (1953) investigated the relative toxicity nitrobenzene derivatives. The concentration of chemical required to halve the growth rate of the mold *Trichoderma viride* was used to determine the relative toxicity. The results of the *T. viride* inhibition tests are shwon in Table II-12.

The change from the parent material (nitrobenzene) to the p-nitro derivative resulted, on the average, in a 30-fold increase in toxicity. The second nitration brought about a slight increase of 1-3 times, while the third nitration (1,3,5-trinitrobenzene) caused the toxicity to increase by a factor of 16. The authors stated that as long as allowance is made of the pH factor, the change of activity in the nitrobenzene series is remarkably consistent irrespective of the test organisms and the nature of the biological response recorded. Their experiments indicated that the p-nitroderivatives were consistently found to be more toxic than the o-nitro derivatives. This difference in toxicity may be related to the fact that the o-nitro is less polar and has a higher volatility and lower solubility in water.

Table II-12. Concentration of Nitrobenzene Required for 50% Inhibition of Growth Rate of *Trichoderma viride* (Simon and Blackman, 1953)

Component	Concentration, mg/l
Nitrobenzene	589
o-dinitrobenzene	50
p-dinitrobenzene	20
1,3,5-trinitrobenzene	21

5. Effects on Plants

a. Phytotoxicity

Specific information concerning the phytotoxicity of 1,3,5-trinitrobenzene is unavailable.

b. Bioaccumulation and Degradation

No information was found on the bioaccumulation or biodegradation of 1,3,5-trinitrobenzene in plants.

F. Regulations and Standards

Since 1,3,5-trinitrobenzene is not used or produced in the U.S., there are no OSHA standards or discharge regulations for this compound. It is, however, listed in EPA's *Toxic Substances Control Act Candidate List of Chemical Substances* (1977). After several toxicity studies with rats, Korolev *et al.* (1977) suggested a maximum limit of 0.4 mg/l in drinking water.

The Department of Transportation lists 1,3,5-trinitrobenzene as a Class A explosive (Federal Register, 1976).

G. Conclusions and Recommendations

1,3,5-Trinitrobenzene is a military unique chemical. It is a by-product in the manufacture of 2,4,6-trinitrotoluene (TNT) and is present in the final TNT product at concentrations ranging from 0.1% to 0.7%. Since there is no civilian usage of 1,3,5-trinitrobenzene in the United States, the entry of this compound into the environment occurs only as a result of TNT manufacture and use. Additionally, photodecomposition of TNT to 1,3,5-trinitrobenzene can occur in the environment. Degradation of 1,3,5-trinitrobenzene in the environment is slow and occurs mainly through biological mechanisms.

1,3,5-Trinitrobenzene is weakly toxic to experimental animals in acute doses. It appears to be more toxic in subacute and chronic doses. The main pharmacologic effect of absorption of this chemical is the production of methemoglobin. Heinz bodies have also been observed. However, 1,3,5-trinitrobenzene is not as potent a methemoglobin former *in vivo* as 1,3-dinitrobenzene. As with 1,3-dinitrobenzene, the little evidence available suggests that 1,3,5-trinitrobenzene can be absorbed through the skin of animals.

Limited evidence suggests that 1,3,5-trinitrobenzene is not carcinogenic to mice nor has it been found to impair the reproductive functions of animals during chronic study. However, a dose-response mutagenic activity has been reported for 1,3,5-trinitrobenzene in the Ames assay.

1,3,5-Trinitrobenzene is moderately toxic to fish in acute exposures. No chronic data were available. Aquatic invertebrates appear to be less sensitive to this compound than fish.

1,3,5-Trinitrobenzene inhibits the growth of many microorganisms in the low ppm levels. Biodegradation of this compound occurs only slowly. The initial step in the biodegradation of 1,3,5-trinitrobenzene is a reduction of the nitro groups to amino groups.

Due to the potential exposure of human and animals to 1,3,5-trinitrobenzene in Waconda Bay, further studies on this compound are recommended in order to clarify its toxicological effects. These studies should include:

- chronic mammalian toxicological study to confirm the Russian study and to determine if levels of 1,3,5-trinitrobenzene in Waconda Bay are potentially dangerous to humans
- degradation study to determine the fate of 1,3,5-trinitrobenzene in the environment
- chronic aquatic toxicity study to determine the effects of long-term, low-level exposure of the compound to fish
- repeat of the Ames assay to confirm the dose-related response and of the yeast assay to score recombinational events.

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PROBLEM DEFINITION STUDY ON DI-N-PROPYL ADIPATE

SUMMARY

Di-n-propyl adipate is only used in the United States at Radford AAP in the production of propellants. This compound is manufactured by the Hatco Chemical Corporation for the Army. Losses at the Hatco plant in New Jersey are not known. However, it is estimated that losses at Radford into the New River are about 10 lb/month. Although this amount of di-n-propyl adipate should not pose a major pollution problem, scarcely any information exists concerning the toxicological and environmental hazards of this compound.

Only one study was available on the mammalian toxicity of di-n-propyl adipate. The compound had a low acute toxicity, and produced mild teratogenesis.

No aquatic toxicity data were available. However, similar adipates produced no toxic effects in fish exposed to low ppm concentrations. The probable degradation products of di-n-propyl adipate, adipic acid and n-propanol, have low toxicity to fish.

The calculated bioconcentration of di-n-propyl adipate is large. However, it is expected to be degraded rapidly to n-propanol and adipic acid. These products have low bioconcentration factors.

Due to the lack of information concerning the toxicological and environmental hazards of di-n-propyl adipate, the following studies are recommended to fill in information gaps.

- acute and chronic aquatic toxicity studies
- bioaccumulation and biodegradation studies with aquatic organisms, plants and microorganisms
- chronic and subacute mammalian toxicological studies
- corroboration of teratogenic effects of di-n-propyl adipate
- mutagenesis testing.

FOREWORD

A. Study Goals

This report presents the results of an evaluation of the available information on the toxicological and environmental hazards of di-n-propyl adipate. The only use of di-n-propyl adipate is at Radford Army Ammunition Plant in propellant production. This compound is manufactured for the Army by the Hatco Chemical Corporation. Entry into the environment should only occur at Radford AAP or at the Hatco Chemical Corporation plant. This evaluation of the toxicological and environmental hazards of di-n-propyl adipate was undertaken in order to aid the Army in identification of research needs and in recommendation of effluent criteria for this compound.

B. Study Methodology

The methodology utilized to gather information for this report included a detailed search of the literature and personal contacts. During the literature search, the following sources were reviewed for pertinent information on di-n-propyl adipate.

- Chemical Abstracts	1940-present
- Biological Abstracts	1950-present
- Excerpta Medica	1950-present
- TOXLINE	1965-present
- National Technical Information Service	1964-present
- Defense Documentation Center	1958-present
- Compendex	1970-present

Personal contacts were made with various civilian researchers. The specific contacts made and results of these contacts are presented below:

1. Contacts with U.S. Manufacturers

Ms. Grace Nartino of the Hatco Chemical Corporation was contacted on October 1, 1979. Material Safety Data Sheets on di-n-propyl adipate, and the related compound, di-2-ethylhexyl adipate, were sent. No toxicological data were present on either of these sheets.

2. Foreign Contacts

Two foreign companies were listed in the 1979 Directory of Chemical Producers of Western Europe. These companies were contacted by Telex in October, 1979.

ENGLAND
Leek Chemicals Ltd.

FRANCE
Manufacture Societe Dauphinoise
se Fabrications Chimiques
tour Manhatten

Leek Chemicals responded on October 23, 1979, but had no information. Ste. Dauphinoise responded on October 30, 1979 and also had no information.

TABLE OF CONTENTS

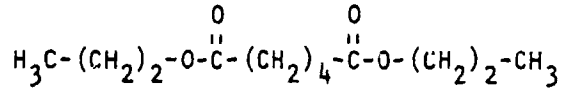
Table of Contents (cont'd.)

LIST OF TABLES

Di-n-Propyl Adipate*

A. Alternate Names

Di-n-propyl adipate is an ester of adipic acid, an aliphatic dicarboxylic acid. It has a molecular formula of $C_{12}H_{22}O_4$ and has the molecular weight of 230.31 g/mole. The structural formula for di-n-propyl adipate is:



$$H_3C\text{-}(CH_2)_2\text{-}O\text{-}\overset{O}{\overset{\|}{C}}\text{-}(CH_2)_4\text{-}\overset{O}{\overset{\|}{C}}\text{-}O\text{-}(CH_2)_2\text{-}CH_3$$

Other pertinent alternate names for di-n-propyl adipate are listed below:

CAS Registry No.: 106-19-4
Synonyms: Hexanedioic acid, dipropyl ester

B. Physical Properties

The physical properties of di-n-propyl adipate are listed in Table III-1.

Table III-1. Physical Properties of Di-n-propyl Adipate*

Physical Form at 20°C:	clear liquid
Color:	straw
Odor:	mild
MP:	-20.3°C
BP:	155°C/10 mm Hg
Density:	0.979_4^{20}
Refractive Index n_D^{20}:	1.4314
Viscosity:	(high)
Solubility:	insoluble in water soluble in ethanol, ether, chloroform
Vapor Pressure:	negligible

*Windholz, 1976; Hawley, 1977

The rate of decomposition of plasticizers when heated is proportional to the rate of change of the acid number (mg KOH/g of sample). When a plasticizer is held at a constant temperature (180°C) in the absence of oxygen, the rate of change of the plot of log of acid number vs. time is constant. Since the plots are linear, the slopes of the plots of a series of plasticizers indicates the relative thermal stabilities. Examples are given below:

diethyl adipate	0.16	diheptyl adipate	0.08
dipropyl adipate	0.15	dinonyl adipate	0.05
dioctyl adipate	0.10	ditridecyl adipate	0.04

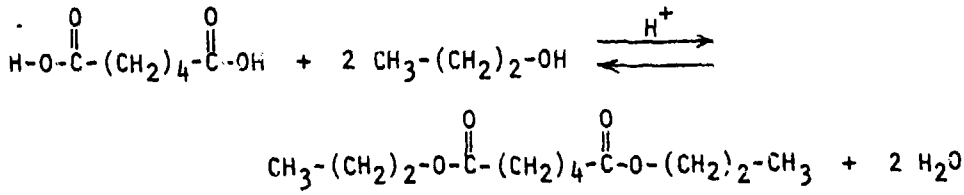
Therefore, it can be seen that di-n-propyl adipate is less stable than diethyl adipate and the stability continues to decrease as the length of the alkyl chain increases.

C. Chemical Properties

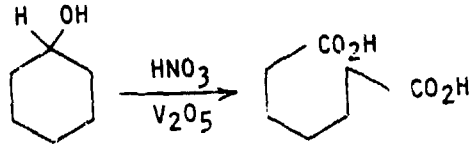
1. General Chemistry

a. Synthesis

Di-n-propyl adipate is prepared by the esterification of adipic acid with n-propanol.

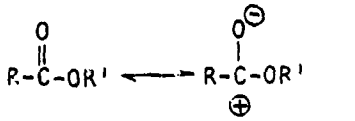


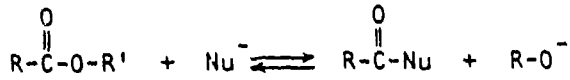
This reaction proceeds well with or without the acid catalyst. Without acid, either a large excess of alcohol is required or water must be removed by distillation. The adipic acid is generally obtained from the nitric acid oxidation of cyclohexanol (Roberts and Caserio, 1964);



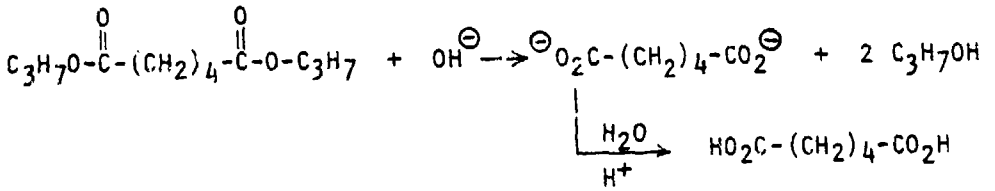
An alternate route could involve the reaction of the acid chloride with n-propanol.

b. Nucleophilic Substitution

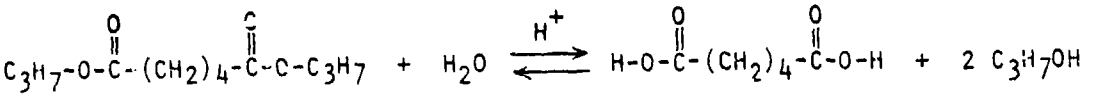
Due to the electron deficient nature of the carbonyl carbon of esters, *i.e.*  , the carbonyl carbon is susceptible to attack by nucleophiles. The major reaction of esters in general is nucleophilic substitution, *i.e.*:



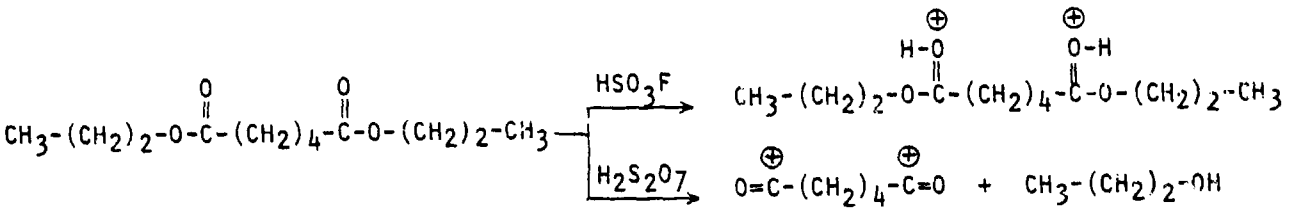
The most common example of this reaction is the hydrolysis or saponification when carried out in base.



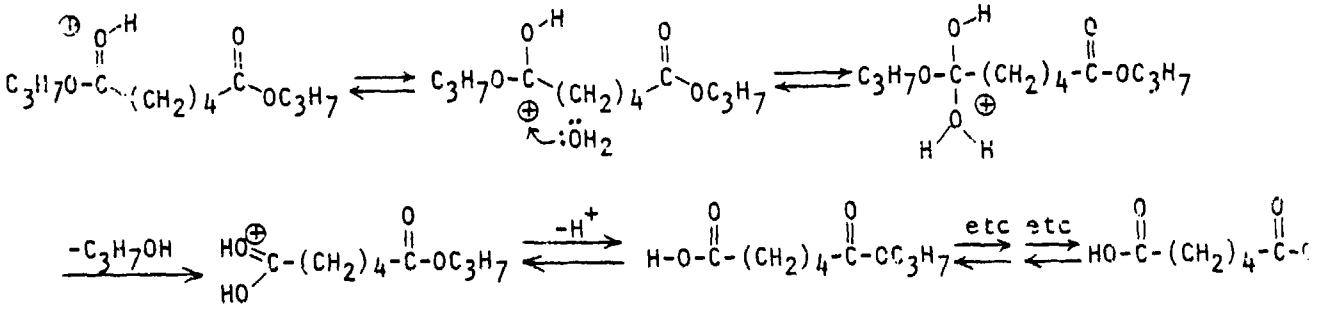
The acid catalyzed hydrolysis involves an initial protonation with formation of an intermediate oxonium ion.



The behavior of di-n-propyl adipate in fluorosulphuric and disulphuric acids has been reported (Malhotra and Sehgal, 1974). Protonation occurs in HSO_3F while in $H_2S_2O_7$ an oxycarbonium ion forms:

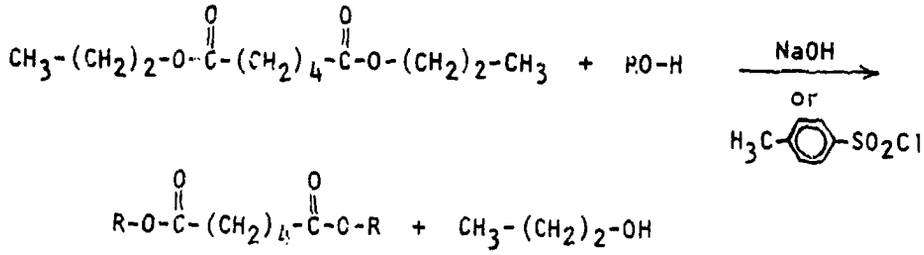


In aqueous acid the oxonium/carbonium ion undergoes nucleophilic attack by water:



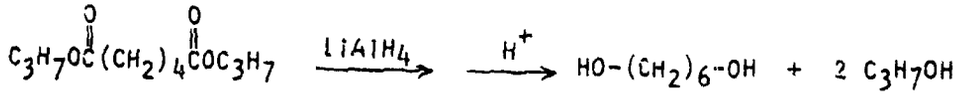
The reaction as indicated is an equilibrium, the reverse of the esterification reaction by which di-n-propyl adipate is formed. In excess water, hydrolysis is favored whereas in n-propanol, formation of the ester is favored.

Transesterification reactions are also examples of nucleophilic substitution reactions and have been reported (Bondar *et al.*, 1971). Propanol was removed by distillation to increase the yield.

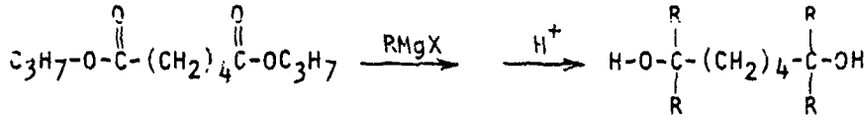


Other nucleophilic substitutions which are typical of aliphatic esters include (Morrison and Boyd, 1973):

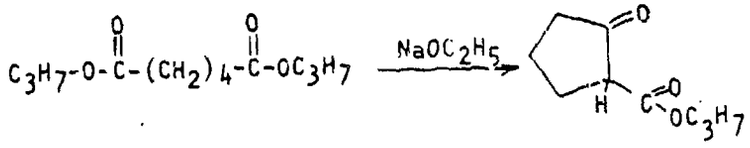
(1) reduction by metal hydrides;



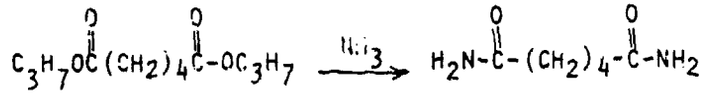
(2) reaction with Grignard reagents;



(3) cyclization *via* the Dieckmann condensation;

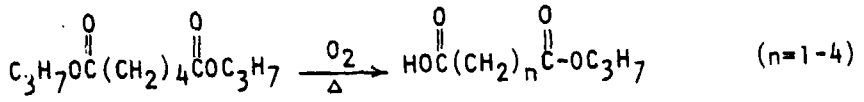


(4) conversion into amides



c. Oxidation

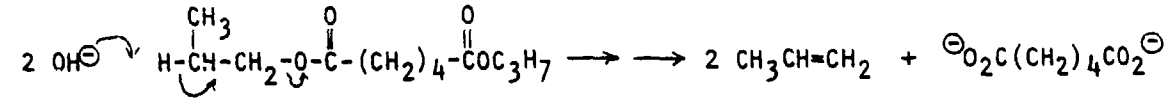
Oxidation of di-n-propyl adipate has been reported to involve mainly C-C bond cleavage with decarbonylation and decarboxylation leading to monoesters of shorter dicarboxylic acid (Fedorishcneva *et al.*, 1974; Fedorishcheva *et al.*, 1972).



$$C_3H_7OC(CH_2)_4COC_3H_7 \xrightarrow[\Delta]{O_2} HOC(CH_2)_nC\text{-}OC_3H_7 \qquad (n=1\text{-}4)$$

d. Elimination Reactions

Di-n-propyl adipate is also likely to undergo E_2 reactions in base due to the adipate anion being a good leaving group. Abstraction of β-protons from the alcohol moieties should give propylene and adipate ion.



$$2\ OH^{\ominus} + H\text{-}\underset{CH_3}{CH}\text{-}CH_2\text{-}O\text{-}C\text{-}(CH_2)_4\text{-}COC_3H_7 \longrightarrow\longrightarrow 2\ CH_3CH{=}CH_2 + {}^{\ominus}O_2C(CH_2)_4CO_2^{\ominus}$$

2. Environmental Reactions

The environmental chemistry of di-n-propyl adipate has not been investigated. Almost certainly, however, the predominant reaction is hydrolysis to adipic acid and propanol. Oxidative decarboxylation of the resultant monopropylester or adipic acid itself is also expected to occur yielding glutamic, succinic, malonic and/or oxalic acids.

3. Monitoring and Analysis

a. Analytical Methods

Analysis for di-n-propyl adipate has been accomplished by gas chromatography. Analytical conditions are listed in Table III-2. Bloom (1975) has also reported the use of thin layer chromatography on silica gel. Solvent systems included (A) methylene chloride/carbon tetrachloride/hexane/ethyl acetate (75:10:10:5), (B) hexane/carbon tetrachloride/ethyl acetate (60:35:15), and (C) isooctane/isoamyl acetate (85:15). Visualization was accomplished by spraying with phosphomolybdic acid and heating at 160°C. Detection of 25 µg samples proved possible.

b. Monitoring

No reference to monitoring studies for di-n-propyl adipate was encountered in the literature.

Table III-2. Gas Chromatography of Di-n-propyl Adipate

Reference	Column	Temperature, °C	Detector	Sensitivity
Strauss, 1973	steel capillary with LB-550X oil or Apiexon M	100-130°C	FID	*
Dykes and Alley, 1974	3.8% OV-101;	120°C (programmed to 220°C)	FID	5-7 µg
Alley and Dykes, 1972	2.5% OV-210;	116°C "	*	*
	1.1% OV-225;	122°C "	*	*
Bloom, 1975	1% QF-1 on Chromosorb Q	160-200°C	FID	2-5 µg
Zulaica and Guiochon, 1962; *Ibid*, 1963	0.5% SE30	200°C	TC	*
	0.5% Neopentylglycol polysebacate	-240°C	*	*

* Not Specified

D. Health Effects

1. Biology

No information on the biochemistry of di-n-propyl adipate was found in the literature. This chemical exists as a liquid with negligible vapor pressure at ambient temperatures (Hatco, 1979a). Therefore, the most likely methods of exposure are through skin absorption and ingestion due to improper personal hygiene.

2. Effects of Human Exposure

No literature could be found on the toxic effects of di-n-propyl adipate to humans. The Materials Safety Data Sheet obtained for this compound from Hatco (1979b) lists no TLV. Di-n-propyl adipate is believed to be essentially non-toxic to humans (Hatco, 1979b). The compound may be a slight skin or eye irritant. This possibility is based on limited skin and occular toxicity studies on rabbits of two similar compounds, diisoamyl adipate and di-2-ethylhexyl adipate (Blaisdell, 1954). The Hatco Chemical Corporation suggests the use of protective gloves for prolonged exposure, but no other special handling procedures, other than those used for handling lubricants, are recommended.

3. Effects on Experimental Animals

Only one article was found which specifically dealt with the toxic effects of di-n-propyl adipate on experimental animals. This limited information is discussed in the following sections.

a. Acute Toxicity

Singh *et al.* (1973) determined the acute toxicity of di-n-proply adipate to Sprague-Dawley rats. The rats weighed between 175 and 225 g. Graded doses of di-n-propyl adipate were administered by intraperitoneal injection. The animals were observed for 7 days after the dosing. The acute LD50, 95% confidence limits and slope were calculated by the method of Cornfield and Mantel (1950). The acute LD50 found for di-n-propyl adipate was 3.7858 ml/kg with the 95% confidence limits between 3.3279 and 4.2087 mg/kg and a slope of 19.55 (probits/log of dose).

b. Subacute and Chronic Toxicity

No information was found on the subacute or chronic toxicity of di-n-propyl adipate to experimental animals.

c. Teratogenicity

Singh *et al.* (1973) evaluated the embryonic-fetal toxicity and teratogenicity of various adipates to rats. Sprague-Dawley virgin females, weighing between 175 and 225 g, were the test animals. A "stud" pool of the same strain of male rats was used. Females were selected only after the

observation of at least two complete estrus cycles. Cycles were monitored by vaginal smears. After females had been selected, 5 were housed with one male in a large cage. The onset of gestation was established by the presence of sperm in the vaginal smear. This day was designated Day 0, with the next day being Day 1 of the gestation period. At Day 1, females were moved to individual cages. Treatment was administered by intraperitoneal injection on the 5th, 10th and 15th days of gestation. Four dosage levels were used, with five females in each group. The dosage levels were 1/30 (0.1262 ml/kg), 1/10 (0.3786 ml/kg), 1/5 (0.7572 ml/kg) and 1/3 (1.2619 ml/kg) of the acute LD50. Controls were injected with either distilled water, normal saline or cottonseed oil. In addition, a "blunt-needle-injected" group was used for control. For this group, a dull needle was inserted in a manner similar to all other injections, but no substance was injected.

On the 20th day of gestation, which is one day prior to expected birth, the rats were sacrificed by ether inhalation. The uterine horns and ovaries were exposed to allow counting of corpora lutea, resorption sites, and viable and dead fetuses. Fetuses were removed, blotted dry and weighed. All of the fetuses were examined for gross abnormalities. A randomly selected number, about 50% of the total number of fetuses were prepared for visualization of skeletal abnormalities. Whenever possible, those showing gross deformities were excluded from this group. The remaining fetuses were examined for visceral abnormalities. A summary of these results is presented in Table III-3. All control and treatment groups had one or more resorption, with the highest incidence, 20% at the highest dose level. From the table, it can also be seen that no fetal deaths occurred at any of the di-n-propyl adipate doses. Malformations tended to show a dose related incidence, and were mostly present as poorly developed small fetuses. One of the fetuses at the 0.757 ml/kg dose had no tail. Other abnormalities noted were hemangiomas, twisted hindlegs, and compact head and neck. No visceral abnormalities were present at any dose level, and skeletal abnormalities only appeared at the highest dose level, 1/3 of the LD50. The only significant ($p<0.05$) decrease in fetal weight occurred in the 1/5 LD50 group.

The teratogenic effects of adipic acid esters were rigorously investigated in this study. The methodology used was sound, however, the number of animals per dose was small(5). The results indicated that di-n-propyl adipate produced few gross, skeletal or visceral abnormalities. The abnormalities observed were mainly at higher dose levels. Only the number of gross abnormalities and resorptions at the highest dose level were greater than the 95% confidence interval of the pooled volume controls (Singh *et al.*, 1973). Since one thirtieth of the LD50 produced no fetal deaths or abnormalities, di-n-propyl adipate may be considered non-teratogenic to rats at this level (Singh *et al.*, 1973).

d. Mutagenicity and Carcinogenicity

No information was found in the literature on the mutagenicity or carcinogenicity of di-n-propyl adipate.

Table III-3. Teratogenic Effects of Di-n-propyl Adipate in the Rat

Treatment Group	Volume Injected, ml/kg	No. of Corpora Lutea	Number of Resorptions (%)	Number of Dead Fetuses (%)	Number of Live Fetuses (%)	Mean Weight of Fetuses, g	Abnormalities		
							Gross (%)(a)	Skeletal (%)(b)	Visceral (%)(c)
Controls									
Blunt Needle	--	69	4(6.0)	0	63(94.0)	3.91±0.02	0	1(3.0)	0
Distilled Water	10.00	59	4(6.8)	0	55(93.2)	4.40±0.33	0	0	-
Normal Saline	10.00	62	7(11.5)	0	54(88.5)	4.10±0.13	1(1.9)	4(14.3)	-
Cottonseed Oil	10.00	71	5(7.5)	0	62(92.5)	3.89±0.09	1(1.6)	2(6.3)	0
Di-n-propyl adipate	0.1262	66	2(3.2)	0	61(96.8)	3.90±0.09	0	0	0
Di-n-propyl adipate	0.3786	61	6(10.9)	0	49(89.1)	3.77±0.17	1(2.0)	0	0
Di-n-propyl adipate	0.7572	64	6(9.8)	0	55(90.2)	3.63±0.11	2(3.6)	0	0
Di-n-propyl adipate	1.2619	56	9(20.0)	0	36(80.0)	3.61±0.22	2(5.6)	1(5.3)	0

Complied from Singh *et al.* (1973)

a. Based on total number of fetuses

b. Based on total number of stained fetuses (50% of total fetuses)

c. Based on total number of unstained fetuses

E. Environmental Effects

1. Entry into the Environment

The only use of di-n-propyl adipate in the United States is at Radford AAP in the production of double base propellants by the solventless process. Current losses of di-n-propyl adipate from the propellant production processes are estimated at 20-25 lb/month (Kitchens *et al.*, 1979). Approximately half of these amounts enter the New River. Potential levels of di-n-propyl adipate in the New River (mean flow of 2380 MGD) resulting from this discharge are:

percent mixing	levels of di-n-propyl adipate
1	2.8 ppb
10	.28 ppb
100	.028 ppb

2. Behavior in Soil and Water

No information is available on the adsorption or leechability of di-n-propyl adipate in soils or sediments. Calculations of the octanol-water partition coefficient of di-n-propyl adipate are presented in Table III-4. The lower coefficient would appear to be more reliable because the starting compound is similar to di-n-propyl adipate. The calculations indicate a relatively high log P. Estimated bioconcentration factors (BCF) for di-n-propyl adipate and its probable breakdown products are presented in Table III-5. Although di-n-propyl adipate has a high estimated BCF, the compound should be rapidly degraded to propanol and adipic acid. The degradation products have very low BCF's.

3. Effects on Animals

a. Mammals

The effects of di-n-propyl adipate on mammals are presented in Section D.

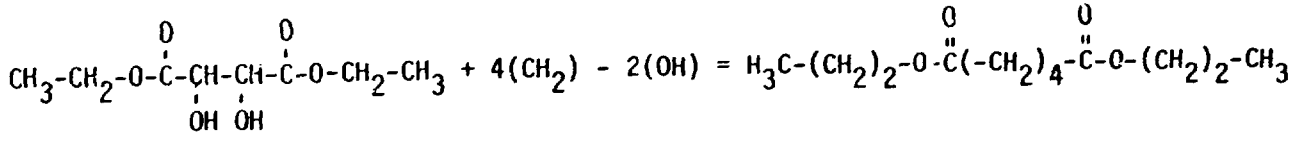
b. Birds

No information is available on the effects of di-n-propyl adipate on birds.

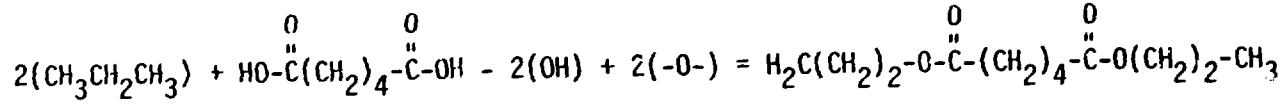
c. Fish

No information was found on the toxic effects of di-n-propyl adipate to fish. However, the aquatic toxicity of some similar compounds is presented in Table III-6. It is likely that di-n-propyl adipate will have a similar toxicity to fish. The probable degradation products, n-propanol and adipic acid were found to have a low toxicity to fish.

Table III-4. Calculations of log P for Di-n-propyl Adipate[1]



- .29 + 4(.50) - 2(-1.16) = 4.03



2(2.36) + .08 - 2(-1.16) + 2(-.98) = 5.16

1. log P values from Lec *et al.* (1971)

Table III-5. Octanol-Water Partition Coefficients Values and Bioconcentration Factors for Di-n-propyl Adipate and its Breakdown Products for Fish

Chemical	Log P	BCF[2]
di-n-propyl adipate	4.03	670
di-n-propyl adipate	5.16	5000
propanol	.34[1]	1.1
adipic acid	.08[1]	0.7

1. Leo *et al.* (1971)

2. log BCF = .76 log P - .23 (Federal Register, 1979)

Table III-6. Aquatic Toxicity of Similar Compounds to Di-n-propyl Adipate

Compound	Fish	Concentration (ppm)	Effect	Reference
Diester 2-ethyl-hexyl adipate	Rainbow Trout *(Salmo gairdnerii)*	5	no Effect	Applegate *et al.* (1957)
" "	Bluegill Sunfish *(Lepomis macrochirus)*	5	"	" "
" "	Lamprey Larvae *(Petromyzon* sp.)	5	"	" "
Monobutyl ester adipic acid	Rainbow Trout *(Salmo gairdnerii)*	5	"	" "
" "	Bluegill Subfish *(Lepomis macrochirus)*	5	"	" "
" "	Lamprey Larvae *(Petromyzon* sp.)	5	"	" "
" "	Squawfish *(Ptychocheilus oregonensis)*	10	"	MacPhee and Ruelle (1969)
" "	Chinook Salmon *(Oncorhynchus tshawytscha)*	10	"	" "
" "	Coho Salmon *(O. Kisutch)*	10	"	" "
n-Propanol	Goldfish *Carassius auratus)*	500	death 24 hr	CHRIS (1974)

d. Amphibians

No toxicity studies were found concerning the effects of di-n-propyl adipate on amphibians.

e. Invertebrates

No information was available on the toxicity of di-n-propyl adipate to invertebrates.

f. Microorganisms

No information was found on the toxicity of di-n-propyl adipate to microorganisms. However, adipic acid at concentrations of 100 mg/l had no effect on bacteria or protozoa (Meinck *et al.*, 1970).

4. Effects on Plants

a. Phytotoxicity

No specific information on the physiological effects of di-n-propyl adipate to vegetation was encountered in the literature search. Several references concerning the phytotoxicity of adipic acid indicate that it is relatively non-toxic to plants in moderate concentrations. Meinck *et al.* (1970) reported that no adverse effects were observed in algae at a concentration of 100 mg/l. Prill *et al.* (1949) observed that a 1.25 M concentration of adipic acid resulted in 50% inhibition in the growth of wheat seedling roots.

b. Bioaccumulation and Biodegradation

No information was found on the bioaccumulation or degradation of di-b-propyl adipate by plants.

F. Regulations and Standards

There are no health or environmental standards in the U.S. specific for di-n-propyl adipate.

G. Conclusions and Recommendations

The only use of di-n-propyl adipate in the United States is at Radford AAP. The estimated amount of this compound entering the New River is about 10 lb/month. Radford AAP is one of only two potential sources of entry into the environment for di-n-propyl adipate.

Di-n-propyl adipate has a large bioconcentration factor. However, the compound should be rapidly degraded to propanol and adipic acid. Both of these compounds have low bioconcentration factors.

Very little information exists on the toxicological and environmental hazards of di-n-propyl adipate. One acute animal study found the compound to be of low toxicity and mildly teratogenic.

No data were available on the effects of di-n-propyl adipate on fish. However, similar adipates produced no toxic effects at low ppm levels. In addition, the probable degradation products of di-n-propyl adipate, n-propanol and adipic acid, have low toxicities to fish.

Although pollution due to di-n-propyl adipate is not presently a major environmental problem, there is a scarcity of information on the properties of this compound. Therefore, the following studies are recommended for di-n-propyl adipate.

- bioaccumulation and biodegradation studies with plants, microorganisms and aquatic organisms
- acute and chronic aquatic toxicity studies
- additional mammalian studies to confirm the acute toxicity data and to determine chronic toxicity
- confirmation of teratogenic effects
- Ames test for mutagenesis.

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ABBREVIATIONS AND SYMBOLS

@	- at
Å	- Angstrom unit
AAP	- Army Ammunition Plant
∿	- approximately
asym	- asymetric
BCF	- bioconcentration factor
B.P.	- boiling point
c	- combination or overtime frequency
°C	- degrees centigrade
cal	- calorie
cm	- centimeter
CM^{-1}	- wavenumber
cm^2	- square centimeters
conc.	- concentration
cps	- cycles per second
d	- density
D	- Debje
δ	- chemical shift in parts per million
dp	- depolarized
ε	- molar absorptivity coefficient
e	- electron
EC	- electron capture detector
EC50	- concentration required to affect 50% of the exposed population
EPA	- Environmental Protection Agency
eV	- electron volt
FID	- flame ionization detector
ft	- feet
g	- gram
H^+	- hydrogen ion

ABBREVIATIONS AND SYMBOLS
(cont.)

ΔHp	- heat of combustion at constant pressure
HMX	- octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
$h\nu$	- light energy
HPLC	- high performance liquid chromatography
hr	- hour
i.p.	- in-plane
J	- coupling constant
kg	- kilogram
l	- liter
$<$	- less than
$\leq$	- less than or equal to
λ_{max}	- wavelength of absorbance maxima
lb	- pounds
LCLo	- lowest lethal concentration
LC50	- concentration required to kill 50% of the exposed population
LC100	- concentration required to kill 100% of the exposed population
LD50	- dose required to kill 50% of the exposed population
log P	- logarithum of the octanol-water partition coefficient
μ	- micro
m	- meter
m	- medium
m^3	- cubic meter
μCi	- microCurie
m/e	- mass to charge ratio
mg	- milligram
mG	- megaGauss
μg	- microgram
MGD	- million gallons per day
min	- minute
ml	- milliliter
μl	- microliter
mM	- millimoles

ABBREVIATIONS AND SYMBOLS
(cont.)

mm Hg	- unit of pressure
μ moles	- micromoles
mm	- millimeter
M.P.	- melting point
NCI	- National Cancer Institute
n_D	- refractive index
nm	- nanometer
NMR	- nuclear magnetic resonance
Nυ	- nucleophile
o	- ortho
o.p.	- out-of-plane
p	- para
p	- probability
p	- polarized
%	- percent
ppb	- parts per billion
ppm	- parts per million
RDX	- hexahydro-1,3,5-trinitro-1,3,5-triazine
redn	- reduction
s	- strong
sh	- shoulder
sp.	- specie
sym	- symmetric
TC	- thermal conductivity detector
TLV	- threshold limit value
TNT	- 2,4,6-trinitrotoluene
USSR	- Union of Soviet Socialist Republics
UV	- ultraviolet
V	- volume
v	- very
vs	- versus
w	- weak
w	- weight

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