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MAMMALIAN TOXICOLOGY AND TOXICITY TO AQUATIC ORGANISMS
OF FOUR IMPORTANT TYPES OF WATERBORNE MUNITIONS POLLUTANTS:
AN EXTENSIVE LITERATURE EVALUATION

ARMY MEDICAL BIOENGINEERING RESEARCH AND
DEVELOPMENT LABORATORY

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The purpose of this work is to provide a summary review and evaluation of the toxicological and related literature on known components of four types of military-relevant wastewaters. These are nitrocellulose and nitroglycerin manufacturing wastes, "phosy water" (from white phosphorus processing), and "pink water" (from TNT production and processing). This report consists of brief descriptions of the wastes along with the most significant toxicological (cont)		

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20. Abstract (Continued)

information concerning them, conclusions and recommendations for future work.

The recommendations indicate the need for the following types of investigations:

(1) Chemical studies, encompassing characterization, development of analytical methods, and the determination of rates of decomposition in aqueous solution.

(2) Mammalian toxicology, to include, on a selective basis: oral acute, chronic and long-term sub-chronic toxicities; dermal acute toxicity; skin and eye irritancy; skin sensitization; and carcinogenicity, mutagenicity and teratogenicity.

(3) Studies on toxicity to aquatic species, to include freshwater biota from receiving waters at major trophic levels. Where appropriate, the metabolites resulting from biological treatment of these wastewaters should be determined. Field studies should be undertaken, as required.

The four Appendices contain detailed literature reviews and evaluations of the toxicology of the known waste constituents (or of mixtures) to mammalian and aquatic species.

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ERRATUM

Due to a paging error prior to printing, this report contains pages 84a through 84j between pages 84 and 85. However, this does not effect the Table of Contents.

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LIST OF ABBREVIATIONS

AAP	Army Ammunition Plant
ACGIH	American Conference of Government Industrial Hygienists
BOD	Biochemical Oxygen Demand
ca	circa (about)
CN	Cyanide
COD	Chemical Oxygen Demand
ct	cutaneous
DNT	Dinitrotoluene
DOD	Department of Defense
EGD	Ethylene Glycol Dinitrate
g	gram
GLC	Gas-liquid Chromatography
gpm	gallons per minute
HAAP	Holston Army Ammunition Plant
IC	Inorganic Carbon
ig	intra gastric
im	intramuscular
ip	interperitoneal
iv	intravenous
kg	kilogram
LC50	Lethal Concentration (kills 50%)
LD	Lethal Dose
LD50	Lethal Dose (kills 50%)
LT	Lethal Time
LT50	Lethal Time (kills 50%)
M	Molar
MAC	Maximum Allowable Concentration

MAO	Monoamine Oxidase
mg	milligram
MGD	Million Gallons per Day
MLD	Minimum Lethal Dose
MLSS	Mixed Liquor Suspended Solids
NG	Nitroglycerin
or	oral
OSHA	Occupational Safety and Health Administration
P, P ₄	Phosphorus
³² P	Phosphorus isotope 32
ppb	parts per billion
ppm	parts per million (mg per liter)
sc	subcutaneous
SS	Settleable Solids
SUS	Suspended Solids
TLC	Thin-layer Chromatography
TLm	Median Tolerance Level
TLV	Threshold Limit Value
TNT	2,4,6-Trinitrotoluene
TOC	Total Organic Carbon
VS	Volatile Solids
WP	White Phosphorus
µg	microgram

INTRODUCTION

The purpose of this work is to provide a summary review and evaluation of the toxicological and related literature on four types of military-relevant wastewaters. This information will contribute towards the development of standards for these wastewater discharges. The four types of wastewaters covered here were selected from a study by Rosenblatt, Small and Barkley (1973)¹, primarily because of the large volume of both products and wastewaters in which they are produced. They are nitrocellulose manufacturing wastes, nitroglycerin manufacturing wastes, "phossy water" (water that has been used to protect white phosphorus from ignition on contact with air), and trinitrotoulene (TNT)-containing "pink water". The available mammalian and aquatic organism toxicology of each type of waste is discussed in a separate Appendix to this report. These appendices represent the effort of a team of toxicologists, aquatic biologists and auxiliary personnel who assembled and evaluated the information during the summer of 1973.

On completion of these reviews, an investigation (Contract No. DAMD 17-74-C4073, Midwest Research Institute) was initiated to supply acceptable toxicological information on the substances of interest. This was essential, since the reviews had clearly shown that there was insufficient existing information on which to establish discharge standards. The information reported in the review was of great importance in identifying the physiological systems to be emphasized, and in documenting cases of human exposure. In the near future, research will be started on the toxicity of the wastes to aquatic organisms.

The objectives of mammalian toxicology and the toxicology of aquatic organisms differ importantly in the present context. Mammalian species are chosen to represent those biological systems and vulnerabilities most closely resembling their counterparts in man. The interest in aquatic biology is derived primarily from the possibility of hazard to human beings via the food chain, and secondarily from consideration of potential damage to the environment. In either case, the effects of the pollutants must be studied as they are related to species and conditions indigenous to the receiving waters.

NITROCELLULOSE MANUFACTURING WASTES

When cellulose is nitrated to produce guncotton, it must be washed and beaten extensively to remove inorganic acids. As a result, the wastewaters contain both the neutralized sulfuric and nitric acids, and suspended nitrocellulose linters (Rosenblatt et al., 1973)¹. It would also appear that these wastes may contain variable amounts of dissolved organic compounds, possibly nitrate esters (Hackley, 1974)². There is no information at present to indicate that the nitrocellulose in wastewaters is either harmless or harmful to man, animals or aquatic biota (Appendix I).

NITROGLYCERIN MANUFACTURING WASTES

In the manufacture of nitroglycerin (glyceryl trinitrate), the washwaters are discarded. These contain not only nitroglycerin, but also two isomeric glyceryl dinitrates, two isomeric glyceryl mononitrates, and a number of degradation products that have yet to be fully characterized (Hackley *et al.*, 1974)³. There is abundant qualitative literature on human exposures to nitroglycerin, and a small body of worthwhile information on the mammalian toxicology (including metabolic fate studies) of nitroglycerin. Essentially no toxicological information on the mono- and dinitrate esters of glycerol could be found.

The acute toxicity of nitroglycerin has been measured in the rat, rabbit, guinea pig, cat and dog by most routes of administration (oral MLD rat 80-100 mg/kg; LD oral man 120-125 mg/kg with survival at 400 mg recorded). The pathological picture at death in animals and man is well documented. Apart from ethylene glycol dinitrate (LD 300 mg/kg) little is known of the acute toxicities of the dinitrates; nor is there any information on the toxicology of nitroglycerin degradation products. Chronic toxicity, short-term, is also well documented but no conventional long-term studies have been carried out in experimental animals. This is true also for all the other compounds considered in this study.

There is no record of carcinogenic, teratogenic or mutagenic studies on nitroglycerin and related compounds. Nor have any data been found on the toxicity of nitroglycerin to aquatic organisms. Nitroglycerin is rapidly metabolized in animals and man by the active nitroreductase enzymes via the known dinitrates and mononitrates and glycerol to carbon dioxide.

The application of gas-liquid chromatography to the microanalysis of nitroglycerin and ethylene glycol dinitrate in human, animal, and environmental exposures has been described in the literature.

Nitroglycerin thus appears to be relatively non-toxic in acute, sub-acute and chronic studies; it is metabolized rapidly to non-toxic compounds in animals and man. This information would indicate that there should be no real hazard in munitions wastes from nitroglycerin, its metabolites or possible breakdown products. Attention, however, is drawn to the possible carcinogenic hazard of these aliphatic nitrates through N-nitrosamine formation in the organism or environment and to the potentiality of interaction of nitroglycerin and its analogs with other chemicals in the wastes.

The literature on nitroglycerin toxicology is reviewed in Appendix II. An additional reference (Kyllin, Englund, Ehrner-Samuel and Yllner, 1964)⁴, obtained subsequent to the compilation of Appendix II, did not affect the conclusions reached here.

"PHOSSY WATER"

At Pine Bluff Arsenal, elemental white phosphorus is loaded into munitions (Rosenblatt *et al.*, 1973)¹. This pyrophoric substance is protected from the atmosphere by a blanket of warm water; the water, containing colloidal white phosphorus and assorted forms of oxidized phosphorus and possibly phosphine is termed "phossy water". It would appear that white phosphorus and phosphine are so much more poisonous than any of the associated derivatives that they alone have been the subject of toxicological studies.

White phosphorus is highly toxic to both experimental animals and man. Ingestion of even small amounts may produce severe gastrointestinal irritation, bloody diarrhea, liver damage, skin eruptions, oliguria, circulatory collapse, coma, convulsions and death. The fatal dose for man is about 1-1.4 mg/kg. No LD50 values have been determined. Acute effects differ considerably from chronic effects. Chronic poisoning (from ingestion or inhalation) is characterized by such effects on the osseous system as bony necrosis ("phossy jaw") and spontaneous fractures, as well as by anemia and weight loss. White phosphorus appears to be noncarcinogenic when fed to experimental animals. Testing for mutagenicity and teratogenicity has not been carried out. Many studies of a biochemical nature have been reported and have been summarized in the report. The primary effect of phosphine is neurological, and in this it differs markedly from white phosphorus.

White phosphorus is also highly toxic to aquatic animals. The 96 hr LC50's are less than 50 micrograms/liter ($\mu\text{g/l}$) for all fish studied, and the incipient lethal level is probably less than 1 $\mu\text{g/l}$ for most fish. Crustaceans and many molluscs are more tolerant, but still succumb to phosphorus concentrations of 1 milligram/liter (mg/l) or less. Phosphorus poisoning appears to be cumulative and irreversible for fish and lobsters, though the cause of mortality in fish has not been determined. There is no information on the toxicity of phosphine to aquatic biota or experimental animals.

A detailed literature review is contained in Appendix III.

TNT, DNT AND OTHER CONSTITUENTS OF "PINK WATER"

Wastewaters from TNT manufacturing and loading facilities contain not only α -TNT (2,4,6-trinitrotoluene), but also DNT (dinitrotoluene) isomers, especially 2,4- and 2,6-DNT. High pH and/or exposure to sunlight produce extensive TNT decomposition to a large number of derived compounds (Burlinson *et al.*, 1973⁵; Hackley *et al.*, 1974³), including both uncharged

and anionic species. The brownish to reddish color of the photodecomposition products gives those aqueous wastes the name "pink water". Not all of the decomposition products have been identified. Work is still in progress on the analytical characterization of "pink water" (Hackley *et al.*, 1974)³. This must proceed at least to the extent that reasonably typical and reproducible examples of "pink water", or selected fractions thereof, may be generated at will for toxicological evaluation. Of the "pink water" constituents, only TNT and (to a lesser extent, and without regard to isomeric form) DNT have been the subjects of toxicological investigation.

Acutely toxic doses of TNT to mammals vary with species and route of administration, starting somewhere below 200 mg/kg. No LD50 values have been determined. Chronic doses in the range of 5-100 mg/kg cause anemia, hemolysis and associated disorders in mammals. In man, hematologic changes are followed by such manifestations as toxic hepatitis, effects on the central nervous system, and, on extremely prolonged exposure, cataract formation.

The order of magnitude for DNT toxicity is about that for TNT. Human beings exposed to DNT (primarily the 2,4-isomer) suffer from a variety of neurological disorders, apparently without hematologic changes.

No evidence has been found for mutagenic, carcinogenic and teratogenic effects or for sensitization, by TNT or DNT. In view of the attention paid to TNT factory workers over long periods of time, it is unlikely that TNT causes sensitization or tumor formation.

The main TNT metabolite identified in biochemical studies is 4-amino-2,6-dinitrotoluene.

Ninety-six hour TLm values for fish lie in or near the range of 2-3 mg/l of TNT. The growth of some aquatic microorganisms appears to be inhibited by TNT concentrations upwards of 1 mg/l; activated sludge biomass can adapt to considerably higher concentrations.

DNT appears to be somewhat less toxic to fish than TNT and "relatively nontoxic" to microorganisms.

Appendix IV contains the literature review on TNT and DNT toxicology. A study found subsequently describes experiments on the inhibition of microorganism growth by TNT and on the metabolism of TNT by certain organisms (Klausmeier, 1973)⁶. This work confirms similar observations by others.

CONCLUSIONS

1. The chemistry of phossey water, and of the wastes from the production of nitrocellulose, nitroglycerin and TNT ("pink water"), is insufficiently known. Research to characterize these wastes is a necessary prerequisite for toxicological investigations.
2. The toxic effects to mammals (including man) of TNT, nitroglycerin and white phosphorus, are well documented, but quantitative information is lacking. These include interference with the hematopoietic system by TNT and nitroglycerin, and with the osseous system by white phosphorus.
3. Little or nothing is known of the toxicity of nitrocellulose, and of the unique constituents accompanying nitrocellulose, nitroglycerin, "pink water" and white phosphorus in the wastes of interest.
4. The literature on the effects of "phossey water" on aquatic organisms is extensive, and indicates that many multicellular species, especially fish, are sensitive to as little as 1 $\mu\text{g/l}$ of white phosphorus. TNT appears to exert its effects on aquatic species above about 1 mg/l. Nothing is known about the effects of nitroglycerin and nitrocellulose wastes on aquatic biota, and especially inhibitory or stimulatory effects of any of the compounds in the wastewaters.
5. The metabolism of TNT and nitroglycerin has been studied in some mammalian species. The disposition and fate of white phosphorus and nitrocellulose in mammalian species is unknown. Moreover, the metabolic breakdown of these compounds as a result of bacterial degradation has not been studied.

RECOMMENDATIONS FOR FUTURE WORK

The following recommendations represent a modified compilation of the recommendations made in the four Appendices. It is realized that some of the recommendations pertaining to TNT and DNT isomers may have to be modified further as a result of characterization studies on "pink water" now in progress.

Chemical Studies

1. Chemical characterization studies of nitrocellulose manufacturing wastewaters, nitroglycerin manufacturing wastewaters, "phossey water" and "pink water" from TNT production and processing should continue. This

is important both to assure the identity and consistency of samples used in long-term toxicological studies and to permit an assessment of improvement in water quality resulting from new wastewater treatment methods.

2. Sensitive and reasonably specific methods must be developed, where needed, for analyzing the major organic components of the above munitions wastewaters, should these components have any toxicological significance. Where appropriate, these should be adapted for use in routine monitoring of wastewaters.

3. The rates of breakdown of nitrocellulose under various simulated natural conditions should be determined.

4. Definitive kinetic information on hydrolysis, chlorination and oxidation by dissolved air should be obtained for the various significant wastewater components, as appropriate to their chemistry.

Mammalian Toxicology

1. Oral LD50's should be determined in two mammalian species for each of the following: Nitrocellulose (particulates from wastewater); glyceryl trinitrate; glyceryl-1-nitrate; glyceryl-2-nitrate; glyceryl-1,2-dinitrate; glyceryl-1,3-dinitrate; white phosphorus; α -TNT; 2,4-DNT; 2,6-DNT; 2,3-DNT; 3,4-DNT; and 2,5-DNT.

2. Dermal LD50's should be determined, and skin and eye irritancy tests should be conducted on all the above except for white phosphorus.

3. Skin sensitization tests should be carried out on: Glyceryl trinitrate; α -TNT; 2,4-DNT; 2,6-DNT; 2,3-DNT; 3,4-DNT; and 2,5-DNT.

4. Ninety-day chronic oral toxicities should be determined in at least two mammalian species for each of the following: Nitrocellulose (particulates from wastewater); glyceryl trinitrate*; 2,4-DNT; and 2,6-DNT. If a suitable consistent dosage form can be prepared, the same determinations should be made with white phosphorus or with the principal component of "phossy water".

5. The effects of subchronic oral dosages, over a two year period, in two species of mammals should be investigated for nitrocellulose (particulates from wastewater) and glyceryl trinitrate*. If a suitable

* The Office of Naval Research has sponsored a 90-day oral toxicity study of TNT in monkeys and dogs (Contract No. 0014-73-C-0162). This work will be extended for a two-year study on rats only.

consistent dosage form can be found, the same studies should be carried out with white phosphorus or with the principal component of "phossy water".

6. Studies on carcinogenicity, mutagenicity, and teratogenicity should be carried out on nitrocellulose (particulates from wastewater) and glyceryl trinitrate. If a suitable consistent dosage form can be found, similar studies should be conducted with white phosphorus or with the principal component of "phossy water".

Toxicity to Aquatic Species

1. Ninety-six hour TLM values should be determined for at least two species of freshwater fish found in the receiving waters of the four munitions manufacturing wastes enumerated above.

2. An investigation should be conducted on the effects of long-term exposure of two suitable freshwater fish species to nitrocellulose production wastewater discharges.

3. The effects of all of the above wastewater discharges should be studied on the following aquatic biota: Algae; diatoms; crustaceans; and other invertebrates representative of the biological communities (i.e., major trophic levels) in the receiving waters.

4. Where biological treatment of munitions wastes is contemplated, the metabolites resulting from such degradative treatment should be determined.

5. Field studies should be undertaken (or continued) to assess the impact of waste discharges on the organisms in receiving waters.

6. Food chain studies of persistent munitions waste components and metabolites should be anticipated. Their institution would await careful assessment of the results of the foregoing recommended actions.

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APPENDIX I

MAMMALIAN TOXICOLOGY AND TOXICITY TO AQUATIC
ORGANISMS OF NITROCELLULOSE, A WATERBORNE
MUNITIONS WASTE POLLUTANT -
A LITERATURE EVALUATION

FINAL COMPREHENSIVE REPORT

BY

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The findings in this report are not to be construed as an
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by other authorized documents

APPENDIX I (Cont)

INTRODUCTION

The objective of this work is to provide part of a data base for standards for military-unique product discharges into waters subject to state and federal regulations.

Nitrocellulose, more properly called "cellulose nitrate", is produced by the nitration of cotton linters or wood pulp cellulose in the presence of sulfuric acid. The crude product is subjected to a number of treatments, involving large quantities of water, to remove the acids used for nitration and to stabilize the product. The wastewaters are known to contain nitrocellulose linters, fine suspended particles that can be partially removed by settling or centrifuging, but cannot be completely eliminated. The extent to which nitrocellulose or lower molecular weight nitric acid esters may be present in solution (rather than suspension) is still to be determined. The recent report by Rosenblatt, Small, and Barkley (1973)¹ summarizes the production process, uses, and wastes generated at US munitions production plants.

EFFECTS OF NITROCELLULOSE ON HUMANS AND OTHER MAMMALS

An extensive review of the literature does not provide any toxicity data on the effects of nitrocellulose on humans and other mammals. Some toxicity studies (US Army Environmental Hygiene Agency) of propellant mixtures of nitroglycerine and nitrocellulose are available, but cannot be extrapolated to pure nitrocellulose. The literature sources and other inquiries on which this conclusion is based are listed in Appendix A.

EFFECTS OF NITROCELLULOSE ON AQUATIC ORGANISMS

The literature search revealed no information on the toxicity of nitrocellulose to aquatic organisms. The search of literature sources and other inquiries carried out in this connection are described in Appendix B. One reference (Malenković, 1907²) was found that suggested possible toxicity of nitrocellulose to molds.

CHEMICAL AND BIOCHEMICAL INFORMATION OF POSSIBLE RELEVANCE

Nitrocellulose as an Adsorbent of Biological Macromolecules. A large number of papers on the use of nitrocellulose filters in biochemistry

APPENDIX I (Cont)

and immunology were encountered in the abstract journals, of which a selected few are discussed here. These publications dealt essentially with interactions of nitrocellulose and biologically active macromolecules, processes that could possibly also take place in the environment.

The chromatographic behavior of proteins, peptides and amino acids on nitrocellulose membrane filters has been discussed by Pristoupil (1967)³. He found that proteins of molecular weight greater than 10^5 or with an uncoiled structure in the denatured state (thyroglobulin, γ -globulin) were strongly adsorbed to the membranes. Proteins with molecular weights less than 10^5 , such as serum albumin, ovalbumin and trypsin were adsorbed only in the presence of acid buffers.

The adsorption of hapten-antibody complexes has been described by Gershman *et al.* (1972)⁴. Cooper (1972)⁵ used nitrocellulose as a supporting matrix for DNA in experiments to determine DNA homology to a given RNA species. Yarns and Berg (1970)⁶ assayed the complex of isoleucyl-tRNA synthetase with isoleucyl AMP with the aid of nitrocellulose filters in studies of isoleucine transfer to tRNA. Hudson (1971)⁷ studied factors involved in the ability of denatured RNA to bind irreversibly to nitrocellulose filters, and found that short chain DNA is lost appreciably during hybridization. Fishman and Schiff (1968)⁸ observed that the amount of DNA removed from solution depends both on the salt concentration of the medium and the filter porosity. Apparently (Daniel *et al.*, 1970⁹) digestion with ribonuclease expedites the removal of RNA from DNA-RNA hybrid and from filters. If the adsorbability of biological macromolecules thus shown is a valid criterion, it may well be that waterborne viruses and viroids are also adsorbed by nitrocellulose, and might thus be accumulated in nitrocellulose-containing sediments.

Nitrocellulose Biodegradation. The literature contains virtually nothing on the degradation of nitrocellulose. In fact, only one reference was found (Malenković, 1907²). According to this paper, cellulose nitrate in contact with decomposing organic matter is itself degraded.

Dr. Elwyn T. Reese, of the US Army Natick Laboratories¹⁰, stated that his group had "looked at" cellulose nitrate and that "nothing would touch it". He said that a cellulose derivative with three acetate or nitro groups per glucose unit would be resistant to attack by cellulase enzymes. Dr. Terry Highly, of the US Forest Products Laboratory¹¹, concurred in this assessment.

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According to Whitaker (1971)¹², cellulose will not resist cellulase action if 1-linked glucose is unsubstituted and 4-linked glucose is either unsubstituted or substituted at carbon 6. In a telephone conversation, this author stated that 2,3,6-substituted glucose in nitrocellulose should be very highly resistant.

Baechler (1959)¹³ and Stamm and Baechler (1960)¹⁴ have evaluated substitution of cellulose as a wood preservative measure. Cyanoethylation has been quite effective.

The literature review revealed only one useful article on degradation of nitrocellulose in AAP wastes (Wendt, 1973¹⁵). This paper was a report of progress on a current program at US Army Natick Laboratories. The Natick workers have developed a bench scale process in which a mixture of alkali digested nitrocellulose, raw sewage, and 0.2% glucose was treated sequentially as follows: (1) denitrification, (2) sedimentation, (3) activated sludge, (4) a second denitrification, and (5) a second sedimentation. The system removed 88.6% of the BOD from the waste entering the first denitrification step and most of the nitrate and sulfate. These results should be accepted with the caution that BOD reduction is not a specific measure of nitrocellulose degradation.

Denitrification, acid neutralization, and BOD reduction during treatment of nitrocellulose industrial wastes in a standard activated sludge unit were discussed by Mudrack (1966)¹⁶. The author made no comments on degradation of nitrocellulose alone or as a component of the waste.

Analysis of Nitrocellulose Particles in Water. Literature on the analysis of nitrocellulose was summarized by Rosenblatt, Small and Barkley (1973)¹. Since then, an automated procedure for nitrocellulose, employing a Technicon Autoanalyzer, has been developed, and is currently being tested (Rosenblatt and Barkley, 1973¹⁷). The aqueous suspension is dialyzed in the Autoanalyzer to remove water-soluble interferences, then treated with sodium hydroxide to split out both nitrate and nitrite ions. The latter are detected by their reaction with sulfanilic acid and coupling of the resulting diazonium salt with 1-naphthylethylenediamine. No interferences are observed when the nitrocellulose is initially suspended in domestic sewage. The method is sensitive to less than 1 mg/liter of nitrocellulose.

RECOMMENDED STUDIES ON NITROCELLULOSE

1. The constituents of nitrocellulose-containing wastewaters from manufacturing plants should be completely characterized. Factors to be

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considered would include particle-size distribution; concentration of particulate nitrocellulose; characterization and concentration of dissolved nitrate esters; and characterization and concentration of nitrocellulose breakdown products. Sensitive analytical methods should be developed for this.

2. The rate profile for chemical breakdown for the relative stability of nitrocellulose in sterile water should be estimated as a limiting condition for production of nitrocellulose sediments accumulation.

3. The LD50 for a typical neutralized nitrocellulose wastewater should be determined for two mammalian species. This may require the selective concentration of organic species (i.e., excluding salts that might obscure biological effects of the organic constituents).

4. A 90-day chronic, feeding study of nitrocellulose (settled solids) and nitrocellulose-waste concentrate (see recommendation 3) should be carried out in several mammalian species with special attention being paid to the hematopoietic system, methemoglobin formation in particular.

5. A determination of 96-hour TLM values (median tolerance level) in neutralized nitrocellulose wastes and waste concentrates, using fish indigenous to the New River (Virginia) and the Wisconsin River is recommended.

6. A methodology analogous to that suggested in recommendation 5 should be developed for algae and invertebrates and applied to nitrocellulose.

7. Nitrocellulose and accompanying or breakdown products should be monitored downstream of Army ammunition plants.

8. The behavior of nitrocellulose in receiving streams should be determined. This would include channeling or layering effects, and precipitation either with or without interaction with colloids.

9. The rate of mineralization of nitrocellulose under simulated natural conditions should be investigated. The use of ^{14}C -labelled compounds is recommended for this work.

APPENDIX I (Cont)

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APPENDIX A

LITERATURE SOURCES, MAJOR REFERENCE DOCUMENTS AND PERSONAL CONTACTS USED IN THE SURVEY ON THE TOXICOLOGY OF NITROCELLULOSE

I. Reference Volumes.

1. Fairhall, R. T.: "Industrial Toxicology". The Williams and Wilkins Co., Baltimore, Md., 1949.
2. Elkins, H. B.: "The Chemistry of Industrial Toxicology". Second Edition. John Wiley and Sons, Inc., New York (Chapman and Hall, Ltd., London), 1959.
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24. US Army Materiel Command AMCP 70G-177. "Engineering Design Handbook: Explosive Series. Properties of Explosives of Military Interest". March 1967.

II. Literature Search Pattern for Mammalian Toxicology of Nitrocellulose.

a. Abstract Journals:

Chemical Abstracts 1973-1940
Biological Abstracts 1973-1943
Toxicity Bibliography 1973-1970
BIOSIS 1973-1972

b. Machine Searches:

MEDLARS (2 searches)
MEDLINE - TOXLINE
DDC (Defense Documentation Center)

III. Other Reference Sources.

1. D/HEW, NIEHS Task Force: "Man and the Environment" - Sub-Task Force on Food and Water Toxicology, collected papers, NLM.

2. Environmental Protection Agency: Water Quality Criteria Data Book, Vol. 1-4.

IV. Contacts.

a. National Academy of Sciences Center for Toxicology (Mr. R. Wands, Tel. 202-389-6751).

b. Department of Health, Education and Welfare.

(1) National Institute for Occupational Safety and Health (Dr. H. Stokinger, Tel. 513-684-2697 and Dr. H. Christensen, Tel. 301-443-3053).

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(2) Communicable Disease Center, Atlanta, Ga. (Mr. Wm. Barthel, Tel. 404-633-3621).

c. Department of the Army.

(1) Aberdeen Proving Ground (Drs. D. H. Rosenblatt, B. McNamara, and B. Hackley and Mr. E. Owens; LTC M. Steinberg).

(2) Army Natick Laboratories (Dr. A. Kaplan, Tel. 8-955-2604, Dr. R. Chalk, Tel. 8-955-2377).

d. Department of the Navy.

(1) Naval Ordnance Laboratory, White Oak, Maryland (Dr. J. Hofsommer, Tel. 8-290-2727).

(2) Navy Industrial Environmental Health Center (Mr. A. Johnson, Tel. 8-989-3947).

(3) Naval Ammunition Depot (Mr. E. Pardee, Tel. 8-956-6011).

(4) Naval Biomedical Research Center, Oakland, California (Dr. Heckley, Tel. 8-836-6343).

(5) Bureau of Medicine and Surgery (LCDR Doptis, Tel. 202-254-4620).

e. Environmental Protection Agency (Dr. Allen, Tel. 703-522-1825; Dr. A. Forziati, Tel. 202-755-0611).

f. Department of Commerce.

(1) National Technical Information Service (NTIS), Springfield, Virginia, (Mr. Joseph Coughlin, Asst. Director, Tel. 703-321-8574).

(2) Joint Publications Research Service (JPRS), (Mr. Gustave Blackett, Tel. 703-557-1431).

g. Non-Governmental Laboratories.

Hazleton Laboratories (Dr. Rutter, Office of Toxicology, Tel. 703-893-5400).

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APPENDIX B

INFORMATION SOURCES CONSULTED FOR TOXICITY TO AQUATIC ORGANISMS

The literature search pattern was arranged to give maximum coverage of key subjects in a minimum of time. Briefly, important recent references were located (1) by way of abstract journals, (2) by telephone, and (3) by local inquiry. Then the literature citations in these references were exploited.

A. Abstracts

Generally, Biological Abstracts and Bioresearch Index were more useful than Chemical Abstracts. A larger number of applicable citations appeared in Biological Abstracts; these were adequately cross-referenced, and the keywords used made it easier to locate compounds of primary interest, as well as related substances.

Descriptors included: nitrocellulose and the names of lakes and streams contiguous with Army Ammunition Plants. When Chemical Abstracts was used, the Guide was consulted for correct nomenclature.

The degree of coverage of each descriptor depended on the relevance of the subtopic. For example, nitrocellulose membranes, immunology of nitro compounds, and lake and stream names were covered only in post-1969 abstracts, while nitrocellulose toxicity and degradation were carried back to 1926 in Biological Abstracts and 1907 in Chemical Abstracts.

B. Telephone and Local Inquiry

Telephone and personal inquiries were made of agencies or individuals known to have knowledge of topics of interest and who might have access to the large body of literature not in the abstracts. Especially helpful were individuals at the Environmental Quality Division of the US Army Medical Bioengineering Research and Development Laboratory; Water Quality Engineering Division, US Army Environmental Hygiene Agency; US Environmental Protection Agency; Tennessee Valley Authority; Illinois State Water Survey; and US Forest Products Laboratory.

APPENDIX II

MAMMALIAN TOXICOLOGY AND TOXICITY TO AQUATIC ORGANISMS OF NITROGLYCERIN, A WATERBORNE MUNITIONS WASTE POLLUTANT - A LITERATURE EVALUATION

FINAL COMPREHENSIVE REPORT

BY

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

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INTRODUCTION

Background. The objective of this work is to provide part of a data base for standards for military-unique product discharges into waters subject to state and federal regulations.

Nitroglycerin is one of a group of aliphatic nitrates that are used as industrial and military explosives and propellants and--in some cases--as therapeutic agents (vasodilators) in medicine.

Nitroglycerin--the trinitrate ester of glycerol--is prepared by nitration of commercial glycerol with a mixture of nitric and sulfuric acids. When the nitration is complete, the nitroglycerin is separated from the largely inorganic layer, washed repeatedly with small amounts of water, and then washed with small amounts of dilute sodium carbonate. A survey and report of the liquid wastes from nitroglycerin manufacture was first made by Smith (1943)¹. The following analytical determinations were made on the wastes from the manufacture of nitroglycerin in a single plant only: color, odor, pH, acidity, alkalinity, total solids, suspended solids, nitrite and nitrate nitrogen, soap hardness, oxygen consumed, and 5 day BOD. No toxicity tests of any kind were carried out on the wastes.

The recent report of Rosenblatt, Small, and Barkley (1973)² summarizes the production process, uses and wastes generated in the two US munitions plants that produce nitroglycerin. In general, analyses of the waste waters from these plants have shown the presence of nitroglycerin and of the two isomeric glyceryl dinitrates (mainly the 1,3-isomer). The authors found no published information concerning the presence or absence of the two isomeric mononitrates in the waste waters.

Ethylene glycol dinitrate, which is often mixed with nitroglycerin to lower the freezing point of commercial dynamite, has been included in the literature search in this investigation.

The compounds considered in this report are as follows:

1. Nitroglycerin (glycerol trinitrate).
2. Glyceryl-1,3-dinitrate and glyceryl-1,2-dinitrate.
3. Glyceryl-1-nitrate and glyceryl-2-nitrate.
4. Ethylene glycol dinitrate and ethylene glycol mononitrate.

Glycerol and nitrite and nitrate ions (as sodium salts) are among the known degradation products of nitroglycerin. However, enough is already known of their effects to permit the formulation of discharge standards; they were therefore not included in this literature evaluation.

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Approach to Mammalian Toxicology. A survey of the chemical and biological literature on the compounds listed above was carried out. Sources for the literature searches are set out in detail in Appendix A. Most of the references for nitroglycerin were obtained from Chemical Abstracts. Several machine searches were also made, but were not very helpful. Some useful documents were obtained from a search by the Defense Documentation Center. All the papers obtained were grouped in the following categories for toxicological evaluation.

1. Acute toxicity studies.
2. Chronic toxicity studies (including carcinogenic, mutagenic, and teratogenic studies).
3. Metabolic fate studies.
4. Related biochemical studies.
5. Analytical and related chemical studies.
6. Clinical studies (as they relate).

Von Oettingen (1946)³ published a very comprehensive bulletin dealing with the effects of aliphatic nitrous and nitric acid esters on the physiological functions, with special reference to their chemical constitution. Among the nitric acid esters were ethylene glycol mononitrate and dinitrate, glycerol 1,3-dinitrate, and glycerol trinitrate. For the toxicological and pharmacological effects of these compounds, this is a key document, apparently being a detailed summary of all the published work prior to 1946.

Because nitroglycerin has been used in medicine, especially for the treatment of angina, there is a fairly extensive literature on its biochemical and clinical aspects. Not all such papers were obtained, but only those that appeared to have useful information of toxicological effects on man or related information. Many recent papers concern biochemical studies, and there are several publications within the last ten years dealing with the metabolism and fate of nitroglycerin in experimental animals.

The use of nitroglycerin purely as a vasodilator drug has been very adequately summarized by Nickerson⁴ in Chapter 34 of Goodman and Gilman (1970). The pharmacological actions on the cardiovascular system, the absorption, fate and excretion, toxicity and untoward responses, tolerance, and therapeutic uses of nitroglycerin in man, have been summarized

APPENDIX II (Cont)

and discussed in some detail. Generally only published papers after 1970, as they relate to the above topics, will be considered in this report.

Documents of possible interest but not cited in the body of this report are listed in Appendix B.

Approach to Toxicity to Aquatic Organisms. The literature search pattern was arranged to give maximum coverage of key subjects in a minimum of time. Briefly, important recent references were to be located (1) by way of abstract journals, (2) by telephone and (3) by local inquiry. For information on sources consulted, see Appendix C.

Current Research on Nitroglycerin Toxicology Laboratories in which research on nitroglycerin (especially biochemical and pharmacological aspects) is currently being pursued are as follows:

1. Warner-Lambert Research Institute (Department of Drug Metabolism, Morris Plains, N. J. 07950, Dr. F. J. DiCarlo *et al.*)
2. J. F. and S. Heymans Institute of Pharmacology, University of Ghent Medical School, Ghent, Belgium. (Dr. M. G. Bogaert *et al.*)
3. The Edward Mallinckrodt Department of Pharmacology, Washington University School of Medicine, St. Louis, Mo. 63110. (Dr. P. Needleman *et al.*)

MAMMALIAN TOXICOLOGY OF NITROGLYCERIN AND RELATED COMPOUNDS

Acute Toxicity in Animals. A summary of acute toxicity data for nitroglycerin and related nitrate esters is given in Table 1. A summary of the acute toxicity of nitroglycerin in animals was also given by Munch and Friedland (1965)⁵.

Bokorny (1896)⁶ claimed that nitroglycerin has little toxicity for lower animals and plants. As shown in Table 1, the toxic dose varies with the route of administration and with the species of animal used. Following intravenous injection of fatal doses, Oltman and Crandall (1931)⁷ noted in rabbits immediate respiratory stimulation, which was closely followed by slowing of the heart beat, muscular twitchings, and clonic and tonic convulsions. Between convulsions the heart rate was accelerated and the respiratory rate decreased; the animals died from respiratory paralysis.

APPENDIX II (Cont)

In contrast to the comparatively high lethal dose of glycerol trinitrate (with subcutaneous injection of 200 mg per kg in the cat and 500 mg per kg in the rabbit), the repeated subcutaneous injection of much smaller doses may cause toxic effects, as demonstrated by Gross, Bock, and Hellrung (1942)⁸.

The toxicological picture of glycerol trinitrate poisoning is similar to that of sodium nitrite poisoning. However, it appears that the toxic action of the former cannot be explained solely on the basis of its nitrite action; the minimal fatal dose of glycerol trinitrate with intravenous administration to rabbits is 45 mg per kg, (cf Table 1) whereas that of sodium nitrite is 80 to 90 mg per kg (Von Oettingen, 1946³). As may be seen from this description, the toxicological picture of glycerol trinitrate poisoning is similar to that of asphyxiation, which may be due either to the fall of the blood pressure or to methemoglobinemia. Nitroglycerin, then has low acute toxicity; in general, methemoglobinemia develops, followed by circulatory collapse, leading then to convulsions and death. Somewhat smaller quantities were reported to produce death by oral than by parenteral administration.

There is no information on the toxicity of glycerol dinitrates except a statement by Will (1908)⁹ that absorption of the 1,3-isomer causes headache similar to that produced by nitroglycerin.

The acute toxicity of ethylene glycol mononitrate has not been investigated. With regard to the toxicity of ethylene glycol dinitrate, Gross, Bock, and Hellrung (1942)⁸ found it to be about twice as toxic for cats as glycerol trinitrate, the certain fatal dose with subcutaneous injection being 100 mg per kg, as compared with 200 mg per kg of the latter. For rabbits, which are less liable to form methemoglobin and are thus less sensitive, the corresponding values are 400 and 500 mg per kg body weight. The death of cats, after doses of 100 mg per kg or more, probably is largely due to methemoglobinemia. Other symptoms observed were lowering of the blood pressure, vomiting, weakness, dyspnea, and salivation. In contrast to the effects of glycerol trinitrate, cats poisoned acutely with ethylene glycol dinitrate did not show convulsions, these being seen only in chronic poisonings with this compound. With prolonged poisoning, the animals became definitely anemic and some also became jaundiced. Wilhelmi (1942)¹⁰ noted that in cats, after intraperitoneal injection of 20 to 24 mg per kg of ethylene glycol dinitrate in oil, the animals became distinctly sick, lost weight, became listless and later dyspneic, and died after 8 to 9 days. During the last day they voided a dark brown urine containing 3+ urobilin, 1+ urobilinogen, but no bilirubin. Even smaller doses caused severe anemia and other blood changes to be described

APPENDIX II (Cont)

below. According to Keeser (1939)¹¹ the subcutaneous injection of 9 cc of a 4-percent suspension of ethylene glycol dinitrate causes severe poisoning in dogs, mainly characterized by ataxia lasting for several days, which is said to be overcome by large doses of ascorbic acid.

The pathological changes observed in experimental animals, as reported by Gross, Bock, and Hellrung (1942)⁸ and Wilhelmi (1942)¹⁰, may be summarized as follows: The heart muscle may show fine fat droplets (possibly due to anemia) and fatty degeneration. The liver may contain large amounts of blood pigment, and, with prolonged poisoning, it may show varying degrees of fatty infiltration and degeneration and hemorrhages. The spleen shows the typical picture seen in anemia, characterized by hemosiderosis and phagocytosis. The kidneys may show fatty infiltration and degeneration, especially of the epithelium of the tubuli contorti, and, in acute poisoning, hemorrhagic inflammation. The mucosa of the stomach and upper intestine may show more or less marked inflammation. In chronic poisoning the bone marrow may show hyperplasia.

The toxicity of the related compound, propylene glycol 1,2-dinitrate, was determined by Clark and Litchfield (1969)¹². The oral LD50 for the rat is 1190 mg/kg.

It should be noted here that the toxicity data in Table 1 is either the lethal dose (LD) or the minimum lethal dose (MLD) that will kill animals. Apart from studies on the mouse, no LD50 values for nitroglycerin have been determined.

TABLE 1. Summary of Acute Toxicity of Nitroglycerin and Related Compounds in Animals.

Animal Species	Route of administration	Dose (mg/kg)	Remarks	References
<u>Nitroglycerin</u>				
Rat	or im	80-100	MLD	Orestano (1937) ¹³
		150-400	MLD	Orestano (1937) ¹³

APPENDIX II (Cont)

TABLE 1. (Cont)

Animal Species	Route of administration	Dose (mg/kg)	Remarks	References
<u>Nitroglycerin</u>				
Mouse	sc	30	LD50	Advisory Center on Toxicology (1968) ¹⁴
	ip	205	LD50	Advisory Center on Toxicology (1968) ¹⁴
Rabbit	sc	500 (tech grade)	LD	Gross et al. (1942) ⁶
	im	400-500	MLD	Orestano (1937) ¹³
	iv	45 (10% in EtOH)	LD	Oltman et al. (1931) ⁷
	ip	25	Survival	Atkinson (1887) ¹⁵
	sc	10	"	Atkinson (1887) ¹⁵
Dog	iv	10	Survival	Crandell (1910) ¹⁶
	or	10	"	Dossin (1911) ¹⁷
	iv	30	"	Dossin (1911) ¹⁷
	sc	30	"	Dossin (1911) ¹⁷
Cat	im	150	MLD	Orestano (1937) ¹³
	sc	200 (tech grade)	LD	Gross et al. (1942) ⁶
	ip	200	LD	Brenton (1876) ¹⁸
Guinea pig	iv	83.5	LD	Orestano (1936) ¹⁹
	im	500	LD	Orestano (1936) ¹⁹
	or	100	Survival	Schott (1920) ²⁰
Frog	im	465	LD	Orestano (1937) ¹³
	sc	475	MLD	Orestano (1937) ¹³

APPENDIX II (Cont)

TABLE 1. (Cont)

Animal Species	Route of administration	Dose (mg/kg)	Remarks	References
<u>Ethylene glycol dinitrate</u>				
Cat	sc	100	LD	Gross et al. (1942) ⁸
Rabbit	sc	400	LD	Gross et al. (1942) ⁸
	sc	300	LD	Christensen (1972) ²¹
<u>Nitroglycerin/ethylene glycol dinitrate (1:1 tech.)</u>				
Cat	sc	100	LD	Gross et al. (1942) ⁸
Rabbit	sc	400	LD	Gross et al. (1942) ⁸
<u>Dynamite mixture (17% glycerol trinitrate and 83% ethylene glycol dinitrate)</u>				
Rat	ip	304	LD50	Phipps (1972) ²²

Acute Toxicity in Man. The acute toxicity of nitroglycerin in humans has been summarized by Hunch and Friedland, 1965⁵. (See Table 2, herewith, which is taken from their paper): "The most common medicinal dose of nitroglycerin is 0.65 mg, and many patients safely and effectively receive 0.65 mg up to 20 times or more daily over substantial periods of time. Survival has been noted after quantities of nitroglycerin up to 400 mg were administered sublingually or orally". The data are all drawn from acute episodes of accidental or deliberate administration of nitroglycerin, mostly by the oral route. The toxic effects produced are listed by von Oettingen (1946)³ on pages 42 and 43 of his Bulletin. While characteristic symptoms develop promptly in humans (and animals) after contact or ingestion of nitroglycerin, including headache or fall in blood pressure,

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available information based on widespread use over a century does not indicate that therapeutic doses of glycerol trinitrate have shown any significant acute toxicity hazard.

The toxic symptoms in man resulting from acute exposure to ethylene glycol dinitrate are essentially the same as those experienced with glycerol trinitrate, namely headache, nausea, vomiting, lowering of the blood pressure, increase of the pulse rate, and cyanosis. Crandall, Leake, Loevenhart, and Muehlberger (1931)²³ determined the minimal dose causing headache when applied to the skin as 1.8 to 3.5 cc of a 1 percent alcoholic solution and found that the application of fractional doses totaling 190 mg (16 cc) caused tolerance within 21 to 36 hours, lasting for 10 to 13 days. Little information is available regarding the chronic toxicity of ethylene glycol dinitrate. Gross, Bock, and Hellrung (1942)⁶ mentioned that a great number of fatalities had occurred among explosives workers, which were presumably due to exposure to ethylene glycol dinitrate, but these never were explained satisfactorily. They believed that the deaths of these men who died suddenly, evidently from circulatory failure after having had no exposure for 24 to 48 hours, were partly complicated by other factors such as the influence of alcohol and excessive physical exercise. In view of the changes of the cardiac muscle observed in experimental animals, the possibility of cardiac muscle weakening, resulting from continued exposure, appears to be within the realm of possibility.

TABLE 2. Summary of Acute Toxicity of Nitroglycerin in Man (Medical Use).

Author	(Year)*	Survived	Died
Hystrom	(1866)	---	3; no details dose
Honert	(1867)	---	1 man; no details dose
Husemann	(1867)	---	Man, 7 scruples (9100 mg)
Holst	(1870)	---	Man, 30; 2 teasp; died 2 hours
Hamilton	(1882)	1 drop	---

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TABLE 2. (Cont)

Author	(Year)*	Survived	Died
Foy	(1886)	---	4
Noer	(1887)	1 female, 20 drops, slow pulse, respiration	---
Lackerstein	(1888)	4 (baby, 3 adults); 10-100 mg	---
McVey	(1894)	---	Criminal, plus alcohol, slum Chicago
Ellery	(1905)	Miners, no deaths, fumes	---
Stewart	(1906)	300-400 mg	---
Loeb	(1905)	Collapse, 0.5 mg	---
Emerson	(1909)	---	Miner, 2 mouthfuls
Peterson	(1926)	300 mg	Few drops surely fatal
White	(1931)	2 collapse 0.65 mg	---
Prodger	(1932)	4/110 symptoms 0.65 mg	---
McNally	(1937)	---	Few drops
Shoun ²⁴	(1942)	---	3-5 drops, 30 mg Exquisitely toxic

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TABLE 2. (Cont)

Author	(Year)*	Survived	Died
Cook	(1945)	0.5 ppm MAC	---
Rabinowitch ²⁵	(1944)	---	Worker, plus alcohol; maniacal, died 30 min.
Mayer	(1951)	---	44 deaths from lit.: doses?
Smith	(1955)	---	1 or 2 drops probable
Sutherland	(1959)	---	Female; 65, 0.6 mg + codeine + morphine
Modi	(1961)	---	Few drops

* See Munch and Friedland (1965)⁵ for authors named in this table. The reference to an author in the table does not mean that the published paper has been obtained and evaluated.

Chronic Toxicity in Animals. Chronic toxicity studies of the accepted long-term duration (2 years in rats) do not appear to have been carried out on nitroglycerin and its analogues. Some of the results of short-term administration and inhalation exposure of nitroglycerin in animals have been reported. (See under Acute Studies in Animals).

Reports on pathological findings in animals exposed to glycerol trinitrate are not numerous. Gross, Bock, and Hellrung (1942)⁸ noted in cats, following 10 daily subcutaneous injections of 15 mg per kg, albumin and bile pigments in the urine, icterus and hemorrhages in the dura, cerebellum, heart, liver, and spleen, and pigment deposits in the two latter organs. Similar pigment deposits were described by Wilhelmi (1942)¹⁰. It may be significant that hemorrhages in the

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heart muscle were also seen in one animal with prolonged cutaneous administration. Wilhelmi (1942)¹⁰ noted central fatty changes in the liver of one animal, and Gross, Bock, and Hellrung (1942)⁸ demonstrated rapid formation of methemoglobin and unrelated Heinz bodies on the red blood cells of cats after inhalation or cutaneous application of glyceryl trinitrate. Subcutaneous administration of 0.1 mg/kg daily for 40 days also produced anemia and fatty degeneration of the liver in one cat and was followed by death 4 days later. Fifty daily doses of 7.5 or 15 mg/kg were survived by most cats, although one died after ten, and another after 20 doses. Albuminuria, icterus and hemorrhage of the cerebellum, heart, liver, and spleen were noted when the animals were sacrificed. Inhalation of air saturated with glyceryl trinitrate (0.005 mg per liter; 0.6 ppm) for 68 days produced only anemia and moderate leukocytosis; inhalation for 156 days produced tolerance in cats. During continued exposure, rabbits developed tolerance and intermittent anemia. Methemoglobinemia, peripheral vasodilation in the brain and the muscles in the vascular walls, with accompanying fall in blood pressure, were noted in animals. Available information did not indicate the toxic or fatal doses. On account of its lower vapor pressure, nitroglycerin is less liable to cause toxic effects from inhalation of its vapors than ethylene glycol dinitrate. With regard to the toxicity of the vapors of ethylene glycol dinitrate, Gross, Bock, and Hellrung (1942)⁸ found that exposure of cats for 8 hours daily to concentrations of 0.013 mg per liter (2 ppm) over a period of 1,000 days caused moderate temporary blood changes, which disappeared later without leaving any apparent aftereffects. Cats exposed in the same way to concentrations of 0.134 mg per liter (21 ppm) developed marked blood changes, but otherwise behaved normally and showed no organic changes, after an exposure for 1,000 days.

Chronic Toxicity in Man. The effects of short-term chronic administration of nitroglycerin to man in therapy are well documented. For example, see both von Oettingen (1946)³ and Chapter 34 (Nickerson)⁴ in Goodman and Gilman (1970), where the effects are described on the cardiovascular system in detail. The two important toxicity symptoms or untoward responses of nitroglycerin are (a) methemoglobinemia and (b) tolerance. Again the reader is referred to Goodman and Gilman (1970) Chapter 34⁴ and to Munch, Friedland and Shepard (1965)²⁶.

Many workers have studied nitroglycerin-induced methemoglobinemia and results are summarized herewith (e.g., Orestano, 1937^{13,27}, Bodansky, 1951²⁸). A series of papers has been published by the school of Hasegawa (National Institute of Industrial Health, Japan) concerning the mechanism of methemoglobin formation in man and animals by nitroglycol (Hasegawa

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and Sato, 1963^{29,30}; Kakizaki, Sato, Tsuruta, and Hasegawa, 1967³¹; Hasegawa and Sato, 1970³²; Tsuruta and Hasegawa, 1970³³; Hasegawa, Sato, and Tsuruta, 1970³⁴).

Development of tolerance and cross-tolerance of nitrites and nitrates is of clinical importance. However, quantitative data concerning tolerance with regard to the cardiovascular effects of these substances in humans and animals are scarce. Bogaert and de Schaepdryver (1968)³⁵ described acutely developing tolerance towards nitroglycerin in dogs and the chronic specific tolerance was quantitatively demonstrated.

A TLV (threshold limit value) of 0.2 ppm, or 2 mg/m³, for nitroglycerin has been established and adopted by ACGIH (American Conference of Governmental Industrial Hygienists)³⁶ for 1972. This value is designated for "skin" and refers to the potential contribution to the overall exposure by the cutaneous route including mucous membranes and eye, either by airborne, or more particularly, by direct contact with the substance. A TLV of 0.2 ppm has also been established for ethylene glycol dinitrate.

For a study of the effects of exposure of workers to mixture of nitroglycerin and ethylene glycol dinitrate see Einert, Adams, Crothers, Moore and Ottoboni (1963)³⁷.

Significant Clinical Studies. The clinical aspects of nitroglycerin and ethylene glycol poisoning dinitrate have been adequately described by von Oettingen (1946)³ and by Nickerson in Goodman and Gilman (1970)⁴. The following notes are in addition to these summaries:

1. Nitroglycerin has been shown in animals to interact with barbiturates by inhibiting their oxidation to inactive metabolites (DiCarlo, Crew and Young, 1967³⁸). Munch and Friedland (1965)⁵ reported the death of a worker who took nitroglycerin and alcohol, and also the death of a woman who ingested nitroglycerin, codeine and morphine.
2. Cases of sudden death among workers exposed to nitroglycerin or ethylene glycol dinitrate are described by Carmichael and Lieber (1963)^{39,40}.
3. Cases with withdrawal symptoms after exposure to nitroglycerin or ethylene glycol dinitrate are described by Lund *et al.* (1968)⁴¹, while Lange *et al.* (1972)⁴² have described cases of nonatheromatous ischemic heart disease following withdrawal from chronic nitroglycerin exposure.

APPENDIX II (Cont)

4. Cases are described by Trainor and Jones (1966)⁴³ of volunteers being exposed to nitroglycerin and ethylene glycol dinitrate over a range of atmospheric concentration. They suffered from "Monday head" (headache), such as is experienced by explosive workers. These results indicate that the TLV for nitroglycerin of 2 mg/m³ needs to be revised.

5. The influence of the thyroid on the acute toxicity of a dynamite-sensitizing mixture (83% EGD, 17% NG) was studied by Phipps (1972)²². Thyroidectomized rats survived a normal LD50 dose of the nitrate mixture.

MAMMALIAN METABOLISM AND BIOCHEMISTRY OF NITROGLYCERIN

The metabolic fate of nitroglycerin in both man and experimental animals has been studied and the pathways essentially elucidated by three groups of workers within the last 8-9 years. (See Current Research on Nitroglycerine, above). The metabolic pathways of nitroglycerin are shown in Figure 1; they have been worked out from studies in the rat, rabbit, dog (oral or intraperitoneal administration) and man (oral administration).

The first step in the biotransformation of nitroglycerin (I) yields inorganic nitrite and the two isomeric glyceryl dinitrates (II, III), according to Needleman and Krantz (1965)⁴⁴ and Needleman and Hunter (1965)⁴⁵ for the rat, and Bogaert *et al.* (1965)^{46,47} for the rabbit. The two isomers are further metabolised to glyceryl mononitrates (IV, V), and ultimately to glycerol (VI) and CO₂ (Needleman *et al.* 1971⁴⁸). Experiments in rats given ¹⁴C-nitroglycerin indicated that the drug was completely hydrolyzed to glycerol, and this compound entered metabolic pools for anabolism as well as catabolism (DiCarlo *et al.*, 1968^{49,50}; DiCarlo *et al.*, 1969⁵¹). Consequently ¹⁴CO₂ was exhaled and radioactive macromolecules were also produced. Nitroglycerin, then, is de-esterified stepwise, with no apparent major initial preference for either the primary or secondary nitrate group. For the metabolism of glycerol see Williams (1959)⁵².

No evidence has been found for the degradation of nitroglycerin by bacteria and other micro-organisms in animals or man.

Nitroglycerin, glyceryl-1,2- and glyceryl-1,3-dinitrates and glyceryl-1-nitrate were administered to human volunteers by capsule (Bogaert, Rosseel and Belpaire, 1971⁵³). Urinary excretion only was investigated and in all cases small amounts of the glyceryl-1-mononitrate was rapidly formed and excreted.

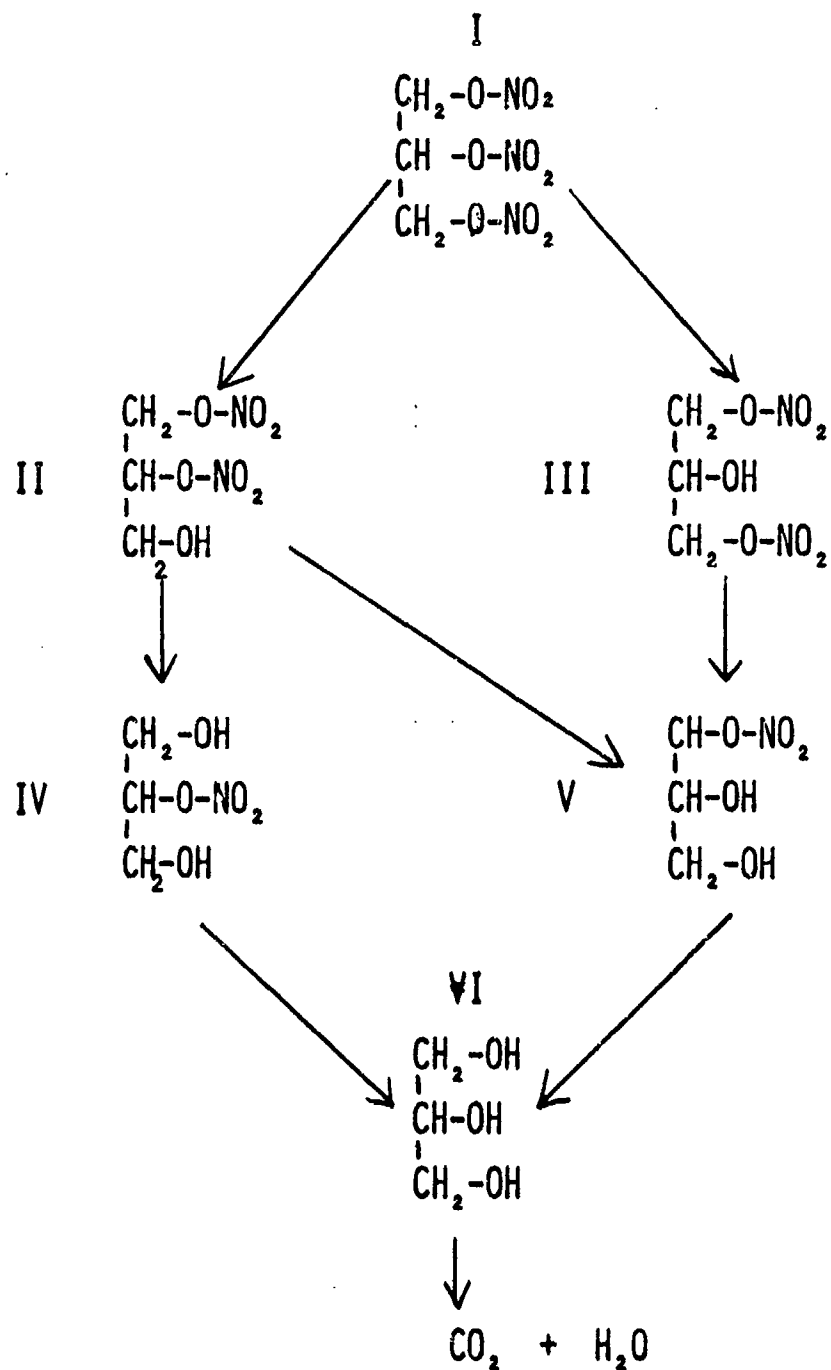
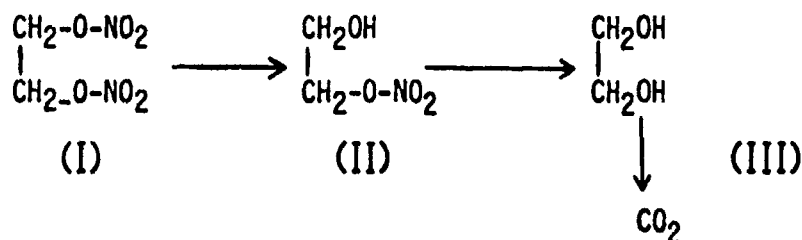
NITROGLYCERIN

FIGURE 1: METABOLIC PATHWAYS FOR NITROGLYCERIN

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Nitroglycerin and other low molecular weight organic nitrates are absorbed through the skin, a route of considerable toxicological importance in the manufacture of explosives (see, e.g., Gross *et al.*, 1960)⁵⁴. The oral administration of nitroglycerin was followed by rapid absorption, transformation and excretion. Turner (1965)⁵⁵ reported that oral nitroglycerin is absorbed from the alimentary tract of the rabbit. DiCarlo *et al.* (1969)⁵¹ showed that at low concentrations, about 60% of nitroglycerin is bound to proteins of rat blood plasma. The dinitrates formed are also bound by plasma proteins, with the 1,2-dinitrate being held more firmly (60%) than the glyceryl-1,3-dinitrate (35%).

The *in vitro* and *in vivo* metabolism of ethylene glycol dinitrate has been studied in the rat and the dog (single and repeated administrations) by Clark and Litchfield (1967)⁵⁶, (1969)⁵⁷. The metabolic pathway is as follows:



Only small amounts of II are excreted in urine and the nitrite formed is oxidized to nitrate and excreted. Clark and Litchfield (1969)¹² studied the metabolism of propylene glycol-1,2-dinitrate *in vitro* and *in vivo* and found that it was very similar to that of ethylene glycol dinitrate.

The following related papers are noted here for reference:

1. Crandall (1933)⁵⁸ and Crandall *et al.* (1939)⁵⁹ on the fate of nitroglycerin in the tolerant and non-tolerant animal.
2. Lee and Belpaire (1965)⁶⁰, (1972)⁶¹ on the kinetic properties of the organic nitrate reductase in rat liver and the influence of phenobarbital and SKF-525 on nitroglycerin metabolism.
3. Bogaert *et al.* (1970)⁶², on the metabolic fate on nitroglycerin in relation to its vascular effects in dogs and rabbits.
4. DiCarlo and Melgar (1970)⁶³ on nitroglycerin biotransformation by rat blood serum.

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5. Needleman and Harkey (1971)⁶⁴ on the role of endogenous glutathione in the metabolism of nitroglycerin by the isolated perfused rat liver.

6. Needleman *et al.* (1972)⁶⁵ on the relationship between biotransformation of nitroglycerin and rational angina pectoris therapy.

7. Lang *et al.* (1972)⁶⁶ on the metabolism of and vascular responses to nitroglycerin in the eviscerated rat.

The effects on mitochondrial monoamine oxidase (MAO) activity of nitroglycerin, propylene glycol dinitrate, ethylene glycol dinitrate, and the metabolites ethylene glycol mononitrate, nitrite ions, and nitrate ions were studied *in vitro* by Kalin and Kylin (1969)⁶⁷. The MAO activity was inhibited by the three explosive compounds, while the metabolites had no such effect. In a second paper, Kalin and Kylin (1969)⁶⁸ reported no MAO inhibiting effect in intact tissues (rat iris) or when animals were treated with the three explosives for a short or long period, neither in whole organs nor in adrenergic nerves. The latter result was confirmed by Waldeck (1970)⁶⁹ when nitroglycerin or the MAO inhibitor nialamide was given to rats or mice which then received ¹⁴C-tyramine. Waldeck concluded that nitroglycerin had no effect on the MAO responsible for the *in vivo* metabolism of tyramine and noradrenaline. Changes of sensitivity to adrenaline in nitroglycerol-poisoned mice have been studied by Yoshihawa (1964⁷⁰, 1965⁷¹). The relationship between nitroglycerol poisoning and alcohol preference in mice has been studied by Yoshitake (1973)⁷². Boime and Hunter (1971)⁷³ studied the effects of nitroglycerin and other aliphatic nitrates on electron transport and phosphorylation in liver mitochondria of rats.

POTENTIAL FOR CARCINOGENICITY, TERATOGENICITY AND MUTAGENICITY IN MAN

Neither nitroglycerin nor any of the other compounds considered in this search appear to have been submitted to the following:

1. A standard carcinogenicity study--2 year feeding to rats, or 80-90 weeks to mice or 7 years to dogs.
2. A mutagenic study in rats.
3. A teratogenic study in mice and rats.

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Nitroglycerin, at least at high pH (and perhaps at lower pH or enzymatically) is hydrolyzed to produce nitrite ions. The latter, under acidic conditions, react with secondary amines to form nitrosamines. Nitrosamines formed in polluted water and soils might pose a danger to the health of man, livestock, or wild animals, since many of them are potent carcinogens and mutagens. The possible formation of nitrosamines from amines present in or derived from the diet occurs by reaction with nitrous acid at pH 4. In man, gastric juice attains a pH of 1.1. The reaction with nitrous acid of high concentrations of H^+ ions gives rise to the nitrosyl cation NO^+ , which is a particularly reactive nitrosating agent. Ferrous ion (Fe^{++}), present in meat, myoglobin, or hemoglobin serves to neutralize this cation. However, fish and cheese do not provide this neutralization, hence permitting the nitrosation of amines to occur.

TOXICITY OF NITROGLYCERIN TO AQUATIC ORGANISMS

The literature review failed to reveal any information on the toxicity of nitroglycerin to any aquatic organism. The possible significance of suspected chemical or metabolic transformations to physiologically harmful or compatible chemical species, e.g., the effect on persistence of a non-sterile environment, was to be considered. No information, however, was obtained on metabolic transformations of nitroglycerin or nitroglycerin wastes by aquatic species to physiologically active chemical compounds.

Only one publication had a possible bearing on nitroglycerin persistence in non-sterile environments. This was a very short summary report, entitled "Biodegradation of Wastes from Nitroglycerine Production", on the status of a current program at Natick Laboratories (see Wendt, 1973⁷⁴). Apparently, wastes from certain unit processes in nitroglycerin production (e.g., NG area, slurry mix water, and "water dry waste") have BOD's exceeding 1000, although neither specific substrates nor the AAP involved were indicated. The only statement on specific substrates was as follows: "Some individual components appear to be readily broken down biologically, but at least one appears recalcitrant to attack".

Smith and Dickenson (1972)⁷⁵ report that NG at the 300 mg/l level in manufacturing wastewater was only slightly degraded in a pilot activated sludge unit of 12 hours retention time, although there was no significant inhibition of BOD removal. Experimental details were not provided, and the very high inorganic nitrate content of the wastewater (72,000 mg/l)

APPENDIX II (Cont)

would be expected to strongly inhibit reduction of organic nitrate groups in any event. Thus the biodegradability of NG in natural waters (as distinct from the treatability of the waste) is still undetermined. According to Malenković (1907)⁷⁶, nitroglycerin does not appear to be attacked by microfungi.

CHEMICAL ANALYSIS AND REACTIONS OF NITROGLYCERIN

Chemical Analysis. This aspect has been adequately summarized by Rosenblatt *et al.* (1973)². Additional relatively recent work, especially as it relates to this report, is detailed herewith:

1. Crew and DiCarlo (1968)⁷⁷ describe the differentiation of glyceryl-1,2- and glyceryl-1,3-dinitrates and glyceryl-1- and glyceryl-2-nitrates using TLC and ¹⁴C labelling in the presence of nitroglycerin and glycerol.
2. A spectrophotometric method for the estimation of ethylene glycol dinitrate (range 0.5-4.2 mg/100 ml) is described by Foulger (1936)⁷⁸ using phenoldisulfonic acid.
3. Litchfield (1968)⁷⁹, in an excellent paper, showed that ethylene glycol dinitrate and mononitrate, propylene glycol-1,2-dinitrate and 1- and 2-mononitrates can be determined by GLC (electron capture detector) in ether extracts of blood with no interference from blood constituents. Recovery was good over the range of 0-25 ug of ester.
4. The detection of microgram amounts of nitroglycerin and related compounds is reported by Lloyd (1967)⁸⁰. The compounds were separated by TLC and identified colorimetrically after reaction with base by means of Griess's reagent.
5. Rosseel and Bogaert (1972⁸¹, 1973⁸²) have studied the GLC analysis (electron capture detector) of nitroglycerin and its nitrated metabolites in the nanogram range in blood plasma.
6. The GLC determination (electron capture detector) of ethylene glycol dinitrate and nitroglycerin in blood and urine (of explosive manufacturing operatives) in the nanogram range is reported by Williams and Murray (1966)⁸³.
7. The colorimetric determination of nitroglycerin and nitroglycol in blood and urine is described by Zurlo, Conti, and Nichelatti (1963)⁸⁴.

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Chemical Reactions. On addition to the reactions listed by Rosenblatt *et al.* (1973)² the following papers considered to be relevant to this report are summarized:

1. The effect of water on decomposition of nitroglycerin at elevated temperatures was studied by Adreev and Bessalov (1963)⁸⁵ (Russian). Nitroglycerin was prepared and purified by molecular distillation at 10^{-6} mm. They concluded that the initial stage of decomposition of nitroglycerin at 100-120° was hydrolysis, which proceeded slowly in neutral solution but was accelerated by the acid decomposition products and their subsequent hydrolysis.

2. The nitroglycerin/sodium hydroxide reaction was studied by Ayres, Whitneck and Merrow (1961)⁸⁶. The reaction was followed spectrophotometrically at various mole ratios.

3. The kinetics and mechanism of the thermal decomposition of nitroglycerin was investigated in the vapor and liquid phases over a temperature range of 115-160° by Waring and Krastins (1970)⁸⁷.

RECOMMENDATIONS FOR LABORATORY AND FIELD STUDIES ON NITROGLYCERIN

1. The determination of the LD50 for glyceryl-1,2- and -1,3-dinitrates and glyceryl-1- and -2-nitrates should be undertaken in two animal species.

2. Chronic 90-day feeding studies of nitroglycerin and some of its degradation products should be undertaken in several animal species. A range of dose levels should be used to especially evaluate the relationships of methemoglobin formation and tolerance development.

3. A two-year chronic feeding study of very low dose levels of nitroglycerin in animal species should be pursued, with special attention paid to the hematopoietic system.

4. A two-year chronic feeding program should be carried out as a carcinogenic study. Administration of nitroglycerin to several animal species may be desirable to evaluate its possible carcinogenic potential thru N-nitrosamine formation.

5. It may be desirable to study the possible mutagenic and teratogenic potential of nitroglycerin and its metabolites.

6. The metabolic breakdown of nitroglycerin and its analogs (and identification of the degradation products) as a result of bacterial

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degradation should be investigated. Both laboratory and field studies would appear to be necessary to evaluate the importance of degradation by micro-organisms in the environment.

7. The toxicity to aquatic organisms of nitroglycerin or waste products of nitroglycerin manufacturing should be investigated. Possible desirable organisms would be indigenous algae, invertebrates, fish and aquatic birds.

8. The monitoring of nitroglycerin and its degradation products in nitroglycerin munitions wastes at all stages of disposal would appear necessary. This could readily be carried out by applying the already developed gas-liquid chromatographic analysis techniques.

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2nd " " 11-20 (1917-1926)
3rd " " 21-30 (1927-1936)
4th " " 31-40 (1937-1946)
5th " " 41-50 (1947-1956)
6th collective " 51-55 (1957-1961)
7th " " 56-65 (1962-1966)
8th " " 66-75 (1967-1971)

Vols. 76, 77 (1972), and 78 (1973) and 79 (1973) to date.

[Key words: nitroglycerin, dynamite, propellants, nitroglycol.
From 1972, 76, Key words: 1,2,3-propanetriol, esters trinitrate
(55-63-0); 1-, 2-mononitrates, 1,2- and 1,3-dinitrates;
1,2-ethanediol, esters dinitrate (628-96-6)].

2. *Biological Abstracts*, from Vol. 1, 1927 to date (1973, 55).

[Key word: glycerol trinitrate].

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3. *Toxicity Bibliography*, from Vol. 1, 1968 to date (Vol. 6, 1973).

[Key words: glycerol trinitrate, ethylene glycol dinitrate].

4. *Current Contents*, Life Sciences Ed., Vol. 14, 1973, No. 1 to date.

[Key word: nitroglycerin].

The search for aquatic toxicology of nitroglycerol was carried back to 1927 in Biological Abstracts and then from 1926 to 1907 in Chemical Abstracts.

C. Computer Searches of Literature for Nitroglycerol:

1. Defense Documentation Center, Defense Supply Agency. Cameron Station, Alexandria, VA 22314, 1940 to date.

[Key words: nitroglycerine, glycerol trinitrate, glycerol dinitrate, ethylene glycol dinitrate].

(See Search Control No. 002788)

2. National Library of Medicine/Medline Retrieval file (1969 to date) and TOXICON (1966 to date).

[Key words: nitroglycerine, glycerol trinitrate, glycerol dinitrate, ethylene glycol dinitrate].

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APPENDIX C

INFORMATION SOURCES CONSULTED FOR TOXICITY TO AQUATIC ORGANISMS

A. Abstracts:

Generally, Biological Abstracts and Bioresearch Index are more useful than Chemical Abstracts for the toxicology of aquatic organisms. Descriptors used include nitroglycerin and the names of lakes and streams contiguous with Army Ammunition Plants. When Chemical Abstracts was used, the Guide was consulted for correct nomenclature. The degree of coverage of each descriptor depended on the relevance of the subtopic. For example, lake and stream names were covered only in post-1969 abstracts, whereas nitroglycerin toxicity and degradation were carried back to 1926 in Biological Abstracts and 1907 in Chemical Abstracts.

B. Telephone and Local Inquiry:

Telephone and personal inquiries were made of agencies or individuals known to have knowledge of topics of interest, who might have access to information not in the abstracts. Especially helpful were individuals in the Environmental Quality Division of the US Army Medical Bioengineering Research and Development Laboratory; the Water Quality Engineering Division of the US Army Environmental Hygiene Agency; the Tennessee Valley Authority; and the Illinois Water Survey.

APPENDIX III

MAMMALIAN TOXICOLOGY AND TOXICITY TO AQUATIC ORGANISMS
OF WHITE PHOSPHORUS AND "PHOSSY WATER", A WATERBORNE
MUNITIONS MANUFACTURING WASTE POLLUTANT -
A LITERATURE EVALUATION

FINAL COMPREHENSIVE REPORT

BY

DICKINSON BURROWS, Ph.D.
JACK C. DACRE, Ph.D.

NOVEMBER 1973

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Nashville, TN 37204

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

INTRODUCTION

The objective of this work is to provide part of a data base for standards for military-unique product discharges into waters subject to State and Federal regulations.

Background. This report is concerned with the toxicology of elemental "white" phosphorus (also known as "yellow" phosphorus) and its probable aqueous decomposition products. The generation of "phossy water" wastes in loading operations at the Pine Bluff, Arkansas, Army Ammunition Plant and some environmental consequences thereof have been reviewed by Rosenblatt, Small and Barkley (1973)¹. White phosphorus (WP) is purchased commercially and unloaded at Pine Bluff Arsenal. It is shipped in rail tank cars and transferred molten, under a warm water blanket, to munitions loading operations.

"Phossy water" is defined as water that has come in direct contact with large quantities of molten phosphorus, including dip-tank overflow, water used to flush white phosphorus from pipes, spray water used for wetting the dip-fill line conveyor and water from periodic cleaning of the dip-fill tanks. The rate of production of this phosphorus-laden water varies greatly, as does the white phosphorus concentration in the water. Elemental phosphorus is continually undergoing reaction with dissolved oxygen (under water), or with atmospheric oxygen to produce oxides of phosphorus that hydrolyze to give phosphorus compounds, perhaps in various oxidation states. Given adequate time, the white phosphorus particulates settle out as a sludge and the dissolved phosphorus is slowly oxidized. However, streams containing white phosphorus remain noticeably polluted over considerable distances.

Acute phosphorus poisoning early in this century was caused by the ingestion of match tips, fireworks, and quack nostrums, and is still encountered with surprising regularity. The literature abounds in cases of human poisonings after the ingestion of rat poisons or roach powders where phosphorus may be present in concentrations up to 4%. The conquest of occupational phosphorus poisoning in lucifer match manufacturing has been described by Oliver (1938)² and by Hamilton and Hardy (1949)³.

Chronic intoxication by inhalation of phosphorus fumes occasionally occurs in industry. A chronic phosphorus poisoning literature review for the period up to 1945 (and a presentation of recent case reports) summarizes the chronic effects of elemental phosphorus on humans (Heimann, 1945)⁴.

Almost all knowledge concerning the toxicity of white phosphorus to aquatic species derives from studies initiated in the late 1960's in response to a massive fish kill caused by the wastewater discharged from a factory manufacturing elemental phosphorus in Newfoundland (Idler, 1969⁵; Malin, 1972⁶). Most of the data concern salt water organisms. A similar

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catastrophic die-off of freshwater fish in Pickwick Reservoir in the early 1950's is believed to have resulted from an accidental discharge of phosphy water from holding ponds at the Muscle Shoals, Alabama, phosphorus manufacturing facility operated by the Tennessee Valley Authority^{7,8,9}. However, phosphorus poisoning was never demonstrated conclusively, and very little research resulted from this episode. Because phosphine, phosphite, hypophosphite and hypophosphate are likely products of the decomposition of white phosphorus in water, the relevant chemistry and toxicology of these compounds are covered in the present report.

Approach to Mammalian Toxicology. A survey was conducted of the chemical and biological literature on white phosphorus and on the related compounds. Sources for the literature search are set out in detail in Appendix A. Most of the references were obtained from Chemical Abstracts. Several machine searches were also made, but were not very helpful. In addition to the references obtained from the sources listed, a paper by Fleming, Miller, and Swayne (1942)¹⁰ on "Some recent observations on phosphorus toxicology" noted that their references had been selected from some 1000 reports on phosphorus toxicology as having special bearing on their reported findings; this bibliography (actually only 444 references) was obtained from the technical library of the Tennessee Valley Authority.

The papers obtained were grouped in the following categories for toxicological evaluation.

1. Acute toxicity studies
2. Chronic toxicity studies
3. Clinical studies (as they relate)
4. Biochemical studies
5. Analytical and related chemical studies.

Further references on the chemistry and mammalian toxicology of white phosphorus, of only peripheral interest, are listed in Appendix B.

Approach to Toxicity to Aquatic Organisms. The principal source of information on the aquatic toxicology of white phosphorus is contained in a collection of reprints from Dr. David Idler of the Memorial University of Newfoundland⁷. Additional information and literature sources were forthcoming in telephone consultations with personnel from Memorial

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University^{11,12}, ERCO Industries, Ltd.^{13,14}, Monsanto Company¹⁵⁻¹⁸, TVA⁷⁻⁹, Fisheries Research Board of Canada²⁰⁻²², American Chemical Society²³, and Ash Stevens, Inc.²⁴.

The published literature was independently surveyed using *Chemical Abstracts*. Appropriate subheadings under phosphorus were scanned from 1937 to August 1973. *Phosphine*, *phosphite*, *hypophosphite*, *hypophosphate*, *phosphorous acid*, *hypophosphorous acid*, and *hypophosphoric acid* were reviewed from 1917 to August 1973.

A machine search for relevant DOD documents was carried out using key words *phosphorus*, *white phosphorus*, *phossy water*, *plasticized white phosphorus*²⁵. Two documents relating to spray treatment of Pine Bluff phossy water, revealed as a result of this search, had not been received at the time this report was compiled. See also Appendix A.

Current Research on WP Toxicology and "Phossy Water". Apart from a few recent studies on the biochemical effects of white phosphorus, there does not appear to be any current research activity on the toxicity and toxicology of WP and phossy water. Laboratories listed in references 11, 12, 20, 21, and 22 are believed to be still active in research on the effects of elemental phosphorus pollution.

MAMMALIAN TOXICOLOGY OF WHITE PHOSPHORUS AND RELATED MATERIALS

Acute Toxicity in Animals. A summary of acute lethal toxicity of phosphorus in the rabbit, dog, rat, and mouse is given in Table 1. No LD50 values have been recorded for phosphorus. The effects of single toxic doses of phosphorus in olive oil given subcutaneously to male rabbits have been studied by Huruya (1928)²⁶. Changes associated with the production of fatty livers by white phosphorus in rats have been investigated by Seakins and Robinson (1964)²⁷ and by Pani, Gravela, Mazzarino and Burdino (1972)²⁸. Love (1934)²⁹ studied the effect of phosphorus on the normal and on the restored liver following partial hepatectomy in the albino rat.

Acute Toxicity in Man. In acute phosphorus poisoning, the predominant effect is fatty degeneration of the tissues. This occurs essentially in the liver, the damage to which is the cause of death in most cases. As little as 1/8 grain (8.1 mg) has been reported as having caused acute toxicity and death but the average fatal dose taken by mouth is generally considered to be of the order of 1.5 grains (97 mg). Christensen (1972)³⁷ gives the oral LD as 1.4 mg/kg. Acute phosphorus poisoning is peculiar because symptoms appear in two stages. During the first 24 hrs, symptoms

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TABLE 1. Summary of Acute Toxicity of White Phosphorus in Animals.

Animal Species	Route of administration	Dose* (mg/kg)	Remarks	References
Rabbit	or	7	LD	Hirz (1913) ³⁰
	or	10	LD	Frank and Isaac (1910-1911) ³¹
	sc	12.5	LD	Santesson and Malmgren (1904) ³²
	sc	30	LD	Santesson and Malmgren (1904) ³²
	or	20.0	LD	Cameron and Patrick (1966) ³³
Dog	sc	2-3	LD	Rubow (1905) ³⁴
	sc	12	LD	Welsch (1905) ³⁵
Rat	or	<0.1 grain [†]	LD	Shepard (1951) ³⁶
	or	0.5	LD	Cameron and Patrick (1966) ³³
Mouse	or	3.5	LD	Cameron and Patrick (1966) ³³

*All administered in oil.

†For a 400 g rat.

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of severe gastrointestinal irritation occur as soon as one-half hour after ingestion. The victim may die of cardiovascular failure within 12 hr. This first stage may be followed by a latent period lasting from a few hrs to a few days depending upon the amount ingested. The systemic stage is characterized by abdominal pain, nausea, vomiting, hematemesis and other hemorrhagic manifestations, jaundice, hepatomegaly, oliguria, toxic psychosis, convulsions, coma, and shock. There may be severe damage to the liver, heart, and kidney, and death may ensue at any time. Cirrhosis of the liver has been reported after recovery from the acute state. The signs and symptoms of acute poisoning in man are also adequately summarized by Gleeson, Gosselin, Hodge and Smith (1969)³⁸.

Fourteen papers on acute human phosphorus poisoning (episodes and case studies) are listed in the references (nos. 39-52) at the end of this report.

Chronic Toxicity in Animals. Sollmann (1925)⁵³ was the first investigator to study the effects of chronic feeding of phosphorus to albino rats. Further similar experiments by Flemming *et al.* (1942)¹⁰ confirmed the retardation in growth and the effects on bone development.

No significant changes were found in the blood, urine and tissues of dogs given subcutaneous injections of phosphorus in peanut oil (0.4, 0.2, and 0.1 mg P/kg/day) according to Buchanan, Sigal, Robinson, Pope, Ferguson and Thomson (1954)⁵⁴. The fate of orally administered choline in dogs with livers damaged through phosphorus poisoning was studied by Sigal, Buchanan and Robinson (1954)⁵⁵.

Ferraro, Jervis and English (1938)⁵⁶ drew attention to the fact that studies on white phosphorus intoxication in rabbits have been focused generally on liver changes and the metabolism of fatty substances. These workers have described the pathological changes in the brains of adult rabbits (nerve cells, blood vessels and glia) given iv injections of a 1% solution of phosphorus in oil (0.20 to 1.0 ml doses), over periods of 2 days to 15 weeks.

Lawrence and Huffman (1929)⁵⁷ noted that sc injections of phosphorus in olive oil into guinea pigs caused a marked increase in the number of the circulating monocytes in the blood.

Chronic Toxicity in Man (Including Potential for Carcinogenicity, Mutagenicity, and Teratogenicity). Chronic poisoning, seen mostly in factory workers exposed repeatedly to phosphorus vapors, is manifest

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by cachexia, anemia, bronchitis, and necrosis of the mandible ("phossy jaw"). The signs and symptoms of chronic poisoning, whether from ingestion or inhalation, have been well documented in the publications of Heimann (1946)⁴ and Gleeson, Gosselin, Hodge, and Smith (1969)³⁸. For summary of chronic toxicity to humans see Table 2.

The TLV for white phosphorus (ACGIH, 1972)⁵⁸ is 0.1 mg/m³. The TLV for phosphine⁵⁸ is 0.3 ppm or 0.4 mg/m³.

Testing for the possible carcinogenic activity of elemental phosphorus in experimental animals has been summarized by Hartwell (1951)⁵⁹, and Shubik and Hartwell (1957⁶⁰, 1969⁶¹). No animals with tumors were found following either oral feeding or subcutaneous injections in oil, even in rats receiving the compound for 33 months.

White phosphorus does not appear to have been investigated for possible mutagenic or teratogenic potential in living systems.

A case of opthalmoplegia due to phosphorus poisoning has been described by Medea (1905)⁶².

The effect of white phosphorus on growing bones and growing teeth of both rabbits and rats has been studied by Adams and Sarrat (1940)⁶³.

Burns due to Phosphorus. Two recent papers describe observations on the burns resulting from elemental white phosphorus. Curreri, Morris, and Pruitt (1970)⁶⁴ reviewed the clinical records of 111 patients, mostly with burns due to white phosphorus, who were admitted to the US Army Institute of Surgical Research. They noted the high incidence of residual ocular damage and suggested a number of specialized requirements for successful systemic and local wound care. No biochemical or toxicological data were evaluated.

Bowen, Whelan, and Nelson (1971)⁶⁵ describe a methodology for the production of a standard white phosphorus burn in rabbits. Although their study offered no suggestions regarding new or improved therapy of the white phosphorus-burned patient, it did elucidate electrolyte aberrations (depression of serum calcium and elevation of serum phosphorus and sometimes a reversal of the calcium/phosphorus ratio), and electrocardiographic changes which may explain early, sudden demise in the seemingly inconsequentially white phosphorus-burned patient.

Toxicity of Phosphine. Because of the possibility that phosphine (PH₃) may be a product of phosphorus metabolism (see p. 15) the toxicology has

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TABLE 2. Summary of Chronic Toxicity of White Phosphorus to Humans (Heimann, 1946)*.

System Affected	Characteristic Effects
Osseous	Jaw necrosis ("Phossy Jaw"). Hyperostosis of entire Skeletal Structure. Ossification of growing centers of bones.
Blood	Low in potassium and inorganic P content, high in chlorides, fat content. Morphological changes in circulating blood cells. Anemia of so-called "secondary" type.
Urinary	General cellular damage. Increase in ammonia N ₂ and unoxidized sulfur content.
Liver	No definite liver diseases described.
Local Irritant Effects	Skin burns, conjunctivitis, skin rashes.
General Cachexia	Exhaustion; loss of appetite; indefinite digestive complaints; garlic-like breath; slight jaundice, bleeding of the mucous membranes and albumin in the urine.

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been summarized herewith (see also Webster, 1946⁶⁶). The toxicology of acute mammalian poisoning by the gas phosphine has not been fully worked out according to Patty, Fassett, and Irish (1963)⁶⁷. It does not alter the blood and its effects are apparently exerted largely through the central nervous system. Some inflammation of the lungs results from its inhalation. The acute action of phosphine does not resemble that of phosphorus; instead, there is marked dyspnea, purgation, weakness, tremor, and finally violent convulsions and death. Mild cases recover without after-effects. Prolonged exposure to small amounts of phosphine is said to give rise to chronic symptoms similar to those of phosphorus poisoning and may be explained by the decomposition of the compound in the body (Meissner, 1924⁶⁸; Müller, 1940⁶⁹; Loewenthal, 1949⁷⁰). A summary of the physiological responses to various concentrations of phosphine is given in Table 3.

BIOCHEMICAL STUDIES

Poisoning due to phosphorus, according to Cutler (1931)⁷², results in an increase of guanidine in the blood of dogs and accompanying hypoglycemia. The results also indicate that the guanidine increase is probably dependent upon liver damage rather than upon kidney failure.

Mizuno (1932)⁷³ reported a relatively large increase (3 to 8 times) in the lactic acid content of the urine of rabbits with liver damage due to phosphorus poisoning.

The glucuronic acid produced by surviving liver slices from guinea pigs given a sc injection of yellow phosphorus (0.75 mg/kg body wt) in olive oil was found by Bueding and Ladewig (1939)⁷⁴ to be considerably reduced. Chloroform poisoning did not affect glucuronic acid production.

Barone, Cittadini, Galeatti, and Terranova (1973)⁷⁵ reported that fatty liver degeneration in rats caused by white phosphorus had no influence on the level of the microsomal respiratory pigments cytochrome bs and cytochrome P₄₅₀.

Rabbits with fatty livers caused by white phosphorus poisoning had three times the amounts of cytochrome c over fasted control animals according to Prader (1947)⁷⁶. Milder poisoning resulted in less cytochrome c and a lag period was also evident.

Gloshal, Porta, and Hartroft (1972)⁷⁷ measured the liver microsomal glucose-6-phosphatase in the fatty livers of rats given oral doses of

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TABLE 3. Summary of the Physiological Responses to Various Concentrations of Phosphine (Henderson and Haggard, 1943)⁷¹.

Response	Phosphene Concentration	
	ppm	µg/m ³
MAC for prolonged exposure	2	2.6
Slight symptoms after several hrs. exposure	7	10,010
Max. concentration that can be inhaled for 1 hr. without serious consequences	100 - 200	143,000 - 286,000
Dangerous after-exposure for 1/2 - 1 hr.	400 - 600	572,000 - 878,000
Fatal after-exposure for 30 mins.	1,000 - 2,000	1,430,000 - 2,860,000

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phosphorus (7.5 mg/kg body wt) and found no significant depression at 6 or 24 hours.

Serological studies on the blood of rabbits given yellow phosphorus (0.1, 1.0, 2.0 mg/kg body wt) were reported by Tanaka (1960)⁷⁸ with the following results: All groups showed an early increase in non-protein nitrogen, amino acid nitrogen and uric acid, and a slight increase in creatine and creatinine. A decrease in erythrocyte count, hemoglobin and red cell fragility was also recorded.

The changes in the serum proteins after blood and plasma losses have been studied in normal and phosphorus poisoned rabbits by Wenner (1943)⁷⁹. He found an increase of the α - and β -globulins after bleeding but no increase of the glucosamine content.

Fleming and Collings (1951)⁸⁰ studied the chylomicron count in the blood of rats given sc injections of white phosphorus (1.1 mg/kg body wt) in peanut oil. No significant changes occurred in fasting rats but significant and reproducible changes were noted 4 hours after resuming food intake.

Recent papers by Truhaut *et al.* (1969⁸¹, 1971⁸², 1972⁸³) describe the effects of both single sc doses and repeated sc doses of white phosphorus in the rat. The effects on the levels of liver phospholipids and triglycerides were reported.

TOXICITIES OF WHITE PHOSPHORUS TO AQUATIC ORGANISMS

Toxicity of Elemental Phosphorus in Water. In the earliest aquatic study of which we are aware, the tolerance of bluegills (*Lepomis macrochirus* Rafinesque) for white phosphorus was measured in freshwater at 26°C (Isom, 1960)⁸⁴. The author concluded that phosphorus was nontoxic to the limit of its solubility (ca. 3 mg/l at 15°C), but paradoxically, that much smaller quantities of colloidal phosphorus were highly toxic. The conclusion concerning colloid-free water is probably invalid, because the author did not have available an analytical procedure sufficiently precise to tell whether elemental phosphorus was present at all; however, the 96 hour median tolerance level of 45 μ g/l, colloidal or dissolved, may reasonably be taken as an upper limit (cf. Table 1). Curiously, a Russian report claims that the toxicity of different forms of elemental phosphorus to freshwater fishes decreases in the order emulsion > solution > suspension > colloid (Kresmov, 1970)⁸⁵.

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The most complete studies on white phosphorus intoxication in a marine environment have been carried out in connection with operation of a plant producing elemental phosphorus in Newfoundland^{5,6}. This plant, constructed by ERCO Industries, Ltd. on Long Harbour, Placentia Bay, began discharging phosphy water in December, 1968. Shortly thereafter large numbers of fish, principally cod and herring, were found dead in Long Harbour and adjacent waters, and in May, 1969, the plant was shut down and part of Placentia Bay was closed to fishing. Subsequently, groups associated with the Fisheries Research Board of Canada have studied the toxicity of white phosphorus in seawater to various fish and crustaceans (Zitko *et al.*, 1970⁸⁶; Fletcher *et al.*, 1970⁸⁷; Fletcher and Hoyle, 1972⁸⁸). Their results are summarized in Table 4. For five genera and four families of fish in saltwater, 96 hour LD 50's are remarkably uniform, $3 \pm .7$ ug/l, although field experience indicates that cod are substantially more tolerant than herring (Fletcher, 1973)¹². Incipient lethal levels are very low, suggesting that phosphorus poisoning is accumulative in these fish. Two separate studies emphasize the importance of the time-dosage relationship (Zitko *et al.*, 1970⁸⁶; Fletcher and Hoyle⁸⁸); a short exposure to a relatively high concentration may be lethal in the same time period as continuous exposure to a low concentration. For crustaceans the tolerance to phosphorus may be several orders of magnitude higher, though the incipient lethal level for lobsters is still quite low in terms of likely pollution levels. The beach flea has the greatest tolerance for white phosphorus of any animal studied, and is the only one for which reversibility of intoxication has been clearly demonstrated.

Similar investigations of other phyla have not been reported. However, a study of the bottom of Long Harbour in the vicinity of the effluent outlet provided evidence of selective mortality among benthic organisms (Peer, 1972)⁹¹. Thus, e.g., mussels (*Modiolus modiolus*) and a burrowing sea anemone (*Edwardsia* sp.) were alive and apparently normal in a region from which scallops and sand dollars (*Echinarachnius parina*) had been nearly exterminated. In a recent study, Black (1973)²² has found no significant variation in annual growth rates for Long Harbour mussels (*Mytilus edulis*) during 1969, the year of significant phosphorus pollution, and preceding and succeeding years (Black, 1973)⁹². On the other hand, no periwinkles (*Littorina littorea*) old enough to have been set before 1970 were found, indicating that all were killed by the 1969 spill.

Accumulation of Phosphorus by Aquatic Organisms. In a 16 hour exposure, cod have been shown to concentrate phosphorus 1000 times in the liver and 10 to 1000 times in muscle tissue from water containing 20 to 80 ug/l (Oyer *et al.*, 1970)⁹³. Concentration factors are roughly proportional to the lipid content of the tissue, and inversely related to the phosphorus content of the water. Other fish have not been studied in such

TABLE 4. Summary of Toxicity of White Phosphorus to Fish Species and Crustaceans.

Organism	Incipient Lethal Level mg/l	96 hr LC50 mg/l	Test	Water	Temp. °C	References
Bluegill (<i>Lepomis macrochirus</i>)		ca. 45	static	fresh	26	Isom (1960) ⁸⁴
Cod (<i>Gadus morhua</i>)	<1.5	2.5	cont.	salt	9-10	Fletcher and Hoyle (1972) ⁸⁸
Atlantic salmon (<i>Salmo salar</i>)	<.8	2.3	cont.	salt	10-13	Zitko et al. (1970) ⁸⁶ Fletcher and Hoyle (1972) ⁸⁸
Herring (<i>Clupea harengus</i>)	<2.5	3.7	static	salt	7-9	Zitko et al. (1970) ⁸⁶
Brook trout (<i>Salvelinus fontinalis</i>)	<.5	2.5	cont.	salt	7-9	Fletcher et al. (1970, 1971) ^{87,89}
Smelt (<i>Osmerus mordax</i>)		2.5	cont.	salt	7-9	Fletcher et al. (1970, 1971) ^{87,89}
Killifish (<i>Fundulus heteroclitus</i>)		>20	cont.	salt	7-9	Fletcher et al. (1971) ⁸⁹
Cunner (<i>Tautoglabrus adspersus</i>)		*	cont.	salt	7-9	Fletcher et al. (1971) ⁸⁹
Lobster (<i>Homarus americanus</i>)	20-40	300	static	salt	?	Zitko et al. (1970) ⁸⁶ Fletcher (1971) ⁹⁰
Beach flea (<i>Gammarus oceanicus</i>)	3000-4000		static	salt	18-21	Zitko et al. (1970) ⁸⁶
Atlantic salmon smolts		ca. 10	cont.	fresh	11-14	Fletcher and Hoyle (1972) ⁸⁸

*LT 50, 3-4 mg/l, 250-300 hr.

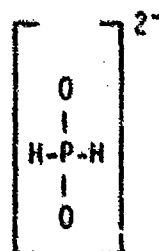
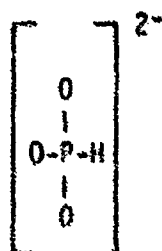
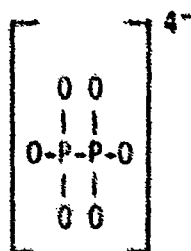
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detail, but from a survey of fish collected in Placentia Bay in 1969-1970, and from limited bioassays, it is known that herring and salmon accumulate phosphorus to the 1 ppm level, and that other fish can accumulate at least measureable quantities (Ackman *et al.*, 1970)⁹⁴.

Fletcher (1971)⁹⁰ has recorded the 48 hour uptake of white phosphorus by several marine invertebrates and seaweed from seawater containing $15 \pm 9 \mu\text{g/l}$ of white phosphorus (cf. Table 5). It may be noted that periwinkles, which are foraging grazers, accumulated greater amounts of phosphorus than the filter feeding molluscs. Fletcher (1971)⁹⁰ suggests that the former may ingest phosphorus with the seaweed. Intoxication was not reported for any of these invertebrates, and elemental phosphorus was eliminated from all the organisms of Table 6 within seven days following transfer to uncontaminated seawater.

Toxicity to Waterfowl. Dabbling ducks of the genus *Anas* feeding in areas of phosphy water discharge have been found to ingest lethal quantities of elemental phosphorus (Coburn *et al.*, 1950)⁹⁵. In a series of experiments conducted with captive black ducks and mallards, chronic and acute responses varied widely among individuals, but a single dose of 3 mg/kg body weight was sufficient to kill all ducks studied in 6 to 33 hours.

Toxicity of the Total Waste. Analysis of a phosphy water sample from Pine Bluff AAP showed it to contain 242 mg/l of phosphate and 208 mg/l of lower phosphorus oxides, as phosphorus (Blumbergs *et al.*, 1973)^{24,96}. These might include hypophosphate, phosphite, and hypophosphite, the latter two being known products of nonaqueous oxidation of phosphorus (Van Wazer, 1958)⁹⁷. No one of these is reported to be very toxic.



Nitrogen fixation by *Azotobacter vinelandii* is completely inhibited by 300 mg/l of phosphite (Bulen and Frear, 1957)⁹⁸ as P, ca. 10^{-2}M , while some other bacteria (e.g. *Escheria coli* and certain *Pseudomonas*, *Agrobacter*, *Rhizobium* and *Agrobacterium* species) can utilize phosphite for growth at the same concentration (Casida, 1960)⁹⁹, and *Pseudomonas fluorescens*

TABLE 5. Phosphorus Accumulation by Seaweed and Invertebrates (Fletcher, 1971)⁹⁰.

Organism	White Phosphorus (mg/g)	Conc. Factor Approx.
Seaweed		
<i>Fucus vesiculosus</i>	.33	22
<i>Fucus distichus</i>	.33	22
Invertebrates		
Clam (<i>Mya arenaria</i>)	.34	23
Quahog (<i>Arctica islandica</i>)	.26	17
Mussel (<i>Mytilus edulis</i>)	.16	11
Periwinkle (<i>Littorina littorea</i>)	.64	43
Starfish (<i>Asterias vulgaris</i>)	.40	27

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converts phosphite to phosphate in excess of that required for growth. Still other micro-organisms, including some yeasts, are unable to utilize phosphite, but are unaffected by it. Robertson and Boyer (1956)¹⁰⁰ report that rat weanlings fed inorganic phosphite at 250 mg/kg on alternate days show some restriction of growth, but 125 mg/kg is without effect. Table 6 summarizes enzyme inhibition data for phosphite and hypophosphite at the $10^{-2}M$ level. The effect is generally small. Van Wazer (1958)¹⁰¹ says that "the phosphites exhibit considerable toxicity, acting on the nerve centers and abdominal glands; he also says "there is no evidence that hypophosphite exerts the slightest effect on mammals, and it passes through the mammalian system unchanged"¹⁰², but he presents no data more recent than 1902. There appear to be no toxicity data of any kind for hypophosphate. At higher pH levels, phosphine is formed from elemental phosphorus, and a small amount may be present in phossy water. Phosphine is a toxic gas (MPC 0.3 ppm for humans) with a moderately low water solubility. It is alleged to be toxic to rainbow trout in concentrations exceeding 3.6 ppm (Daudoroff and Katz, 1950)¹⁰⁶. A few papers report the generation of phosphine in natural waters by anaerobic sediments (Datsko, 1958¹⁰⁷; Luning and Brohm, 1932¹⁰⁸, 1934¹⁰⁹), and one implies that it is highly toxic to fish¹⁰⁷, but otherwise there does not appear to be a literature of aquatic toxicology. It is rapidly degraded by the microorganisms in wet soil (Hilton and Robison, 1972)¹¹⁰.

Polyphosphoric acid, if formed in phossy water, would not be expected to present a problem. Although it is a sequestering agent, like NTA, and might enhance the toxicities of any heavy metals in the water, it is readily biodegradable to orthophosphoric acid (Metcalf, 1973¹⁵, Karl-kroupa *et al.*, 1957)¹¹¹.

The phossy water discharged in the manufacture of phosphorus is a more complex waste than that released in filling operations, such as that at Pine Bluff AAP^{5,15-19}. The latter consists only of dissolved and suspended elemental phosphorus and oxidation and hydrolysis products thereof (principally phosphoric acid). The manufacturing wastewater includes as well a large amount of suspended solids, fluosilicic acid and sulfur dioxide, and some ammonia and cyanide. A typical wastewater analysis for the ERCO Industries, Ltd. plant, prior to shutdown in 1969, is given in Table 7. Thus although extensive mortality studies were carried out in the area, it was necessary that early investigators consider that the catastrophic effect of the Long Harbour discharge may have been due in part to waste components other than phosphorus. Bioassays of the various waste constituents, however, have shown phosphorus to be the most toxic by several orders of magnitude (Fletcher *et al.*, 1971)⁸⁹. For example, Figure 1 illustrates the comparative toxicities of several substances to brook trout maintained in seawater. The total wastewater

TABLE 5. Enzyme Inhibition by Phosphite and Orthophosphite.

Enzyme	Anion	Conc	& Inhibition	References
Intestinal phosphatase	orthophosphite	$10^{-2}M$	5	Robertson and Boyer (1956) ¹⁰⁰
3-Phosphoglyceraldehyde dehydrogenase	orthophosphite	$2 \times 10^{-2}M$	salt effect only	Robertson and Boyer (1956) ¹⁰⁰
<i>E. coli</i> formic dehydrogenase	hypophosphite	$10^{-2}M$	~20	Crewther (1956) ¹⁰³
<i>E. coli</i> formic hydrogenlyase	hypophosphite	$10^{-2}M$	~40	Crewther (1956) ¹⁰³
<i>E. coli</i> hydragenase	hypophosphite	?	0	Crewther (1956) ¹⁰³ Gruenberg-Monago <i>et al.</i> (1951) ¹⁰⁴
Horse serum cholinesterase	hypophosphite	10^{-2}	~35	Frommel <i>et al.</i> (1944) ¹⁰⁵

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TABLE 7. Analysis of Long Harbour Wastewater (Idler, 1969)⁵.

Flow	ca. 8000 gpm	11.5 MGD
Fluoride	22800 lb/day	245 mg/l
SO ₂	6250 lb/day	67 mg/l
Total P	1630 lb/day	18 mg/l
P ₄	1250 lb/day	13 mg/l
CN	96 lb/day	1 mg/l
NH ₃	455 lb/day	5 mg/l
Solids	38360 lb/day	410 mg/l

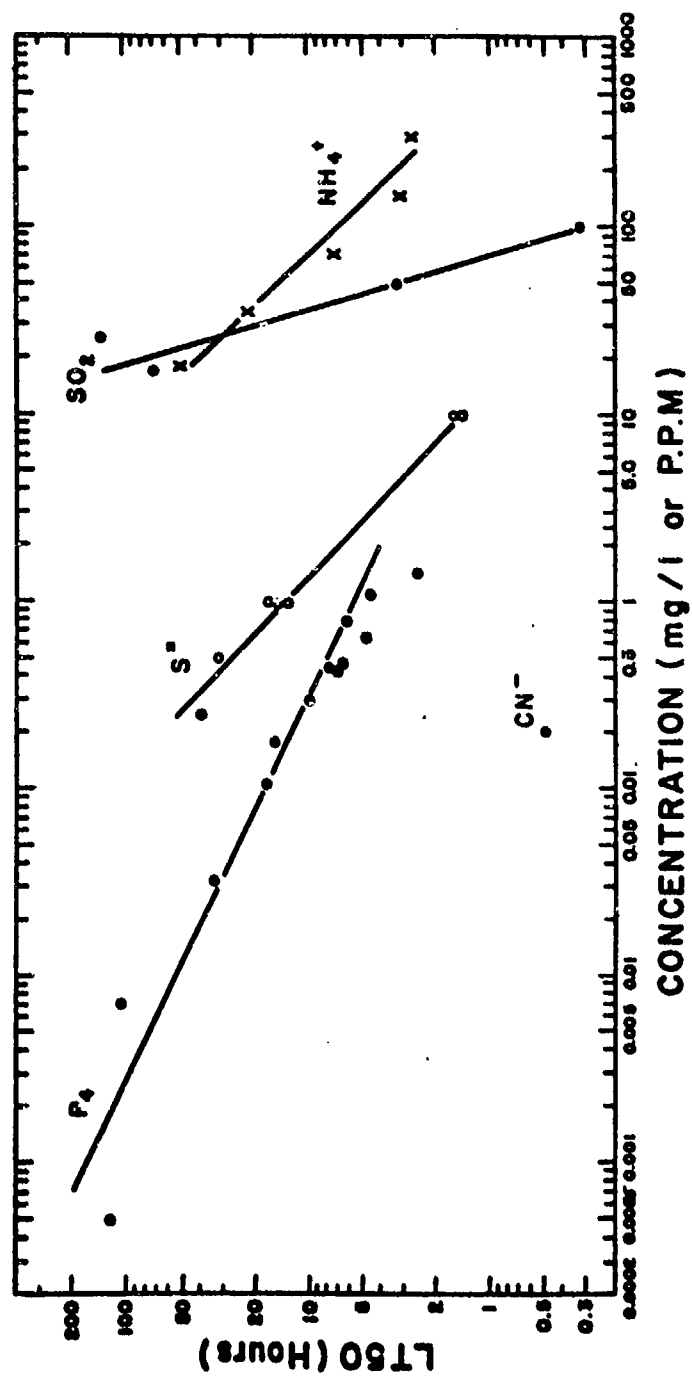


FIGURE 1: TOXICITIES OF VARIOUS SUBSTANCES TO SEAWATER MAINTAINED BROOK TROUT (Fletcher, *et al*, 1971)⁸⁹

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exhibits no enhanced toxicity to cod beyond that to be predicted on the basis of its white phosphorus content alone (Idler, 1965)⁵.

Other constituents of the waste may affect the distribution and dispersal of phosphorus (Jangaard, 1970)¹¹². Fluosilicic acid reacts with calcium and magnesium in seawater to produce a flocculant precipitate, which can coagulate and thereby immobilize colloidal white phosphorus in the immediate vicinity of the outfall. This greatly reduces the efficacy of tidal dilution, and may result in the creation of permanent toxic zones. In the case of the Long Harbour operation, much of the phosphorus contamination resulted from accidental spills in the course of loading ships with liquid phosphorus¹³. Phossey water from this source would not be expected to differ greatly from that from ammunition plants.

Behavioral Effects. The most obvious symptom of phossey water poisoning, other than death of the victim, is the red color acquired by the belly and head of certain fish, particularly herring. All fish eventually become lethargic, and on the approach of death may swim in circles (Zitko *et al.*, 1970)⁸⁶. Lobsters exposed to lethal levels of white phosphorus "lose muscle tone, become lethargic, move the pereopods in a jerky, uncoordinated and aimless manner, and eventually lose all neuromuscular response"⁸⁶. beach fleas become paralyzed when subjected to lethal doses, but may recover when transferred to clean water⁸⁶.

Among waterfowl, acute poisoning results in depression, followed by leg weakness and finally violent convulsions and death. Birds suffering from chronic poisoning steadily lose weight and eventually show signs of paralysis. Field cases, usually suffering from acute poisoning, are described as having "ungainly flight" (Coburn *et al.*, 1950)⁹⁵.

Pathology. White phosphorus can be expected to cause extensive tissue damage wherever it accumulates. Tissues of the gills, kidneys, liver and spleen may undergo substantial disintegration (Odense *et al.*, 1972)¹¹³. However, the symptoms of phosphorus intoxication, including death, are not necessarily related to tissue destruction.

The cause of fish mortality from phossey water has yet to be determined. White phosphorus levels low enough to permit survival of the fish for more than a few hours may cause extensive hemolysis of erythrocytes for some species, viz. herring (Zitko *et al.*, 1970⁸⁶; Fletcher *et al.*, 1970⁸⁷), Atlantic salmon (Zitko *et al.*, 1970⁸⁶; Fletcher and Hoyle, 1972⁸⁸), and brook trout (Fletcher *et al.*, 1970⁸⁷). Some of the hemoglobin released may then leave the circulatory system and pass into the interstitial areas, temporarily imparting a bright red color to the head and belly. Hematocrits

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may drop to a very low value, and in latter stages the gills may turn pale, but loss of red cells is apparently not the cause of death. Cod and smelt exhibit no evidence of hemolysis at any level of phosphorus exposure, and trout and salmon expire without hemolysis if the dosage is great enough (Fletcher *et al.*, 1970⁸⁷, 1972⁸⁸). Recent Russian studies suggest that the central nervous system may be involved (Krasnov, 1970⁸⁵; Mazmanidi, 1970¹¹⁴). Fletcher (1973)¹¹⁵ has found that brook trout fed cod poisoned by elemental phosphorus develop the same symptoms as trout exposed to phosphorus in water. Whatever the cause, phosphorus intoxication of fish appears not to be reversible.

In lobsters, phosphorus intoxication brings about degeneration of the hepatopancreas and antennal gland tissue (Aiken and Byard, 1972)¹¹⁶. Although this damage should eventually kill the lobster, death is probably due to asphyxiation resulting from blood coagulation. Toxic effects are cumulative and irreversible or very slowly reversible.

For waterfowl, the histopathology of phosphorus intoxication is inconsistent. The kidneys are usually congested, the liver commonly shows signs of damage, and duodenal ulcers may be present, but it is not certain that any of these disorders are related to the early death of the bird (Coburn *et al.*, 1950)⁹⁵.

Summary and Conclusions. White phosphorus is highly toxic to fish. The 96 hour LC 50's are less than 50 ppb for all fish studied, and the incipient lethal level is probably less than 1 ppb for most fish. Phosphorus poisoning appears to be accumulative and irreversible, though the cause of mortality has not been determined. While phosphorus is readily taken up by fish and other aquatic organisms directly from the water, fish may also acquire lethal quantities of elemental phosphorus through the food chain, since the few macroinvertebrates studied have a much higher tolerance for white phosphorus than fish. The symptoms of phosphorus intoxication are passed onto brook trout when they are fed muscle tissue from phosphorus-poisoned cod (Fletcher, 1973)¹¹⁵. Furthermore, elemental phosphorus can be passed on to humans, since a considerable portion, 25% or more, remains in the muscle of the fish after processing, storage, and cooking (Dyer *et al.*, 1972)¹¹⁷.

So toxic is white phosphorus to some fish that it is difficult to set maximum safe levels, although there are indications that fish acquire a tolerance after exposure (Fletcher, 1973)¹². If it is assumed that the minimum incipient lethal level is 0.1 µg/l (1/5 the lowest measured), then it would seem advisable to set a maximum of 0.01 µg/l in the mixing zone of any outfall. Zero discharge of phosphorus manufacturing process water has been recommended (Anon., 1973)¹¹⁸.

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Other components of phosphy water are probably inconsequential in terms of their likely concentrations, but there is uncertainty concerning phosphine.

CHEMISTRY OF WHITE PHOSPHORUS AND DERIVED SUBSTANCES

The Fate of Elemental Phosphorus in Water. Although there are no definitive published reports on the kinetics of oxidation of elemental phosphorus in water, it appears that the rate is highly dependent on the degree of dispersion. At concentrations (ca. 10 $\mu\text{g/l}$) well below the accepted solubility limit of 3 mg/l, with the dissolved oxygen content unspecified, elemental phosphorus disappears by a first order process with a half-life of 2 hours at ca. 10°C (Zitko *et al.*, 1970)⁸⁶ 0.85 hour at 30°C (Addison, 1971)¹¹⁹. At concentrations (50 to 100 mg/l) well above the solubility limit, with a dissolved oxygen content of 6 to 7 mg/l, the same reaction has a half-life of 80 hours at 30°C and 240 hours at 0°C (Bullock and Newlands, 1969)¹²⁰. The relatively small temperature effect combined with the large inverse concentration effect is consistent with a diffusion controlled process. The oxidation of colloidal phosphorus in seawater is reported to be measurably slower than in fresh water, suggesting that the high salt content brings about agglomeration of the phosphorus particles¹²⁰. Thus, rapidly moving fresh water should lose elemental phosphorus faster than quiescent seawater.

Addison has presented evidence indicating that 1 ppm aqueous elemental phosphorus is oxidized to phosphate in a single step¹¹⁹. Likely intermediates, though stable under the reaction conditions, could not be isolated. Blumbergs *et al.*, 1973^{24,96} on the other hand, report that phosphy water from Pine Bluff AAP contains as much phosphorus in intermediate oxidation states as orthophosphate. A synthetic mixture prepared with several hundred ppm white phosphorus gave the same results. It is possible that the products of oxidation of suspended phosphorus are different from those of dissolved phosphorus.

The rapid reaction of white phosphorus with chlorine in water to form phosphorus acid *via* phosphorus trichloride is well documented (Blumbergs *et al.*, 1973⁹⁶; Anon, 1952¹²¹; Barber, 1969¹²²).

ANALYTICAL METHODOLOGY

Elemental Phosphorus. Until recently, the usual method for determining elemental phosphorus involved extraction of the sample with an organic

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solvent such as benzene, oxidation of the extracted phosphorus to orthophosphate, and estimation of the latter by the molybdenum blue or other colorimetric method. Under ideal conditions, a detection limit of about 0.01 ppm of phosphorus in water is attainable by this technique, but of course it is not possible to distinguish P_4 from benzene-soluble phosphorus derivatives, phosphine in particular (McGilvery, 1973)¹⁴.

Addison and Ackman have devised a gas-liquid chromatographic procedure for elemental phosphorus capable of detecting as little as $10^{-12}g$ (Addison, 1971¹¹⁹; Ackman and Addison, 1969¹²³; Addison and Ackman, 1970¹²⁴). Benzene extracts of mud, water, or biological samples are injected onto a gas-liquid chromatograph equipped with a flame photometric detector. Techniques have been contrived to permit detection of 0.01 $\mu g/l$ of elemental phosphorus in water. (The solubility of P_4 in benzene is 10^4 times its solubility in water). Some interference from organics has been noted (Addison, 1971)¹¹⁹; for natural water samples, $\pm 0.1 \mu g/l$ is probably the limit of precision, and for mud and biological samples, $\pm 0.1-1 mg/kg$; depending on the nature of the sample. Using their methods, the authors and associates have analyzed for phosphorus in water (Addison *et al.*, 1971¹²⁵, 1972¹²⁶), mud (Ackman *et al.*, 1972^{126,127}), and fish (Ackman *et al.*, 1970^{94,126}) samples from Long Harbour.

More recently, Bohl and Kaelble (1972)¹²⁸ of Monsanto have devised a method for the determination of white phosphorus in air, also employing flame photometric gas chromatography. They cite a detection limit of $10^{-11}g$ for P_4 dissolved in xylene. Gorzny (1972)¹²⁹ has reviewed methods for elemental phosphorus through early 1970. It appears at this time that flame photometric gas chromatography is the method of choice for environmental samples.

Phosphine. Methods for phosphine have been reviewed by Fetchin and Grayson (1972)¹³⁰. Because phosphine is usually encountered as a gas, analytical procedures are based on air sampling. A typical procedure utilizes formation of a colored complex with silver diethyldithiocarbamate (Dechant *et al.*, 1966)¹³¹, the sensitivity corresponding to a level of ± 50 ppm in water. Berck *et al.*, 1970¹³² employing flame photometric gas chromatography, are able to detect quantities of gaseous phosphine in the picogram ($10^{-12}g$) range. Extraction and gas chromatography should be applicable to water samples containing phosphine.

Oxyacids of Phosphorus. Van Wazer (1958)¹³³ and Ohashi (1972)¹³⁴ have reviewed analytical methods for the oxyacids of phosphorus. The preferred method is separation by paper on thin layer (Seiler, 1965)¹³⁵ chromatography and estimation of the developed spots. More recently, Addison

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has employed a gas chromatographic technique based on formation of trimethylsilyl derivatives and utilizing the flame ionization detector (Addison, 1971¹¹⁹; Butts, 1970¹³⁶). Sensitivities in the 0.1 ppm range are implied.

RECOMMENDATIONS FOR FUTURE EXPERIMENTAL AND FIELD STUDIES ON WHITE PHOSPHORUS

1. The determination of the LD50 for white phosphorus should be undertaken in two animal species.
2. Because there is no information on the long-term effects of phosphorus, both chronic 90-day studies of low dose levels of phosphorus in 2 species of animals as well as a 2 yr long-term feeding study of very low dose levels of phosphorus in animal species should be undertaken, with special attention being paid to bone development.
3. The metabolism of phosphorus using radio active ^{32}P in animal species should be studied especially to determine retention in bone and possibly in other organs. The form of urinary and fecal excretion of phosphorus should be investigated.
4. Teratogenic (especially for possible interference with bone development) and mutagenic studies of white phosphorus might be desirable.
5. An investigation of phosphine (PH_3) production from elemental phosphorus by microorganisms in both field and laboratory studies should be undertaken.
6. The recommendation made by Rosenblatt, Small, and Barkley (1973)¹ in their report (p. 25) viz. "Procedures for monitoring white phosphorus in water should continue to be developed. The toxicity and analysis of lower oxides of phosphorus and polyphosphates should be investigated", is fully supported in this report.
7. Because elemental phosphorus is by far the most toxic constituent, the primary data required for the assessment of phosphy water pollution is the rate of oxidation of dissolved phosphorus in the concentration range of 1-1000 $\mu\text{g/l}$. A definitive kinetic study should be initiated to determine the effects of phosphorus concentration, dissolved oxygen content, temperature, pH and dissolved solids. From such data the time required to achieve safe phosphorus levels could be estimated for any particular body of water.

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8. Pine Bluff AAP phosphy water should be analyzed for the presence of intermediate oxyacids of phosphorus and for phosphine. Acute toxicities, based on LD 50's, should be measured for those present at the mg/l level, using appropriate freshwater target species, such as fish and crayfish. For any substance found to be toxic in terms of the quantities present in the waste microbiological studies should be initiated to measure inhibition and utilization of phosphorus.

9. Although toxicity data for elemental phosphorus is available for numerous species of sea-water fish and a few lower animals, further bioassay studies on phosphy water using freshwater fish, lower animals and algae is desirable. Chronic toxicity data for white phosphorus levels below 0.1 ppb would be useful for establishing maximum safe concentrations, but the rapid oxidation of dissolved phosphorus at these levels and uncertainties in the present best analytical method would appear to present awesome experimental complications.

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[Key words: phosphorus, white]

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[Key words: phosphorus, white]

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[Key words: phosphorus, white phosphorus, phossy water, plasticized white phosphorus].

(See Search Control No. 002787)

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[Key words: phosphorus, white]

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APPENDIX IV

**MAMMALIAN TOXICOLOGY AND TOXICITY TO AQUATIC ORGANISMS OF TNT,
DNT, AND OTHER MUNITIONS MANUFACTURING WASTE CONSTITUENTS
OF PINK WATER - A LITERATURE EVALUATION**

FINAL COMPREHENSIVE REPORT

BY

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official Department of the Army position unless so designated
by other authorized documents**

APPENDIX IV (Cont)

INTRODUCTION

The objective of this work is to provide part of a data base for standards for military-unique product discharges into waters subject to state and federal regulations.

Background. "Pink waters" are aqueous effluents from plants that manufacture or process TNT (α -TNT or 2,4,6-trinitrotoluene). Initially almost colorless to yellow, such effluents assume their characteristic color when brought to pH 7 or above and exposed to sunlight, or when made fairly alkaline. The color, due to α -TNT degradation products, is visible even at initial TNT concentrations as low as 0.5 ppm. The nature of the TNT degradation products is as yet ill-defined (Rosenblatt, Lauterbach and Davis, 1971¹). The "pink" constituents are a group of water-soluble (and organic-insoluble) anions, some of which display free-radical character, and appear to constitute the major proportion of the products of α -TNT photolysis². Another group of photolytic degradation products is extractable from water with organic solvents; compounds so far identified³ are 1,3,5-trinitrobenzene, 2,4,6-trinitrobenzaldehyde, 2,4,6-trinitrobenzonitrile, 4,6-dinitroanthranil, and a group of four azoxy compounds of which a typical example is 4,4'-dimethyl-3,3',5,5'-tetranitroazoxybenzene. In addition to α -TNT, other constituents initially present in the wastewaters remain after the "pink" color becomes manifest and the α -TNT content has been drastically decreased. These constituents vary with the source, as shown in Table 1; the most important appear to be DNT (dinitrotoluene) isomers, especially 2,4-DNT and 2,6-DNT.

The recent report by Rosenblatt, Small, and Barkley (1973)⁴ summarizes the production process, uses, and wastes generated at US munitions production plants and load, assemble and pack plants.

Approach to Mammalian Toxicology. A survey was conducted of the chemical and biological literature on TNT, on DNT, and on the related compounds listed above (for which nothing was found). Sources for the literature search are set out in detail in Appendix A. Most of the references were obtained from Chemical Abstracts. Several machine searches were also made, but were not very helpful. The papers obtained were grouped in the following categories for toxicological evaluation.

1. Acute toxicity studies
2. Chronic toxicity studies
3. Clinical studies (as they relate)
4. Metabolic fate studies
5. Related biochemical studies
6. Analytical and related chemical studies.

APPENDIX IV (Cont)

The comprehensive reports on TNT poisoning and toxicity by von Oettingen *et al.* (1944)⁵ and by Voegtlin, Hooper and Johnson (1921-1922)⁶ provide much of the information summarized here.

Additional literature references, not cited in the body of the report or in Appendix A, are listed in Appendix B.

Approach to Aquatic Toxicity. The literature search pattern (Appendix C) was arranged to give maximum coverage of key subjects in a minimum of time. Briefly, important recent references were located a) by way of abstract journals, b) by telephone, and c) by local inquiry. Then the literature citations in these references were exploited. Generally, Biological Abstracts and BioResearch Index were more useful than Chemical Abstracts. A large number of applicable citations appeared in Biological Abstracts; these were adequately cross-referenced, and the key words used made it easy to locate compounds of primary interest and related substances.

Current Research on TNT Toxicology and Pink Water Chemistry. Through a contract with Litton Bionetics, Inc., Falls Church, VA (Contract No. N00014-73-C-0162), the US Navy Toxicology Unit, Bethesda, MD, is currently conducting mammalian toxicological tests on munitions grade α -TNT. The project monitor is LCDR Lawrence J. Jenkins, Jr.

Research on the chemistry of "pink water" is in progress at the US Army Natick Laboratories (Dr. Ronald Chalk), and, under contract with Natick, at Tufts University, Boston, MA (Dr. Grant Urry, Dept. of Chemistry, Contract No. DAAG17-73-C-0073), and at the University of Idaho, Moscow, ID (Dr. Budalur S. Thyagarajan, Dept. of Chemistry, Contract No. DAAA15-73-C-0248). Work is also going on at Edgewood Arsenal (Drs. Harold Sommers, Brennie Hackley, and George Davis) to characterize "pink water" sufficiently to permit samples to be reliably reproduced for toxicological testing, and at the Naval Ordnance Laboratory, White Oak, MD (Dr. J. Hofsommer).

MAMMALIAN TOXICOLOGY OF TNT

Acute Toxicity* in Animals. Animals vary considerably in their sensitivity to acute TNT poisoning not only from one species to another but also within any species (Voegtlin *et al.*, 1919⁷, 1920⁸, 1921-1922⁶; Wyon, 1921⁹). Voegtlin *et al.* (1919)⁷ indicated that cats and dogs were somewhat more sensitive than humans to TNT poisoning, and that all of these were in turn more sensitive than rabbits, rats and guinea pigs to TNT poisoning. Wyon⁹ conducted lethality (not LD₅₀) studies on a number of

APPENDIX IV (Cont)

animal species. While the exact numbers of specimens used for these studies were not always indicated in the report, it appears that very few animals were used for each species, with the exception of cats. In thirteen experiments with cats, Wyon⁹ found the lethal dose to be 200 mg/kg given subcutaneously in oil or 480 mg/kg administered orally. He determined the subcutaneous lethal dose of TNT in rabbits to be 500-700 mg/kg. Death occurred in one rabbit within three hours after receiving a dose of 700 mg/kg. In TNT mortality studies of six rats, he found the subcutaneous lethal dose to be 700 mg/kg with death occurring as early as 28 hours. There have been no comparative LD₅₀ studies (as opposed to LD studies) of the toxicity of TNT to various animal species.

Voegtlin *et al.* (1921-1922)⁶, exposed a large number of dogs to daily TNT dosages ranging from 5 mg/kg to 100 mg/kg, administered orally, subcutaneously in oil, or by inhalation. Marked cyanosis developed within 12 hrs after the administration of a single 100 mg/kg dose, and severe incoordination appeared within the second or third day. The authors noted that the striking feature of TNT poisoning in dogs was the important part played by individual susceptibility. Certain animals receiving a large dose (such as 100 mg/kg) did not show the symptoms of those receiving 50 or 75 percent less TNT.

It should be stressed that all the above workers determined the lethal dose for TNT that will kill experimental animals. No LD₅₀ values for TNT have been described.

Chronic Toxicity in Animals. Voegtlin *et al.* (1920⁸, 1921-1922⁶) administered dosages of 5-33 mg TNT daily to 39 dogs either by mouth (gelatin capsules), subcutaneously (dissolved in olive oil), or by inhalation for various periods up to 150 days. In addition to the acute symptoms (cyanosis and incoordination), notable icterus was observed in the mucous membrane of the mouth and in the conjunctiva of 6 of 39 dogs, but the salient features of chronic TNT poisoning were anemia and the rapid destruction of red blood cells. Every animal developed anemia of varying intensity. Voegtlin *et al.* (1919⁷, 1921-1922⁶) found that the exposed animals showed a reduction in the total blood volume, as well as in hemoglobin and red corpuscle volumes, and an increase in reticulated red blood cells during the first 15 days. Anisocytosis (inequality in the size of the red blood corpuscles) was very common. Blood destruction was due to hemolysis within the circulating blood, leading to increased phagocytosis of these cells and breakdown products in the spleen, liver and bone marrow.

* Acute toxicity will be used to denote effects resulting from a single dose.

APPENDIX IV (Cont)

Von Oettingen *et al.* (1944)⁵, in a series of experiments, administered daily oral doses of 5, 15, 25 and 100 mg TNT/kg respectively to 8 dogs (2 in each dosage category) for 4 weeks. The 6 dogs receiving the larger doses (15, 25 and 100 mg/kg) developed ataxia and incoordination, diarrhea and a dark-colored urine after the second dose of TNT. The two animals that received 5 mg/kg daily manifested the same signs after the third daily administration. During the first 7-10 days all of the animals appeared extremely sick, with ataxia, asynergia and incoordination, and developed irregular nystagmoid eye movements and diarrhea, as well as cyanosis of the mucous membranes. By the end of the second week, however, all animals showed marked improvement. A slight ataxia persisted, however, while the mucous membrane pallor increased to become ashen pale or "lilac" colored. Neurological studies of the dogs revealed slight body tremors, slight to moderate weakness, moderate ataxia, asynergia, incoordination, poor righting reflexes and occasional nystagmoid eye movements in all of the animals. The entire group of animals also showed chronic inflammatory changes in the mucosa of the small intestine. Half of the number of dogs developed involuntary urination. Both dogs that had received 100 mg/kg daily manifested marked weakness and paresis of the hind quarters.

In another series of tests, von Oettingen *et al.* (1944)⁵ fed female dogs 50 mg TNT/kg with their food daily for 12 weeks. The animals developed diarrhea, incoordination, nystagmus, and other symptoms referable to the central nervous system. The blood changes were similar to those described by Voegtlin *et al.* (1921-1922)⁶; a temporary decrease in red blood cells and hemoglobin was paralleled by an increase in reticulocytes during the first 3 weeks, indicating that the reduction of erythrocytes was due to a peripheral destruction rather than to injury of the blood-forming organs. Upon autopsy after 12 weeks, the TNT-fed dogs showed inflammatory changes of the intestines; the spleens were dark in color, and in most animals the bone marrow was hyperplastic. Hemosiderosis was found in the liver, bone marrow and lungs, usually in the phagocytes. Some of the animals died during the course of the experiment.

In long-term experimental studies by Kleiner (1969)¹⁰, dogs were exposed to 0.1 mg/kg and 5-20 mg/kg of TNT administered subcutaneously in 1 or 5% oil solution every other day for 2.5 yrs. Kleiner found elevated levels of ammonia, phosphate, and lactic acid in the gastric juices, which may be considered indications of early gastric secretory disorder. The presence of ammonia may have been due to inhibition of ammonia enzyme systems, while increased lactic acid content may have arisen in response to disruption of redox processes, with a compensatory increase in glycolysis.

APPENDIX IV (Cont)

In a parallel investigation of six dogs exposed to levels of 0.1-1 mg/kg and 5-20 mg/kg for two years, Kleiner (1972)¹¹ found predominantly functional secretory disorders and slight morphological alterations in the gastric mucosa after exfoliative gastritis.

Kleiner (1971)¹² exposed three dogs for periods up to 1 1/2 years to small doses of TNT (0.1-1 mg/kg and 5-20 mg/kg) and found that such doses caused changes in the volume of secretion and in the activity of pancreatic enzymes, as well as mild morphological changes in the acinous apparatus (lobules) of the pancreas.

Lillie (1943)¹³ found that cats given a dose of 50 mg TNT/kg body weight daily, subcutaneously, died within 4-9 days. Their livers showed fatty degeneration with slight to moderate centrilobular fine fat droplet deposit in the liver cells, atrophy of the cell cords, and slight to marked Kupffer cell hemosiderosis. The spleens showed slight to marked myelosis and slight to moderate pulp hemosiderosis. Lillie also found that cats given a dose of 20 mg TNT/kg of body weight daily by subcutaneous injection for 10-30 days showed fairly marked hemosiderosis of the spleen pulp reticuloendothelium and of the hepatic Kupffer cells. Cats given similar doses of TNT daily in their food or by stomach intubation for 21-30 days, and guinea pigs given TNT orally, 200 mg/kg for 16 days then 400 mg/kg for a further 43 days, showed similar manifestations.

Lillie (1943)¹³ observed that rabbits given subcutaneous injections of 200 mg TNT/kg body weight every second day survived for 17-57 days. Their livers showed slight to moderate centrilobular fatty degeneration. Their spleens regularly showed quite noticeable pulp hemosiderosis. Vitamin C in their diet made little difference in the foregoing symptoms.

Pidensky et al. (1968)¹⁴ observed that rats given TNT orally at 30 mg/kg daily for 6 days exhibited progressively decreased phagocytosis. Similar rats, when administered 30 mg/kg TNT simultaneously with 1 mg/kg niacin subcutaneously, showed no decrease in phagocytosis and even increased the phagocytic activity of the leucocytes to 1.5 times the level of control rats.

The most noteworthy point in the above discussion is the striking difference in individual susceptibilities, as well as the generally greater sensitivity of cats, dogs and humans, as compared to rabbits and rats. The effects of chronic TNT intoxication of animals are summarized in Table 2.

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Chronic Toxicity in Man. Among the first signs of TNT intoxication in man are hematologic changes, which are prominent effects of both chronic and acute exposures to TNT. Reduction of the red blood cell count and of the hemoglobin content may be associated with chemical and physical changes in the blood: polychromasia (variation in the hemoglobin content of the erythrocytes), poikilocytosis (presence of irregular erythrocytes of abnormal size and shape), and anisocytosis (the presence of nucleated red blood cells), reticulocytosis and eosinophilia (Voegtlin *et al.*, 1921-1922⁶). Changes in the white blood cell picture due to TNT exposure may consist of leukocytosis or relative lymphocytosis.

Stewart *et al.* (1945)¹⁵ in studies on student volunteers exposed in a munitions plant to an air concentration of 0.3 mg TNT/m³ for 8 hrs/day, 5-6 days/wk for an average of 33 days, found that the hemoglobin content and circulating red blood cell count was reduced in 85 percent of the subjects. The hemolytic effects were more pronounced in the male subjects. Most of these volunteer subjects also had a slight rash on the hands, while a few had a somewhat more severe rash.

The most serious systemic sequelae to acute TNT poisoning in man are toxic jaundice and toxic hepatitis, which may end in acute yellow atrophy of the liver (Evans, 1941¹⁶; von Ottingen *et al.*, 1944⁵; McConnell and Flinn, 1946¹⁷), and hyperplasia of the bone marrow leading to aplastic anemia (Evans, 1941¹⁶; Crawford, 1954¹⁸; Sievers, Stump and Monaco, 1946¹⁹; Hayhoe, 1953²⁰; and McConnell and Flinn, 1946¹⁷).

In a study of twenty-two fatalities due to TNT poisoning during World War II, McConnell and Flinn (1946)¹⁷ listed eight persons who died of toxic hepatitis, thirteen who died of aplastic anemia and one who died from a combination of the two conditions. They found that toxic hepatitis occurred more frequently in the younger age group (average age = 30 yrs), and aplastic anemia among the older age group (average age = 45 yrs). One-third of the total who died were exposed to average concentrations in excess of the OSHA maximum allowable contact concentration of 1.5 mg of TNT/m³. The amount of TNT ingested or absorbed through the skin, which may have been considerable, was not known. In addition, McConnell and Flinn indicated that aside from the amount of TNT entering the body, individual differences, undernourishment or nutritional disturbances, excesses (of alcohol), acute illness, and other factors may have rendered the individuals more susceptible to the toxic action of TNT.

The most important laboratory findings in toxic jaundice were elevation of the icterus index, which rose rapidly, indicating early liver damage (McConnell and Flinn, 1946¹⁷). Palmer *et al.* (1946)²¹ found that

APPENDIX IV (Cont)

in workers with hepatic necrosis, the severity of the hepatic injury was evident from the intensity and duration of the jaundice and was indicated further by the change in percentage of cholesterol esters and prothrombin, and in the excretion of hippuric acid. Hamblin (1963)²² considers the prothrombin level to be a more sensitive index of toxic jaundice and necrosis (atrophy) of the liver than the icterus level. Sievers *et al.* (1946)¹⁹, however, found no single test to be reliable for early diagnosis of severe TNT intoxication.

In the anemia cases, a rapid reduction of the number of red blood cells and of the hemoglobin are perhaps the most conspicuous signs of significant bone marrow damage. It is not unusual to find a depression of all cellular elements, and a marked reduction in the platelets is a grave sign. A hypoplastic or aplastic appearance of the bone marrow is a common finding. The marked tendency to, or presence of petechiae (small colored spots on the skin) is often associated with aplastic anemia. The course of the disease commonly progresses more or less rapidly to fatal termination. In these cases, the elapsed time from the onset of definite symptoms until death ranges from 6 to 185 days with a median figure of 40 days (Sollmann, 1957²³; von Oettingen, 1958²⁴).

Von Oettingen *et al.* (1944)⁵ studied two subjects who ingested 1 mg TNT/kg body weight daily for 4 days. Only a slight increase was found in the turbidity of the hemolyzed blood samples measured spectrophotometrically. No other physical or chemical changes in the blood were observed. The significance of the blood turbidity, if any, has not been established. At no time did the methemoglobin content exceed 1 percent of the total hemoglobin, nor were any Heinz bodies observed in the erythrocytes. No subjective symptoms were experienced by either of the subjects. The daily excretion of TNT metabolites (4-amino-2,6-dinitrotoluene and 2,4-diamino-6-nitrotoluene) in the urine amounted to about 3 percent of the ingested TNT.

In another short-term study on humans where the concentrations were known, von Oettingen *et al.* (1944)⁵ observed two human volunteers who each rubbed 500 mg of fine TNT powder into the palms of their hands to test the degree of absorption of TNT by the skin (= 7.1 mg/kg assuming 70 kg subjects). They covered their hands with rubber gloves and the TNT was kept on the skin for 8 hours. The only effect noted was a moderately bitter taste during the course of the experiment. Only traces of the TNT metabolite, 4-amino-2,6-dinitrotoluene, were found in the urine during the first 23 hours.

Many studies have been made of humans suffering occupational exposure to TNT. Cone (1944)²⁵ and Brill *et al.* (1944)²⁶ reported that workers in

APPENDIX IV (Cont)

a munitions plant inhaling an average of 2.0 mg/m^3 TNT daily in 8-hr daily work shifts over a two-year period showed transitory initial leukocytosis accompanied by moderate eosinophilia, which did not appear to affect the well-being of the workers. No other changes were seen in the formed elements of the blood by these investigators.

Soboleva (1969)²⁷, in a chronic exposure study of TNT workers, found occupational cataract formation, pathological changes in the peripheral blood, neurasthenia and polyneuritis, as well as a statistical relationship between the functional state of the myocardium and the degree of intoxication.

Hassman and Hassmanova (1968)²⁸ found that changes in serum enzymes and prothrombin complex factors were useful as possible early diagnostic tests of toxic liver damage. In another study of TNT intoxication, Hassman (1971)²⁹ found that the first symptoms of intoxication are toxic gastritis, reduced secretion of trypsin in the pancreas and increased renal glomerular filtration. Kleiner (1966)³⁰ also found that the secretion of the pancreatic enzymes - trypsin, amylase and lipase - were reduced in volume.

Vychub (1970)³¹ examined 82 TNT workers exposed for periods ranging from 3-32 years. Permeability and resistance of capillaries showed significant change even when intoxication was mild. These parameters were suggested as auxiliary means for early detection of intoxication.

Distinctive features of chronic TNT poisoning were studied by Zakharova and Manoilova (1971)³², who examined 360 workers exposed occupationally to TNT for 5 or more years; they found eye lesions that developed into cataracts in 45.3% of the cases, chronic gastritis with subnormal acidity, and mild forms of hepatitis. Hassman and Juran (1968³³, 1971³⁴) also noted the occurrence of cataracts in workers who had been exposed to TNT for four years or longer. Ermakov et al. (1969)³⁵ observed the long-term effects on 574 persons of TNT in doses far exceeding the Russian maximum permissible inhalation level of 0.001 mg/l , and found symptoms of chronic poisoning in 122 of them; these included lesions in the central nervous and vascular systems. Anatskovskaya and Liman (1972)³⁶ used EEG and other devices on 140 patients with chronic TNT intoxication to detect the frequency and character of neurological symptoms. They described three successive stages in the development of central nervous system intoxication. Some effects of chronic TNT intoxication of man are summarized in Table 3, and the clinical symptoms are listed in Table 4.

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In the absence of any water quality standard for TNT, an Ad Hoc Committee of the National Academy of Sciences has recommended a TNT Emergency Drinking Water Limit (EDWL) of 0.75 mg/kg for three days duration, based on a formula for converting air inhalation data to estimates for ingestion via water (Appendix D).

Modes of Entry. Most clinical cases of TNT poisoning have resulted from: (a) percutaneous absorption (Voegtlin *et al.*, 1921-1922⁶, Schwartz, 1944³⁷, Haythorn, 1920-1921³⁸; McCausland and Hawkins, 1944³⁹; Sollmann, 1957²³, Goodwin, 1972⁴⁰); or (b) ingestion (von Oettingen *et al.*, 1944⁵, Haythorn, 1920-1921³⁸; Voegtlin *et al.*, 1921-1922⁶, Sollmann, 1957²³; McCausland and Hawkins, 1944³⁹). Great variations among individuals exist in regard to the permeability of the skin to TNT (Moore, 1917⁴¹). Oily skin (Barnes, 1916-1917⁴²) and excessive sweating (Livingstone-Learmonth and Cunningham, 1916⁴³) enhance the rate of absorption. Skin absorption takes place most readily through the palms of the hands and the ankle region (Voegtlin *et al.*, 1921-1922⁶).

The low solubility of TNT in water (130 mg/liter at 20°C) limits the danger of ingesting dissolved TNT. Another, although less important mode of entry is by the respiratory route (Voegtlin *et al.*, 1921-1922⁶, von Oettingen *et al.*, 1944⁵, McCausland and Hawkins, 1944³⁹, Cone, 1944²⁵; Brill *et al.*, 1944²⁶; Stewart *et al.*, 1945¹⁵; and Crawford, 1954¹⁸).

MAMMALIAN TOXICOLOGY OF DNT

Toxicity to Man. McGee *et al.* (1942)⁴⁴ observed 154 men exposed to DNT (a mixture in which the 2,4-isomer predominated). A total of 42 workers reported not feeling well; none required hospitalization. The most common symptom was an unpleasant metallic or slightly bitter taste. This was closely followed in frequency by sensations of muscular weakness, headache, loss of appetite, giddiness, dizziness or drunkenness. Other symptoms were nausea and vomiting, difficulty in sleeping, pain, numbness and tingling in the extremities. The chief clinical findings with DNT-intoxicated workers were pallor and cyanosis, found in one-third of the group studied, and anemia, present in nearly one quarter of these workers. Both pallor and cyanosis may be present in workmen particularly sensitive to DNT without significant changes in the blood counts. An occasional workman was so sensitive to DNT exposure that acute symptoms of illness together with cyanosis appeared after but a few hours of exposure. Sixty percent (23 out of 36) of the anemia cases were normocytic-normochromic,

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or slightly hyperchromic. No instances of permanent physical impairment were found in this study.

In a follow-on study over a 3-year period in the same plants on 714 workers after improved ventilation, personal protective devices, and better personal hygiene, McGee *et al.* (1947)⁴⁵ reported a reduction in the number of complaints and clinical signs.

Toxicity to Animals. The early literature on DNT toxicity to animals has been reviewed by von Oettingen (1941)⁴⁶. Data for cats are contradictory. One report⁴⁶ states that cats may tolerate without effect repeated oral doses of 20 to 40 mg of DNT in cod liver oil to a total of 240 mg; another states that the oral MLD for cats is 27 mg/kg (White, 1917⁴⁷). Subcutaneous administration of a single dose of 50 to 500 mg of DNT in oil is reported to be fatal in 2-23 days⁴⁶. Cutaneous administration of 300 mg/kg was without effect in one study while two 5 g doses were fatal to a cat in 8 hours in another study⁴⁶. DNT appears to be less toxic than dinitrobenzene.

Clayton and Baumann (1944)⁴⁸ found that mice fed various diets with 0.3 percent DNT for 2-3 week periods are more resistant to DNT in a diet of 5 or 30 percent fat than in a low fat diet (0.46%). This was evident both by the better growth and better survival of the animals. In later experiments (1948)⁴⁹, they showed that the growth and survival of mice fed *ad lib* and receiving DNT varied with the percent of fat in the diet. The effect of DNT on restricted caloric diets was more complicated. However, it was apparent that most natural fats confer increased tolerance of DNT. Shills and Goldwater (1953)⁵⁰ found that a high fat intake was beneficial to the survival of rats receiving DNT in a low-protein diet. There was no effect of fat intake on the resistance to DNT of rats on a high-protein diet, and no effect on the growth rate, irrespective of diet. Himsworth and Glynn (1942)⁵¹ had earlier reported an adverse effect of high-fat diets on rats receiving TNT.

Galuzova (1963)⁵² found that rabbits which ingested 0.5 mg and 0.05 mg/kg body weight daily for 8 months gained weight at the normal rate when compared to control animals. No recognizable pattern of changes between the experimental and control animals could be observed. However, a microscopic study of the organs of the exposed rabbits disclosed well-defined protein and fatty dystrophy of the liver cells.

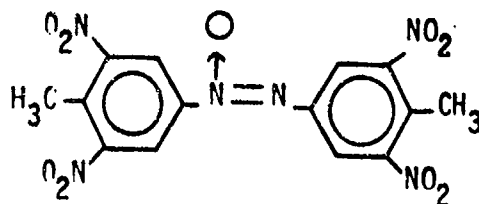
No tumors were found in 15 mice that were treated on the skin with 0.3% (in acetone) DNT three times weekly for 2 months and then 2% (in acetone) daily for 2 months according to Clayton and Baumann (1944)⁴⁸.

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MAMMALIAN METABOLISM AND BIOCHEMISTRY OF TNT

When the dose is small, TNT is rapidly detoxified in the liver, the metabolic products are excreted mainly by the kidneys, and no harm is done to the organism (Crawford, 1954¹⁸). However, when the dose is larger, or for some reason the detoxifying mechanism in the body is unable to cope completely with the invasion, then TNT or more probably the toxic products of its metabolism (Channon *et al.*, 1944⁵³) accumulate and circulate in the blood stream, inflicting damage upon susceptible tissue (Crawford, 1954¹⁸). There is some indication that the toxicity of TNT is due to its metabolic products, all of which have probably not been identified (Bueding and Jolliffe, 1946⁵⁴, Channon *et al.*, 1944⁵³, and Wyon, 1921⁹). Studies by Moore, 1917⁴¹; Dale, 1921⁵⁵; and Voegtlin *et al.*, 1921-1922⁶, showed that urine of TNT workers and of experimental animals receiving TNT orally or subcutaneously contained both tetranitroazoxytoluene and an aminodinitrotoluene as metabolites.

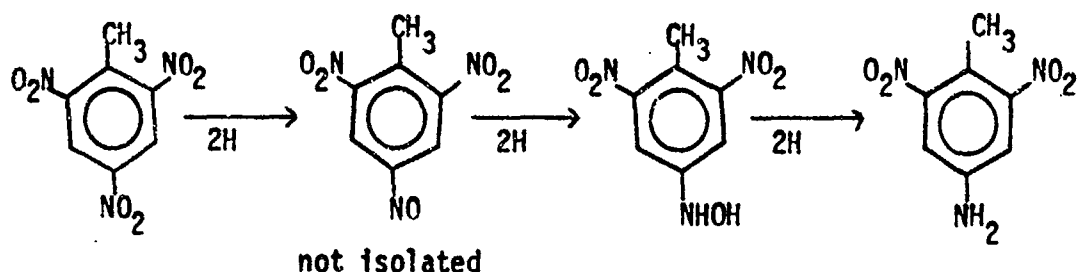
From the urine of rabbits receiving TNT intragastrically, Channon *et al.* (1944)⁵³ isolated 4-amino-2,6-dinitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene and 6-amino-2,4-dinitrotoluene. Because the hydroxylamine is converted on standing to 2,2',6,6'-tetranitro-4,4'-azoxytoluene, which was not isolated from the urine, they concluded that the azoxytoluene reported by earlier workers was an artifact.



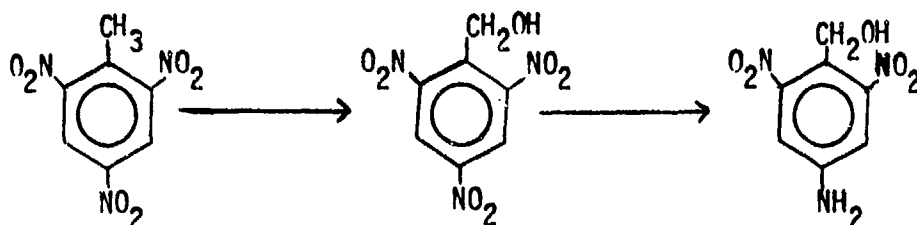
2,2',6,6'-Tetranitro-4,4'-azoxytoluene

Lemberg and Callaghan (1944)⁵⁶ similarly isolated 4-amino-2,6-dinitrotoluene and 6-amino-2,4-dinitrotoluene from human urine, but only the 4-amino isomer from rat urine which, however, also contained 2,4-diamino-6-nitrotoluene. Bueding and Jolliffe⁵⁴ have achieved nearly quantitative reduction of TNT to 4-hydroxylamino-2,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene *in vitro* by treatment with xanthine-xanthine oxidase in the first case and pig liver extract in the second. The following reduction mechanism is indicated.

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TNT also causes an increased output of a glucuronide, equivalent to nearly half the dose. The nature of the glucuronide formed is uncertain, but the evidence points to the formation of either trinitrobenzyl alcohol or aminodinitrobenzyl alcohol, which could form glucuronides in the body. This is supported by the finding that TNT causes the excretion of a red pigment by rabbits and rats, and, of a number of related nitro compounds tested, only 2,4,6-trinitrobenzyl alcohol will produce a similar pigment (Channon *et al.*, 1944⁵³). The following oxidation-reduction pathway is suggested:



The formation of trinitrobenzyl alcohol is supported by the finding of Lemberg and Callaghan (1944)⁵⁶ that rats excrete 5-nitro-*m*-phenylenediamine after receiving TNT. The formation of this compound most likely involves oxidation of trinitrobenzyl alcohol to trinitrobenzoic acid, followed by decarboxylation to trinitrobenzene and reduction to the nitrophenylenediamine.

TNT is metabolized by bacteria as well as by mammals. Several studies have been conducted on TNT degradation (Table 5), but only two contained data on the identity of the products. Won, Heckley and Hofsommer (1973)⁵⁷ stated that 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene, nitrite

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and nitrate accounted for only a very small percentage of the initial TNT nitrogen. It was found by Fowler (1965)⁵⁸ that 2,4-diamino-6-nitrotoluene, 4-amino-2,6-dinitrotoluene, and 2,2',6,6'-tetrinitro-4,4'-azoxytoluene are present in *Pseudomonas* culture filtrates. Hydrolyzates of cells incubated with TNT labeled with ¹⁴C at the 1-ring position yielded succinic acid, glycine, aspartic acid, cystine, glutamic acid, other amino acids undistinguishable from each other on the basis of R_f and CO₂. The author postulated a scheme for the complete dissimilation of TNT.

POTENTIAL FOR CARCINOGENICITY, TERATOGENICITY, MUTAGENICITY AND SENSITIZATION IN MAN

In the TNT chronic studies of Himsworth and Glynn, 1942⁵¹ (rats fed 0.15g/kg TNT daily for 120 days), Velling, 1943⁵⁹ (guinea pigs, SC TNT 1 mg/kg daily for 9-12 months) and von Oettingen *et al.*, 1944⁵ (dogs, 25-50 mg/kg TNT by insufflation 5 days/wk for 17 weeks, 50 mg/kg oral daily for 12 weeks) no evidence was found for tumor formation in the experimental animals (see Hartwell, 1951⁶⁰). Despite long-term observation of human beings, including female workers (see above), there have been no indications in the literature of the carcinogenicity, mutagenicity or teratogenicity of TNT. Perhaps the potential mutagenic or teratogenic danger from exposure to unusual chemicals was not recognized during the period of intense interest in TNT in the Western world. Nevertheless, the discovery of such efforts might have been expected in the more recent Eastern European literature; no such discoveries appear to have been recorded.

Trinitrotoluene is structurally related to several compounds which have been used as haptens to study immediate and delayed hypersensitivity and other aspects of experimental immunology. Since some of the symptoms of toxicity of TNT in man resemble hypersensitivity reactions, and since these reactions may be induced by trace quantities of haptens, a few appropriate references seemed worthwhile:

Brody and Greenberg (1972)⁶¹ induced delayed hypersensitivity in humans by applying 2 mg dinitrochlorobenzene to the forearms of 14 patients, all of whom showed reactions to 0.1 mg 14 days later. They state that the response to this compound is predictable and that it has been used clinically to define cellular immunity. Cohen *et al.* (1972)⁶² used dinitrochlorobenzene to study delayed cutaneous hypersensitivity in guinea pigs in the presence of a growing, transplanted, methylchloanthrene-induced sarcoma. Liacopoulos *et al.* (1969)⁶³

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demonstrated delayed and immediate hypersensitivity while studying genetic heterogeneity of the immune response in guinea pigs immunized with dinitrophenyl poly-l-lysine.

TOXICITY OF TNT TO AQUATIC ORGANISMS

Fish. An excellent starting point for evaluating the literature on TNT toxicity to fish is a recent paper by Pedersen (1971)⁶⁴, who used 10 bluegills (ave. wt. 0.39g) in 10 liter test containers in two 96 hour experiments. In the first, or "preliminary" bioassay, he employed initial TNT concentrations of 5.6, 3.2, 0.8 and 0.56 ppm. In the final bioassay 10, 7.5, 4.9, 3.7, 2.8, 2.4, 2.1 and 1.8 ppm TNT were used, and the test solutions were renewed daily. Assays showed that TNT levels fell to less than 10% of the original in uncovered containers in which test solutions were not renewed. During the preliminary bioassay, TNT was determined by GLC, and in the final bioassay by spectrophotometry. TNT concentrations were prepared in deionized water reconstituted to 60 or 180 ppm hardness as CaCO_3 . Fish were not fed during or for two days prior to the bioassays.

The TLM for bluegills was 2.3 at 180 ppm hardness and 10°, 2.3 at 90 ppm and 10°, 2.8 at 180 ppm and 25°, and 2.7 at 90 ppm and 10°. Statistical analysis of the data showed that hardness did not affect toxicity but that temperature did. The mortality curve was very steep in the concentration range tested; this may have prompted the recommendation that an interim standard of 0.25 ppm α -TNT be adopted.

A thesis by Gring (1971)⁶⁵ was read only in abstract. Twenty-four- and 96-hour TLM's of approximately 2.6 ppm and 2.0 ppm TNT were found for bluegills.

The following data for the toxicity of TNT and related compounds to 5-8 cm "minnows" at 21-23°C are cited from Leclerc (1960)⁶⁶:

<u>6 Hour Minimum Lethal Dose</u>		
<u>Compound</u>	<u>Hardness, 12.5 ppm CaCO_3</u>	<u>Hardness, 150 ppm CaCO_3</u>
Trinitrotoluene	4.0-5.0	4.0-5.0
m-Dinitrophenol	0.5-1.0	35-38
Dinitro-o-cresol	1.5-2.0	3.0-4.0

APPENDIX IV (Cont)

<u>Compound</u>	<u>Hardness, 12.5 ppm CaCO₃</u>	<u>Hardness, 150 ppm CaCO₃</u>
<i>o</i> -Mononitrotoluene	18-20	35-40
<i>m</i> -Mononitrotoluene	14-18	25-30
<i>p</i> -Mononitrotoluene	20-22	45-50

The data indicate that the nitro-compounds tested (other than TNT) were less toxic at a hardness equivalent of 150 ppm CaCO₃ than at levels less than 12.5 ppm as CaCO₃, and that TNT was more toxic than mononitrotoluenes but less so than *m*-dinitrophenol or dinitro-*o*-cresol.

However, the data are weakened by the lack of statistical evaluations of significance. The experiments were carried out in "diffused light", which was not defined further, and no assays were reported for test substances; thus, no idea of changes of concentration could be obtained. Apparently, 10 fish were used per concentration of test compound, but the actual concentrations used were not given. Finally, no statement was made on the addition of food before and during the experimental period.

Mckee and Wolfe (1963)⁶⁷ list the lethal concentration of TNT for "fish" as 1.5-2.0 ppm. According to Mathews *et al.* (1954)⁶⁸, 2 ppm TNT was the highest concentration not producing symptoms in 4.5-6 cm goldfish. The experimental plan and results with four fish per TNT concentration were as follows:

<u>TNT, ppm</u>	<u>1 day</u>	<u>% Mortality</u>			
		<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
		<u>As TNT</u>			
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	25	25	25
4	0	100	--	--	--
5	50	75	100	--	--
6	75	100	--	--	--

APPENDIX IV (Cont)

TNT, ppm	1 day	2	3	4	5
	as Colored TNT Complex				
0	0	0	0	0	0
2	0	0	0	25	25
3	0	0	0	0	0
4	0	0	25	25	50

The investigators suggest that the 25% mortality at 2 ppm TNT complex was not significant, and that the data show a lesser toxicity of the complex than of TNT itself. (Actually, 25% mortality was one fish out of four, a situation which prejudices the data). Mathews and his colleagues noted symptoms in fish after five minutes of exposure to 3 ppm or more TNT but not until after 8 hours for 2 ppm or less. The data from this paper are difficult to interpret in terms of the toxicity of TNT itself for two reasons: (1) no TNT assays were performed, and (2) little information was included on conditions of the experiment, i.e., illumination, which might influence the persistence of unaltered TNT.

Mohlman (1945)⁶⁹ states that 1/400 dilutions of combined TNT wastes were lethal in a program involving "hundreds of fish". Ruchhoft *et al.* (1945)⁷⁰ mention briefly that "5 mg/l TNT kills fish". Toxicity data for fish are included in Table 6.

Algae. A single reference constituted the extent of the literature found on the toxicity of TNT to blue green algae. Fitzgerald (1952)⁷¹ states that 8 ppm TNT killed 100% of *Microcystis-aeruginosa* cells in suspensions containing 10^6 to 2×10^6 cells/ml growing in a mineral salts solution under continuous fluorescent illumination. Five ppm m-dinitrobenzene killed 50% of the cells, while 2,4-dinitrophenol was without effect at 20 ppm. The solutions were not assayed for TNT, the time to kill was not given, and the means of determining death not defined.

According to Gring (1971)⁶⁵ 3 ppm TNT is quite toxic to the unicellular green alga *Chlamydomonas reinhardtii*. The methods and results sections of Gring's paper did not become available for critical review.

Bacteria. Ruchhoft *et al.* (1945)⁷⁰ stated that more than 1.17 ppm TNT retarded BOD reduction in mineralized water containing 1% sewage, and that 30 to 40 ppm reduced the rate of sewage purification by activated

APPENDIX IV (Cont)

sludge. Schott *et al.* (1943)⁷² claimed that 5% TNT waste reduced the efficiency of the activated sludge process. Mathew *et al.* (1954)⁶⁸ stated that 2 ppm of α -TNT retarded the utilization of oxygen by sewage organisms. According to Rogovskaya (1951)⁷³, 0.5-1.0 ppm TNT retarded the self purification of streams.

Yet, Enzinger (1971)⁷⁴ was able to adapt cells of *Zoogloea ramigera*, an important activated sludge bacterium, to utilize 100 ppm TNT, although this concentration retarded growth of non-adapted cultures. He and other authors (Won *et al.*, 1973⁵⁷; Gring, 1971⁶⁵) obtained pseudomonads from sewage treatment waters, or from feces of rats fed TNT, which could degrade 100 ppm TNT efficiently. See Table 6.

Only one paper was found dealing with the toxicity of TNT to organisms not studied in connection with degradation processes. This publication (Usov *et al.*, 1967⁷⁵) in an obscure Russian journal was read in abstract. The authors, who were mainly concerned with TNT as a potential antiseptic, claimed that 250-1000 ppm applied in ethyl cellosolve was bacteriostatic and 125,000 ppm bactericidal (solubility in water 200 ppm approximately) to *Staphylococci*, *Proteus*, yeast, *Pseudomonas aeruginosa*, coliforms, and bacilli.

TOXICITY OF DNT TO AQUATIC ORGANISMS

For an unspecified isomer or mixture of isomers of DNT, Burton (1971)⁷⁶ reports 24, 48 and 96 hour TLM values of 50, 27 and 16 mg/l, resp., for bluegills maintained at 20°C. On the basis of Warburg studies, Randall and King (1971)⁷⁷ concluded that DNT is relatively nontoxic to microorganisms, but is only slowly biodegraded. Bringmann and Kuehn (1971)⁷⁸, on the other hand, found that 2,4- and 2,6-DNT (as well as other nitro-bodies, including α -TNT) were completely removed at the 100-150 mg/l level by a 2-stage activated sludge treatment unit using *Azotobacter agilis* in the first stage.

OCCURRENCE OF DNT IN TNT WASTEWATERS

A portion of the TNT waste stream is made up of water from the Mahon fog filter system used to control air pollution. The Mahon water contains ca 270 mg/l of toluene extractable material, of which the major

APPENDIX IV (Cont)

component is reported to be 2,4-DNT, as shown below (Siele and Ribaudo, 1971⁷⁹).

2,4-DNT	72.7%
2,5-DNT	0.6
2,6-DNT	5.0
3,4-DNT	1.2
2,4,6-TNT	20.5
2,4,5-TNT	trace

ANALYTICAL METHODOLOGY FOR TNT METABOLITES

Webster Test. The Webster test (1921)⁸⁰ is based on the production of a purple color in urine samples when the acidified urine is extracted with ether and the ether extract is treated with an alcoholic solution of KOH. It has been the most widely used method in the past for determining the presence of TNT in urine and hence of TNT intoxication. This procedure, while commonly used, is only a qualitative indication of exposure to TNT and is considered to be poor proof of incipient or manifest TNT poisoning (Snyder and von Oettingen, 1943⁸¹; Pinto and Wilson, 1943⁸²). The colors formed in the Webster test are unstable and may lead to erroneous results. As shown by Voegtlin *et al.* (1921-1922)⁶, Putnam and Herman (1919)⁸³, and Silver (1938)⁸⁴, negative results may be obtained in severe cases of liver injury resulting from TNT poisoning. The Webster test is thought to be characteristic for the presence of 4-hydroxylamino-2,6-dinitrotoluene.

More definitive and quantitative analysis of urine samples for the presence of TNT metabolites, 4-hydroxylamino-2,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene, is obtained through the spectrophotometric method of Snyder and von Oettingen (1943)⁸¹ or the photoelectric colorimetric method of Pinto and Wilson (1943)⁸².

Snyder and von Oettingen Method for Determination of 4-Amino-2,6-dinitrotoluene. Snyder and von Oettingen (1943)⁸¹ have described in detail the preparation and treatment of urine samples and the preparation of color standards for determination of the TNT metabolite, 4-amino-2,6-dinitrotoluene. Amines present in the urine are diazotized and coupled with α -naphthylamine; then the color intensity of a toluene extract is compared

APPENDIX IV (Cont)

spectrophotometrically with standard methyl orange solutions. The color thus developed is much more stable than that of the Webster test.

Pinto and Wilson Test for Determination of TNT Derivatives in Human Urine.
Pinto and Wilson (1943)⁸² described the quantitative determination of TNT derivatives or metabolites that may be present in the urine of persons exposed to TNT. The TNT derivatives are extracted from urine with ethyl ether, reduced in aqueous solution to triaminotoluene by titanium chloride ($TiCl_3$), diazotized, and coupled with dimethyl- α -naphthylamine.

The color developed is measured spectrophotometrically and compared with samples derived from known concentrations of TNT. This method is quantitative and can be used for estimating the TNT at the micrograms per liter level.

RECOMMENDATIONS FOR FUTURE LABORATORY AND FIELD STUDIES

1. Analysis studies of "pink water" for the identification of all its components. TNT isomers and all phenolic conversion products should be identified using the most sensitive analytical methods that are available.
2. The LD50 for TNT (α -TNT) and the 2,4- and 2,6-isomers of DNT should be determined for two mammalian species.
3. A 90-day chronic feeding study of TNT and the 2,4- and 2,6-isomers of DNT should be carried out in several mammalian species with special attention being paid to the hematopoietic system, methemoglobin formation in particular.
4. A study of the metabolic fate of TNT, 2,4- and 2,6-isomers of DNT in experimental animals should be undertaken.
5. Laboratory studies of the degradation of TNT by bacterial species, preferably using ^{14}C -labelled TNT, should be undertaken.
6. The 96 hr TLM values using fish should be determined for TNT and the 2,4- and 2,6-isomers of DNT.
7. The behavior of TNT, DNT and breakdown products in receiving waters should be determined. This would include channeling or layering effects, build-up in idealized detention situations such as hypolimnia, and precipitation either with or without interaction with colloids.

APPENDIX IV (Cont)

8. The rate of mineralization of TNT, DNT and breakdown products under simulated natural conditions should be investigated. The use of ¹⁴C-labelled compounds would be recommended for this work.
9. Methods for assessing the toxicity of TNT, DNT and breakdown products to aquatic organisms, other than fish, should be developed.
10. In the event that this literature search is to be used as a basis for industrial health standards, it would be highly desirable to extend it to include a translation and subsequent evaluation of the many Russian publications that have only been seen in abstract.

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TABLE 1. Constituents of Pink Water (Other Than Those Derived from α -TNT) from Various Sources

SOURCE	CONSTITUENTS ^a
Manufacture	
Nitrator fume scrubbers	DNT's, (MNT's), (all TNT isomers), (dinitro-m-cresols ^b)
Red water concentrator distillate	DNT's
Mahon fog filter effluent ^c	DNT's, (MNT's), (all TNT isomers), (dinitro-m-cresols ^b)
Finishing building air scrubbers and washdown ^d	(DNT's)
RDX/TNT incorporation ^e	RDX, HMX, and products associated with their manufacture
Load, assemble and pack plants	RDX, HMX

a. Parentheses mean constituents are believed present but not positively identified

b. Nitrocresols could arise by displacement of a nitro group on any of the isomers of α -TNT

c. Volunteer AAP only

d. α -TNT from this source is rather pure

e. Holston AAP

TABLE 2. Summary of Chronic Effects of TNT on Mammalian Systems

Animal Species	Dose (mg/kg)	Route of Administration	Exposure Period	Effects	References
Dog	0.1-1 & 5-20	or	Daily, 1 1/2 yrs	Changes in volume of secretion and activity of pancreatic enzymes. Mild morphological changes in the acinous apparatus (lobules of the pancreas).	Kleiner, 1971 ¹²
Dog	0.1-1 & 5-20	or	1 or 5% oil soln every sec-ond day up to 2 1/2 yrs	Increased NH_3 , PO_4 , and lactic acid levels in the gastric juice (may be due to inhibition of enzyme systems).	Kleiner, 1969 ¹⁰
Dog	5-33	or, sc inhalation	Daily 6 day/wk, up to 150 days	Incoordination, cyanosis, anemia; reduction in total blood, red corpuscle, hemoglobin and pigment of blood; increase in reticulated blood cells; jaundice and liver degeneration in some animals.	Voegtlin et al., 1919 ⁷ , 1921-1922 ⁶
Dog	5-100	or, sc	Daily for 1 week	Incoordination, cyanosis, anemia, vomiting, salivation; some jaundice.	Voegtlin et al., 1921-1922 ⁶

TABLE 2. (continued)

Animal Species	Dose (mg/kg)	Route of Administration	Exposure Period	Effects	References
Dog	5-100	or	Daily, 6 day/wk, 4 wks	Ataxia, incoordination, nystagmus, diarrhea, cyanosis, dark urine, inflammation of small intestine mucosa.	Von Nettingen <i>et al.</i> , 1944 ⁵
Dog	50	or	Daily, 6 day/wk, 12 wks	As above; higher leucocyte and reticulocyte, lower blood cell and hemoglobin; hemosiderosis of spleen, liver, lungs and bone marrow.	Von Nettingen <i>et al.</i> , 1944 ⁵
Dog	25 & 50	inhalation	Daily, 6 day/wk, 17 wks	Salivation, vomiting, diarrhea, incoordination, weakness, mod. anemia, reduction in red blood cell and hemoglobin; no cyanosis.	Von Nettingen <i>et al.</i> , 1944 ⁵
Cat (two animals)	20-130	or, sc, (in oil)	Every 1-3 days, 6-15 weeks	Wasting, cyanosis, death.	Wyon, 1921 ⁹
Cat	20 mg-200 (approx)	sc	2-23 days	Death in 2-23 days.	Von Nettingen, 1941 ⁴⁶

TABLE 2. (continued)

Animal Species	Dose (mg/kg)	Route of Administration	Exposure Period	Effects	Reference
Cat	2000	ct	2 doses in 8 hours	Death of animal.	Von Nettingen, 1941 ⁴⁶
Cat	300	ct	?	No untoward effects.	Von Nettingen, 1941 ⁴⁶
Cat	20	sc	Daily, 10-30 days	Hemosiderosis of spleen pulp reticuloendothelium and hepatic kupffer cells.	Lillie, 1943 ¹³
Cat	20	or, then ig	Daily, 21-30 days	Similar to above.	Lillie, 1943 ¹³
Cat	50	sc	Daily, 4-9 days	Lethal to some; slight to moderate hemosiderosis of spleen and liver; moderate fatty degeneration of liver; centrilobular congestion.	Lillie, 1943 ¹³
Guinea pig	200 for 16 days then 400 for further 43 days	or	Daily, 59 days	Lethal to some in 30-40 days; effects as described for the cat.	Lillie, 1943 ¹³

TABLE 2. (continued)

Animal Species	Dose (mg/kg)	Route of Administration	Exposure Period	Effects	References
Rabbit	0.5 & 0.05	ig (in aqueous solution)	Daily, 8 months	Well-defined protein and fatty dystrophy of liver cells. No other changes noted in any other organs.	Galuzova, 1953 ⁵²
Rabbit (two animals)	ca 300	sc	Every 2 days, 18 days	Convulsions and death.	Wyon, 1921 ⁹
	ca 300	ct	13 doses in 25 days	No important changes.	
Rabbit	200	sc every other day.	17-57 days	Survived 17-57 days. Slight to moderate centrilobular fatty degeneration of the liver. The kidneys contained many hemoglobin casts in the collecting tubules. The spleen showed marked pulp hemosiderosis.	Lillie, 1943 ¹³

TABLE 2. (continued)

Animal Species	Dose (mg/kg)	Route of Administration	Exposure Period	Effects	References
Rabbit	30	ig	Daily, 60 days	Nerve-muscle reduced in irritability on 15th day, then raised irritability on 25 day. On 60th day, a reduction in phospholipids and a rise in diphenylamine reaction in brain.	Mul'menko et al., 1966 ⁸⁵
Rat	30	or	Daily, 6 days	Progressive decrease in phagocytosis (of leukocytes). Niacin administered simultaneously at 1 mg/kg prevented this decrease.	Pidemsky et al., 1968 ¹⁴

TABLE 3. Summary of Chronic Toxicity of TNT in Man

Dose or Exposure	Route of Administration	Exposure Period	Effect	References
1 mg/kg	or	Daily for 4 days	No subjective symptoms. Slight increase in turbidity of hemolyzed blood. No hemoglobin or other detectable changes in the blood. Daily excretion of metabolites (4-amino-2,6-dinitrotoluene and 2,4-diamino-6-nitrotoluene) in the urine amount to about 3%.	Von Oettingen, 1944 ⁵
		3-32 yrs	Change in permeability and resistance of capillaries exposed to mild intoxication.	Vychub, 1970 ³¹
3 mg/m ³	Inhalation	8 hrs/day, 6 day/wk, 33 day (ave.)	Slight rash on hands in most student volunteers, moderate rash (dermatitis) on a few. Hunger symptoms. Hemolytic effects: hemoglobin and circulating red blood cells reduced in 85% of subjects; hemolytic effects more pronounced in males.	Stewart <i>et al.</i> , 1945 ¹⁵
2 mg/m ³	Inhalation of dust	Daily, 8 hrs/day, 6 day/wk for 2 yrs	Transitory initial leukocytosis and moderate eosinophilia in blood cells. Did not appear to affect the general well-being of workers.	Cone, 1944 ²⁵ ; Brill <i>et al.</i> , 1944 ²⁶

TABLE 3. (continued)

Dose or Exposure	Route of Adminis- tration	Exposure Period	Effect	References
		Occupational	Reduced secretion of pancreatic enzymes.	Hassman <i>et al.</i> , 1968 ²⁸ ; 1971 ²⁹ ; Kleiner, 1966 ³⁰

APPENDIX IV (Cont)

TABLE 4. Symptoms of TNT Poisoning

Symptoms	References
1. Irritative Symptoms	
a. <u>Respiratory</u>	
Mucous membrane irritation (throat irritation, sneezing irritant cough, etc.)	Livingstone-Learmonth <i>et al.</i> , 1916 ⁴³ ; Smith, 1918 ⁸⁶ ; Putnam and Herman, 1919 ⁸³ ; Voegtlin <i>et al.</i> , 1920 ⁸ ; Barnes, 1916-1917 ⁴² ; Stewart <i>et al.</i> , 1945 ¹⁵
b. <u>Gastro-intestinal</u>	
Constant bitter taste	Moore, 1918 ⁴¹ ; Noro, 1941 ⁸⁷
Salivation, nausea and vomiting	Putnam and Herman, 1919 ⁸³ ; O'Donovan, 1921 ⁸⁸ ; von Oettingen, 1941 ⁴⁶ , 1958 ²⁴
Spasmodic epigastric pain	O'Donovan, 1921 ⁸⁸
c. <u>Cutaneous</u>	
Dermatitis; dermatoses	White, 1916 ⁸⁹ ; Livingstone- Learmonth <i>et al.</i> , 1916 ⁴³ ; Legge, 1917 ⁹⁰ ; Lewin, 1921 ⁹¹ ; Schwartz, 1944 ³⁷ ; Noro, 1941 ⁸⁷
2. Toxic Symptoms	
a. <u>Gastro-intestinal</u>	
Epigastric distress (nausea, vomiting, diarrhea and anorexia)	Gregerson and Taylor, 1918 ⁹² ; Voegtlin <i>et al.</i> , 1920 ⁸ , 1921- 1922 ⁶ ; Brill <i>et al.</i> , 1944 ²⁶

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TABLE 4. (continued)

Symptoms	References
Spasmodic epigastric pain	O'Donovan, 1921 ⁸⁸ ; von Oettingen <i>et al.</i> , 1944 ⁵ ; Evans, 1941 ¹⁶
Inflammation of small intestine	Von Oettingen <i>et al.</i> , 1944 ⁵ ; Crawford, 1954 ¹⁸
b. <u>Circulatory</u>	
Cyanosis (of the lips, ears, tongue and mucosae)	Barnes, 1916-1917 ⁴² ; Smith, 1918 ⁸⁶ ; Moore, 1918 ⁴¹ ; Brill <i>et al.</i> , 1944 ²⁶ ; McConnell and Flinn, 1946 ¹⁷ ; Voegtlin <i>et al.</i> , 1920 ⁸ ; 1921-1922 ⁶
<u>Hematological Changes</u>	
Red blood cell abnormalities (Reduction in red blood cells and hemoglobin volume; increase in nucleated cells, reticulocytosis; poikilocytosis, polychromasia)	Minot, 1919 ⁹³ ; Montfort-Sales, 1937 ⁹⁴ ; Velling, 1956 ⁹⁵ ; Moore, 1918 ⁴¹ ; von Oettingen, 1941 ⁴⁶ ; 1958 ²⁴ ; Stewart <i>et al.</i> , 1945 ¹⁵ ; Brill <i>et al.</i> , 1944 ²⁶ ; Cone, 1944 ²⁵ ; Voegtlin <i>et al.</i> , 1920 ⁸ , 1921-1922 ⁶ ; Panton, 1917 ⁹⁶ , 1921 ⁹⁷ ; von Oettingen <i>et al.</i> , 1944 ⁵ ; Noro, 1941 ⁸⁷
White blood cell changes Leucocytosis (increase in number of leucocytes)	Cone, 1944 ²⁵ ; Brill <i>et al.</i> , 1944 ²⁶ ; Voegtlin <i>et al.</i> , 1921-1922 ⁶ ; Smith, 1918 ⁸⁶ ; von Oettingen <i>et al.</i> , 1944 ⁵
Vasomotor irregularities (palpitation, brachycardia)	Putnam and Herman, 1919 ⁹³ ; von Oettingen, 1941 ⁴⁶ , 1944 ⁵

APPENDIX IV (Cont)

TABLE 4. (continued)

Symptoms	References
Anemia - hemolytic; aplastic (see bone marrow)	Germini <i>et al.</i> , 1956 ⁹⁸ ; Voegtlin <i>et al.</i> , 1919 ⁷ , 1921-1922 ⁶ ; Hayhoe, 1953 ²⁰ ; McConnell and Flinn, 1946 ¹⁷ ; Noro, 1941 ⁸⁷
c. <u>Neurological</u>	
Slight body tremors	Voegtlin <i>et al.</i> , 1920 ⁸ , 1921- 1922 ⁶
Weakness; stupor; lassitude	Kramer and Meierhoff, 1916- 1918 ⁹⁹ ; McConnell and Flinn, 1946 ¹⁷ ; Voegtlin <i>et al.</i> , 1920 ⁸ , 1921-1922 ⁶ ; Crawford, 1954 ¹⁸
Ataxia; incoordination	Voegtlin <i>et al.</i> , 1920 ⁸ , 1921- 1922 ⁶ ; von Oettingen, 1944 ⁵
Nystagmus; circus movements	Von Oettingen <i>et al.</i> , 1944 ⁵
Involuntary urination	Von Oettingen <i>et al.</i> , 1944 ⁵
Central nervous system intoxication	Anatovskaya and Liman, 1972 ³⁶ ; Ermakov <i>et al.</i> , 1969 ³⁵ ; von Oettingen <i>et al.</i> , 1944 ⁵
d. <u>Systemic</u>	
Anemia	Von Oettingen <i>et al.</i> , 1944 ⁵
Hem siderosis in spleen, liver, lungs and bone marrow	Voegtlin <i>et al.</i> , 1920 ⁸ , 1921- 1922 ⁶ ; von Oettingen <i>et al.</i> , 1944 ⁵ ; Lillie, 1943 ¹³

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TABLE 4. (continued)

Symptoms	References
Liver-icterus (toxic jaundice; necrosis, yellow atrophy of liver (in very severe cases - fatal)	O'Donovan, 1921 ⁸⁸ ; Voegtlin <i>et al.</i> , 1920 ⁸ , 1921-1922 ⁶ ; Crawford, 1954 ¹⁸ ; Haythorn, 1920-1921 ³⁸ ; Al'pern, 1944 ¹⁰⁰
Spleen-hemosiderosis	Voegtlin <i>et al.</i> , 1920 ⁸ , 1921-1922 ⁶
Loss in body weight	Panton, 1921 ⁹⁷ ; von Oettingen <i>et al.</i> , 1944 ⁵ ; Crawford, 1954 ¹⁸
Bone marrow-hyperplasia; hemosiderosis (aplastic anemia)	Voegtlin <i>et al.</i> , 1920 ⁸ , 1921-1922 ⁶ ; Hayhoe, 1953 ²⁰ ; von Oettingen <i>et al.</i> , 1944 ⁵ ; McConnell and Flinn, 1946 ¹⁷ ; Crawford, 1954 ¹⁸
Lymph nodes-phagocytes loaded with granules of hemosiderin	McCausland and Hawkins, 1944 ³⁹
e. <u>Special</u>	
Irregular menstruation	Putnam and Herman, 1918 ⁸³ ; von Oettingen, 1953 ²⁴
Dark, scanty urine, dysuria	Putnam and Herman, 1918 ⁸³
Yellow-orange skin discoloration	Schwartz, 1944 ³⁷ ; Horo, 1941 ⁸⁷
Conjunctivitis	Horo, 1941 ⁸⁷
Cataract formation	Krol' and Kolevatykh, 1965 ¹⁰¹ ; Pen'kov, 1965 ¹⁰² ; Scholeva, 1969 ²⁷ ; Hassman and Juran, 1968 ³³ , 1971 ³⁴

TABLE 5. Dissimilation of TNT by Bacteria

Genus	Growth Conditions	Substrate Added, ppm	% Dissimilation	Assays	References
<i>Pseudomonas</i>	Shake flasks	100	99	TNT and derivatives by GLC. Respirometry.	Mon et al., 1973 ⁵⁷
<i>Pseudomonas</i> , <i>Alcaligenes</i>	Laboratory activated sludge	0.5-33 in HAAP waste 20% system volume per day. 2.5 days holding time	98.4	TNT by GLC. DOC, IC, BOD, COD, SS, SUS, % VS, acetone, cyclohexanone. Plate counts.	Green, 1972 ¹⁰³
<i>Pseudomonas</i>	Shake flasks, "Bio-oxidation Unit"	29-100	98.75	TNT and derivatives by GLC. Total carbon, pH, MLSS, O ₂ uptake COD. Respirometry.	Enzinger, 1970 ⁷⁴
<i>Pseudomonas</i>	Continuous flow columns	0.39 mg/10 ⁶ bacteria/day	53	TNT by Ruchoff-Neckler. Derivatives by paper chromatography w/ and w/o ¹⁴ C labeling. Respirometry.	Fowler, 1965 ⁵⁸

TABLE 5. (continued)

Genus	Growth Conditions	Substrate Added, ppm	% Dissimilation	Assays	References
<i>Pseudomonas</i> (<i>Achromobacter</i> , <i>Flavobacterium</i> , <i>Xanthomonas</i>)	Respirometers	100	32/180 min (computed)	TNT by spectropho- tometry of alka- line solutions. Nitrite. Respirometry.	Tabak <i>et</i> <i>al.</i> , 1964 ¹⁰⁴

TABLE 6. Toxicity of TNT to Various Aquatic Organisms

	Toxicity	References
<u>Bacteria</u>		
<i>Pseudomonas</i>	100 ppm bactericidal at pH 2-3.5, not at pH 6-7.2	Mon et al., 1973 ⁵⁷
<i>Escherichia coli</i>	50 ppm not toxic	Gring, 1971 ⁶⁵
<i>Zoogloea ramigera</i>	100 ppm bacteriostatic to non-acclimated cells; pH approx. 7	Enzinger, 1970 ⁷⁴
<i>Staphylococci</i> , <i>Proteus</i> , yeasts, <i>Pseudomonas aeruginosa</i> , coliforms, bacilli	250-1000 ppm bacteriostatic, 125,000 ppm bactericidal (solubility, 200 ppm approx.)	Usov et al., 1966 ⁷⁵
<u>Blue Green Algae</u>		
<i>Microcystis aeruginosa</i>	8 ppm killed 100% of 1×10^6 to 2×10^6 cells/ml	Fitzgerald et al., 1952 ⁷¹
<u>Green Algae</u>		
<i>Chlamydomonas reinhardtii</i>	3 ppm "toxic"	Grina, 1971 ⁶⁵

TABLE 6. (continued)

<u>Fish</u>	Toxicity	References
Bluegills, .39g.	2.3-2.8 ppm 96 hour TLM	Pedersen, 1971 ⁶⁴
Bluegills	2.6 ppm 24 hour MLD 2.0 ppm 96 hour MLD	Nay, 1971 ¹⁰⁵
"Minnow"	4-5 ppm 6 hour MLD	Leclerc, 1960 ⁶⁶
"Fish"	1.5-2.0 ppm "lethal concentration"	McKee and Wolfe, 1963 ⁶⁷
Goldfish, 4.5-6 cm	3 ppm, 25% mortality, 3 days 5 ppm, 100% mortality, 3 days	Mathews <i>et al.</i> , 1954 ⁶⁸
"Fish"	"5 ppm kills fish"	Ruchhoft <i>et al.</i> , 1945 ⁷⁰
Minnows Carp Bullheads	40 ppm MLD, 87-110 min 40 ppm MLD, 455 min 50 ppm MLD, 556 min	Deqani, 1943 ¹⁰⁶
"Fish"	1/400 combined TNT waste lethal	Mohlman, 1943 ⁶⁹

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B. Abstract Journals Searched for TNT, etc.:

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[Key words: Trinitrotoluene, dinitrotoluene].

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4. BIOSIS, from 1972 to 1973.

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C. Computer Searches of Literature for TNT, etc.:

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(See Search Control Nos. 002825, T19564)

2. National Library of Medicine/Medline Retrieval file (1969 to date).

3. Medlars (Common Research Computer Facility, Houston) 1956 to date.

D. Contacts:

1. National Academy of Sciences, Center for Toxicology (Mr. R. Wands).

2. Department of Health, Education and Welfare.

a. National Institute for Occupational Safety and Health (Dr. H. Stokinger, Tel. 513-684-2697 and Dr. H. Christensen, Tel. 301-443-3053).

b. Communicable Disease Center, Atlanta, GA. (Dr. Wm. Barthel, Tel. 404-633-3621).

3. Department of the Army.

a. Aberdeen Proving Ground. (Drs. D. Rosenblatt, McNamara, Hackley and Owens; LTC Steinberg).

b. Army Natick Laboratories. (Dr. T. Kaplan, Tel. 8-955-2604 and Dr. R. Chalk, Tel. 8-955-2377).

4. Department of the Navy.

a. Naval Ordnance Laboratory, White Oak, Maryland. (Dr. J. Hoffsommer).

b. Navy Industrial Environmental Health Center. (Mr. A. Johnson, Tel. 8-989-3947).

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- c. Naval Ammunition Depot. (Mr. E. Pardee, Tel. 8-956-6011).
- d. Naval Biomedical Research Center, Oakland, California.
(Dr. R. Heckley, Tel. 8-836-6343).
- e. Bureau of Medicine and Surgery. (Lt. CDR L. Doptis, Tel. 202-254-4620).
- 5. Environmental Protection Agency. (Dr. Allen, Tel. 703-522-1825 and Dr. A. Forziati, Tel. 202-755-0611).
- 6. Department of Commerce.
 - a. National Technical Information Service (NTIS), Springfield, Virginia, Mr. Joseph Coughlin, Asst. Director, Tel. 703-321-8574).
 - b. Joint Publications Research Service (JPRS). (Mr. Gustave Blackett, Tel. 703-557-1431).
- 7. Non-Governmental Laboratories.
 - Hazleton Laboratories. (Dr. Rutter, Office of Toxicology, Tel. 703-893-5400).

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APPENDIX C

INFORMATION SOURCES CONSULTED FOR TOXICITY TO AQUATIC ORGANISMS

The literature search pattern was arranged to give maximum coverage of key subjects in a minimum of time. Briefly, important recent references were located (1) by way of abstract journals, (2) by telephone, and (3) by local inquiry. Then the literature citations in these references were exploited.

A. Abstracts.

Generally, Biological Abstracts and Bioresearch Index were more useful than Chemical Abstracts. A larger number of applicable citations appeared in Biological Abstracts; these were adequately cross-referenced, and the key words used made it easier to locate compounds of primary interest, as well as related substances.

Descriptors included: trinitrotoluene (TNT); derivatives indicated in the introduction to this report and others possibly formed by chemical or biological means from TNT or "pink water"; and the names of lakes and streams contiguous with Army ammunition plants. When Chemical Abstracts was used, the Guide was consulted for correct nomenclature.

The degree of coverage of each descriptor depended on the relevance of the subtopic. For example, immunology of nitro compounds, and lake and stream names were covered only in post-1969 abstracts, while TNT toxicity and degradation were carried back to 1926 in Biological Abstracts and 1907 in Chemical Abstracts.

B. Telephone and Local Inquiry.

Telephone and personal inquiries were made of agencies or individuals known to have knowledge of topics of interest and who might have access to the large body of literature not in the abstracts. Especially helpful were individuals at the Environmental Quality Division of the US Army Medical Bioengineering Research and Development Laboratory; the Water Quality Engineering Division of the US Army Environmental Hygiene Agency; the Tennessee Valley Authority; the Illinois State Water Survey; and the US Forest Products Laboratory.

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APPENDIX D

GENERAL FORMULA FOR ESTIMATING EMERGENCY DRINKING WATER LIMITS FROM THRESHOLD LIMIT VALUES FOR AIR*†

$$EDWL = \frac{TLV_{(air)} (mg/m^3) \times 10 \times \text{Absorption Factor (inhalation)}}{2 \times \text{Absorption Factor (ingestion)} \times \text{Safety Factor}}$$

Application: Those substances with TLV based on systemic toxicity

Exclusion: Those substances with TLV based on local or pulmonary irritation or sensitization

Absorption Factors: In instances in which these are not known, estimation should be made by experienced toxicologist

Safety Factor: Should include term for health protection for general population, as well as one for taste and odor†† where applicable, i.e. TLV is based on adult, health population

The factors used in the development of the TNT Emergency Drinking Water Limit (EDWL) for 3 days duration using the aforementioned formula are as follows:

$$\begin{aligned} EDWL &= \frac{1.5 \text{ mg/m}^3 (\text{TLV for TNT/air}) \times 10 \times 100\% (\text{Absorption Factor})}{2 \times 100\% (\text{Absorption Factor/ingestion}) \times 10 (\text{Safety Factor})} \\ &= \frac{1.5 \text{ mg} \times 10 \times 100\%}{2 \times 100\% \times 10} = \frac{1.5 \text{ mg}}{2} = 0.75 \text{ mg/l} \end{aligned}$$

Thus 0.75 mg TNT/l will be considered to be safe for an emergency drinking water limit for 3 days duration as determined by the National Academy of Science Ad Hoc Committee, in the absence of the evaluation of TNT toxicity data based on ingestion.

*Adapted from J. Amer. Water Works Assoc., 50, 515 (1958)

†Developed by the National Academy of Sciences Ad Hoc Committee on Emergency Drinking Water Limits (Dr. H. Stokinger, Chairman)

††Subsequent decision by the PHS Drinking Water Standard Committee disregarded the taste and odor problem for EDWLs (unless overpowering!)