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

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The literature search revealed the following facts:

a. The acute toxicity 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

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degradation products.

b. 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.

c. There is no record of carcinogenic, teratogenic or mutagenic studies on nitroglycerin and related compounds.

d. No data was found on the toxicity of nitroglycerin to aquatic organisms.

e. Nitroglycerin is rapidly metabolized in animals and man by the active nitroreductase enzymes via the known dinitrates and mononitrates and glycerol to carbon dioxide.

f. 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, subacute 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.

Recommendations for studies on nitroglycerin and its derivatives (metabolites) in experimental animals and wastewaters are outlined.

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LIST OF ABBREVIATIONS

AAP	Army Ammunition Plant
BOD	Biological Oxygen Demand
EGD	Ethylene Glycol Dinitrate
GLC	Gas-Liquid Chromatography
im	intramuscular
ip	interperitoneal
iv	intravenous
LD	Lethal Dose
LD50	Lethal Dose (kills 50%)
MAC	Maximum Allowable Concentration
MAO	Monoamine Oxidase
MLD	Minimum Lethal Dose
NG	Nitroglycerin
or	oral
sc	subcutaneous
TLC	Thin-Layer Chromatography
TLV	Threshold Limit Value

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 hardnes, 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 commerical 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.

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 Nickerson[4] 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

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. DiCarlc *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.

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, 1946[3]). 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

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
		Nitroglycerin		
Rat	or	80-100	MLD	Orestano (1937)[13]
	im	150-400	MLD	Orestano (1937)[13]

TABLE 1. (Cont)

Animal Species	Route of administration	Dose (mg/kg)	Remarks	References
		Nitroglycerin		
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)[8]
	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)[8]
	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]

TABLE 1. (Cont)

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

Acute Toxicity in Man. The acute toxicity of nitroglycerin in humans has been summarized by Munch and Friedland, 1965[5]. (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,

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)[8] 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 (Medicinal 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	---

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
Shcun[24]	(1942)	---	3-5 drops, 30 mg Exquisitely toxic

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 Heilrung (1942)[18] 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

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 34[4] 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, 1951[28]). 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

and Sato, 1963[29],[30]; Kakizaki, Sato, Tsuruta, and Hasegawa, 1967[31]; Hasegawa and Sato, 1970[32]; Tsuruta and Hasegawa, 1970[33]; Hasegawa, Sato, and Tsuruta, 1970[34]).

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/m^3, 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, 1967[38]). 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.

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/m^3 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 CO_2 (Needleman *et al.* 1971[48]). Experiments in rats given ^{14}C-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.*, 1969[51]). Consequently $^{14}CO_2$ 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, 1971[53]). Urinary excretion only was investigated and in all cases small amounts of the glyceryl-1-mononitrate was rapidly formed and excreted.

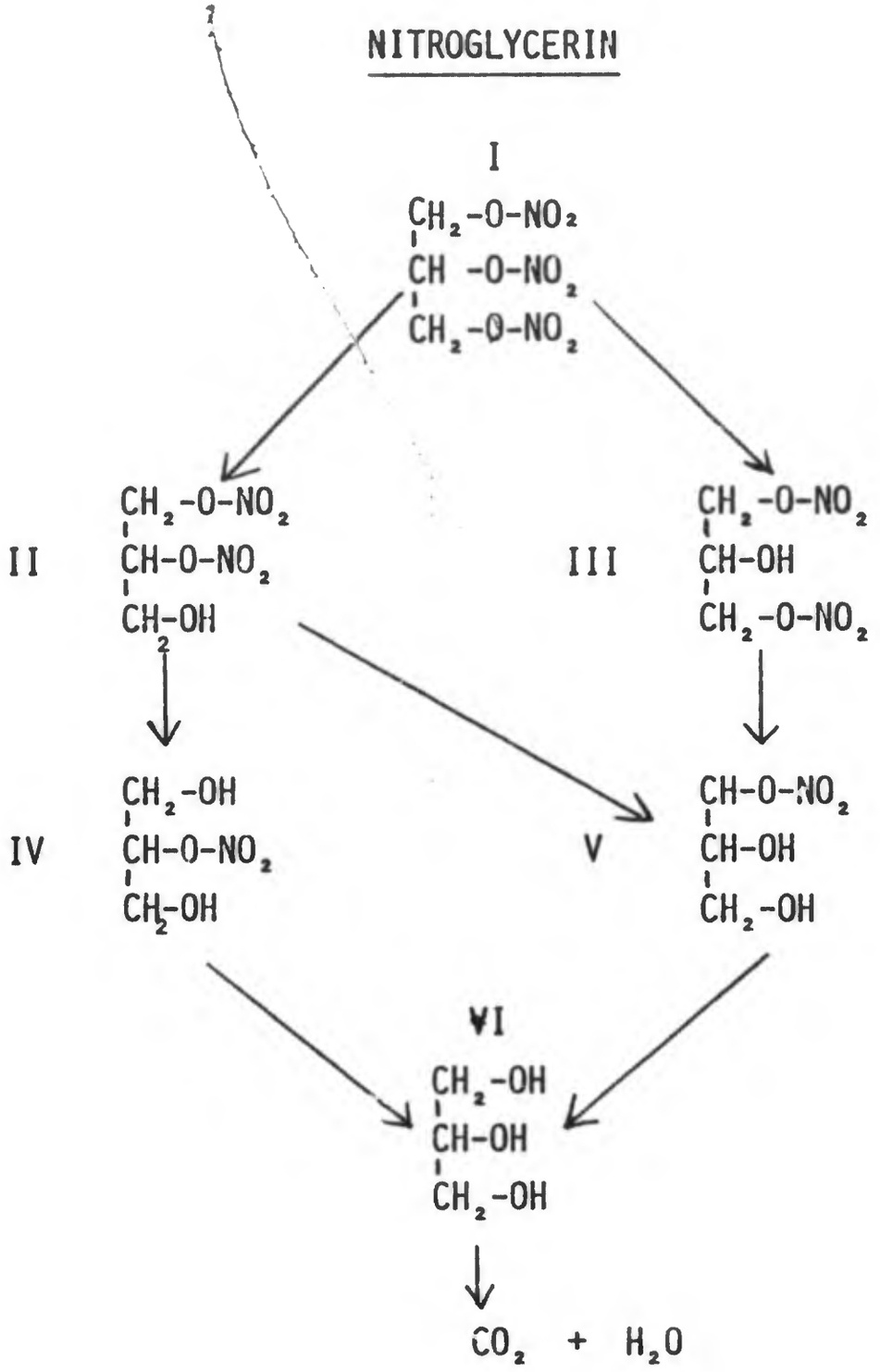
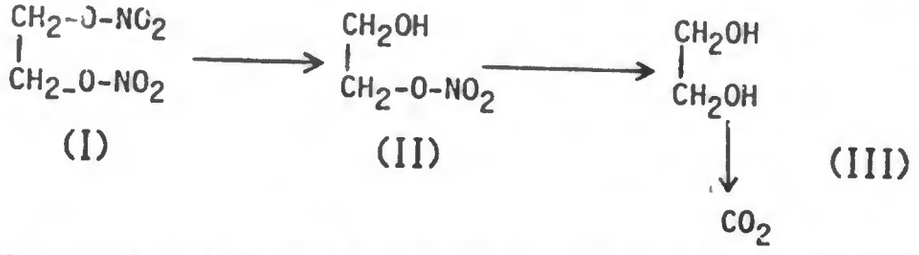


FIGURE 1: METABOLIC PATHWAYS FOR NITROGLYCERIN

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.

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 ^{14}C-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 nitroglycol-poisoned mice have been studied by Yoshihawa (1964[70], 1965[71]). The relationship between nitroglycol 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 mitchondria 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.

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, 1973[74]). 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 not 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)

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 attached 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 ^{14}C labelling in the presence of nitroglycerin and glyerol.

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 µg 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 (1972[81], 1973[82]) 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].

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 Bespalov (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

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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APPENDIX A

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B. Abstract Journals Searched for Nitroglycerol:

1. *Chemical Abstracts*, from Vol. 1, 1907 to date.

Includes 1st decennial index, 1-10 (1907-1916)
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].

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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[Key words: glycerol trinitrate, nitroglycerin].

APPENDIX B

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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.