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PROBLEM DEFINITION STUDY ON THE HEALTH EFFECTS OF DIETHYLENEGLYCOL DINITRATE, TRIETHYLENEGLYCOL DINITRATE, AND TRIMETHYLOLETHANE TRINITRATE AND THEIR RESPECTIVE COMBUSTION PRODUCTS

PREPARED BY

James W. Holleman
Robert H. Ross
Captain James W. Carroll

April 1983

SUPPORTED BY

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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This report is an evaluation of the available literature on the health effects and toxicity of three nitrate esters, diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), and trimethylolethane trinitrate (TMETN) and their respective combustion products. Information on the combustion products of munitions containing these three nitrate esters was inexistent but some data was available on the products of combustion of munitions in general. Toxicologic and health effects literature dealing directly with DEGDN,

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TEGDN, and TMETN was very sparse and accordingly an evaluation of the toxicology of other nitrate esters used in the explosives industry is presented, along with recommendations for future medical research to develop the necessary scientific data base to recommend occupational health and environmental quality protection criteria for these compounds in their production, use, and disposal.

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FINAL REPORT

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EXECUTIVE SUMMARY

Nitrate esters, especially diethyleneglycol dinitrate (DEGDN), were introduced in Europe to replace nitroglycerin as a plasticizer for nitrocellulose in so-called double-base explosives used in military weaponry, nitroglycerin being in short supply since glycerin was derived mainly from animal fats. A number of other nitrate esters have also been introduced for various explosive or propellant uses. Not only did the nitrate esters turn out to have some superior properties to nitroglycerin from a purely ballistic standpoint, but they were also found to present lower handling hazards and offer potentially lower health hazards. This report summarizes and analyzes information available on three of the nitrate esters, namely diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), and trimethylolethane trinitrate (TMETN). These three compounds are being considered for broader use in new military munitions such as the 120 mm shell to be used in the M-1 Abrams tank. Health hazards in the production, processing and packing and disposal of these munitions are of concern. In the field, concern focuses on the nature and levels of the combustion products which will be produced by the burning of these munitions, and the potential effects on the weapons system crew members.

Toxicological and health effects literature dealing directly with DEGDN, TEGDN, and TMETN was very sparse; accordingly, as well as discussion of the effects of DEGDN, TEGDN, and TMETN, an evaluation of the toxicology of other nitrate esters used in the explosives industry was made, selecting information which would likely be pertinent to the production and use of the three nitrate esters in question. Information on the combustion products of munitions containing these three nitrate esters was inexistent, but some information was available on the products of combustion of other munitions. It is believed that the products of combustion of munitions containing the three compounds of concern will fall within these ranges. It is nonetheless imperative that sampling and analysis be done to determine what the products actually will be, and to further study those combustion products representing uncertain health hazards. Carbon monoxide will certainly be among them, as it is usually the chief product of combustion of many explosives and propellants. As shown by research results with other explosives, some reactive and/or irritating compounds (aldehydes, acids, nitrogen oxides, free radicals) could also be present, but research is necessary to determine this for nitrate ester-containing explosives and propellants.

In general, although information is sparse, the nitrate esters in question seem to show less toxicity than nitroglycerin or ethyleneglycol dinitrate, which are commonly used in explosives. The most urgent recommendation that has been made for these, and which can also be made for DEGDN, TEGDN, and TMETN, is that industrial workplace exposure levels should be set so that the adverse pharmacological effects of exposure to the nitrate esters not occur — that is, no headaches and no blood-pressure lowering. To achieve this, the TLVs for NG and EGDN have been lowered from 0.2 to 0.02 ppm, and NIOSH has recommended an even lower level of

0.01 ppm (measurable in any 20 minute sampling period). This represents a significant reduction, and it is felt that this level for DEGDN, TEGDN, and TMETN would be more than adequate; however, the levels at which effects become manifest for the three nitrate esters in question compared to NG, EGDN and other compounds, must be determined by empirical study.

The underlying reason for the strict control mentioned above is that exposure to the nitrate esters results in accommodation, and this accommodation is associated with hemodynamic changes which result in a narrowing of the pulse pressure. On withdrawal from exposure, a worker, particularly one addicted to alcohol or already suffering from a cardiovascular disorder, may collapse and even die from lack of blood to the heart. A few cases of this have been reported where DEGDN was being produced. Keeping levels low and keeping sensitive workers from the high-risk workplaces were applied as measures to avoid these incidents.

The LD_{50}s of DEGDN, TEGDN, and TMETN, are quite high, of the order of 700-2500 mg/kg, the differences reflecting mostly the routes of administration. These levels are much higher than ones to which humans would be exposed – in the human case exposure would be mostly by inhalation, with a possibility of absorption through the skin from contaminated gloves, clothing, or equipment.

Organic nitrate esters, including the three of concern here, can be absorbed through the skin, and avoidance of the use of contaminated gloves, clothing, and protective devices is recommended.

Only limited information was available on the behavioral effects, neurotoxicity, and effects on performance of exposure to the three compounds of concern. Exposure of a monkey to vapors of TEGDN at a level of 2.4 ppm for 4 hr caused effects suggesting interference with sensorimotor integration. In subchronic feeding tests with rats exposed at levels of from 0.5 to 5.0 mg/kg/day for 6 months it was concluded that DEGDN has the capacity of influencing the central nervous system (e.g., changes in the conditioned reflex activity) in very small doses during prolonged intoxication. In a test with human volunteers exposed to propyleneglycol dinitrate (PGDN), a nitrate ester generally more toxic than DEGDN, TEGDN, or TMETN, effects similar to moderate alcohol intoxication were noted after exposure to 0.5 ppm by inhalation for 6-8 hr.

Organic nitrate esters, including DEGDN, TEGDN, and TMETN, induce methemoglobin formation; but while respiratory collapse due to the massive formation of methemoglobin is the cause of death in acute poisoning, at usual occupational exposure levels the production is so slight that the body's methemoglobin reductase system easily takes care of it. Some neurological effects are, however, considered to be cumulative. Also, long-continued exposure to nitrate esters, with the associated hemodynamic changes and effects on the blood vessels, may lead to an arteriosclerosis which would add to the gravity of the situation in the withdrawal syndrome.

Liver tumors, cholangiofibrosis, and interstitial cell tumors of the testes have resulted in rats exposed to high doses of NG for long periods of time. Chronic toxicity tests including carcinogenicity have not been conducted on DEGDN, TEGDN, and TMETN. Likewise, while nitrites have been tested for teratogenicity (the results were negative), teratogenicity studies with DEGDN, TEGDN, and TMETN have not been reported. Some evidence exists of reproductive effects in humans on exposure to organic nitrate esters, but no reproductive or mutagenicity studies were found with respect to the three title compounds. However, the parent glycol of TMETN has been tested for mutagenicity, with and without metabolic activation, and found to be negative.

Preliminary study data was found on the biodegradability of four nitrate esters PGDN, DEGDN, TEGDN, and TMETN to support the evaluation of microbiological treatment techniques for waste waters containing these materials. One specific biological treatment process under evaluation for NG was also found to be effective in eliminating these four esters without essential process modification. Very limited environmental fate data was also available on the degradation of the glycols formed from nitrate ester propellants when treated by biological and chemical means.

In conclusion, the available scientific data on the health and environmental quality effects of DEGDN, TEGDN, and TMETN is very limited, and further comprehensive medical research studies are now necessary to establish occupational health and environmental quality protection criteria for production, use, and disposal of these compounds and associated munition items.

This medical research must be supported by a thorough evaluation of workplace exposure settings and potential environmental emission forms and routes. Health effects research requirements include the full range of toxicologic studies necessary to establish safe workplace exposure levels. These studies should begin with mutagenicity and acute toxicity evaluation, and proceed through subchronic or repeated exposure toxicity studies, metabolism studies, and teratogenicity and reproductive effects studies; and finally include chronic toxicity and carcinogenicity studies. Environmental quality research studies are necessary to evaluate environmental transformation, transport and fate issues, and aquatic toxicity evaluation. In reference to military field workplace exposure settings, the composition and levels of combustion products arising from these nitrate esters and their associated propellant formulations need to be thoroughly assessed. If necessary, medical research studies should then be planned and conducted on the health effects of those combustion product contaminant hazards identified and not under active research or protection standard coverage for this field workplace setting typified by short-term, episodic exposure during weapons firings.

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CONTENTS

LIST OF TABLES

1. INTRODUCTION AND BACKGROUND

1.1. PURPOSE

The U.S. Department of Defense (DOD) community is currently considering the broader use of new propellant formulations which will use diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), and trimethylolethane trinitrate (TMETN) in place of nitroglycerin (NG). In particular, DEGDN may be used as a major component (21-35%) of the propellant for the 120 mm main gun round of the Army's M-1 (Abrams) Main Battle Tank. DEGDN is more volatile than NG, and while TEGDN and TMETN are less volatile than NG, the compounds may require higher processing temperatures in the formulation of military explosives and propellants, and thus present hazards which must be evaluated. The purpose of this problem definition study is to identify and evaluate the known biological and toxicological effects of these compounds and their potential combustion products because of the possibility of exposure to workers involved in their manufacture and handling and to troops in the field (primarily the armored vehicle crew compartment) who may use munitions containing these compounds.

Specifically the scope and objectives of this study are to:

(1) Characterize the known physical and chemical properties of DEGDN, TEGDN, and TMETN compounds.

(2) Define the range of biological effects of exposures to DEGDN, TEGDN, and TMETN as indicated by data developed through toxicologic evaluations, clinical, and epidemiological study experiences.

(3) Define the range of biological effects of exposure to DEGDN-based (and TEGDN, TMETN) propellants and of the gases and aerosols potentially generated during combustion of such propellants.

(4) Identify gaps in the data base regarding the potential health hazards of these compounds, and recommend medical research needed to fill these gaps.

As well as considerations of health effects from short-term high level military exposure, munition specific health criteria are also to be developed if possible for exposures in the industrial production workplace setting. Thus the focus of concern is U.S. Army munitions production and use exposure settings.

1.2. BACKGROUND ON DEGDN, TEGDN, AND TMETN

DEGDN, TEGDN, and TMETN, and some other glycol nitrate esters came into use as replacements for nitroglycerin as co-energetics/plasticizers for nitrocellulose and other solid nitro energetics in gun propellants

due to shortage of glycerin and also because of superior properties of the glycol nitrate esters. Thus Camp, Csanady, and Mosher (1980), in a patent concerning plateau propellant compositions, state that propellants plasticized with metriol trinitrate (TMETN) and triethyleneglycol dinitrate are "vastly superior in health, stability, and storage considerations over those made with nitroglycerin."

The introduction of DEGDN, TEGDN, and TMETN into explosive/propellant formulations can be followed to some extent by references to previous work in the patent abstracts. As reported by Ayerst (1968), the nitration of glycols to provide alternatives to nitroglycerin in propellants had been under consideration prior to 1939 both in Germany and in Great Britain; in Germany, DEGDN had been made on a plant scale before World War II, and from German documents it appears that TEGDN had also been made by the Westphalian Anhalt Explosives Company in 1938. The work in Britain was on a very small scale, and although DEGDN was eventually (1945) produced on a pilot scale, the nitration of triethylene glycol (TEG) was restricted to laboratory work and a batch nitration. The glycol nitrate esters have a lower caloric yield than that of nitroglycerin, and partly because of this and partly because of greater difficulties of supply and nitration, further work on TEGDN was restricted in spite of its low sensitivity to friction and impact and its low vapor pressure. After 1945 it was found that TEGDN had been made in Germany for small arms powders and some interest was shown in Britain for a few years. A small quantity of TEGDN was made from some British TEG in 1947 and a substantial quantity of German TEG was obtained and stocked. TEG nitrations were carried out in 1950 (Varrato, Picatinny Arsenal Report No. 1767, 1950; as cited in Ayerst, 1968) in the United States principally for colloidal propellant work but interest in this material diminished and, apart from a single nitration in 1954 in Britain and some investigations in the U.S. in 1956, interest waned until 1957.

The need to produce castable composite propellants led to the examination of suitable nitroplasticizers and in 1957 TEGDN was considered for polyurethane mixes as it had been shown to give good physical properties to the propellant at low temperatures. However, problems arose. Information on preparative methods was inadequate, especially with the knowledge that the higher homologues of ethylene glycol gave unstable waste acids. The dangerous instability of the nitration mixture itself was only partially appreciated, and there was a complete absence of information on optimum processing conditions, as this information had been obtained for DEGDN only. Finally, there was only a limited supply of German TEG and it was deteriorating. The importance of the glycol supply became apparent when it was found that even the best glycol available would not nitrate to give a storable product; it was not appreciated until this time that there had been a change in the manufacturing method for glycol production from the chlorhydrin route as used in Germany to the ethylene oxide route, with a resultant change in the extent and nature of the impurities; the impurities leading to changes in processing characteristics and stability of the product. Satisfactory TEGDN

was finally made with TEG purified by vacuum distillation, and instabilities were avoided by the use of a continuous tubular reactor for nitration.

Lindner (1980) states that DEGDN is the most widely used explosive plasticizer, other than nitroglycerin, in the formulation of military propellants for gun use. The lower caloric yield than that of nitroglycerin, mentioned above (Ayerst, 1968), is taken advantage of to obtain cooler propellants without the use of relatively inert diluents such as vaseline, used in some earlier formulations (Phillips, 1943), thereby decreasing erosion in gun tubes and increasing tube life. The composition of solid propellants having a wide mass impetus range potential exceeding that of standard double-base gun propellants and a low flame temperature and which evolve noncorrosive combustion products is given in a patent of Haury and Frankel (1975). This explosive contained 5-10% of a nitrocellulose binder, 5-30% of a 23/2.5 or 4.8/15 TMETN/TEGDN blend, and 40-80% of an oxidizer, such as an 80/0 or 50/30 triaminoguanidine nitrate mixture with RDX, plus a stabilizer such as ethyl centralite plus resorcinol. In this composition, the use of triaminoguanidine nitrate to replace nitrocellulose is noteworthy. Nitroguanidines produce ammonia on combustion of the propellant, and this limits the use of such compounds, especially for large caliber weapons, because of the effects of ammonia on the gun crew (Phillips, 1943). Legters (1980) has reviewed for the Army the biological effects of short, high-level exposure to ammonia in the munitions context.

As further stated by Lindner (1980), DEGDN decreases muzzle flash and minimizes the need for flash reducers in propellant charges. It is superior to nitroglycerin in terms of stability and handling safety (however, its greater volatility may pose problems during prolonged high temperature storage), has a lower freezing point than nitroglycerin (-11.3 vs 13.2°C), and has a high and low stable detonation rate (6760 m/s and 1800-2200 m/s, respectively). Lindner states that DEGDN does not appear to have harmful physiological effects, but this conclusion must be modified in view of studies which will be discussed later in this report.

As stated by Lindner (1980), TEGDN is an explosive plasticizer of low sensitivity that has been used in some nitrocellulose-based propellant compositions, often in combination with TMETN. TMETN alone is not very satisfactory as a plasticizer for nitrocellulose and must be used with other plasticizers such as metriol triacetate (acetate analog of TMETN). Mixtures with nitroglycerin tend to improve the mechanical properties of double-base cast propellants at high and low temperatures.

Use of TMETN as a plasticizer for nitrocellulose to produce an explosive suitable for either sheet explosive or as a warhead filler which retains flexibility at -50°C was described by Wells (1976). A difficulty in producing the explosive was that TMETN coated the propellant surface and impeded its own diffusion into the body of the propellant mix; this

difficulty was circumvented by using differential rolling to constantly expose fresh surface.

While nitrocellulose is notable in providing mechanical strength as well as readily available energy to gun and rocket propellants (Lindner, 1980), the nitrated plasticizers can also contribute to the physical integrity of the propellant. This is exemplified by a patent of Zucker, Trask, and Costa (1975), in which nitrocellulose double-base propellants which do not disintegrate upon firing in high-pressure systems are obtained with TMETN, TEGDN, and DEGDN.

Phillips (1943), during wartime, reported on the composition of foreign propellants, in calibers from 13 mm to 105 mm. Use of DEGDN instead of nitroglycerine and of nitroguanidine to replace nitrocellulose were differences noted between the foreign powders and the U.S. formulations, the use of DEGDN reflecting the continental, particularly German, lack of glycerin from animal fats. The powders were also characterized by a high content of centralite in place of the diphenylamine generally used in U.S. powders as a stabilizing agent, the centralite in the foreign powders acting not only as a stabilizer but also as a gelatinizing (to substitute for nitroglycerin) and flash-reducing agent. The amounts of centralite and of DEGDN were in a reciprocal relation — the amount of DEGDN being increased in powders in which the centralite concentration was low. There was similarly an inverse relationship between the amount of DEGDN used and the nitration level of the nitrocellulose — a low nitrate concentration of the nitrocellulose being compensated for by increased DEGDN. Some differences in secondary additives (metal salts, graphite, etc.) were also noted. At the time, only experimental work with powders containing DEGDN had been done in the United States, and it had been found that (a) DEGDN was more difficult to stabilize after nitration than nitroglycerin, (b) it had a more pronounced physiological effect on workers coming in contact with it than had nitroglycerin, (c) it was less sensitive to shock and flame than nitroglycerin and therefore powders containing it were more difficult to ignite, and (d) powders containing DEGDN were more volatile than nitroglycerin powders.

The earliest U.S. work with a powder containing DEGDN was found in a study by Cameron (1934) of the storage stability and ballistic performance of a powder which had first been made at Picatinny Arsenal in 1929 for a 75 mm gun loading. The composition of the powder was: nitrocellulose (12.6% N), 74.0%; DEGDN, 20.0%; ethyl abietate (flash reducer), 5.0%; diphenylamine, 1%; and a similar lot without the abietate. The powders were tested under a variety of conditions of storage and temperature. The powders showed after four years of storage some loss of extractive matter and release of volatiles and increase in hygroscopicity, and what were considered to be excessive ballistic changes; and thus, although the chemical stability was acceptable, it was decided, in view of the procurement situation at that time, that further consideration of DEGDN as a powder ingredient was not warranted. Lack of a commercial producer of DEGDN was also a consideration.

Cooley (1946) reviewed the Axis manufacture of explosives during World War II. Explosives manufacture in Germany compared favorably with the best American practice, with Japan generally behind both Germany and the United States. While American production engineering was definitely superior to that of the Japanese and perhaps even to German procedures, Germany was possibly ahead in methods of preparation. Of interest was the increasing tendency of the Germans to use continuous processing and their development of DEGDN as a replacement for nitroglycerin in double-base powders. It may be noted that whereas glycerin was obtained theretofore generally only from fats or in poor yield from sugar, the first production of synthetic glycerin on a large scale (30,000,000 lb/yr) by a petroleum refiner occurred in 1946. Glycol had been nitrated during World War I by the Germans but it proved too toxic to workers, too volatile, and there was slow but noticeable evaporation from finished powders. About 1929 it was proved that diethyleneglycol (DEG) could be nitrated on an industrial scale (see first characterization of DEGDN by Rinkenbach in 1927) and was free of the above drawbacks. The raw materials for DEG were present in Germany in large quantities, namely coal and lime and ethylene from waste gases of coke ovens, and even diglycol from potatoes. DEGDN-containing powders were used in the Spanish Civil War, and it was notable that because of the lower caloric yield of the DEGDN, the German artillery pieces suffered considerably less barrel erosion than artillery using other formulations. As well as DEGDN, Germany also manufactured TEGDN, TMETN, other alkyl nitrates, and several cyclic nitrated explosives components.

A report by Norden in 1953 (Norden, 1953) describes the preparation of TEGDN on a semiplant scale at Picatinny Arsenal. A surveillance study of propellants made from a regular commercial grade of triethyleneglycol (TEG) was done. It was found that the standard grade TEG available from the Dow Chemical Company had been improved so that it was approximately equal to the refined TEG (vacuum distilled) used in an earlier study and gave a product similar in purity and stability to that prepared from the redistilled material.

Patterson et al. (1976) have described the state-of-the-art of the military explosives and propellants production industry, as part of a U.S. Environmental Protection Agency (USEPA) program for miscellaneous chemical industries to establish a baseline of information concerning the industry, the magnitude of its waste problems, and the adequacy of the industry's treatment technology. Because of the small amounts of DEGDN, TEGDN, and TMETN made, they do not receive special treatment in the report, but numerous conditions of the production and handling of other components would also apply to them. The results of the study indicate that many of the wastes do present significant problems of toxicity and/or resistance to treatment. The study covered both manufacturing and LAP (Load, Assemble, and Pack) activities. Application of better treatment methods in some cases, and further research, to be performed by USEPA and/or DOD to control pollutants generated by certain sectors of the industry are recommended.

1.3 APPROACH TO THE PROBLEM

The initial step to define and characterize the biological and health effects of DEGDN, TEGDN, and TMETN was a comprehensive search of the literature by both computerized and manual methods. This search indicated that very little information was available on these compounds. This literature was summarized (Section 4) and addressed in detail in Appendix A and B. Since there was such a small amount of toxicological information on these three nitrate esters, a brief analysis of the literature documenting the toxicology of other nitrate esters was conducted and is included as Appendix C in this report to provide further insight into the possible health effects of exposure to DEGDN, TEGDN, and TMETN. In some instances studies on the toxicology of DEGDN, TEGDN, and TMETN summarized in Section 4 were also cited in Appendix C for comparison.

1.4 ORGANIZATION OF THE REPORT

The main body of the report (Sections 1-7) summarizes and discusses the principal findings concerning the health effects of DEGDN, TEGDN, and TMETN and their respective combustion products. Appendix A is a detailed analysis of the key references and Appendix B is a tabular summary of the health effects of the three nitrate esters. The last part of the report, Appendix C, is a discussion of the health effects of nitrate esters other than DEGDN, TEGDN, or TMETN, and in some instances, comparisons with these three compounds.

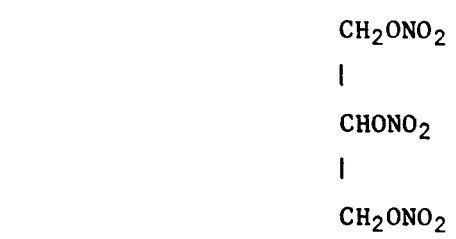
2. CHEMICAL AND PHYSICAL PROPERTIES OF MUNITION COMPOUND

2.1 DEGDN, TEGDN, AND TMETN

The chemistry of nitrate esters in general has been treated by Boschan, Merrow, and Van Dolah (1955) and with consideration of DEGDN in particular by Kitchens et al. (1978) in a preliminary problem definition study of 48 munitions-related chemicals; most of the information in the study of Kitchens et al. being taken from the Boschan, Merrow, and Van Dolah work. Only data on the physical chemical properties is presented; the reader is referred to Boschan, Merrow, and Van Dolah for further information.

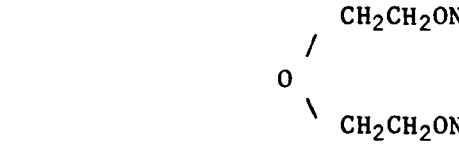
The Chemical Abstract Service's (CAS) Registry numbers, molecular and structural formulas, and CAS names for DEGDN, TEGDN, and TMETN, and for nitroglycerin (NG) for comparison are as follows:

NG CAS No. 55-63-0 $C_3H_5N_3O_9$



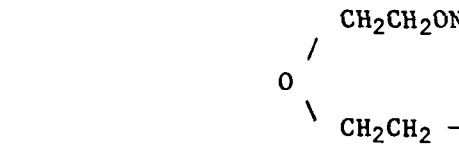
7th CI (Collective Index, 1962-1966) and
8th CI (1967-1971) names: Nitroglycerin.
9th CI (1972-1976) name: 1,2,3-Propanetriol, trinitrate

DEGDN CAS No. 693-21-0 $C_4H_8N_2O_7$



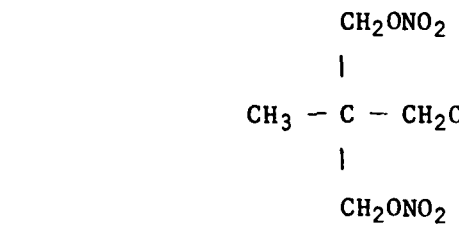
7th and 8th CI names: Diethyleneglycol, dinitrate
9th CI name: Etnanol, 2,2'-oxybis-, dinitrate

TEGDN CAS No. 111-22-8 $C_6H_{12}N_2O_8$



7th and 8th CI names: Triethylene glycol, dinitrate
9th CI name: Ethanol, 2,2'-[1,2-ethanediylbis(oxy)]bis-, dinitrate

TMETN CAS No. 3032-55-1 $C_5H_9N_3O_9$



7th and 8th CI names: 1,3-Propanediol, 2-(hydroxymethyl)-2-methyl-, trinitrate
9th CI name: 1,3-Propanediol, 2-methyl-2-[(nitroxy)methyl]-, dinitrate (ester)

It may be noted that not only are DEGDN and TEGDN ethers (TEGDN being a di-ether) but also in them the nitro groups are well removed from each other. In NG they are on adjacent carbons and in TMETN also quite near each other. These structural features reflect themselves in the chemistry, both organic and biological, of the compounds, with respect to reaction rates and mechanisms of denitration, and mechanisms of other reactions. It may also be noted that NG and TMETN, carrying three nitro groups, have more oxidizing potential than the other two compounds.

Table 1 gives chemical and physical characteristics of NG, DEGDN, TEGDN, and TMETN.

Vapor pressures at various temperatures of NG, ethyleneglycol dinitrate (EGDN), TEGDN, TMETN, and DEGDN at various temperatures are given in Table 2. The difference in vapor pressure of EGDN compared to that of the other compounds is striking. While EGDN is used in explosives, particularly in blasting explosives, its high vapor pressure renders it more hazardous from a production and use standpoint than other organic nitrates. Its physiologic effects are believed to be closely similar to those of NG (ACGIH, 1980).

2.2 COMBUSTION PRODUCTS

Information identifying combustion products of DEGDN, TEGDN, or TMETN containing munitions under field use was not available. However, theoretical studies of the mechanisms of combustion of DEGDN, TEGDN, and some other polyol nitrates under various conditions of temperature and pressure have been done by Lur'e and Svetlov (1963, 1967, 1968, 1974). Free radicals are prominent in the reaction chains (free radicals have also been implicated by Salter and Thrush, 1981), peroxides may be formed, and nitrogen oxides, although they exist transitorily, hardly appear in the products, their low levels and even total absence being due to the fact that the oxidation process of decomposition is considerably faster than the primary splitting off of the NO_2 (Svetlov, 1963). This is in contrast with the behavior of NG, EGDN, and PETN (pentaerythritol tetranitrate) where the rate differences are less dramatic. Andreev and Plyasunov (1963) found that a number of secondary explosives produced some NO; the composition of products from DEGDN was found to be: carbon dioxide (CO_2), 9.4%; carbon monoxide (CO), 45.3%; nitric oxide (NO), 30.1%; hydrogen (H_2), 10.9%; methane (CH_4), 2.7%; and nitrogen (N_2), 1.5%. This combustion was, however, at atmospheric pressure. Boschan, Merrow, and Van Dolah (1955) give NO_2 and NO as intermediates in the explosive decomposition of nitrate esters, these compounds then being used up in the oxidation of other components of the explosive formulation.

Lindner (1980) gives the major combustion products and their amounts for a number of munitions formulations. These range, in moles/100 g, as follows: CO, 1.13 to 2.33; CO_2, 0.14 to 0.74; H_2, 0.15 to 0.88; H_2O, 0.07 to 1.10; and N_2, 0.44 to 1.30. The relatively high concentration

TABLE 1. CHEMICAL AND PHYSICAL CHARACTERISTICS OF NG, DIGDN, TEGDN, AND TMETN[a]

Characteristic	NG	DEGDN	TEGDN	TMETN
Molecular weight	227	196	240	255
Color	Pale yellow	Colorless		
Density, g/cm^3	1.59	1.38	1.32[b]	1.47
Vapor specific gravity relative to air[c]	7.9	6.8	8.3	7.8
Crystal form	Rhombic			
Melting point, °C	13.2	-11.3		-3
Hygroscopicity, % at 90% rh, 20°C	0.06			0.07
Solubility in water at 20°C, g/100 g	0.2	0.4		0.05
Oxygen balance, % to CO_2	3.5	-41	-53[c]	-35
Viscosity at 20°C, cP	36	8[d]		156
Vapor pressure, µm Hg	1.8[d]	5.9[d]	0.188[e]	0.183[f]
Heat of formation, kJ/g[g]	1.63	2.7		1.60
Heat of combustion, kJ/g[g]	6.80	11.68		11.05
Heat of detonation, kJ/g[g]	6.29	4.86		5.17
Heat of vaporization, kJ/g[g]			0.37 ± 0.18[h]	0.35 ± .018[h]
Gas volume at STP, cm^3/g	715	880		855
Detonation velocity, km/s	7.60	6.75		7.05
Vacuum stability at 100°C, mL gas/g per 40 h at STP	Explode	<1		1-3
Weight loss at 100°C, %	3-5			1-3
Explosion temperature at 5s, °C	220 (dec) 240	240		235
Effect of prolonged storage	dec 3-4 d at 75°			
Relative impact test value, % TNT	15	100		45
Friction pendulum	Explode	Explode		
Relative energy output, % TNT, lead block	180	150		140
Refractive index			1.45[i]	1.47[b]

[a]Data from Lindner (1980) except as noted.
[b]Woodman and Adicoff (1963). At 25°C.
[c]Calculated.
[d]At 25°C.
[e]At 30.27°C.
[f]At 26.51°C.
[g]To convert J to cal, divide by 4.184.
[h]Woodman and Adicoff (1963).
[i]Woodman and Adicoff (1963). At 24.5°C.

TABLE 2. VAPOR PRESSURE-TEMPERATURE DATA FOR NG, EGDN, DEGDN, TEGDN, AND TMETN. PRESSURE IN μm Hg

Temperature, °C	NG	Temperature, °C	EGDN	Temperature, °C	DEGDN	Temperature, °C	TEGDN	Temperature, °C	TMETN
15.00	1.28	15.00	23.18	15.00	2.25	30.27	0.188	26.51	0.183
25.00	1.80	25.00	70.43	25.00	5.93	37.30	0.407	29.78	0.268
35.00	4.50	35.00	218.25	35.00	14.78	43.88	0.838	30.91	0.305
45.00	12.9	45.00	447.75	45.00	28.35	50.55	1.675	35.46	0.503
55.00	35.78	55.00	960	55.00	86.55	55.69	2.797	40.25	0.863
						60.44	4.450	46.16	1.623
						65.57	7.166	50.78	2.616
						70.29	11.06	56.25	4.494
						74.89	16.55	64.04	9.370
								67.08	12.35
								70.26	16.59
								72.15	19.46

Source: Adapted from Lindner (1980) and Woodman and Adicoff (1963).

of carbon monoxide is noteworthy. Since the composition(s) of the explosive formulation(s) to be used in the M-1 tank are not given, it is not possible to match products with products of some of the formulations given by Lindner.

Mechanisms of combustion, giving theoretical insight into the formation of combustion products, have been studied by numerous authors. The burning of explosives has classically been considered to go through three stages (Parr and Crawford, 1950; Lindner, 1980). These stages are (a) a primary solid-phase decomposition giving organic fragments and nitrogen dioxide, (b) nonluminous gas-phase reactions of the products of (a) to give nitric oxide and aldehydic molecules, and (c) luminous gas-phase reactions of the products of (b) to give the final products. These stages are called the foam, fizz, and flame reactions, respectively. Dauerman and Tajima (1968) present a more elaborate scheme for the stages of the progression of combustion events, namely solid, foam, fizz, dark, explosion, and flame stages or zones. They also present evidence that NO_3, not NO_2, is the primary oxide of nitrogen evolved.

All authors are in accord in mentioning the presence of aldehydic compounds, some even attributing their formation to stage (a). These compounds are so reactive, and the conditions of burning so intense, that it does not seem likely any aldehydic compounds would be present in the final product mix. DEGDN is an explosive that melts and burns during heating (Babaitsev and Kondrikov, 1967). The combustion process is accompanied by violent decomposition of the surface of the powder grains. The rate-determining step in the burning of the propellant is the decomposition of the molecules at the surface (Wilfong, Penner, and Daniels, 1950), fragments of NO_2 and organic radicals being thrown out from the surface and then reacting in the gas phase (Stages a and b); the primary event is the breaking of the O-N bond of the linkage $-ONO_2$, this bond being weaker than C-H, C-C, C-O, and N=O bonds. See, however, Dauerman and Tajima (1968), for evidence of the primary production of NO_3, not NO_2. Dauerman and Tajima have determined the products evolved from the decomposition of two double-base explosives termed D-4 and D-6 in a research situation where decomposition was initiated by radiation from an arc-image furnace. Table 3 gives a comparison of products expected based on the literature and potential products based upon the mass spectra obtained in the study. Compounds of concern which can be mentioned are: nitric oxide (NO); nitrogen dioxide (NO_2); carbon monoxide (CO); formaldehyde (HCHO); formic acid (HCOOH); acetaldehyde (CH_3CHO); nitrous oxide (N_2O); acetylene (C_2H_2); cyanogen (CN_2); and hydrogen cyanide (HCN). In the second half of the table, one notes a number of the compounds already named, plus formic and acetic free radicals. The compounds listed, assuming that they actually would be present in the combustion gases from the M-1 munition, could be of concern because of their reactivity and potentially irritating nature.

Some propellants have metal powders (e.g., aluminum; Griffith, 1971; Lindner, 1980) added for extra explosive power. Ballistic modifiers are

TABLE 3. COMPARISON OF COMBUSTION PRODUCTS EXPECTED BASED ON THE LITERATURE AND POTENTIAL PRODUCTS BASED UPON MASS SPECTRA OBTAINED[a]

A. Expected Products Based on Literature			
Major (over 10%)		Minor (1 to 10%)	Trace (less than 1%)
NO, nitric oxide	H_2O, water	N_2O, nitrous oxide	$(CN)_2$, cyanogen
NO_2, nitrogen dioxide	HCHO, formaldehyde	N_2, nitrogen	HCN, hydrogen cyanide
CO, carbon monoxide	HCOOH, formic acid	CH_4, methane	
CO_2, carbon dioxide		C_2H_2, acetylene	$(CHO)_2$, glyoxal
CH_3CHO, acetaldehyde		H_2, hydrogen	
B. Potential Products Based on Spectra[b]			
Triacetin	$CH_3CO\bullet$, acetic free radical	CO_2, carbon dioxide	NO_3, nitrogen trioxide
C_2H_5OH, ethanol	HCOOH, formic acid	CO, carbon monoxide	NO_2, nitrogen dioxide
CH_3OH, methanol	HCHO, formaldehyde	O_2, oxygen	NO, nitric oxide
CH_3CHO, acetaldehyde	$HCO\bullet$, formic free radical	H_2O, water	
CH_4, methane		HCN, hydrogen cyanide	

[a] Adapted from Dauerman and Tajima (1968).
[b] Amounts not specifically stated.

also added. These may be lead or copper organic chelates, for instance, copper resorcylate (Camp, Csanady, and Mosher, 1980). The use of copper resorcylate eliminates the need for known modifiers which are efficient but contain environmentally objectionable lead. The presence of modifiers changes the dynamics of the decomposition of the explosive. Thus Androsov, Denisyuk, and Tokarev (1978) found, in studying the effects of PbO_2, CuO, and CuO-PbO as additives on the combustion of a propellant containing nitrocellulose and DEGDN, that CuO accelerates the oxidation of intermediate decomposition products with nitrogen oxides. The addition of lead oxides results in an increase in N_2O and CO_2, a decrease in CO, and appearance of H_2. In this way the concentration of the most active oxidizer (N_2O) increases and the possibility of poisoning the CuO catalyst with CO diminishes. In the light of the above, the presence of a modifier in the M-1 propellant could affect the nature and amounts of specific combustion products.

The igniter is part of the munition. Black powder is commonly used in igniter formulations. Table 4, adapted from Lindner (1980) gives the approximate composition of reaction products of black powder.

Table 5 gives some of the principal physical and chemical characteristics of carbon monoxide (CO) and nitric oxide (NO), the combustion products that probably are of most concern among those specifically identified from combustion of DEGDN in research situations.

TABLE 4. APPROXIMATE COMPOSITION OF REACTION PRODUCTS OF BLACK POWDER

Component: Gases	Wt %	Component: Solids	Wt %
Carbon dioxide	49	Potassium carbonate	61
Carbon monoxide	12	Potassium sulfate	15
Nitrogen	33	Potassium sulfide	14.3
Hydrogen sulfide	2.5	Potassium thiocyanate	0.2
Methane	0.5	Potassium nitrate	0.3
Water	1	Ammonium carbonate	0.1
Hydrogen	2	Sulfur	9
		Carbon	0.1
Total	44	Total	56

Source: Adapted from Lindner (1980).

TABLE 5. PHYSICAL AND CHEMICAL PROPERTIES OF CARBON MONOXIDE AND NITRIC OXIDE

	Carbon Monoxide	Nitric Oxide
Molecular weight	28.01	30.01
Density	1.250^{0} g/L; liquid 0.793 g/mL	1.3402 g/L; liquid $1.269^{-150.2}$ g/mL
Melting point (°C)	-199	-163.6
Boiling point (°C)	-191.5	-151.8
Solubility (cm^3/100 cm^3)	3.5 at 0°C, in water 2.32 at 20°C, in water soluble in alcohol benzene, acetic acid	7.34 at 0°C, in water 2.37 at 60°C, in water 3.4 in H_2SO_4, 26.6 in alcohol; soluble in CS_2

Source: Weast and Astle (1981-1982).

2.3 DETERMINATION AND ANALYSIS OF EXPLOSIVES AND THEIR COMBUSTION PRODUCTS

2.3.1. Determination of Combustion Products and Combustion Events

Because of the extreme conditions involved, determining the progression of events of explosive decomposition has been a difficult exercise. Optical methods — ultraviolet, visible, infrared (Levy, 1954; Lindner, 1980) have been largely used, under controlled pressure conditions or in reaction vessels. Temperature profiles have been obtained by the use of thermocouples (Goodman, Gray, and Jones, 1972; Kubota et al., 1974) or by measurements of flame intensities (Salter and Thrush, 1981). White and Reynolds (1975) describe the development of an apparatus involving a molecular beam sampling system with a time-of-flight mass spectrometer used as a detector for detecting interior ballistic combustion products. Trapping methods have been developed by Burns (1962), and the products studied by infrared spectroscopy, gas-solid partition chromatography, and mass spectroscopy.

2.3.2. Analysis of Explosives and of Combustion Products

Gas-liquid chromatographic determination of nitrate esters, stabilizers, and plasticizers was described by Alley and Dykes (1972). Earlier chromatographic (column) methods were described by Schroeder

(1948) and by Ovenston (1949). Asselin (1978) has described the development of instrumentation for the detection of EGDN vapor by plasma chromatography. Bandelin and Pankratz (1958) applied the colorimetric ferrous sulfate method, specific for the $-ONO_2$ grouping, to the determination of a number of organic nitro compounds. Camera and Pravisani (1964) have described a gas chromatographic system for the analysis of polyol polynitrates, including DEGDN and TEGDN, and have applied this to the analysis of a number of nitrates used in the explosives industry (Camera, Pravisani, and Ohman, 1965; Camera and Pravisani, 1967). A feature of their protocol is the low temperature at which the columns are run, to minimize decomposition of the compounds during analysis. Den Hartog and Shafer (1967) used thin-layer chromatography (TLC) and infrared spectroscopy for analysis of propellant mixtures. Pinchas (1951) also analyzed DEGDN by infrared spectroscopy. TLC was used by Elie-Calmet and Forestier (1979) for characterization of explosive traces following an explosion, and was used by Rao, Bhalla, and Sinha (1964) and by Parihar, Sharma, and Verma (1967) for analysis of explosive components. Evendijk (1968) used two-dimensional TLC for separation and identification of 18 components encountered in explosive powder mixes. DEGDN and TEGDN were among them. DEGDN and other components of explosives were analyzed by Fariwar-Mohseni, Ripper, and Habermann (1979) by high-pressure liquid chromatography (HPLC) on columns of silica gel, this method avoiding the destruction of nitro compounds resulting from the temperature programming of gas chromatography. HPLC was also used by Juhasz and Dowli (1977) for analysis of components in U.S. single-, double-, and triple-base gun propellants. Vich, Churacek, and Kucera (1970) compared paper chromatography, TLC, and spectrophotometry for analysis of nitric esters, including DEGDN. Rogers, Yasuda, and Zinn (1960) used pyrolysis curves for identification and quantification of explosives and their decomposition products. Rucci et al. (1975) used a chemical-potentiometric technique for the analysis of DEGDN, and Sarson (1958) described an antomated differential potentiometric technique combined with nonaqueous titration for analysis of explosive mixtures.

2.3.3. Monitoring Techniques

In contrast to the extensive literature on the analysis of components of explosives, the literature on monitoring methods is sparse, although some of the methods described in Section 2.3.2 would be applicable. Some methods more specifically aimed at monitoring are described in this section. The NIOSH document on criteria for a recommended standard on occupational exposure to nitroglycerin and ethyleneglycol dinitrate (NIOSH, 1978) describes monitoring practices for NG and EGDN which should be applicable to monitoring for DEGDN, TEGDN, and TMETN also.

It is mentioned that early battery-operated sampling pumps were considered potential detonation hazards because of their electrical wiring and exposed metal surfaces. A compressed gas-driven (fluorocarbon) ejector-aspirator apparatus was used but was erratic in performance and is no longer commercially available. Battery-operated personal

sampling pumps that can be used safely are now available. Bubblers and impingers and adsorption on Tenax-GC resin are used in collecting the vapors. Generation of static electricity is avoided. Following collection of the vapors, a number of techniques, including several of those described in Section 2.3.2, are used for analysis. Direct-reading color reaction tubes for on-the-spot monitoring are also used. One such device is the U.S. Navy's "Otto Fuel Detector, Mk-15", developed for monitoring propyleneglycol dinitrate (PGDN), a chief component of several torpedo propellants. Fluorescent spot tests are described, as is a portable gas chromatograph and a polarographic detector-monitor.

The sensitivity of dogs for detection of nitrate esters was tested by Gage and Wall (1974). Trained dogs gave a result of 10 correct responses in 18 tests of EGDN at 100 ppb, and 7 correct responses of 12 tests of negative samples. Some positive results were obtained at the 40 ppb level.

Detection of explosive vapors using thin metallic films as sensors was described by Lootens (1973). Metal oxide gas sensors were coated with platinum, gold, or chromium, or left uncoated, and changes in the surface resistance were measured when the sensors were exposed. EGDN and NG were detected at a level of 1 ppm in ambient air.

A dual-channel bioluminescent sensor was evaluated by Wall and Gage (1974). In this apparatus air is sampled and passed through a cartridge containing bacteria which luminesce, and the fluctuations in luminescence are recorded. Response signatures were obtained for EGDN and some other substances. Detection of EGDN at a level of 26 ppb was demonstrated, and of TNT at 65 ppb.

The two most effective methods examined by NIOSH (1978) for collecting samples of airborne NG and EGDN have been the use of midget impingers or bubblers and the use of a Tenax-GC (porous polymer material based on 2,6-diphenyl-p-phenylene oxide) resin adsorption tube. The bubblers show nearly 100% collecting efficiency at a sampling rate of 0.5 to 1.0 L/min. In potentially explosive environments, the glass impinger units can be enclosed in cardboard containers with cellophane windows, to avoid a static electricity detonation hazard. The Tenax resin tube, combined with a nonsparking sampling pump, is used as a personal sampling device. Analysis may be by several methods, but usually by gas chromatography following elution of the adsorbed nitrate ester(s). By gas chromatography, amounts of the order of 0.25 ng (for EGDN) and 2.5 ng (for NG) can be analyzed. For on-the-spot tests, the direct-reading color reaction tube mentioned above can provide approximate measurements of NG at concentrations as low as 1.5 ppm and of EGDN as 0.25 ppm. Figures for the response of the various monitoring devices for DEGDN, TEGDN, and TMETN, and the applicability of the devices for monitoring of these compounds, remain to be determined.

2.3.4. Monitoring of Workers

Nitrite and nitrate are formed in the body from organic nitrate esters. Litchfield (1967) has described a method for the automated analysis of nitrite and nitrate in blood which uses the Technicon Auto-Analyzer sampling, manifold, reaction, and readout systems. A dialysis unit separates the nitrite and nitrate from blood protein. The sample is then diazotized (nitrate is reduced to nitrite by a zinc column), then coupled with reagent for color development and readout. Recovery from blood is reproducible and almost 100%. The limit of detection is 0.1 μg/mL for nitrite and 0.2 μg/mL for nitrate, from 1 mL blood samples.

A method for determination of nitrates in the urine as an exposure test in work with DEGDN was described by Vasak (1965). Nitration of 1,2,4-xylenol with nitrate in sulfuric acid yields a deep yellow nitro derivative which is determined colorimetrically at 450 nm after distillation or extraction with an organic solvent. The examination of 250 employees in a plant producing DEGDN was performed, and 200 mg nitrate/24 hr was taken as a statistically significant proof of exposure.

Morikawa et al. (1967) have recommended the use of pulse-wave monitoring (plethysmography), examination of the pulse wave being made on the tip of the index finger, as a means of monitoring workers for exposure to organic nitrates. Not only does this measurement pick up the diastolic and systolic pressures, but the pulse wave also shows changes in profile associated with exposure. Characteristic anomalies were noted at levels (of ethylene nitrate) in a plant where the highest average concentration in the workshops did not exceed 0.066 ppm.

3. ENVIRONMENTAL ASPECTS

Although Krasovsky, Korolev, and Shigan (1973) reported DEGDN to be relatively stable in reservoir waters, the ester structure and the formation of alcohol functions on denitration suggests that organic nitrate esters should be biodegradable, and this was shown to be true in a study by Cornell et al. (1981) of the biodegradation of nitrate esters used as military propellants. These authors, in connection with a study directed toward the elimination of NG from waste streams by microbial action, investigated the biodegradation of the four esters PGDN, DEGDN, TEGDN, and TMETN. The authors' organization, the U.S. Army Natick Laboratories, had demonstrated the basic feasibility of elimination of NG from simulated waste waters on a bench scale using an activated sludge system under aerobic conditions. It was of great interest to determine whether the proposed biological treatment for NG would also be effective in eliminating the four esters without essential modification in operating conditions. When exposed to activated sludge inocula, mineral salts, and ethanol as an additional carbon source, the four esters were found to undergo sequential hydrolytic cleavage of nitrate groups under aerobic

batch or continuous fermentation, with the concentrations of the nitrate esters being reduced below detectable limits by choice of retention time. A program to obtain bench scale data for development of a microbiological treatment process for waste waters containing nitrate ester propellants was recommended.

In a parallel study, Kaplan, Walsh, and Kaplan (1981) studied the degradation of the glycols formed from nitrate ester propellants. The authors give the history of elimination of glycols, whether by biological or chemical means, and then describe a system using careful concentration of the glycols followed by derivatization and gas chromatography for the determination of glycols in aqueous systems in the low ppm range. Propylene glycol (PG), diethylene glycol (DEG), triethylene glycol (TEG), and trimethylolethane glycol (TMEG), which are produced by the microbiological denitration of the corresponding ester propellants, were found to be degraded by microbiological and/or chemical/physical factors. The relative rates of disappearance were PG > DEG > TEG > TMEG. TMEG was tested for mutagenicity in the Ames tests, with and without metabolic activation, and was found to be negative, and in general there seemed to be a minimum of toxicological hazards associated with the compounds.

Krasovsky, Korolev, and Shigan (1973) have evaluated the hygienic status and hazard potential of DEGDN in connection with its standardization in water reservoirs. DEGDN was found to give the water a bitter-astringent taste, intensity 1 ball, at a concentration of 130 mg/L. At $\leq$ 10 mg/L, DEGDN did not influence the biochemical oxygen uptake, the processes of mineralization of organic impurities, or the dynamics of growth of saprophytic microflora. DEGDN was found to be a compound with moderate toxicity and a medium-degree accumulative tendency. The authors concluded that DEGDN is capable of changing the organoleptic properties of water in concentrations above 100 mg/L; that in concentrations up to 10 mg/L, it has no detectable effect on the sanitary regimen of water reservoirs; that it is a moderately toxic substance with a medium tendency to accumulate in animal tissues; and that the maximum permissible level of DEGDN in the water of reservoirs should be set at 1 mg/L.

The state-of-the-art of the military explosives and propellants production industry is examined by Patterson et al. (1976) in a three-volume work, Volume I describing the industry, as well as the production processes and technology, Volume II detailing the wastewater effluents of manufacture and Load-Assembly-Pack operations by product, process, and military installation, to the extent of availability of the data, and Volume III describing and evaluating the effectiveness of various treatment technologies of water pollution abatement in the industry. While DEGDN, TEGDN, and TMETN are not specifically treated, a great number of the conclusions in the report apply to them as well as to the munitions which are treated.

4. BIOLOGICAL EFFECTS SUMMARY

Appendix B summarizes in tabular form the health effects of exposure of humans and animals to DEGDN, TEGDN, and TMETN. Discussion of these effects follows below.

4.1 EFFECTS IN LABORATORY ANIMALS

4.1.1. Immediate and Delayed Effects

The LD_{50} of DEGDN has been found to be similar in several species. As studied by Krasovsky, Korolev, and Shigan (1973), the oral LD_{50} of DEGDN in white mice was 1250 mg/kg; in white rats 1180 mg/kg; and in guinea pigs 1250 mg/kg. In all these species, acute poisoning was characterized by cyanosis (blockage of cellular respiration by massive formation of methemoglobin) and by symptoms of damage to the central nervous system. In tests with rabbits Krasovsky and coworkers also noted a decrease in blood pressure after iv injection of 0.4 mg/kg DEGDN. NIOSH (1979a) gives an oral LD_{50} of DEGDN in rats of 777 mg/kg.

The 24 hr intraperitoneal LD_{50} of TEGDN in rats as determined by Andersen, Koppenhaver, and Jenkins, (1976) was 995 (932-1063, 95% confidence limits) mg/kg. However, in rats anesthetized with pentobarbital, it was only 321 (170-605, 95% confidence limits) mg/kg. Significant increases in pentobarbital sleep times were observed in rats pretreated with 312 mg/kg/day for 4 days before testing. Higher doses ameliorated Metrazole toxicity and increased zoxazolamine paralysis times. In vivo, severe tremors and clonic convulsions occurred, and in vitro the compound selectively blocked nerve-stimulated contractions of a cholinergic rat phrenic nerve diaphragm preparation. Thus, as well as causing formation of methemoglobin resulting in death, TEGDN also acted in a way suggesting blocking of cholinergic transmission in the peripheral nerve system. Doses of TEGDN causing these effects were at the level of 50-75 mg/kg for the drug studies, 312-600 mg/kg for the pretreatment study, and 2.4-4.8 mM for the in vitro studies. The level of plasma TEGDN in the rat following ip administration of TEGDN at a level of 1000 mg/kg was 0.84 ± 0.09 mM at first convulsion and 1.23 ± 0.08 mM in extremis, and in the brain 163 ± 21 µg/g tissue at first convulsion and 315 ± 12 µg/g tissue in extremis. The oral LD_{50} of TEDGN in rats is listed by NIOSH (1979b) as 1000 mg/kg.

Andersen and Mehl (1973) determined the 24 hr LD_{50} of TEGDN in mice, rats, and guinea pigs. When given intraperitoneally TEGDN was most toxic to guinea pigs (LD_{50} of 700 mg/kg) and least toxic to mice (LD_{50} of 945 mg/kg). In rats, TEGDN was least toxic when administered subcutaneously (LD_{50} of 2520 mg/kg) and most toxic when given intraperitoneally (LD_{50} of 796 mg/kg). Toxic signs included ataxia, lethargy, respiratory depression, tremors, and hyperreaction to auditory and tactile stimuli. The activities of several enzymes in the plasma of

rats were determined at 24 hr intervals over a 168 hr period in animals that had survived the intraperitoneal administration of the calculated LD_{50} dose. Long-lasting increases were found in the activities of alkaline phosphatase, creatinine kinase, lactic dehydrogenase, and aspartate aminotransferase, and measurements of lactic dehydrogenase isoenzyme distributions in the plasma and homogenates of rat brain and spinal cord showed increases in these enzymes 24 hr after exposure to TEGDN.

Andersen and Mehl (1973) also found that injection of small doses (2.4 mg/kg) of TEGDN into anesthetized rats resulted in the immediate lowering of blood pressure (41 mm Hg reduction) followed by a fairly rapid recovery. Dermal application of 21 mmole/kg TEGDN daily to 11 rabbits for 3 weeks resulted in the deaths of 9 of the 11 rabbits, the mean time to death being 17 days. There was a 20% weight loss in the rabbits during the exposure period. In a parallel experiment guinea pigs exposed to TEGDN (100-400 mg/kg) lost weight and showed reduced food consumption.

Vasodilation and changes in hemodynamics are characteristic of exposure to organic nitrate esters (Carmichael and Lieben, 1963; Clark and Litchfield, 1969; Needleman, 1976). Valachovic (1965) attempted to determine if any of these effects is due to changes in the heart itself by studying the effect of DEGDN on the work output of the heart muscles of the dog in an acute exposure experiment. A heart-lung machine was used to supply controlled amounts of blood and air to the dogs (23 dogs of both sexes weighing 14-16 kg). The greater circulatory system of the dogs was replaced by an adjustable resistance system with a device for measuring the volume of the blood pumped. The lesser circulatory system was retained in order to oxygenate the blood, but the air volume for respiration was predetermined and maintained by a mechanical pump. Following calibration of the system and establishment of baseline values, DEGDN in distilled water was added at a rate of 6 mg/kg body weight and heart output was measured at 6 resistance and 2 height settings. Results showed no significant differences between baseline and treatment values. It was concluded that DEGDN did not directly affect the heart muscle metabolism or its output, nor was the lumen of the cardiac arteries altered, especially not vasoconstricted; and it was suggested that any stenocardiac difficulties associated with exposure to DEGDN result from a secondary effect of DEGDN which causes cardiac muscle hyperemia.

No delayed effects (i.e., effects which show up days or weeks following administration of a single dose or a regimen of doses) as a result of exposure to DEGDN, TEGDN, and TMETN were identified. However, there are effects which occur on interruption of accommodation to exposure to nitrates, which will be discussed under Human Effects.

4.1.2 Reversible and Irreversible Effects

The effects of exposure to DEGDN, TEGDN, and TMETN seem to be largely reversible. In the case of acute doses with resulting massive

formation of methemoglobin, even though this formation can be reversed by the action of the animal body's methemoglobin reductase system, time does not permit this and death ensues from respiratory failure. With lower doses, there is a balance, and one of the features of chronic exposure to nitrates is a compensated anemia (Krasovsky, Korolev, and Shigan, 1973). In studies by these authors, cardiovascular and central nervous system symptoms were more evident in long-term treatment with small doses of DEGDN than was methemoglobinemia.

Other effects, which may be considered as cumulative or quasi-irreversible have also been described by Krasovsky, Korolev, and Shigan (1973). White male rats were administered DEGDN in doses of 0.05, 0.5, and 5 mg/kg/day for a period of 6 months. The indices studied were as follows: conditioned reflex activity; blood cholinesterase activity; erythrocyte, leukocyte, and reticulocyte counts; hemoglobin and methemoglobin amounts; diameter of erythrocytes; blood pressure; 17-ketosteroids in the urine; organ weight; kidney handling of sodium sulfobromophthalein; ascorbic acid content of some organs; and level of thiol groups in liver homogenates. Immunobiological and pathomorphological studies were also done. The experiment showed that on intoxication with DEGDN doses of 5 and 0.5 mg/kg/day there were changes in the conditioned reflex activity and the immunobiological condition of the animals (details not given). A dose of 5 mg/kg/day provoked a certain decrease in blood pressure by the 5th to the 6th month and also a change in the mitotic activity of the bone marrow. No substantial changes were found in the other indices. The authors considered 0.5 mg/kg/day to be the minimum effective dose and 0.05 mg/kg/day to be the noneffective dose. On the basis that the earliest and most substantial changes were observed in the conditioned reflex activity of the animals, the authors consider that DEGDN, like a number of other nitro compounds, has the capacity of influencing the central nervous system in very small doses under the conditions of prolonged intoxication.

An effect which might show some persistence is the acquired tolerance to the vasodilating effect of organic nitrates. This was studied with nitroglycerin by Needleman and Johnson, Jr. (1973), but since the nitrates all possess oxidizing ability, this effect likely exists with them also; and in fact, Needleman and Johnson observed cross-tolerance on the part of a number of vasodilating nitrates. It was proposed that the tolerance involves the oxidation of a critical sulfhydryl of a cysteine residue in a "receptor," the disulfide form of this vascular protein receptor having a lowered affinity for the nitrate(s). Proof of this was that the tolerance induced in vivo by chronic administration of a nitrate could be reversed by treatment with sulfhydryl reagents such as dithiothreitol.

In general, while recovery from most symptoms of exposure to nitrates seems to occur, systematic studies on the recovery from effects of treatment with nitrates and especially with DEGDN, TEGDN, and TMETN under varying regimens have apparently not been conducted.

4.1.3. Behavioral and Neurotoxicological Effects of DEGDN, TEGDN, and TMETN

Mattson, Crock, and Jenkins (1977) studied the effects of NOSET-A on rhesus monkey visual evoked response and Sidman avoidance task. NOSET-A is a material which contains TEGDN (percent not quantified). A male rhesus monkey (*Macaca mulatta*) was exposed for 4 hr in a continuous-exposure inhalation chamber to a 2.4 ppm aerosol of NOSET-A on two separate occasions. The monkey was monitored for changes in electroencephalogram (EEG), and visual evoked response (VER) and Sidman avoidance task (free operant avoidance) data were collected after 2 hr and 4 hr of exposure. There were no significant changes in the EEG or any component of the VER but the Sidman avoidance response was significantly increased on both test days. The lack of an effect in the EEG or VER rules out any diffuse impairment of the central nervous system and indicates that the peripheral nervous system or sensory detection system might have been altered. The authors consider that the data indicate that NOSET-A has neurobehavioral effects of potentially serious consequences, and that further testing, such as measurement of peripheral nerve conduction velocities, and behavioral tests requiring a high degree of sensorimotor integration, should be conducted.

4.2. HUMAN EFFECTS

4.2.1. Immediate and Delayed Effects

Immediate effects of exposure to organic nitrates, including DEGDN and no doubt also TEGDN and TMETN, can include headache, vasodilation, lowered blood pressure, changes in pulse patterns, palpitation, nausea, and sometimes electroencephalographic changes and chest pain (Boschan, Merrow, and Van Dolah, 1955; Prerovska and Teisinger, 1965; Morikawa et al., 1967; Sax, 1975; ACGIH, 1980). The effects of DEGDN in causing headache and some other symptoms in subjects sensitive to nitroglycerin (NG) poisoning were found to be considerably less than in the case of NG, when tested by absorption through the skin (Rinkenbach, 1927). These symptoms can be compensated by the body and soon pass.

An effect which can be considered to be delayed is collapse on interuption of accommodation to organic nitrate esters. Deaths have resulted from this exposure regime; one reference in which DEGDN was implicated was Prerovska and Teisinger (1965).

The mechanism of this collapse, as studied in Sweden with workers in a plant producing EGDN (Forsmann et al., 1958; as cited in Carmichael and Lieben, 1973), seems to be that following the immediate and acute effect of lowering of both the systolic and diastolic blood pressure, continued exposure to organic nitrate esters produces a progressive rise in the diastolic blood pressure from the previously depressed levels without a comparable rise in the systolic pressure, the net result being a narrowing of the pulse pressure. As a result of this a myocardial

ischemia may ensue, which can give rise to an incident, or which, continued over a long time, may contribute to cardiac and circulatory insufficiency.

4.2.2. Reversible and Irreversible Effects

Exposure to organic nitrate esters is generally not considered to cause permanent effects (Sax, 1975; Lindner, 1980). However, the changes in hemodynamics mentioned in Section 4.2.1. could, it would seem, leave some sequelae. Thus, Carmichael and Lieben (1973) mention the hypothesis of Hueper (Hueper, 1941, 1953; as cited in Carmichael and Lieben, 1973) that exposure to vasodilating substances (e.g., organic nitrate esters) over long periods of time may compromise the nutrient arterioles of the walls of arteries causing the deposition of hyaline connective tissue, ultimately resulting in sclerosis of the coronaries indistinguishable at autopsy from ateriosclerosis from other causes.

A feature of poisoning with nitrate esters is formation of methemoglobin (Boschan, Merrow, and Van Dolah, 1955; Andersen and Mehl, 1973; Andersen and Smith, 1973; Krasovsky, Korolev, and Shigan, 1973). In contrast with poisoning by carbon monoxide where the carboxyhemoglobin formed is refractory to transformation and persists until replaced by fresh hemoglobin, methemoglobinemia can be reversed by the action of the body's methemoglobin reductase system; and in fact, this process is going on continually (White, Handler, and Smith, 1964). Thus the formation of methemoglobin caused by exposure to nitrate esters may be considered reversible, at least as long as it is within the body's capability to reduce the methemoglobin.

Prerovska and Teisinger (1965) have presented the clinical picture of chronic intoxication with DEGDN. Employees studied were in a plant producing and processing NG and DEGDN. Four sudden deaths had occurred in workers having had 5 to 7 yr of exposure to the compounds. These subjects showed, on necroscopy, mild to marked signs of coronary sclerosis but no signs of coronary occlusion. Of 45 employees, 37 reported precordial pain, headaches, and more rarely, collapse conditions with loss of consciousness. Three showed obvious signs of coronary sclerosis, 8 symptoms of intermediary coronary syndrome, and 1 myocardial infarction. In most subjects examined the cholesterol blood level was at the upper borderline of the normal range, i.e., 220 mg%, and in some it reached 300 mg%. After measures were taken to eliminate from the high-risk workplaces those employees with diseases of the cardiovascular system, liver, or kidneys, with any neural disorders, with ulcers, or with any disease causing general weakness, only specific subjective difficulties (e.g., intolerance towards alcohol or headaches on return from a holiday) were reported. It was recommended that persons with a disposition to atherosclerosis, elevated blood cholesterol, ocular fundus, or abnormal electrocardiogram be examined regularly.

Prerovska and Teisinger (1965) further state, that while in the cases of human exposure mentioned in the Swedish, Italian, or American literature the EGDN concentration together with that of NG did not exceed 5 mg/m^3, the exposure concentrations (presumably of DEGDN and NG) in the cases studied by them were much higher. Actual concentrations were not given. In another study by an investigator from the same clinic (Vasak, 1965), concentrations are again not stated, but it is mentioned that workers in the rolling mill part of the plant had higher nitrates in the urine than workers in the control part of the plant, and it is pointed out that the rolling mill workers would be more subjected to absorption of the nitrate esters through the skin.

A level of 5 mg/m^3 seems quite high. In a Japanese study, plethysmographic disturbances (pulse wave/volume changes in the peripheral blood) were noted in workers where the gas concentrations (of NG and ethylene nitrate) were in the range of 0.02 to 0.066 ppm, with a history of a few excursions over 0.5 ppm in the plant in question; and in the NIOSH (1978) publication on NG and EGDN, representative work station concentrations are generally below 0.2 or 0.3 ppm, sometimes as low as 0.02 ppm, with rare levels as high as 1-2 ppm.

4.2.3. Behavioral and Neurotoxicological Effects of DEGDN, TEGDN, and TMETN

No information was available to indicate that DEGDN, TEGDN, or TMETN cause behavioral and neurotoxicological effects in humans. Stewart et al. (1974), however, have done a study on the effects on human performance and physiology of exposure to vapors of PGDN (propyleneglycol dinitrate). This substance is used by the U.S. Navy as a torpedo propellant and is also used in other propellant/explosive formulations (Lindner, 1980). It resembles EGDN very closely in having its two nitro groups on adjacent $-CH_2O-$ groupings; and of the three compounds which are of concern in this report, perhaps resembles most closely TMETN which, although its nitro groups are not strictly on adjacent glycol groupings, has them in close proximity to each other. In the Stewart et al. study, human volunteers were exposed in a controlled-environment chamber to PGDN vapor at concentrations of 0.03 to 1.5 ppm. Exposure to concentrations of 0.2 ppm or greater produced disruption of the organization of the visual evoked response (VER) and headache in the majority of subjects. Subjects repeatedly exposed to 0.2 ppm for 8 hr on a daily basis developed a tolerance to the induction of headaches, but the alterations in VER morphology appeared cumulative. Marked impairment in balance became manifest after exposure to 0.5 ppm for 6.5 hr, while 40 min of exposure to 1.5 ppm added eye irritation to the list of symptoms. The impairment of the ability of the subjects exposed to 0.5 ppm for 6-8 hr to perform the heel-to-toe and modified Romberg test was similar to the incoordination of the same subjects tested in the same setting when intoxicated with ethyl alcohol at blood alcohol concentrations in the 100-150 mg/100 mL range. The authors consider that this disturbance of equilibrium and sense of balance presents a serious safety hazard.

Cognitive tests were performed, but only in the case of the Flanagan coordination test (arithmetic, coordination, inspection test) was a decrement noted, after 3 hr of exposure at a level of 1.5 ppm.

The exposures in the above study were not of sufficient magnitude to elevate the blood nitrate or methemoglobin concentrations above control values.

5. METABOLISM

As discussed by Needleman (1976), the metabolism of organic nitrate esters centers around an initial redox reaction – oxidation of reduced glutathione and reduction of nitrate to nitrite with concomitant denitration. The nitrite formed is converted by the action of catalase to nitrate, largely in the kidney, and excreted. The denitration of the parent di- or trinitrate may go only to the stage of removal of a first nitrate, and the denitration of subsequent nitrates may be a slower process. The effect of the denitration is to convert the potent lipo-soluble vasodilator intact nitrates into water-soluble metabolites which have a much lower biological potency and are readily excreted in the urine. Blood clearance data explicitly demonstrate that the parent nitrate ester (following intravenous administration) has a very transient lifetime, whereas the nitrate metabolites circulate for hours. The liver is the chief organ for degradation of the nitrates. Pathways for a number of organic nitrate esters are given by Needleman, but DEGDN, TEGDN, and TMETN were not studied. It is only mentioned that linear-chain polynitrate esters were rapidly transformed by rat-liver supernatant in the presence of GSH whereas branched-chain alcohol nitrates, e.g., TMETN, were only slowly transformed.

In a study of Needleman and Hunter, Jr. (1965) using a rat liver enzyme preparation, the biotransformation of NG was the primary focus of the investigation; however, some values were given for DEGDN, TEGDN, and TMETN. Compared to NG, which showed a V_{max} of 120 mmole/kg protein/min for denitration of an initial nitrate, DEGDN showed a V_{max} of 1.2, TEGDN of 1.7, and TMETN of 11.1 mmole/kg protein/min.

These values seem in conflict with the statement by Needleman (1976) concerning the slowness of transformation of branched-chain polynitrates as compared to linear-chain polynitrates. What is meant is in comparison with a compound such as mannitol polynitrate, which has a series of adjacent nitro groups; not necessarily TMETN compared with DEGDN or TEGDN, as it is seen by the V_{max} values given above that TMETN is more quickly transformed than DEGDN or TEGDN.

The oxidation of the iron of hemoglobin to the met state by organic nitrates seems to be nonenzymatic (Wilhelmi, 1942; Clark and Litchfield, 1969). This oxidation mechanism has been studied by Andersen and Smith

(1973) using rat and human blood. They found that oxygen, inhibitory at high concentration, must nonetheless be present for the reaction to occur, and it is deoxyhemoglobin, not oxyhemoglobin, which reacts. Oxidation by TEGDN was mentioned as being slower than oxidation by PGDN.

With both compounds tested in the study of Andersen and Smith (1973), only one of the nitro groups of either compound seemed reactive in the oxidation of the hemoglobin to methemoglobin. The stoichiometry of 1.5 hemes oxidized per ester bond broken indicated involvement of the nitrite produced by the oxidation. While the reaction is at first chemical, not enzymatic, it can be considered that hemoglobin in vivo together with the methemoglobin reductase system acts catalytically to metabolize the nitrates in question to nitrite (or nitrate), producing mononitrates which are further broken down, presumably by the the denitrifying enzymes found in various tissues (Heppel and Hilmoe, 1950; Needleman and Hunter, Jr., 1965). Andersen and Smith consider that for the toxic effects caused by intact dinitrates, hemoglobin would play an important role in detoxifying these compounds. Thus, Andersen and Smith state that it would be expected that humans, because of the slow rate of methemoglobin formation by TEGDN, would be less susceptible to methemoglobinemia from this dinitrate than is the rat (difference in rate of methemoglobin formation). However, humans would be more susceptible to TEGDN-produced nervous disorders which are apparently caused by the intact dinitrate (Andersen and Mehl, 1973).

6. DISCUSSION AND CONCLUSIONS

6.1. COMPOUND TOXICOLOGY

6.1.1. DEGDN, TEGDN, and TMETN

Very little information was available on the toxicology of DEGDN, TEGDN and especially TMETN. No information was available on the carcinogenic, mutagenic, or teratogenic potential of the three compounds. The available data suggest that the toxicology of these compounds is similar to that of other nitrate esters used in explosives, such as NG, EGDN, PGDN, etc.; the potential adverse health effects being generally, however, of lesser degree. Effects noted on exposure are headache, vasodilation, formation of methemoglobin, changes in behavioral reflexes, and impairment in balance and in performance of some tasks. A danger with any of the nitrate esters is collapse on withdrawal from exposure or on re-exposure following withdrawal. This effect is due to changes in hemodynamics (narrowed pulse pressure) resulting in ischemia of the heart muscles.

6.1.2. Combustion By-Products

Information documenting the combustion products formed from the munition(s) which will contain DEGDN, TEGDN, or TMETN, or in fact to

munitions which have contained them, was not available. However, some data was found on the combustion products from a number of other munitions formulations and ranges were given for the chief products (Lindner, 1980), and it is likely that the combustion from the munitions which will contain DEGDN, TEGDN, or TMETN will result in many of the same products and that the amounts will fall within the ranges given.

Carbon monoxide (CO) was identified as one of the major combustion products of explosives by Lindner (1980) and could be of concern. A voluminous literature exists on the effects of CO. One such effect described by Hosko (1970) is the effect of CO on the visual evoked response in man and the spontaneous electroencephalogram. Existing human data, cited by this author, suggests that exposure to very low concentrations of CO may exert a detrimental effect upon cerebral function, and studies with experimental animals have revealed that CO exposure can result in alteration of the visual cortical response evoked by strobe light stimulation of the eye. Alteration in this response is considered to be a sensitive means for detecting the central nervous system depressant effect of compounds. In the study, human volunteers were exposed to carbon monoxide at concentrations ranging from 1 to 1,000 ppm for 0.5 to 24 hr. Carboxyhemoglobin levels greater than 20% produced changes in the VER similar to those previously described in animals. The amplitude of the 2-3-4 wave complex of the VER profile was increased and was accompanied by a negative-going shift in the 5a-6 waves. Carboxyhemoglobin levels approaching 33% did not alter gross spontaneous electroencephalographic activity. The authors consider that the CO-induced negative-going shift involving late waves of the VER may be associated with general CNS depression, or it may represent a specific pharmacological action of CO.

With respect to the exposures that might occur in the munitions use situation, levels of 20% and 33% carboxyhemoglobin seem high. However, the author of the article just cited noted that the exposure levels used by him, 1 to 1000 ppm for periods of 0.25 to 24 hr, were designed to simulate those encountered in industry and in urban areas where CO is absorbed over a period of several hours. How close the concentrations in the tank would approach these is a matter for speculation, in the absence of data on the level of CO in the actual combustion products of the munition, the amount of air exchange, and other weapons system crew space conditions. The same may be said for other potentially hazardous combustion products or intermediates or unburned explosive components which might be present in trace amounts but which nonetheless could have an effect. Recent studies have been completed on five prominent munition combustion products and their potential health hazards during short-term, intermittent high concentration exposures typifying weapons firing as a unique military workplace exposure. These studies include evaluation of carbon monoxide (CO) (Nightingale, 1980), ammonia (NH_2) (Legters, 1980); nitrogen oxides (NO_x) (Morton, 1980), sulfur dioxide (SO_2) (Normandy, Szlyk, and Brienza, 1980), and hydrogen chloride (HCl) gas and mist (Cohen and Strange, 1982). Also included is a Summary Report of the

Biological Effects of Short, High-Level Exposure to Ammonia, Carbon Monoxide, Sulfur Dioxide and Nitrogen Oxides (Legters et al., 1980).

A question with the combustion of explosives is whether any nitrous/nitric oxides are produced or if these are consumed in the oxidation of the other components of the munitions formulation. While most studies (e.g., Wilfong, Penner, and Daniels, 1950) indicate that oxidation by NO_2 or NO_3 of the fuel components of the munition is faster than the release of the nitro entities, a research study of the decomposition of DEGDN (Lur'e and Svetlov, 1974) indicates that some nitrogen oxides may be formed. On the other hand, these same authors (Lur'e and Svetlov, 1967) present evidence showing that in the decomposition of several dinitrates, including DEGDN and TEGDN, NO_2 does not accumulate in the gaseous by-products, as it does in the case of NG. These studies were performed in the laboratory and therefore are only speculative concerning the actual composition of gases resulting from firing of the munitions containing DEGDN or TEGDN. Another research study (Dauerman and Tajima, 1968) gives a considerable number of products from the decomposition of a standard munition. Some of these, as discussed in Section 2.2 are highly reactive and potentially irritating, and even though present in only small amounts, could present problems. Odors, cordite headache, eye irritation, and other effects noted at firing ranges are clearly indicative of release of a number of physiologically active compounds in munitions firing. Clearly, monitoring studies are needed to determine the combustion products resulting from the firing of munitions containing DEGDN, TEGDN, or TMETN and especially to determine if any of the by-products are unique to munitions containing these compounds, and to account for this militarily unique short, high level episodic exposure environment such as in weapon system crew compartments.

6.2. INTERPRETATION OF EFFECTS MATRIX

6.2.1. Concentration and Concentration-Time Relationships

No information was available to determine possible concentration and concentration-time health effects relationships for DEGDN, TEGDN, or TMETN. It is known that methemoglobin is formed by the action of the nitrates; however, at industrial exposure levels, which are of the order of 0.02 to 0.2 ppm (as reported in NIOSH, 1978, for NG and EGDN), the amounts formed are sufficiently slight that the body's methemoglobin reductase can compensate for this production fairly well. At the concentrations mentioned, headache and vasodilation, lowering of blood pressure and perhaps nausea, may be evident as effects.

6.2.2. Dose-Response Relationships

Pharmacological responses to organic nitrate esters can occur at very low levels. Thus, a dose of 0.001 mL (1 μL) of NG in man can cause a severe headache (Rabinowitch, 1944; as cited in Carmichael and Lieben,

1973). It is also reported in Carmichael and Lieben (1973) that controlled absorption of fractional doses totaling 170 mg caused tolerance within 24-36 hr lasting 10-13 days. There was considerable variability among persons, however. Similar tests apparently have not been conducted with DEGDN, TEGDN, or TMETN. Rinkenbach (1927), however, who was the first to characterize DEGDN, tested its toxicity in guinea pigs. A dosage of 0.006 mg/kg animal weight, a dosage proportional to the standard dosage (of NG) prescribed for injection in humans, was injected intramuscularly. Although in some cases double this dosage was used, none of the guinea pigs showed untoward symptoms. To determine if DEGDN would, when absorbed through the skin, cause blood pressure changes and headache, a drop of the pure substance was placed on the forearm of each of three subjects, spread, and allowed to absorb. In no case were any unusual symptoms apparent, although each of these persons was sensitive to nitroglycerin poisoning and similar application of pure NG produced violent headaches.

The LD_{50} values of DEGDN, TEGDN, and TMETN, where they have been determined, are quite high (see Appendix B Tabular Summary). It may be noted, however, that in studies with animals anesthetized with pentobarbital (Andersen, Koppenhaver, and Jenkins, Jr., 1976) the LD_{50} of TEGDN was about 1/3 of what it would have been normally, suggesting neural involvement in the death syndrome along with the formation of methemoglobin, and also perhaps suggesting that persons on drugs should be particularly careful about exposure to organic nitrates.

In subchronic experiments with animals given DEGDN orally for 6 months, Krasovsky, Korolev, and Shigan (1973) found a no-effect level of 0.05 mg/kg/day and a minimum effect level of 0.5 mg/kg/day for the effects studied, which included neurological, behavioral, enzymatic, and other biochemical/physiological effects or indices. This article is analyzed in detail in Appendix A.

It may be noted that whereas the formation of methemoglobin increased more than proportionally with increasing dose of TEGDN (Andersen and Mehl, 1973), very likely due to the cooperativity of reaction of hemes, the blood-pressure lowering effect [of PGDN, but the effect would likely occur in a similar manner with other nitrates (Clark and Litchfield, 1969)] fell off with increasing dose. Blood levels of organic nitrates are what one would expect of a substance which is not stored in the body; i.e., the levels peak and then decline in a concave hyperbolic manner (Clark and Litchfield, 1969).

Exposure of a monkey for 4 hr to a level of 2.4 ppm TEGDN in a preparation called NOSET-A caused an increased response rate in an avoidance task but no change in the visual evoked response test. Neurobehavioral effects of potentially serious consequences were indicated.

A dose of 6 mg/kg of DEGDN in dogs gave no reduction of cardiac output, nor did it affect the lumen of the blood vessels or cause vasoconstriction. Thus the heart's metabolism seemed not to be affected, and it was suggested that difficulties arose rather from a secondary effect of DEGDN causing cardiac muscle hyperemia.

6.2.3. Human Performance Effects

No studies documenting the effects of humans exposed to DEGDN, TEGDN, or TMETN were available. The effects of TEGDN in the preparation called NOSET-A were studied in the rhesus monkey by Mattson, Crock, Jr., and Jenkins, Jr. (1977), and are discussed in Section 4.1.3. A study with human volunteers using PGDN conducted by Stewart et al. (1974) indicated that exposure to PGDN vapor at a concentration of 0.2 ppm for 4 hr produced disruption of the visual evoked response, and marked impairment of balance became manifest after exposure to 0.5 ppm for 6-8 hr.

6.2.4. Data Gaps

Toxicologic, health effects, and metabolism data gaps are numerous. Information documenting the carcinogenicity, teratogenicity, mutagenicity, eye and skin irritation, skin sensitization, non-oncogenic chronic toxicity, metabolism, inhalation toxicology, and dermal toxicology, with the exception of one study on TEGDN, was totally lacking. Levels of the release of the compounds during production, processing, transport, and use in the field are also not well documented. With respect to the munitions use situation, there is a total lack of data identifying the combustion products which will result from the firing of munitions containing DEGDN, TEGDN, or TMETN and on the levels of these by-products to which the weapons crews will actually be exposed. Finally, environmental quality effects information including transformation, transport, fate, and aquatic toxicology is almost totally absent.

6.3. MEDICAL RESEARCH NEEDS

Extensive medical research is needed to fill the data gaps outlined in Section 6.2.4. necessary to recommend scientifically based occupational health and environmental quality protection criteria for the production, use, and disposal of DEGDN, TEGDN, TMETN, and propellants containing these nitrate esters. This research must be supported by a thorough chemical/physical characterization of the materials in actual production and use; and a thorough evaluation of industrial and field use workplace exposure settings, and potential environmental emission forms and routes. Identification and quantification of the combustion products resulting from the firing of nitrate ester containing munitions are component needs to assess potential health hazards associated with this short duration, episodic and potentially high concentration exposure setting facing the field soldier during weapons use.

Monitoring techniques and methods that have been used for measuring levels of NG and EGDN in the industrial workplace, as discussed in Sections 2.3.3. and 2.3.4. can likely be applied to monitoring levels of DEGDN, TEGDN, and TMETN, but these methods require empirical validation. Nitrate ester based propellant combustion product testing may be initially performed using available thermodynamic equilibrium combustion models and controlled laboratory combustion simulation testing to qualitatively identify and quantitatively estimate the presence of potential toxic contaminant hazards during their use. This combustion product evaluation also requires field monitoring of contaminants during actual weapons firing to establish definitive field workplace exposure data on contaminants requiring health effects assessment. Based on technology limitations affecting field testing capabilities, development of monitoring techniques and instrumentation may be necessary for selected combustion products identified to represent potential health hazards.

Given a thorough characterization of the production, handling, and disposal workplace settings for these nitrate esters, health effects research requirements include the full range of interactive toxicologic studies outlined in Section 6.2.4. These sequenced studies should begin with mutagenicity and acute toxicity evaluation, and proceed through subchronic or repeated exposure toxicity studies, toxicokinesis and metabolism studies, and teratogenicity and reproductive effect studies; and finally include chronic toxicity and carcinogenicity evaluation, and additional studies addressing areas of observed health effect concern such as neurobehavioral performance effects.

Environmental quality research studies are necessary for evaluation of environmental transformation, transport, fate, and aquatic toxicity.

7. RECOMMENDED OCCUPATIONAL HEALTH PROTECTION CRITERIA

7.1. EXISTING HEALTH CRITERIA/STANDARDS

No health protection criteria or standards are available for DEGDN, TEGDN, or TMETN. Values listed for EGDN and NG in ACGIH (1980) are a TLV of 0.02 ppm (about 0.2 mg/m^3) and an STEL of 0.04 ppm (about 0.4 mg/m^3) for both EGDN and NG, reduced from a previous TLV of 0.2 ppm. The reduction by ACGIH is based on the differences in dose/effects manifestations noted on regular exposure where accommodation is a fact, and on intermittent exposure where effects appear at lower concentrations. The National Institute for Occupational Safety and Health in their 1978 criteria document (NIOSH 1978) recommended that exposure to NG, EGDN, or a mixture of both not exceed a ceiling value of 0.1 mg/m^3 (about 0.01 ppm) for any 20 minute sampling period. This value is lower than the TLV of 0.02 ppm proposed by ACGIH (as noted above) and was recommended because, in the opinion of NIOSH, it is necessary to prevent workers from experiencing vasodilation, as indicated by throbbing headache and/or

decreases in blood pressure. The present OSHA standard for both NG and EGDN is a ceiling limit of 2 mg/m^3 (about 0.2 ppm) and has a "skin" notation. These standards are based on TLV's for workplace exposure adopted by ACGIH in 1968 and are thus higher than either the current ACGIH TLV (ACGIH 1980) or the 1978 NIOSH recommendation (NIOSH 1978).

7.2. RECOMMENDED HEALTH CRITERIA

7.2.1. In the Industrial Workplace

The most cogent recommendation that can be made is that the TLV for the compounds be set at levels at which no significant pharmacological action occurs. This is the conclusion in the NIOSH (1978) criteria document that workplace exposure to nitroglycols should be controlled so that workers do not absorb amounts (of EGDN and NG combined, but the same could be said for DEGDN, TEGDN, and TMETN) that will cause vasodilation, as indicated by throbbing headaches or decreases in blood pressure, on either regular or intermittent exposure. The medical research requirements outlined in Section 6.3. must be fulfilled in order to base scientifically sound occupational health protection criteria for these compounds.

7.2.2. Short-Term High-Level Military Exposure Setting

Since it is not known exactly what the combustion products of munitions containing either DEGDN, TEGDN, or TMETN will be, it is not possible to recommend standards. Further research is necessary to characterize these combustion products with regard to their chemistry, and concentrationtime profiles of exposures from weapons firing. This information is essential in order to recommend health protection criteria for contaminant exposures in the unique short, episodic, high level military weapons system workplace setting.

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APPENDIX A

REVIEW OF KEY LITERATURE

Andersen M.E, R.E. Koppenhaver, and L.J. Jenkins, Jr. 1976. Some neurotoxic properties of triethylene glycol dinitrate: a comparison with decamethonium. Toxicol. Appl. Pharmacol. 36:585-594.

Review:

Andersen, Koppenhaver, and Jenkins (1976) investigated some of the neurotoxic properties of triethylene glycol dinitrate in NMRI rats. Intraperitoneal LD_{50} values were established for the compound; pentobarbital sleep time, zoxazolamine paralysis time, and Metrazol toxicity were determined after treatment with the compound; the effect of the compound in nerve-stimulated contraction of rat phrenic nerve diaphragm preparations was tested; and levels of triethylene glycol dinitrate in brain and plasma were measured.

All compounds were administered intraperitoneally, and the drugs were given so that peak plasma concentrations of triethylene glycol dinitrate would coincide with the peak physiological activity of the test compound. For the faster-acting compounds this was approximately 30 min after triethylene glycol dinitrate.

The 24 hr LD_{50} values and 95% confidence limits for rats, under various conditions, were as follows: 995 (932-1063) mg/kg body weight for rats housed in nonisolated (partitioned) cages; and 321 (170-605) mg/kg for pentobarbitol-anesthetized rats. The intoxicated rats displayed hyperactivity to both auditory and tactile stimuli; accompanied by tremors and severe clonic convulsions.

The sleeping time in pentobarbital-anesthetized rats was progressively increased by pretreatment with increasing concentrations of triethylene glycol dinitrate. The normal sleep time of 82 min was increased to 175 min with 250 mg/kg, which was 0.25 of the LD_{50}. Pretreatment with triethylene glycol dinitrate also increased the one-hr (but not the 24 hr) LD_{50} of Metrazol, a convulsant, and increased zoxazolamine paralysis times. These effects indicate depressant activity of triethylene glycol dinitrate on the central nervous system.

The ability of triethylene glycol dinitrate to interfere with nerve- and muscle-stimulated contraction of a phrenic nerve diaphragm preparation was compared to that of decamethonium, a muscle relaxant. Both compounds selectively blocked nerve-stimulated contractions of the preparations, decamethonium being the more potent; and when both were administered simultaneously the inhibition of force of contraction occurred more rapidly and was greater than that expected from either compound

applied alone. Triethylene glycol dinitrate had no effect on muscle-stimulated contractions. The authors concluded that both compounds acted at the same site in the peripheral nervous system to block cholinergic transmission.

Rats injected with 1000 mg/kg of triethylene glycol dinitrate were sacrificed either at the first sign of tremors or when moribund, and plasma and brain samples were analyzed for triethylene glycol dinitrate content. The plasma concentration of the moribund animals was 1.23 µg/g tissue, which corresponded with the concentration which interfered with the contraction of phrenic nerve diaphragm in vitro. This supports evidence that the triethylene glycol dinitrate-poisoned rats die from respiratory arrest, secondary to neuromuscular blockade. Formation of methemoglobin was only 35-45%, in contrast to the case with other nitrates, where the level is generally higher.

Analysis:

The experiment appeared to be conducted carefully and thoroughly with the proper application of statistics to the data, in spite of the small number of animals per group. The results of the study indicate that triethylene glycol dinitrate interferes with normal cholinergic transmission at the neuromuscular synapse but the site and mechanisms of action in the central nervous system were not identified.

Andersen M.E. and R.G. Mehl. 1973. A comparison of the toxicology of triethylene glycol dinitrate and propylene glycol dinitrate. Amer. Ind. Hyg. Assoc. J. 34:526-539.

Review:

A comparative toxicological study of triethylene glycol dinitrate (TEGDN) and propylene glycol dinitrate (PGDN) is described in which several species of animals were used including: NMRI:O (SD) Sprague-Dawley-derived rats; FTD:Hartley-derived guinea pigs; New Zealand albino rabbits; and NIH:NMRI mice. Twenty-four hour LD_{50}'s were determined for mice, guinea pigs, and rats in groups of six, with various routes of administration. PGDN was significantly more toxic than TEGDN in all cases except in mice, for which the toxicity of TEGDN was much greater. When given intraperitoneally, TEGDN was most toxic to guinea pigs (LD_{50} of 700 mg/kg) and least toxic to mice (LD_{50} of 945 mg/kg). In rats, TEGDN was least toxic when administered subcutaneously (LD_{50} of 2520 mg/kg) and most toxic when given intraperitoneally (LD_{50} of 796 mg/kg). PGDN given intraperitoneally was most toxic to guinea pigs (LD_{50} of 402 mg/kg) and least toxic to mice (LD_{50} of 1047 mg/kg). In rats, PGDN was most toxic when given orally (LD_{50} of 250 mg/kg) and least toxic when given subcutaneously (LD_{50} of 530 mg/kg). Toxic signs caused by PGDN included ataxia, lethargy, and respiratory depression. Toxic signs from

TEGDN included those caused by PGDN as well as violent tremors and hyperactive auditory and tactile stimuli.

Results from a determination of the effect of both compounds on the in vitro phrenic nerve-diaphragm preparation indicated that the tremors caused by TEGDN may have resulted from the compound's ability to interfere with nerve-muscle communication. Concentrations of 1.5 mM TEGDN blocked nerve stimulated contraction of the phrenic nerve-diaphragm preparation while greater than 4 mM PGDN was necessary to block the contraction.

Both compounds were found to produce metHb in rats in vivo, but PGDN was found to produce metHb at a much faster rate than TEGDN. The activities of several enzymes in the plasma of rats (six animals per group) were determined for both compounds at 24 hr intervals over a 168 hr period in animals that had survived the intraperitoneal administration of the amount calculated to be the LD_{50}. The activities of the following enzymes were measured: alkaline phosphatase (AP), creatine kinase (CK), lactic dehydrogenase (LD), and aspartate aminotransferase (AsT). TEGDN was found to cause long-lasting increases in all of these measured enzyme activities, while only moderate increases in CK and AP were observed after PGDN treatment. Measurements of LD isoenzyme distributions in the plasma and homogenates of rat brain and spinal cord showed increases in the enzymes 24 hr after exposure to TEGDN, while changes in the enzymes following PGDN exposure were not mentioned.

Injection of small doses (2.4 mg/kg) of TEGDN into anesthetized rats resulted in the immediate lowering of blood pressure (41 mm Hg reduction) followed by a fairly rapid recovery. Dermal application of 21 mmole/kg TEGDN and PGDN to groups of 11 rabbits for 3 weeks resulted in the deaths of 9 of 11 rabbits in the TEGDN group and 6 of 11 rabbits in the PGDN group. Mean times to death were 17 and 16 days for TEGDN and PGDN exposure groups, respectively. Rabbits of the PGDN group were found to gain weight (about 10% gain) during the exposure period while rabbits of the TEGDN group were found to lose weight (about 20% loss). A continuation of this experiment using guinea pigs and exposing them to TEGDN showed that food consumption was reduced and that the animals lost weight.

Analysis:

In general, the experimental results appear to be valid; however, experimental details were lacking in some instances, particularly in the analysis of the in vitro phrenic nerve-diaphragm preparation and in the analyses of the plasma enzyme activities. In addition, the number of animals used in analyses of plasma enzyme activities were usually too low for good statistical analyses. The use of such a small number of animals usually results in wide variation as was the case in several instances.

Andersen, M.E. and R.A. Smith. 1973. Mechanism of the oxidation of human and rat hemoglobin by propylene glycol dinitrate. Biochem. Pharmacol. 22:3247-3256.

Review:

The authors state that the ability of organic polynitrates such as propylene glycol dinitrate (PGDN) to oxidize hemoglobin has been known for a long time but that the nature of this reaction is largely unknown. It is known that PGDN, on incubation with rat blood, is broken down to NO_2^-, NO_3^-, and propylene glycol mononitrate. This hydrolysis occurs in the erythrocytes, not in the plasma. It also is known that PGDN produces larger amounts of metHb in vivo than do equivalent amounts of triethylene glycol dinitrate (TEGDN). To understand the biochemical basis of the difference in the heme oxidizing potency of the two dinitrates and their metabolism, studies on the kinetics, stoichiometry and the mechanism of the oxidation of human and rat oxyhemoglobin in vitro were done by Andersen and Smith.

Erythrocytes were isolated from human blood and Sprague-Dawley rat blood by spinning down at 5000 rpm in a Sorvall RC-2B centrifuge for 10 min and then washed three times with 0.9% saline. Hemolysates were prepared by adding five parts of distilled water to one part of erythrocytes. After stirring for several minutes and standing at 4° for 5 min, the stroma were removed by centrifugation at 15,000 rpm for 15 min. Potassium phosphate buffer, pH 7, was added to the supernatant to a concentration of 0.01 M. The hemolysate was recentrifuged at 15,000 rpm for 30 min after standing for 30 min. The concentration of hemoglobin was determined spectrophotometrically. Lyophilized bovine metHb was reduced to deoxyhemoglobin with 0.1% dithionite and purified by running through a column of G-25 Sephadex and reoxygenated by gentle swirling.

Stock solutions of PDGN and TEGDN contained small amounts of insoluble stabilizers. Solubility of PGDN in normal saline was found to be 13 mM (2.16 g/L). These dinitrate solutions were assayed spectrophotometrically at 220 nm.

Spectrophotometric assay of methemoglobin at 630 nm and analysis of NO_2^- and NO_3^- were carried out by published methods. A tonometer was custom-made for regulating the O_2 concentration in the reaction mixture. De-oxygenated dinitrate solution and the hemoglobin solutions were introduced into the tonometer. A measured volume of air or O_2 was injected through the septum on the end of the side arm of the tonometer. The solution was equilibrated with the gas phase by vigorous swirling for 2-5 min and the oxidation reaction was monitored spectrophotometrically by taking the absorbance readings in the cuvet fused to the bottom of the tonometer.

Reactions of PGDN and TEGDN with human hemolysate oxyhemoglobin (O_2Hb) at pH 7 and 37° were performed at constant dinitrate and varying

O_2Hb concentrations and vice versa. Lineweaver-Burk plots were constructed. The plotted lines passed through the origin. These results indicated that the reaction is not enzyme catalyzed and is first-order in both dinitrate and O_2Hb concentrations. The reaction was repeated with purified bovine O_2Hb and PGDN with essentially similar results.

The pH dependence of the reaction was studied. The results indicated that the reaction is zero-order in hydrogen ion concentration above pH 7 but markedly pH dependent at lower values.

Carboxyhemoglobin was found to be resistant to oxidation by PGDN. The PGDN reaction with O_2Hb was found to depend on the oxygen concentration in a complex manner.

The stoichiometry of the reaction was determined by preparing a series of reaction mixtures, each containing equal O_2Hb concentrations and varying amounts of PGDN. 1.5 mole of O_2Hb was oxidized per mole of PGDN. A similar study with erythrocytes indicated 2.30 moles of O_2Hb oxidized per mole of PGDN.

The PGDN solution in 0.9% saline has little NO_2^-. In reaction mixtures in which oxidation of 500 μM O_2Hb was half complete, less than 20 μM NO_2^- was present.

The identity of metHb produced by PGDN with that produced by ferricyanide was indicated by their ability to form a complex with NO.

The oxidation of both rat O_2Hb and human O_2Hb by TEGDN were found to be slower than oxidation by PGDN. Furthermore, the reaction between rat O_2Hb and TEGDN was 7-8 times faster than that between human O_2Hb and TEGDN.

The presence of oxygen is absolutely essential for O_2Hb to be oxidized by PGDN but the reaction is inhibited by high concentrations of oxygen.

A tentative reaction mechanism for the oxidation of O_2Hb by PGDN was proposed. The dinitrate reacts at or near the heme site with deoxyHb, not with O_2Hb. This PGDN-deoxyHb complex in the presence of O_2 is oxidized, and the dinitrate is coverted into mononitrate and other products. Attempts to find spectrophotometric evidence for the formation of a PGDN-deoxyHb complex did not succeed.

The difference in the biological activity of PGDN and TEGDN was also reflected in the higher rate of alkaline hydrolysis of the former.

For toxic effects caused by the intact dinitrate, hemoglobin may act as a scavenger. Thus it is expected that humans, because of the slower rate of metHb formation by TEGDN, will be less susceptible to methemoglobinemia from this dinitrate than is the rat. However, humans

will be more susceptible to TEGDN-produced nervous disorders which are apparently caused by the intact dinitrate.

Analysis:

This paper could have been written with more clarity. Extinction coefficients for O_2Hb and metHb used in calculating the results were not given. In addition, the data generated from the experiments with oxygen are not very convincing.

Cornell, J.H., T.M. Wendt, N.G. McCormick, D.L. Kaplan, and A.M. Kaplan. 1981. Biodegradation of Nitrate Esters Used as Military Propellants: A Status Report. Technical Report NATICK/TR-81/029. U.S. Army Natick Research and Development Laboratories, Natick, MA.

Review:

The purpose of the research described in this report was three-fold: (1) to determine whether propyleneglycol dinitrate (PGDN), diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), and trimethylolethane trinitrate (TMETN) are biodegradable, (2) to characterize the products and intermediates resulting from their biodegradation, and (3) to synthesize and characterize those intermediates which were not otherwise available. The work described was an initial study to determine if the proposed biological treatment for nitroglycerin (NG) would also be effective in eliminating the four esters without essential modification in operating conditions.

The inocula for cultures was obtained by inoculating nutrient broth with fresh activated sludge from a domestic sewage treatment plant. The progress of the biotransformation was monitored by thin-layer chromatography (TLC). The experiments were conducted aerobically.

Each of the four propellant esters gave the corresponding mononitrates as a result of biologically mediated denitration occurring in steps (Figure 1). Thus, TMETN is degraded initially to the dinitrate, then to the moninitrate, and then to the parent glycol. These degradations proceeded via a sequential stepwise hydrolytic cleavage of the nitrate groups as Figure 1 illustrates. Although Figure 1 indicates that the esters were transformed to their respective parent glycols, this was not confirmed experimentally. The identities of the intermediates were confirmed, however, by comparison with synthesized standards.

Analysis:

This report describes a well designed and thoroughly conducted study of the biodegradation of four nitrate esters used as military propellants. The results indicate that PGDN, DEGDN, TEGDN, and TMETN are biodegradable.

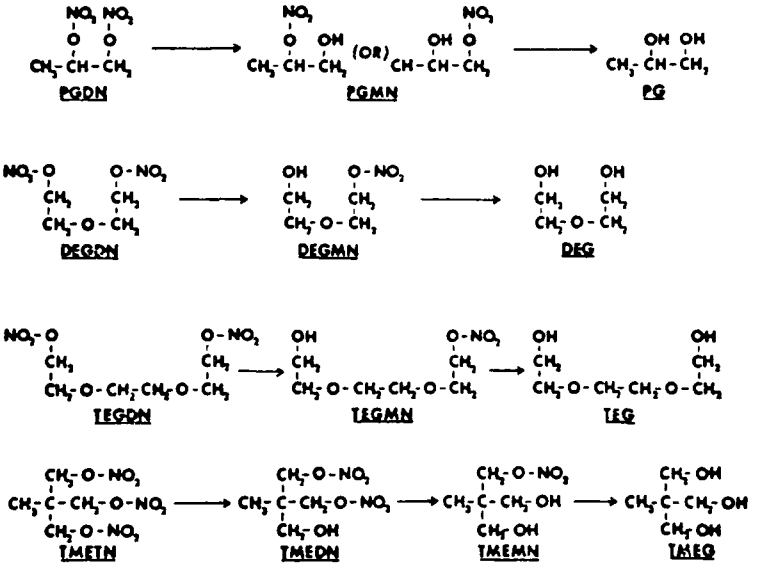

Figure 1. Biotransformation of the nitrate esters.

Dacre, J.C, C.C. Lee, H.V. Ellis, III, and C.-B. Hong. 1980. Chronic and carcinogenic study of trinitroglycerin in rats, mice and dogs. Arch. Toxicol., Suppl. 4:88 (Abstract only).

Review:

Four groups of 38 male and 38 female Charles River CD albino rats and of CD-1 mice were fed diets containing 0 (control), 0.01, 0.1, or 1.0% trinitroglycerin (TNG) for 2 years. Rats fed the high dose had decreased food consumption and weight gain. Periodic hematological examination found compensated anemia with reticulocytosis, elevated serum transaminases and occasional methemoglobinemia in high dosage rats. At the 1 year necropsy, 8 high-dose rats examined had cholangiofibrosis and some had hyperplastic foci in the liver. At 2 years, all 13 surviving high-dose rats and 6 of the 16 middle-dose rats had enlarged and grossly abnormal livers which on microscopic examination releaved severe cholangiofibrosis and hepatocellular carcinomas. One-half of the high-dose males had interstitial cell tumors in the testes. No apparent toxic effects were observed in the low-dose animals. Mice fed the high-dose of TNG had decreased food consumption and weight gain. At 1 year, the high-dose mice had heme-derived pigment deposits in various organs, and liver dysplasia. At 2 years, the pigmentation occurred in most high-dose and some middle-dose mice while minimal liver dysplasia was

found in all the treated groups. Four groups of 6 male and 6 female beagle dogs were given capsules containing 0 (control), 1, 5 or 25 mg/kg/day of TNG for 1 year. Only transient methemoglobinemia was observed in most of the dogs at all the dose levels.

Analysis:

Based on the information in this abstract, the study appears to be well designed. Additional information on the statistical treatments would be helpful. TNG is chemically similar (and should be toxicologically similar) to the compounds of interest.

Kaplan, D.L., J.T. Walsh, and A.M. Kaplan. 1981. Decomposition of glycols from nitrate ester propellants. Technical Report NATICK/TR-81/017. U.S. Army Natick Research and Development Laboratories, Natick, MA.

Review:

This paper assessed the biodegradability of four glycols [propylene glycol (PG), diethyleneglycol (DEG), triethyleneglycol (TEG), and trimethylolethane glycol (TMEG)] which are dervied via successive denitration steps from four esters (PGDN, DEGDN, TEGDN and TMETN) often found as propellants in waste streams from munition plants. A gas chromatographic method was developed that detected the glycols in the low ppm range in aqueous media and allowed an assessment of their biodegradability. PG was relatively stable to nonbiological forces at low concentrations in aqueous solutions but microbiological decomposition occurred in rich media and when it was the sole carbon source. DEG and TEG decomposed nonbiologically via mechanisms which presumably produce low molecular weight fragments. TMEG was stable under the test conditions and showed at best only very slow rates of decomposition. Relative rates of disappearance of the parent compounds indicate a sequence of PG > DEG > TEC > TMEG from high to low. At concentrations up to 500 µg per plate, TMEG was not bactericidal in five *Salmonella* strains nor was it mutagenic in those same organisms.

Analysis:

Based on the descriptions in the Methods section of the procedures used, this was a well designed study. The main focus of the paper is on decomposition products derived from the esters of interest.

Krasovskii, G.N., A.A. Korolev, and S.A. Shigan. 1973. Toxicological and hygienic evaluation of diethylene glycol dinitrate in connection with its standardization in water reservoirs. J. Hyg., Epidemiol., Microbiol., Immunol. 17(1):114-119.

Review:

Krasovsky, Korolev, and Shigan (1973) conducted a set of experiments to determine the admissible level of diethyleneglycol dinitrate (DEGDN) in the water of reservoirs.

The experiments included: the study of the effect of DEGDN on the odor, taste, and color of the water; determination of biochemical oxygen uptake$_5$ (BOU_5) and biochemical oxygen uptake$_{20}$ (BOU_{20}); and acute, subacute and chronic assays in warm-blooded animals.

DEGDN gave the water a bitter-astringent taste; the perception threshold (1 ball) was 130 mg/L. The perception threshold of odor was approximately five times that of taste. In water solutions, DEGDN was highly stable and did not color the water, even at the solubility border and upon prolonged exposure to light. In concentrations of up to 10 mg/L DEGDN did not perceivably affect the biochemical oxygen uptake (BOU_5 and BOU_{20}), nitrification processes of organic impurities, or the development and dying of saprophytic microflora.

Acute oral studies were performed in various species to determine LD_{50} values. The mean lethal doses (LD_{50}) were as follows: 1250 mg/kg for white mice; 1180 mg/kg for white rats, and 1250 mg/kg for guinea pigs. In all species tested acute poisoning was characterized by acute cyanosis and symptoms of damage to the central nervous system.

In a rabbit experiment, intravenous administration of 0.4 mg/kg produced a prolonged decrease in blood pressure, but no changes in the electrocardiogram.

To study accumulation of the compound, rats were given DEGDN in daily oral doses of 1/5, 1/25, or 1/125 of the LD_{50}. On days 1, 5, 10, 15 and 20 of the experiment blood samples were collected just before, and 1/2, 1-1/2, and 2-1/2 hr after, the introduction of the compound. The blood content of methemoglobin, erythrocytes, hemoglobin, and glutathione were determined in the animals. This protocol allowed the investigators to study the effect of DEGDN on the functional condition of the animals, as well as the dynamics of the changes in and recovery of, the blood parameters following successive exposures to the compound. In these experiments DEGDN displayed a medium-degree cumulative capacity.

"Chronic" tests were carried out in groups of eight rats each. One group served as vehicle controls; the other groups were given DEGDN in oral doses of 0.05, 0.5, or 5.0 mg/kg six times per week for six months.

Doses of 0.5 and 5.0 mg/kg produced changes in the conditioned reflex activity and in the immunobiological status of the animals. Five mg/kg provoked both a decrease in blood pressure by the 5th and 6th months, and a change in mitotic activity of the bone marrow. No significant differences were observed in levels of blood cholinesterase, erythrocytes, leucocytes, reticulocytes, hemoglobin and methemoglobin; in the average diameter of the erythrocytes; in 17-ketosteroid levels; in bromsulfalein load; in weights and ascorbic acid content of some of the organs; or in the content of thiol groups in liver homogenates.

The investigators concluded that DEGDN is capable of changing the organoleptic properties of water in concentrations above 100 mg/L; that in concentrations up to 10 mg/L, DEGDN has no detectable effect on the sanitary regimen of water reservoirs; that DEGDN is a moderately toxic substance with a medium degree of cumulative capacity; and that the maximum admissible level of DEGDN in the water of reservoirs should be set at 1 mg/L.

Analysis:

The report of Krasovsky, Korolev, and Shigan (1973) is lacking in experimental details and data. Also, the "chronic" study only covers a period of six months, instead of the usual 18-24 months. Therefore, the long-term effects (or lack of long-term effects) of the compound should not be considered as conclusive.

Mattsson, J.L., J.W. Crock, Jr., and L.J. Jenkins, Jr. 1977. Effects of NOSET-A on Rhesus Monkey Visual Evoked Response and Sidman Avoidance Task. Technical Report AFRRI-SR77-1, AD A045416. Armed Forced Radiobiology Research Institute, Bethesda, MD.

Review:

In this study NOSET-A, which contains triethyleneglycol dinitrate, was tested for effects on selected behavioral responses of the Rhesus monkey (*Macaca mulatta*). A 10 kg male was exposed for four hours in a Rochester-type, continuous-exposure inhalation chamber to a 2.4 ppm aerosol of NOSET-A on two separate occasions. The monkey was monitored for changes in electroencephalogram (EEG), and visual evoked response (VER), and Sidman avoidance task data were gathered. Three 1 hr testing periods were used, one an hour before exposure, one after two hours of exposure and a final one after four hours of exposure. Control data were obtained the previous day. There were no significant changes in the EEG or any component of the VER, but the Sidman avoidance responding was significantly increased on both test days. The lack of an effect in the EEG or VER rules out any diffuse impairment of the central nervous system and indicates that the peripheral nervous system or sensory detection system may have been altered. These data indicate that NOSET-A

has neurobehavioral effects of potentially serious consequence, and that further testing, such as measurement of peripheral nerve conduction velocities and behavioral tests requiring a high degree of sensorimotor integration, is necessary.

Analysis:

Although some question might be raised regarding the use of only one test animal, in general the methods appear to be satisfactory. Additionally, no percentage is given for the amount of triethyleneglycol dinitrate in NOSET-A.

Needleman, P. and F.E. Hunter, Jr. 1965. The transformation of glyceryl trinitrate and other nitrates by glutathione-organic nitrate reductase. Mol. Pharmacol. 1(1):77-86.

Review:

This paper reports the development of a rapid, sensitive enzymic assay based on the reaction of organic nitrates with reduced glutathione to yield oxidized glutathione. Although the main focus of the paper is on transformation and denitration of glyceryl compounds, comparative information is provided on the enzymic transformation rates of the alkyl nitrates: diethyleneglycol dinitrate, triethyleneglycol dinitrate and trimethylolethane trinitrate. Denitration, with formation of inorganic nitrite, occurs, this denitration resulting in the formation of products with greater water solubility. Trinitrate compounds were generally transformed at rates greater than the dinitrate ones.

Analysis:

The descriptions in the Methods section indicate a well designed study, although the main discussion is on chemicals similar to those of interest.

National Institute for Occupational Safety and Health. 1978. Occupational exposure to nitroglycerin and ethylene glycol dinitrate. Technical Report NIOSH-78-167, AD B042966L. National Institute for Occupational Safety and Health, Morgantown, W.VA.

Review:

This document recommends exposure standards for manufacture, storage, and use and handling of nitroglycerin (NG) and ethyleneglycol dinitrate (EGDN). These chemicals are potent vasodilators. Short term exposure results in headache, dizziness, nausea, palpitations and

decrease in blood and pulse pressure. These symptoms disappear in workers exposed on successive days indicating that the vasodilatory activity has been counteracted by compensatory vasoconstriction. Withdrawal from long-term exposure has been associated with angina pectoris and sudden death. Standards are provided for the following areas: environment, medical, labeling and posting, personal protective clothing and equipment, informing employees of hazards, work-practices, sanitation, and monitoring and record keeping.

Analysis:

Although there is no discussion of the health effects of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), trimethylolethane trinitrate (TEMTN), or their respective combustion products, the structural similarity between these compounds and NG and EGDN will enable possible toxicological analogies.

Prerovska, I. and J. Teisinger. 1965. Clinical picture of chronic intoxication with dinitrodiglycol. Pracovni Lekar. 17(2):41-43.

Review:

This paper discusses the clinical observations and findings of employees in a dinitroglycol and nitroglycerin producing and processing plant. Four employees of the plant, with 5-7 years of past exposure to the compounds, died suddenly between 1958 and 1961 (three of them during after-work hours). Autopsies revealed slight to significant signs of coronary sclerosis, but no signs of blockage of coronary arteries. This prompted the examination of 37 workers, most of whom had reported precordial pain, headaches, and in some cases breakdowns with a loss of consciousness. Three of these employees had clear signs of coronary sclerosis, eight had symptoms of intermediary coronary syndrome, and one had evidence of an infarct of the myocardium. In almost every examined employee, the cholesterol level was at the upper limit of the normal range, around 220 mg %, and in some the level was as high as 300 mg %. Unlike literature reports, there were no definite trends of elevated diastolic pressures, increased methemoglobin levels, or liver damage. After measures were made to eliminate from the high-risk workplaces those employees with diseases of the cardiovascular system, liver, or kidney, with any neural disorders, with ulcers, or with any disease causing general weakness, only specific subjective difficulties (e.g., intolerance for alcohol or headaches on return from a holiday) were reported. Objectively, no cardiogram changes or deviations in the serum lipid spectrum were found. It was recommended that people with a disposition to atherosclerosis, elevated blood cholesterol level, ocular fundus, or electrocardiogram be examined regularly.

Analysis:

No Methods section is included in this report, therefore it is difficult to analyze the integrity of the study. However, based on the authors discussion of the pertinent literature and their consideration of potential interfering factors, the paper appears to be an acceptable epidemiological analysis.

Valachovic, A. 1965. Effect of dinitrodiglycol on the work of the heart muscle of dog in acute experiment. Pracovni Lekar. 17(2):50-53.

Review:

This experiment deals with the acute effects of dinitrodiglycol [(DNDG) also known as DEGDN] on the work output of heart muscles in the dog. A heart-lung machine was used to supply controlled amounts of blood and air to the dogs. A total of 23 dogs of both sexes weighing 14-16 kg were used as test subjects. The greater circulatory system of the dogs was replaced by an adjustable resistance system (20-120 mm Hg) with a graduated burette for measuring the minute volume of the blood. The lesser circulatory system was retained in order to oxygenate the blood, but the air volume for respiration was predetermined and maintained by a mechanical pump. Blood pressure was measured by a mercury manometer placed in the arterial duct. The heart output was calculated using the following formula:

$$V = \frac{7}{6} Q \cdot R + \frac{7\ m\ v^2}{g} \cdot K$$

V = output expressed in watts, Q = quantity of blood in kg/hr, R = mean arterial pressure in meters, m = weight of blood in kg/hr, g = acceleration, v = velocity of the blood flow in meters per second, K = factor used for coverting kg/hr into watts.

$$K = \frac{9.8}{3600}$$

The system was first tested by measuring the normal output of the heart at six different peripheral resistances and at two different blood heights. These data were used as the baseline values. DNDG in distilled water was then added at a rate of 6 mg/kg body weight and heart output measured at each of the 6 resistance and 2 height settings. These values were expressed as a percentage of the baseline values. A comparison was also made with a control group to verify that no equipment or procedural interference occurred. Results showed no significant differences between baseline and treatment values. The author concluded that DNDG did not directly affect the heart muscle metabolism or its output, and the lumen of the cardiac arteries were not altered, especially

not vasoconstricted. The author also suggested that any stenocardiac difficulties associated with DNDG exposure result from a secondary effect of DNDG which causes cardiac muscle hyperemia.

Analysis:

The descriptions in the Methods section indicate that this is a well designed study. However, more information on the statistical treatment used and on the test animals (e.g., strain, age) would be useful.

Vasak, V. 1965. Determination of nitrates in the urine as exposure test in work with dinitrodiglycol. Vnitrni Lekar. 17(2):47-50.

Review:

In this paper, a urinalysis detection method was developed that allowed a more accurate appraisal of occupational exposure to diethyleneglycol dinitrate (DEGDN). This method was felt to be more informative than just establishing the presence of nitroesters in the work atmosphere since it includes the potentially considerable exposure by resorption through the skin.

In preliminary experiments with groups of three rats injected with doses of 50 mg, 75 mg, 100 mg and 150 mg of DEGDN, a dose-related increase in the quantity of inorganic nitrates in the urine was found. This finding was used to develop an exposure test for humans in which the hygienic evaluation of the workplace was added to previously performed analysis of the atmosphere. Verification of the method was obtained by examining the urine of a group of employees in an explosives plant exposed to DEGDN as well as those of two control groups (a total of 250 urine samples); a statistically significant increase in the content of nitrates in urine of the exposed persons was found. The limit of the increased content was considered to be a concentration of 200 mg NO_3^-/24 hr.

A detailed method of determining the content of nitrates in urine by the xylenol method was also described.

Analysis:

The detailed descriptions of the method used and developed in this study, indicate that it was well designed.

APPENDIX B

TABULAR SUMMARY OF THE HEALTH EFFECTS OF DEGDN, TEGDN, AND TMETN

TABLE B-1. SUMMARY OF HEALTH EFFECTS, METABOLISM, AND DETOXICATION OF DIETHYLENEGLYCOL DINITRATE (DEGDN)

Species	Exposure: Concentration, Route, and Duration	Effects and Remarks	Reference
		Lethality	
Guinea pigs	1250 mg/kg; perorally in vegetable oil	LD_{50}.	
White mice	1250 mg/kg; perorally in vegetable oil	LD_{50}. For all species tested, acute poisoning was characterized by symptoms of damage to the central nervous system and acute cyanosis.	Krasovskii, Korolev, and Shigan, 1973
White rats	1180 mg/kg; perorally in vegetable oil	LD_{50}.	
Rats	777 mg/kg, orally	LD_{50}.	Marhold, 1972
		General Toxicity	
Humans	Not stated, but above 5 mg/m^3. Four workers who died had 5 to 7 yr exposure. Exposure times for other workers not stated.	Workers in DEGDN and nitroglycerin-producing plant. Four workers, 29-47 yr old, who died suddenly showed on necroscopy mild to marked signs of coronary occlusion. Of 45 other employees, 37 reported precordial pain, headaches, and rarely collapse conditions with loss of consciousness. Three showed signs of coronary sclerosis, 8 symptoms of intermediary coronary syndrome, and 1 myocardial infarction. In most the cholesterol blood level was at the upper borderline of normal, i.e., 220 mg%, and in some it reached 300 mg%.	Prerovska and Teisinger, 1965
Dogs	6 mg/kg in heart-lung experimental setup. Duration of experiment long enough to get heart pressure-work data.	DEGDN did not directly affect the heart metabolism or its output, the lumen of the cardiac arteries were not altered, and there was no vasoconstriction. Stenocardiac difficulties associated with DEGDN exposure probably resulted from secondary effects causing cardiac muscle hyperemia.	Valachovic, 1965
Rabbits	0.4 mg/kg; iv injection	Fairly long hypotensive effect. No effect on ECG.	Krasovskii, Korolev, and Shigan, 1973
White male rats	0.05 mg/kg, perorally, 6 times/wk over 6 months	No effects noted.	Krasovskii, Korolev, and Shigan, 1973
	0.5 mg/kg, perorally, 6 times/wk over 6 months	Minimum effective dose. Changes in conditioned reflex activity and immunobiological responses.	

Table B-1 (continued)

Species	Exposure: Concentration, Route, and Duration	Effects and Remarks	Reference
		General Toxicity (continued)	
White male rats (continued)	5.0 mg/kg, peroally, 6 times/wk over 6 months	Decrease in blood pressure by the 5th to 6th month. Change in the mitotic activity of the bone marrow No substantial changes over controls with respect to blood cholinestrase activity, erythrocyte, leukocyte, and reticulocyte counts, HB and metHb amounts, erythrocyte diameter, 17-ketosteroids in urine, organ weights, ascorbic acid content of some organs, level of SH groups in liver homogenates, and liver handling of bromsulfalein load.	
White rats	Doses of 1/5, 1/25, and 1/125 of LD_{50}; daily peroral administration	metHB, hematocrit, Hb content, and GSH content monitored at ½, 1½, and 2½ hr after introduction of DEGDN on days 1, 5, 10, 15, and 20. Results showed DEGDN to be a substance with a medium-degree cumulative capacity.	Krasovskii, Korolev, and Shigan, 1973
		Metabolism	
Humans	Exposure levels not stated	Workers in explosives production plant. Excretion of nitrates. 250 workers were examined. 200 mg nitrate/24 hr taken as statistically significant proof of exposure.	Vasak, 1965
Rats	50, 75, 100, 150 mg/rat, by injection	60% of injected quantity appeared in urine during first four days. Some nitrate was apparently reduced.	Vasak, 1965
		Detoxication	
Rat liver enzyme	Not applicable. In vitro enzyme assay.	1.2 mmoles/kg protein/min detoxication. Formation of nitrite from organic nitrates by action of organic nitrate reductase results in oxidation of 2 GSH to GSSG. GSH oxidation measured spectrophotometrically by disappearance of TPNH.	Needleman and Hunter, 1965

TABLE B-2. SUMMARY OF HEALTH EFFECTS AND DETOXICATION OF TRIETHYLENEGLYCOL DINITRATE (TEGDN)

Species	Exposure: Concentration, Route, and Duration	Effects and Remarks	Reference
		Lethality	
FTD-Hartley male guinea pigs	700 (586-835) mg/kg; single ip injection	24 hr LD_{50}.	Anderson and Mehl, 1973
New Zealand albino male rabbits	21 mmole/kg; dermally, daily for 3 wk	9 of 11 died; mean time to death was 17 days. Rabbits lost 20% of starting weight and appeared strikingly emaciated.	Anderson and Mehl, 1973
NIH:NMRI male mice	945 (792-1126) mg/kg; single ip injection	24 hr LD_{50}. (95% confidence limit)	Anderson and Mehl, 1973
NMRI:(O)SD male rats	796 (785-807) mg/kg; single ip injection 1000 (733-1365 mg/kg; perorally 2520 (1720-3660) mg/kg; single sc injection	24 hr LD_{50}. (95% confidence limit) 24 hr LD_{50}. (95% confidence limit) 24 hr LD_{50}. (95% confidence limit)	Anderson and Mehl, 1973
NMRI:(O)SD male rats	995 (932-1063 mg/kg; single ip injection 776 (470-1283 mg/kg; single ip injection 321 (170-605 mg/kg; single ip injection	24 hr LD_{50}. (95% confidence level) 24 hr LD_{50}; rats in nonisolated cages. 24 hr LD_{50}; rats anesthetized with pentobarbital.	Anderson, Koppenhaver, and Jenkins, Jr., 1976
NMRI:(O)SD male rats	4600 mg/kg; single sc injection	Time to death: 597 ± 125 min. With methylene blue pretreatment, 1582 ± 235 min, showing the importance of metHb in causing death from TEGDN poisoning.	Anderson and Mehl, 1973
NMRI:(O)SD male rats	1000 mg/kg; single ip injection	In vivo plasma concentration 0.84 ± 0.09 mM at first convulsion; 1.23 ± 0.08 mM in extremis. Brain TEGDN 163 ± 21 µg/g at first convulsion; 315 ± 12 µg/g in extremis.	Anderson, Koppenhaver, and Jenkins, Jr., 1976
		General Toxicity	
Humans — outdated blood from blood bank and fresh blood from authors	1.1 and 2.2 mM in hemolysate preparations	Oxidation of HbO_2 to metHb. No accurate estimation of stoichiometry because of auto-oxidation of HbO_2.	Andersen and Smith, 1973
Rhesus monkey (Macaca mulatta)	2.4 ppm aerosol in inhalation chamber. Two hours exposure and 4 hours exposure.	Monkey was monitored for changes in electroencephalogram (EEG), visual evoked response (VER), and Sidman avoidance task data. No significant changes in EEG or VER, but Sidman avoidance response rate was significantly increased following both exposures.	Mattson, Crock, and Jenkins, Jr., 1977

Table B-2 (continued)

Species	Exposure: Concentration, Route, and Duration	Effects and Remarks	Reference
		General Toxicity (continued)	
FTD-Hartley male guinea pigs	100 mg/kg; daily ip injections	95% of control weight at 15 days.	Andersen and Mehl, 1973
	200 mg/kg; daily ip injections	82% of control weight at 15 days.	
	400 mg/kg; daily ip injections	40% of control weight at 15 days.	
NMRI:(O)SD male rats	LD_{50}dose (796 mg/kg; single ip injection	Plasma enzyme activities in rats surviving the LD_{50} dose (enzyme activities in international units/L):	Anderson and Mehl, 1973
		Aspartic transaminase – from high of 261 ± 104 at 48 hr to 26 ± 6 at 168 hr. Control: 43 ± 3.	
		Lactic dehydrogenase – from high of 134 ± 38 at 48 hr to 48 ± 20 at 168 hr. Control: 42 ± 5.	
		Creatine kinase – from high of 375 ± 91 at 48 hr to 101 ± 3 at 168 hr. Control: 90 ± 15.	
		Alkaline phosphatase – from high of 144 ± 12 at 48 hr to 49 ± 14 at 168 hr. Control: 83 ± 7.	
NMRI:(O)SD male rats	50 mg/kg; single ip injection	Increase in pentobarbital sleep time starting at dosage given; progressive with increasing concentrations of TEGDN.	Anderson, Koppenhaver, and Jenkins, Jr., 1976
	600 mg/kg; single ip injection	Raised the 1 hr LD_{50} of Metrazol (centrally acting convulsant) from 89 (76-100) mg/kg to 164 (144-182) mg/kg and 24 hr LD_{50} from 89 (76-100) mg/kg to 112 (98-129) mg/kg.	
	300 mg/kg; single ip injection	Increased zoxazolamine (centrally acting relaxant) paralysis time from 100 ± 7 min to 227 ± 27 min.	
	600 mg/kg; single ip injection	Increased zoxazolamine paralysis time from 110 ± 7 min to 475 ± 75 min.	
	1000 mg/kg; single ip injection	In vivo plasma concentration 0.84 ± 0.09 mM at first convulsion; 1.23 ± 0.08 mM in extremis. Brain TEGDN 163 ± 21 µg/g at first convulsion; 315 ± 12 µg/g in extremis.	
	All levels	Hyperactivity to both auditory and tactile stimuli; tremors; severe clonic convulsions.	
NMRI:(O)SD female rats	13.0 mmoles/kg; single sc injection	20% metHb at 200 min; 28% at 600 min.	Andersen and Mehl, 1973

Table B-2 (continued)

Species	Exposure: Concentration, Route, and Duration	Effects and Remarks	Reference
		General Toxicity (continued)	
NMRI:(O)SD male rats	1.1 and 2.2 mM in hemolysate preparations	Oxidation of HbO_2 to metHb. Seven to 8 times faster than in case of human HbO_2.	Andersen and Smith, 1973
NMRI:(LE) male rats; phrenic nerve diaphragm preparations	2.4 to 4.8 mM in Krebs-Henseleit buffer	Reduction in twitch height. Preparations became refractory to stimulation at 60-70% twitch-height reduction at the higher concentrations. Effects reversible by washing out.	Anderson, Koppenhaver, and Jenkins, Jr., 1976
Male rat phrenic nerve diaphragm preparation	1.5 mM in Krebs-Henseleit buffer	Above this level, diaphragm did not contract on phrenic nerve stimulation but did when stimulated directly.	Andersen and Mehl, 1973
	4.0 mM in Krebs-Henseleit buffer	Above this level both nerve- and muscle-stimulated contractions were abolished. Effects reversible by washing out.	
NMRI:(O)SD male rat	2.4 mg/kg; single iv injection	Mean blood pressure fell from 90 to 49 mm Hg immediately, and recovered to 96 mm Hg in 15 min. One hour later on iv injection of 0.8 mg/kg, pressure fell from 86 to 59 mm Hg, recovering to 84 mm within 2 min.	Anderson and Mehl, 1973
		Detoxication	
Rat liver enzyme	Not applicable. In vitro enzyme assay.	1.7 mmoles/kg protein/min detoxication. Formation of nitrite from organic nitrates by action of organic nitrate reductase results in oxidation of 2 GSH to GSSG. GSH oxidation measured spectrophotometrically by disappearance of TPNH.	Needleman and Hunter, 1965

TABLE B-3. SUMMARY OF HEALTH EFFECTS AND DETOXICATION OF TRIMETHYLOLETHANE TRINITRATE (TMETN)

Species	Compound	Exposure: Concentration, Route, and Duration	Effects and Remarks	Reference
			Mutagenicity	
Salmonella tryphimurium strains TA98, 100, 1535, 1537, and 1538	TMEG (parent glycol of TMETN)	Up to 5000 ug/plate	No toxic effects. Ames tests for mutagenicity were also performed, with and without metabolic activation. No mutagenic effects were observed.	Kaplan et al., 1981
			Detoxication	
Rat liver enzyme preparation	TMETN	Not applicable. In vitro enzyme assay.	11.1 moles/kg protein/min detoxication. Formation of nitrite from organic nitrates by action of organic nitrate reductase results in oxidation of 2 GSH to GSSG. GSH oxidation measured spectrophotometrically by disappearance of TPNH.	Needleman and Hunter, 1965

APPENDIX C

NITRATE ESTER TOXICOLOGY

INTRODUCTION

A large section of the NIOSH document on criteria for a recommended standard for occupational exposure to nitroglycerin (NG) and ethyleneglycol dinitrate (EGDN) (NIOSH, 1978) is devoted to the history of studies on the effects, exposure levels, incidents, epidemiology, etc. of NG and EGDN. The literature on those two compounds is extensive, particularly for NG. Literature on diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), trimethyolethane trinitrate (TMETN), and other nitroglycols is considerably less extensive. Generally, the effects of the nitrated glycols diminish as the molecular weights increase. A search of the literature does not reveal any outstanding differences in effects, but mainly differences in degree; for instance, the faster metabolic denitration of TMETN as compared to some straight-chain nitrate esters (Needleman and Hunter, Jr., 1965) and the greater production of metHb by polyglycol dinitrate (PGDN) than by TEGDN (Andersen and Smith, 1973), and it may be assumed that the effects and behavior of DEGDN, TEGDN, and TMETN are basically similar to those of NG and EGDN and of some organic nitrate esters used therapeutically.

GENERAL TOXICOLOGY

Sax (1975) gives the toxicities of NG and EGDN on a scale of 0 (none), 1 (slight), 2 (moderate), and 3 (high) as: acute local — NG irritant 2, EGDN irritant 1; acute systemic — NG ingestion 3, inhalation 3, skin absorption 3; acute systemic — EGDN ingestion 2, inhalation 2; chronic local — NG irritant 2, EGDN irritant 1; chronic systemic — NG ingestion 2, inhalation 2, skin absorption 2; chronic systemic — EGDN ingestion 2, skin absorption 2.

It is stated for EGDN (Sax, 1975) that it can cause lowered blood pressure leading to headache, dizziness, and weakness. For NG, the effects are more severe: headaches and reduced blood pressure, excitement, vertigo, fainting, respiratory rales and cyanosis. Taken internally respiratory difficulties occur, and death may ensue, due to respiratory paralysis. Confusion, pugnaciousness, hallucinations, and maniacal behavior are manifestations of severe poisoning. In the work situation the most common complaint is headache, which is noted upon commencing work but soon passes. A practice among explosives workers was to moisten their hat bands with NG when off the job to maintain accommodation. Sax also mentioned that NG can be absorbed through uninjured skin (also true for other organic nitrates) and may produce eruptions on the palms and intradigital spaces. It is further mentioned that in normal manufacture and use of explosives, the physiological effects of NG cause only temporary discomfort and are not injurious to

health, and this is generally even more true for the other organic nitrate esters, but the details of the effects of the compounds require study.

Rinkenbach (1926; 1927) in preparing and characterizing EGDN and DEGDN, also studied their physiological properties. Experience in the laboratory handling of EGDN showed that its effects upon the human system when absorbed through the skin or inhaled in vapor form were almost identical with those of NG. It produced dilation of the blood vessels, acceleration of the heart action, and a severe headache. The effects seemed not as prolonged, however, as in the case of NG. However, effects were more likely to occur, because of the higher vapor pressure of EGDN. Persons constantly working in proximity to the material soon developed an immunity to its usual effects just as is the case with NG. The effects of DEGDN were much less marked (Rinkenbach, 1927).

Further description of the effects of exposure to nitrate esters is given by Boschan, Merrow, and Van Dolah (1955). The most characteristic symptom of poisoning (particularly with NG) is severe headache and a feeling of pressure on the front and back of the head. The esters depress muscles in the vascular walls, which causes a peripheral vasodilation resulting in lowered systolic blood pressure and increased pulse and respiratory rates. This dilation of the peripheral blood vessels results in a shift of blood to the splanchnic area and may cause a decreased supply of blood to the brain (in spite of cerebral vasodilation), resulting in vertigo and fainting.

Withdrawal following a period of accommodation represents a danger with respect to poisoning by organic nitrate esters. Incidents have occurred in which workers have collapsed and even died after leaving an atmosphere of exposure, for instance, over a weekend. Heavy drinkers (Boschan, Merrow, and Van Dolah, 1955) and persons with heart disorders (Carmichael and Lieben, 1963) are particularly susceptible. Fatal cases have been cited by Valachovic (1965) for both EGDN and DEGDN. Prerovska and Teisinger (1965) have reported on the clinical picture of chronic intoxication with DEGDN. Four sudden deaths in a plant processing DEGDN and NG showed on necropsy mild to marked signs of coronary sclerosis but no signs of coronary occlusion. Of 45 employees, 37 reported precordial pain, headaches, but only rarely collapse conditions with loss of consciousness. Three showed obvious signs of coronary sclerosis, 8 symptoms of intermediary coronary syndrome, and 1 myocardial infarction. In most subjects examined, the cholesterol blood level was at the upper borderline of the normal range, i.e., 220 mg %, and in some it reached 300 mg %. After measures were taken to eliminate from the high-risk workplaces employees with diseases of the cardiovascular system, liver, or kidneys, with neural disorders, with ulcers, or with any diseases causing general weakness, only specific subjective difficulties (e.g., intolerances for alcohol or headaches on return from a holiday) were reported. Objectively, no cardiogram anomalies or deviation in the serum lipid spectrum were

found. It was recommended that people with a disposition to atherosclerosis, elevated blood cholesterol, ocular fundus, or abnormal electrocardiogram be examined regularly.

While people with heart and circulatory disease seem prone to incidents following disruption of tolerance it seems that long-continued exposure to nitrate esters also leads in some measure to cardiac or at least circulatory difficulties. Thus, in work in Sweden (Forssman et al., 1958, as cited in Carmichael and Lieben, 1963) it was found that, following the immediate and acute effect of lowering of both the systolic and diastolic blood pressure, continued exposure (to EGDN in the cases studied) produces a progressive rise in the diastolic blood pressure from the previously depressed levels without a comparable rise in the systolic pressure. The net result is a narrowing of the pulse pressure. As a result of this a myocardial ischemia may ensue, which can give rise to an incident or which, continued over a long time, may contribute to cardiac and circulatory insufficiency. To what extent this becomes permanent and what is the risk run with specific compounds, are questions for further research.

For the short term, work with dogs has shown that the output of the heart and the immediate metabolism of the heart are not affected by exposure to DEGDN (Valachovic, 1965). Dogs were put into a heart-lung machine so that respiration rates, pressure against which the heart beat, etc. could be controlled. DEGDN at a level of 6 mg/kg infused into the blood did not cause any diminution in the work output of the heart at a number of pressures, nor did it affect the lumen of the cardiac arteries nor cause them to vasoconstrict. The author suggested that any stenocardiac difficulties associated with exposure to DEGDN result from a secondary effect of it which causes cardiac muscle hyperemia.

METABOLISM OF NITRATE ESTERS AND THE VASODILATING EFFECT

Nitrite has a vasodilating, blood pressure lowering effect similar to that of organic nitrate esters, and so the question of whether it is the intact molecule of a nitrate in question or the nitrite produced from it which is chiefly responsible for the effect(s) has occupied the attention of investigators for almost 100 years. Some articles which shed light on the question and which are concerned with other aspects of the metabolism of the nitrates are summarized in the following paragraphs.

Krantz et al. (1939) studied the pharmacology of isomannide dinitrate and found that this compound promptly dilated the coronary vessels of the heart of the rabbit, steer, and dog. The authors attributed this to the action of the intact molecule and not to any product of cleavage. Furthermore, nitrite was hardly found in the blood. Compounds of higher fat solubility showed a greater vasodilation effect than less lipid-soluble ones.

In a 1940 study Krantz et al. investigated the mechanism of action of organic nitrate esters and found that the depressor response was greater in nitrates that were freely soluble in oil, whereas the water-soluble compounds were less active but the responses lasted longer. No evidence was found that the compounds needed to be hydrolyzed for depressive action. Krantz et al. (1962) studied the mechanism of action of glyceryl nitrate esters and discovered that while the coronary vessels and vascular bed of the ear (rabbit heart and ear) can reduce a fraction of the NG perfused through them to nitrite, this action is not necessary to elicit the depressor response, which seems to be mainly due to the intact molecule.

The metabolism of EGDN and its influence on the blood pressure of the rat were investigated by Clark and Litchfield (1967). The dinitrate was broken down to nitrite and mononitrate. An initial fall in blood pressure was thought to be due to the intact molecule of EGDN followed by the effects of nitrite and EGMN (mononitrate) released during the breakdown of the EGDN molecule. Clark and Litchfield (1969) found that injection of PGDN gave rise to a fall in blood pressure lasting several hours that seemed to be due to the combined influence of the intact molecule and its metabolites.

In a 1971 article, Litchfield reviewed the aspects of nitrate ester metabolism and concluded the following: (1) the metabolism of nitrate esters in the blood occurs almost entirely in the red cells, and that injected nitrite is quickly excreted as nitrate in the urine, reflecting the action of the enzyme catalase, particularly kidney cell catalase; (2) while the main depressor action in the blood seems to be due to the intact molecule, the situation may be different in other tissue cells; (3) nitrate esters stimulate mitochondrial respiration; (4) nitrite and some nitrate esters are uncouplers of oxidative phosphorylation; however, the ATP-ATPase reaction does not seem to be the target enzyme system of nitrate esters; (5) some nitrate esters are monoamine oxidase inhibitors, but their metabolites show no such effect; and (6) nitrate esters break down fairly rapidly in contact with tissues.

Needleman (1976) reviewed organic nitrate ester metabolism with the main emphasis being degradation pathways. He pointed out that the biotransformation of organic nitrate esters is initiated by a redox reaction and is manifested by the conversion of potent lipid-soluble vasodilating compounds into water-soluble metabolites which have much lower biological potency and which are readily excreted in the urine. Linear-chain polynitrate esters were rapidly transformed by rat liver supernatant preparations in the presence of glutathione, whereas anhydrides and branched-chain alcohol nitrate esters (example: TMETN) were only slowly transformed.

The oxidizing ability of nitrate esters vis-à-vis -SH groups is shown by a study of Needleman and Johnson, Jr. (1973) on the mechanism of the development of tolerance to the vasodilating effect of organic

nitrates. This tolerance seems to involve an alteration in the responsiveness of a receptor site in vascular smooth muscle. Clark and Litchfield (1969) had shown that this tolerance is not due to increased biotransformation of the molecule [however, this can occur on stimulation, as shown by a study of Lee and Belpaire (1972), in which degradation of nitrates was increased by pretreatment with phenobarbital, this regimen increasing the activity of both the organic nitrate ester reductase and GSH-generating systems]; rather, as Needleman and Johnson, Jr. (1973) showed, the nitrate ester oxidizes a sulfhydryl of a receptor enzyme to a disulfide, changing the receptor into a form having a much lower affinity for the nitrate than had the -SH form.

FORMATION OF METHEMOGLOBIN

A chief effect of exposure to organic nitrate esters is formation of methemoglobin (metHb) in which the iron is in the 3^+ state and is no longer able to carry oxygen reversibly. While the body has an enzyme system that reduces metHb back to Hb with the iron in the 2^+ state, this system cannot cope with the amount of metHb produced in massive poisoning, and death results, from respiratory failure (Clark and Litchfield, 1969). The mechanism of the oxidation of the iron of hemoglobin by organic nitrate esters has received recent intensive investigation. It was known (Clark and Litchfield, 1969) that organic polynitrate esters, on incubation with rat blood, are broken down in the erythrocytes into nitrite, nitrate, and the corresponding mononitrate, with differences between the nitrate esters — PGDN, for instance, producing larger amounts of metHb than did an equivalent amount of TEGDN (Andersen and Mehl, 1973). Earlier, Wilhelmi (1942) had suggested that the reaction of oxidation was molecular, not enzymatic. Andersen and Smith (1973) found that oxygen had to be present for the oxidation to occur; however, a high concentration of oxygen was inhibitory, as was the presence of CO, which would form carboxyhemoglobin, thus blocking access of the nitrate or nitrite to the iron. The authors further found that the oxidation proceeded as well with separated hemoglobin as with the whole cells, showing that enzyme involvement was minimal. It was postulated that the organic dinitrate interacts at or near the heme site with deoxy-Hb, not with HbO_2. This nitrate-deoxy-Hb complex in the presence of O_2 is oxidized (Fe^{2+} to Fe^{3+}) and the dinitrate is converted to the mononitrate and other products.

Smith (1967) found evidence for the formation of a reversible complex between excess free nitrite and the ferric groups of metHb, and postulated that at least in mice, this complex formation may contribute to the unusually sustained methemoglobinemia produced by the nitrite.

The fact that formation of metHb in the presence of nitrate esters does not seem necessarily to involve enzyme action does not mean that metabolism of nitrate esters does not ensue, as denitration can occur in the red cells and other tissues and particularly in the liver (Heppel and Hilmoe, 1950). Reduced glutathione (GSH) was found to be necessary

for this metabolism. As shown by Heppel and Hilmoe (1950), glutathione was oxidized and organic nitrates were reduced under the influence of an enzyme from hog liver, this reduction occurring before the liberation of nitrite, with consequent liberation of nitrite, leaving a nitrate of lesser degree of nitration, which could undergo further transformation. The enzyme involved has been termed "hepatic GSH-organic nitrate reductase," and further studies of this system are reported in Needleman and Hunter, Jr. (1965), Litchfield (1971), and Needleman (1976). For some nitrate esters, the mononitrate was further metabolized, with the result that only small amounts were excreted, the major urinary metabolite being inorganic nitrate (Needleman, 1976). For some others the entire dose could not be thus accounted for, and it was postulated that some more refractory metabolites were formed, to be degraded and excreted more slowly.

NEUROTOXIC AND BEHAVIORAL EFFECTS OF ORGANIC NITRATE ESTERS

Andersen and Mehl (1973) compared the toxicology of TEGDN and PGDN (in the TEGDN the nitro groups are on the ends of a triglycol unit; in the PGDN they are on adjacent carbons) using Sprague-Dawley derived rats, FTD:Hartley-derived guinea pigs, New Zealand albino rabbits, and NMRI mice. Except for the mice, for which TEGDN was the more toxic nitrate ester, the LD_{50} for TEGDN was two to five times as high as for the PGDN. Both compounds produced methemoglobinemia and hypotension in rats. The TEGDN-poisoned rats, however, tremored violently and expired, apparently in respiratory arrest, while the PGDN-poisoned rats were lethargic and did not convulse. Results from a determination of the effect of both compounds on an in vitro phrenic nerve-diaphragm preparation indicated that the tremors caused by TEGDN may be the result of the compound's ability to interfere with nerve-muscle communication.

Andersen, Koppenhaver, and Jenkins, Jr. (1976) compared the neurotoxic properties of TEGDN with those of decamethonium (C_{10}). This compound has two quaternary amine trimethyl units, $-N^{+}(CH_3)_3$, separated by 10 $-CH_2-$ or methylene units. It thus resembles TEGDN in having its active groupings at some distance from each other. Both compounds injected into rats caused severe tremors and clonic convulsions, and in vitro both selectively blocked nerve stimulated contractions of the cholinergic rat phrenic nerve diaphragm (PND) preparation, with C_{10} being 60 times more potent in this respect than was TEGDN (note its similarity to acetylcholine). When TEGDN and C_{10} were applied to PND preparations in combination, the inhibition of the force of contraction occurred more rapidly and was greater than that expected from either compound applied alone. It appears that TEGDN and C_{10} act at the same site in vivo in the peripheral nervous system to block cholinergic transmission.

Jones, Strickland, and Siegel (1972) examined the toxicity of propylene glycol 1,2-dinitrate (PGDN) in experimental animals. This polyol nitrate ester is the major volatile constituent of Otto Fuel II, a

torpedo propellant used by the U.S. Navy. In the study, the peroral toxicity of PGDN in rats and the ocular and dermal irritation effects of PGDN on rabbits were determined. Rats, guinea pigs, rabbits, squirrel monkeys and dogs were exposed to the vapors of PGDN in acute, repeated, and continuous inhalation exposures. Physiological and biochemical changes noted were anemia, pigment deposition in various organs, fatty changes in the liver, methemoglobin formation, and greatly increased serum and urinary inorganic nitrates. Prior to the start of inhalation exposures, an acute iv study was carried out using 12 untrained rhesus monkeys. A total of 6 dose levels using between 0.028 and 0.41 g/kg were administered to 2 monkeys each to determine the minimum dose at which toxic signs could be noted. Doses exceeding 0.041 g/kg produced emesis, retching, ptosis, ataxia and apnea of increasing degree and duration, with death occurring to the 2 monkeys at the 0.41 g/kg level. Following this, 4 monkeys previously trained to perform in a visual discrimination test (VDT) were injected with 10% of the dose which produced a toxic effect, i.e., 0.004 g/kg, and after a 1 wk rest were injected with 0.007 g/kg. Minimal behavioral effects were noted in 1 animal at the lower dose group only.

While other monkeys exposed by inhalation at a level of 500-700 mg PGDN/m^3 of air for 6 hr showed signs of toxicity, trained monkeys exposed continuously for 90 days to 262 mg PGDN/m^3 and tested weekly showed only mydriasis (dilation of the pupils, presumably caused by the anticholinergic activity of the PGDN), and no changes in the avoidance behavioral pattern in the monkeys as indicated by the VDT and visual activity threshold tests.

Based on the general effects of nitrate esters and on the fact that headaches have been reported for man at levels of 1.2 to 4.8 mg PGDN/m^3, a tentative confined space guideline of zero was recommended by the Committee on Toxicology, National Academy of Sciences — National Research Council (as cited in Jones, Strickland, and Siegel, 1972). This level was believed to be necessary to prevent a degradation of performance; and a limit of 1.2 mg/m^3 was recommended for a 40 hr week industrial-type exposure.

Stewart et al. (1974) exposed human volunteers in a controlled-environment chamber to PGDN vapor at concentrations of 0.03 to 1.5 ppm. Physiological and central nervous system responses to the exposures were monitored. Exposure to concentrations of 0.2 ppm or greater produced disruption of the organization of the visual evoked response (VER) and headache in the majority of subjects. Subjects repeatedly exposed to 0.2 ppm for 8 hr on a daily basis developed a tolerance to the induction of headaches but the alteration in VER morphology appeared cumulative. Marked impairment in balance became manifest after exposure to 0.5 ppm for 6.5 hr, while 40 min of exposure to 1.5 ppm added eye irritation to the list of symptoms.

Mattson, Crock, Jr., and Jenkins, Jr. (1977) studied the effects of NOSET-A on rhesus monkey visual evoked response and Sidman avoidance task. NOSET-A is a preparation which contains TEGDN. In the study, a male rhesus monkey (*Macaca mulatta*) was exposed to NOSET-A aerosol for 4 hr at a concentration of 2.4 ppm. Visual evoked response and Sidman avoidance task (free operant avoidance) data were collected after 2 hr and 4 hr of exposure. The VER was not affected, but a significant increase in response rate in the Sidman avoidance task occurred. The lack of an encephalographic effect (normal EEG) and lack of influence on the VER rules out any diffuse impairment of the central nervous system and indicates that the peripheral nervous system or sensory detection system may have been altered. The authors consider that the data indicate that NOSET-A has neurobehavioral effects of potentially serious consequences, and that further testing, such as measurement of peripheral nerve conduction velocities and behavioral tests requiring a high degree of sensorimotor integration, is necessary.

CARCINOGENICITY OF ORGANIC NITRATE ESTERS

No studies investigating the carcinogenicity of DEGDN, TEDGN, or TMETN were available; however, some information is available on the carcinogenicity of NG. Takayama (1975; as cited in Ellis et al., 1982) gave rats drinking water containing 0.03% NG, and found no adverse effects. Suzuki et al. (1975; as cited in Ellis et al., 1982) gave mice drinking water containing 0.033% NG, and found a small, but statistically significant, increase in pituitary tumors in female mice.

A preliminary study with rats, mice, and dogs (Dacre et al., 1980) and a more extended study with the same species (Ellis et al., 1982) indicated that there were quantitative and qualitative differences among species with respect to the toxic effects of NG. Oral doses of up to 200 mg/kg/day for 5 days or 25 mg/kg/day for 12 months produced a transient methemoglobinemia with no other serious adverse effects in the dogs. In the rats, life-time feeding of 363 mg/kg/day for males or 434 mg/kg/day for females caused toxic effects on the liver and blood, and 31.5 or 38.1 mg/kg/day for males and females, respectively, caused mild effects on the liver of susceptible individuals. Mice were the least affected. Life-time feeding of 1,022 mg/kg/day for males or 1,058 mg/kg/day for females produced a compensated toxic anemia and some pigment deposits. In the rat, however, some neoplastic effects were seen. At 2 years, all 13 surviving high-dose rats and 6 of the 16 middle-dose rats had enlarged and grossly abnormal livers which on microscopic examination revealed severe cholangiofibrosis and hepatocellular carcinomas. One-half of the high-dose males had interstitial cell tumors in the testes. On the other hand, there were decreases in the incidences of pituitary chromophobe adenoma and mammary tumors, primarily fibroadenomas, which often occur spontaneously in these rats. The conclusion is that at high doses NG has the capability of producing tumors in rats, particularly in the liver. It should be noted that when NG is given orally, it enters the liver through the portal vein, and is there processed, denitrated, and conjugated.

TERATOGENIC AND REPRODUCTIVE EFFECTS OF NITRATE ESTERS

No references were found to any studies on teratogenic or reproductive effects of DEGDN, TEGDN, or TMETN. The NIOSH (1978) report on criteria for a recommended standard for occupational exposure to NG and EGDN mentions some turn-of-the-century reports noting that wives of dynamite workers often experienced heavy menstrual bleeding and had fewer children than did other women. It was added that often the children of dynamite workers were born prematurely, were cyanotic, or were not as strong as other children. It was stated that no study of the effects of exposure to NG or EGDN on reproduction in animals was found.

Nitrite is formed as a consequence of the metabolism of organic nitrate esters. Sleight, Sinha, and Uzoukwu (1972) produced severe toxicosis in pregnant sows with 21 to 35 mg of sodium nitrite/kg subcutaneously. The treatment performed on various single days during the first 100 days of gestation did not produce any fetal defects. In spite of high maternal methemoglobin, fetal methemoglobin was low. The authors concluded that nitrite does not easily cross the placenta.

Globus and Samuel (1978) administered sodium nitrite orally to pregnant CD-1 mice at a concentration of 0.5 mg/mouse/day. Embryotoxic and teratogenic effects on the hemopoietic tissues and skeletons of the offspring were evaluated. Fetal mortality, resorptions, the mean number of offspring per litter, the mean weight per embryo, and the incidence of skeletal malformations were not significantly different from controls. Sodium nitrite administered to the mothers did, however, seem to stimulate fetal hepatic erythopoiesis.

The above studies would seem to indicate that teratogenic effects from administration of nitrite are slight. However, as indicated in Section 6.3, the potential for teratogenic and reproductive effects of exposure to TEGDN, DEGDN, and TMETN should be investigated in laboratory animals.

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LIST OF ABBREVIATIONS

ACGIH	–	American Conference of Governmental Industrial Hygienists
AP	–	alkaline phosphatase
AsT	–	aspartate aminotransferase
ATP	–	adenosine triphosphate
ATPase	–	adenosine triphosphatase
BOU_5	–	biochemical oxygen uptake$_5$
BOU_{20}	–	biochemical oxygen uptake$_{20}$
°C	–	degrees Celsius
C_{10}	–	decamethonium; decamethylene bis(trimethyl ammonium)
CAS	–	Chemical Abstracts Service
$-CH_2-$	–	methylene unit
CH_4	–	methane
C_2H_2	–	acetylene
CH_3CHO	–	acetaldehyde
$CH_3CO\bullet$	–	acetic free radical
$(CHO)_2$	–	glyoxal
CH_3OH	–	methanol
C_2H_5OH	–	ethanol
CI	–	Collective Index
CK	–	creatine kinase
cm^3/g	–	cubic centimeters per gram
$(CN)_2$	–	cyanogen
CNS	–	central nervous system
CO	–	carbon monoxide
CO_2	–	carbon dioxide
cP	–	centipoise
CS_2	–	carbon disulfide
CuO	–	copper oxide
d	–	days, or day
dec	–	decomposes
DEG	–	diethylene glycol
DEGDN	–	diethyleneglycol dinitrate

LIST OF ABBREVIATIONS (continued)

DEGMN	—	diethyleneglycol mononitrate
DNDG	—	dinitrodiglycol = DEGDN
DOD	—	Department of Defense
ECG	—	electrocardiogram
EEG	—	electroencephalogram
EGDN	—	ethyleneglycol dinitrate
EGMN	—	ethyleneglycol mononitrate
Fe^{2+}	—	ferrous iron
Fe^{3+}	—	ferric iron
FTD: Hartley	—	Fort Detrick: Hartley strain of guinea pigs
g	—	acceleration of gravity
g/cm^3	—	grams per cubic centimeter
g/100 g	—	grams per 100 grams
g/kg	—	grams per kilogram
g/mL	—	grams per milliliter
GSH	—	reduced glutathione
GSSG	—	oxidized glutathione
H_2	—	hydrogen
Hb	—	hemoglobin
HbO_2	—	oxyhemoglobin; sometimes O_2Hb
HCHO	—	formaldehyde
HCN	—	hydrogen cyanide
HCO•	—	formic free radical
HCOOH	—	formic acid
Hg	—	mercury
H_2O	—	water
HPLC	—	high-precision liquid chromatography
hr	—	hour
H_2SO_4	—	sulfuric acid
ip	—	intraperitoneal
iv	—	intravenous
kJ/g	—	kilojoules per gram

km/s	–	kilometers per second
LAP	–	load, assembly, pack
lb/yr	–	pounds per year
LD	–	lactic dehydrogenase
LD_{50}	–	dose required to kill 50% of a group of test animals
m/s	–	meters per second
metHb	–	methemoglobin
µg/g	–	micrograms per gram
µg/mL	–	micrograms per milliliter
µg/plate	–	micrograms per plate
µm Hg	–	micrometers of mercury
mg	–	milligrams
mg %	–	milligrams percent (milligrams per 100 milliliters)
mg/kg	–	milligrams per kilogram
mg/L	–	milligrams per liter
mg/m^3	–	milligrams per cubic meter
mg/100 mL	–	milligrams per 100 milliliters
mL	–	milliliters
mm	–	millimeters
mM	–	millimolar
mmole	–	millimole
mmole/kg	–	millimoles per kilogram
min	–	minutes
N, N_2	–	nitrogen
NG	–	nitroglycerin
$-N^+(CH_3)_3$	–	trimethylammonium radical
NIH: NMRI	–	National Institutes of Health: Naval Medical Research Institute strain of mice
NMRI: (LE)	–	Naval Medical Research Institute: Long-Evans strain of rats
NMRI: O(SD)	–	Naval Medical Research Institute: Osborne-Mendel Sprague-Dawley strain of rats
NIOSH	–	National Institute of Occupational Safety and Health

nm	—	nanometer
NO	—	nitric oxide
N_2O	—	nitrous oxide
NO_2^-	—	nitrite ion
NO_2	—	nitrogen dioxide
NO_3^-	—	nitrogen dioxide
NO_3	—	nitrogen trioxide
O_2	—	oxygen
O_2Hb	—	oxyhemoglobin = HbO_2
PbO	—	lead oxide
PbO_2	—	lead dioxide
PETN	—	pentaerythritol tetranitrate
PG	—	propylene glycol
PGDN	—	propyleneglycol dinitrate
PGMN	—	propyleneglycol mononitrate
pH	—	potential hydrogen
PND	—	phrenic nerve diaphragm
ppb	—	parts per billion
ppm	—	parts per million
Q	—	quantity of blood; kilograms per hour
RDX	—	cyclotrimethylene trinitramine
rh	—	relative humidity
rpm	—	revolutions per minute
RTECS	—	Registry of Toxic Effects of Chemical Substances
s	—	seconds
sc	—	subcutaneous
SH and -SH	—	sulfhydryl groups
STEL	—	short term exposure limit
STP	—	standard temperature and pressure
TA	—	Test, Ames. Strains of *Salmonella* used in the Ames test
TEG	—	triethylene glycol
TEGDN	—	triethyleneglycol dinitrate

TEGMN	—	triethyleneglycol mononitrate
TLC	—	thin layer chromatography
TLV	—	threshold limit value
TMEDN	—	trimethylolethane dinitrate
TMEG	—	trimethylolethane glycol
TMEMN	—	trimethylolethane mononitrate
TMETN	—	trimethylolethane trinitrate
TNG	—	trinitroglycerin = NG, nitroglycerin
TNT	—	trinitrotoluene
TPNH	—	triphosphopyridine nucleotide, reduced
USEPA	—	U.S. Environmental Protection Agency
v	—	velocity of blood flow in meters per second
V	—	heart output in watts
VDT	—	visual discrimination test
VER	—	visual evoked response
V_{max}	—	enzyme reaction velocity, maximum
wk	—	week
wt %	—	weight percent
yr	—	year

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