September 1992

DATA DEFICIENCIES, PROBLEM AREAS, AND RECOMMENDATIONS FOR ADDITIONAL DATABASE DEVELOPMENT FOR DIETHYLENE GLYCOL DINITRATE (DEGDN)

AUTHORS

Mary B. Deardorff, Ph.D.
B. Ram Das, Ph.D., DABT
Welford C. Roberts, Ph.D.

PROJECT OFFICER

Krishan Khanna, Ph.D.
Office of Water
Health and Ecological Criteria Division
Office of Science and Technology
U.S. Environmental Protection Agency
Washington, DC 20460

September 1992

DATA DEFICIENCIES, PROBLEM AREAS, AND RECOMMENDATIONS FOR ADDITIONAL DATABASE DEVELOPMENT FOR DIETHYLENE GLYCOL DINITRATE (DEGDN)

AUTHORS

Mary B. Deardorff, Ph.D.
B. Ram Das, Ph.D., DABT
Welford C. Roberts, Ph.D.

PROJECT OFFICER

Krishan Khanna, Ph.D.
Office of Water
Health and Ecological Criteria Division
Office of Science and Technology
U.S. Environmental Protection Agency
Washington, DC 20460

PREFACE

This report was prepared in accordance with the Memorandum of Understanding between the Department of the Army, Deputy for Environmental Safety and Occupational Health (OASA (IL&E)), and the U.S. Environmental Protection Agency (EPA), Office of Water (OW), Office of Science and Technology (OST) for the purpose of developing drinking water Health Advisories (HAs) for selected environmental contaminants, as requested by the Army.

Health Advisories provide specific advice on the levels of contaminants in drinking water at which adverse health effects would not be anticipated and which include a margin of safety so as to protect the most sensitive members of the population at risk. These advisories normally are prepared for One-day, Ten-day, Longer-term, and Lifetime exposure periods where available toxicological data permit.

This report is the product of the foregoing process. Available toxicological data, including that provided by the Army, on the munitions chemical diethylene glycol dinitrate (DEGDN) have been reviewed, and relevant findings are presented in a manner so as to allow for an evaluation of the data without continued reference to the primary documents.

The available data are not sufficient to develop a HA; therefore, this report identifies deficiencies and recommends research that will enhance and optimize the database. When the recommended research has been conducted, it is expected that the data will allow the development of a drinking water HA for DEGDN.

I would like to thank the authors, Dr. Mary B. Deardorff, Dr. B. Ram Das, and Dr. Welford C. Roberts, who provided the extensive technical skills required for the preparation of this report. I am grateful to the members of the EPA Tox-Review Panel who took time to review this report and to provide their invaluable input, and I would like to thank Dr. Edward Ohanian, Chief, Human Risk Assessment Branch, and Ms. Margaret J. Stasikowski, Director, Health and Ecological Criteria Division for providing me with the opportunity and encouragement to be a part of this project.

The preparation of this Health Advisory was funded in part by Interagency Agreement (IAG) between the U.S. EPA and the U.S. Army Medical Research and Development Command (USAMRDC). This IAG was conducted with the technical support of the U.S. Army Biomedical Research and Development Laboratory (USABRDL), Dr. Howard T. Bausum, Project Manager.

Krishan Khanna, Ph.D.
Project Officer
Office of Water

CONTENTS

Page

EXECUTIVE SUMMARY v

I. OBJECTIVE I-1

II. BACKGROUND II-1

III. DISCUSSION III-1

IV. CONCLUSIONS AND RECOMMENDATIONS IV-1

V. REFERENCES V-1

APPENDIX A: REVIEW OF HEALTH EFFECTS AND OTHER DATA: DIETHYLENE GLYCOL DINITRATE

I. GENERAL INFORMATION A-1

II. SOURCES OF EXPOSURE A-3

III. ENVIRONMENTAL FATE A-4
A. PHOTOLYSIS A-4
B. BIOTRANSFORMATION A-4
C. HYDROLYSIS A-5
D. SORPTION ON SEDIMENT AND SOIL A-5

IV. TOXICOKINETICS A-6
A. ABSORPTION A-6
B. DISTRIBUTION A-6
C. METABOLISM A-6
D. EXCRETION A-6

V. HEALTH EFFECTS A-7
A. HUMANS A-7
B. ANIMAL EXPERIMENTS A-8
1. Short-term Exposure A-8
a. Acute A-8
b. Primary Irritation, Dermal Sensitization, and Ophthalmologic Effects A-13
c. Subacute A-14
2. Longer-term Exposure A-15
3. Reproductive Effects A-15
4. Developmental Toxicity A-15
5. Carcinogenicity A-16
6. Genotoxicity A-16

7. Other Effects A-17

C. CARCINOGENIC POTENTIAL A-18

VI. OTHER CRITERIA, GUIDANCE, AND STANDARDS A-19

VII. ANALYTICAL METHODS A-20

VIII. TREATMENT TECHNOLOGIES A-22

IX. REFERENCES A-24

TABLES for APPENDIX A

Page

I-1. General Chemical and Physical Properties of Diethylene Glycol Dinitrate A-2
V-1. Oral Median Lethal Dose (LD_{50}) for DEGDN A-9
V-2. Mortality of Sprague-Dawley Rats Dosed by Gavage with DEGDN A-10
V-3. Mortality of ICR Mice Dosed by Gavage with DEGDN A-12
VII-1. HPLC Conditions for Quantification of DEGDN in Water A-21

EXECUTIVE SUMMARY

Diethylene glycol dinitrate (DEGDN), a nitrate ester, is a highly water soluble liquid at room temperature. Because of its stability and resistance to shock, it is used as a plasticizer in place of nitroglycerin in some military gun propellants. DEGDN is produced by nitrating diethylene glycol with mixed acid and water. The Naval Ordinance Station, Indian Head, MD, currently manufactures DEGDN, and though treated, wastewater ultimately may enter the Potomac River. No studies of environmental levels of DEGDN were found. Photolysis is the major chemical transformation process for DEGDN loss from the aquatic environment, with a half-life less than 35 days. Microbial biotransformation is also an important fate process in water containing organic nutrients. Diethylene glycol dinitrate does not adsorb readily to soil but appears to bind irreversibly to sediment.

The available toxicology data are not sufficient to develop drinking water Health Advisories. Additional health and environmental research is necessary to develop the database. Needed research includes an acute (≤ 10 day), oral, dose-response study of DEGDN toxicity in rodents and subacute (less than 30 days), subchronic, and chronic oral studies that demonstrate dose-response relationships and No-Observed-Adverse-Effect Levels (NOAELs) in both sexes of at least two mammalian species. In addition, lifetime cancer bioassays that also evaluate systemic, noncarcinogenic effects in rats and mice of both sexes are required to adequately assess the potential health effects of DEGDN. Other areas where adequate data are lacking include potential reproductive and developmental effects, genotoxicity, toxicokinetics, and DEGDN levels in the environment.

Published reports in Czechoslovakia describe the human health effects from chronic occupational exposures to nitric esters of glycerine including DEGDN, but these reports do not differentiate the effects of DEGDN from those of other compounds produced at the factories. The major health effects associated with chronic exposures to unknown concentrations of primarily DEGDN and dinitroglycol were sudden death, precordial pain, headaches, coronary sclerosis, intermediary coronary syndrome, myocardial infarction, and elevated (200-300 mg%) blood cholesterol levels. No quantitative data on the toxicokinetics of DEGDN from oral, inhalation, or dermal exposures in humans or test animals were found in the available literature.

Acute oral doses of DEGDN were moderately toxic to a variety of laboratory animals. It produced signs of neurotoxicity, characterized primarily by behavioral and reflexive abnormalities in

male and female Sprague-Dawley rats and in ICR mice. For rats, the median lethal dose (LD_{50}) ± S.E. (at the 95% confidence limit) was 990.4±30.0 mg/kg for males and 753.1±35.9 mg/kg for females. For mice, the LD_{50} ± S.E. (at the 95% confidence limit) was 1,394.7±59.3 mg/kg for males and 1,320.7±73.5 mg/kg for females. In other DEGDN toxicity studies that lacked experimental information and data, oral LD_{50}s were reported for mice (1,250 mg/kg), rats (1,180 mg/kg), and guinea pigs (1,250 mg/kg), and symptoms typical of central nervous system damage and acute cyanosis were observed in all three species.

In studies of acute dermal toxicity and primary dermal irritation potential with New Zealand white rabbits, DEGDN did not produce any systemic or dermal signs of toxicity and was classified as a nonirritant. In guinea pigs, no evidence of dermal sensitization to DEGDN was obtained using the Buehler dermal sensitization method. Diethylene glycol dinitrate produced no primary eye irritation in New Zealand white rabbits.

A subacute cumulative toxicity study of DEGDN was located in the literature; however, the study lacked both experimental detail and data. In a 6-month oral toxicity study with white male rats, the earliest and most substantial DEGDN effect in the mid- and high-dose groups was a change in conditioned reflex activity. Changes in immunobiological status (not specified) were also reported. This study is limited by the use of one sex of a single species and a small number of animals per dose group. Although results were not given in detail and did not include histopathology, the study suggested a NOAEL of 0.05 mg/kg/day and a Lowest-Observed-Adverse-Effect level (LOAEL) of 0.5 mg/kg/day. DEGDN was not found to be toxic *in utero* when dermally administered to pregnant rats on days 6-15 of gestation, although an aberrant right subclavian artery seen in one fetus (1/254) of a DEGDN treated dam was judged by the authors to be a compound-related effect.

No *in vivo* studies on the carcinogenicity of DEGDN were located. In an *in vitro* mammalian cell transformation assay for the detection of potential chemical carcinogens, DEGDN did not cause cells to transform. In the absence of other data, DEGDN is classified in Group D: Not classifiable as to human carcinogenicity

An assessment of the mutagenic potential of DEGDN using the mouse lymphoma cell, a forward mutation assay, indicated that DEGDN is a weak mutagen. Diethylene glycol dinitrate was not mutagenic in the Ames *Salmonella*/mammalian microsome mutagenicity assay.

High performance liquid chromatography (HPLC) appears to be the method of choice for analysis of DEGDN. High performance liquid chromatography is preferred over gas chromatography because it avoids the destruction of nitro compounds resulting from the temperature programming of gas chromatography.

Biotransformation and photolysis are the only methodologies found in the literature for the treatment of DEGDN in water or sludge. Studies have shown that hydrolysis with lime or sodium sulfide does not decompose DEGDN effectively in wastewater.

I. OBJECTIVE

The objective of this document is to provide an evaluation of data deficiencies and problem areas encountered after a careful review of the literature on diethylene glycol dinitrate (DEGDN) and to make recommendations for additional database development. This document is presented as an independent analysis of the current data related to DEGDN in drinking water, and it includes a summary of the background information that was considered for the development of a Health Advisory (HA). For greater detail on the toxicology of DEGDN, the *Review of Health Effects and Other Data: Diethylene Glycol Dinitrate* (Appendix A) should be consulted.

II. BACKGROUND

Diethylene glycol dinitrate exists as a pale, yellow liquid at room temperature and is used as an explosive plasticizer in military gun propellants (Holleman *et al.*, 1983). It is replacing the more commonly used plasticizer, nitroglycerin, in gun propellants because DEGDN is more stable and less shock-sensitive than nitroglycerin (Kirk-Othmer, 1980; Holleman *et al.*, 1983; Burrows *et al.*, 1989). Diethylene glycol dinitrate is produced in the United States at the Naval Ordinance Station, Indian Head, MD, (Fischer *et al.*, 1987) by nitrating diethylene glycol with mixed acids and water (Rinkenbach, 1927). No measures of DEGDN in the environment are available. Once in water, DEGDN may be degraded mainly by sunlight, but also by microorganisms (Spanggord *et al.*, 1985, 1987). Photolytic chemical transformation occurs with a half-life less than 35 days. Microbial biotransformation is significant only in water containing organic nutrients. Diethylene glycol dinitrate does not readily adsorb to soil but appears to bind irreversibly to sediment.

Published reports in Czechoslovakia describe human health effects from chronic occupational exposures to nitric esters of glycerine including DEGDN, but these reports do not differentiate the effects of DEGDN from those of other compounds produced at the factories. Prerovska and Teisinger (1965) found that the major health effects associated with chronic exposures to unknown concentrations of mainly DEGDN and dinitroglycol were sudden death, precordial pain, headaches, coronary sclerosis, intermediary coronary syndrome, and elevated (200-300 mg%) blood cholesterol levels. No data on the toxicokinetics of DEGDN from oral, inhalation, or dermal exposures in humans or test animals have been reported.

Acute oral doses of DEGDN in male and female Sprague-Dawley rats and in ICR mice produced signs of neurotoxicity, characterized primarily by behavioral and reflexive abnormalities (Brown *et al.*, 1989; Ryabik *et al.*, 1989). For the rats, the median lethal dose (LD_{50}) ± S.E. (at the 95% confidence limit) was 990.4±30.0 mg/kg for males and 753.1±35.9 mg/kg for females. For the mice, the LD_{50} ± S.E. (at the 95% confidence limit) was 1,394.7±59.3 mg/kg for males and 1,320.7±73.5 mg/kg for females. Krasovsky *et al.* (1973) reported oral LD_{50}s of 1,250 mg/kg for white mice, 1,180 mg/kg for white rats, and 1,250 mg/kg for guinea pigs. Clinical signs in these three species included symptoms typical of central nervous system damage and acute cyanosis. Acute dermal exposure of rabbits to DEGDN did not result in any systemic toxicity (Brown and Korte, 1989b).

In studies of primary dermal irritation potential with New Zealand white rabbits, DEGDN did not produce any dermal signs of toxicity and was classified as a nonirritant (Brown and Korte, 1988a). In guinea pigs, no evidence of dermal sensitization to DEGDN was obtained using the Buehler dermal sensitization method (Hiatt *et al.*, 1988). Diethylene glycol dinitrate produced no primary eye irritation in New Zealand white rabbits (Hiatt and Korte, 1988).

A subacute cumulative toxicity study of DEGDN was located in the literature, but the report was inadequate in that it lacked both experimental detail and data (Krasovsky *et al.*, 1973). In a 6-month oral study with white male rats, the earliest and most substantial DEGDN effect in the mid-level and high dose groups was a change in conditioned reflex activity (Krasovsky *et al.*, 1973). Unspecified changes in the animals' immunobiological condition also were reported. However, the authors provided minimal experimental detail and no data to support their results. Although this study was less than adequate, it suggested a No-Observed-Adverse-Effect Level (NOAEL) of 0.05 mg/kg/day and a Lowest-Observed-Adverse-Effect Level (LOAEL) of 0.5 mg/kg/day. DEGDN was not found to be toxic *in utero* when dermally administered to pregnant rats on days 6-15 of gestation, although an aberrant right subclavian artery seen in one fetus (1/254) of a DEGDN treated dam was judged by the authors to be a compound-related effect (Mitala and Boardman, 1981).

Although no *in vivo* studies on the carcinogenicity of DEGDN were located, the results of an *in vitro*, mammalian cell assay for the detection of potential chemical carcinogens demonstrated that DEGDN did not transform cells (Kawakami *et al.*, 1988).

Diethylene glycol dinitrate was not mutagenic in the Ames *Salmonella*/mammalian microsome mutagenicity assay (Sano and Korte, 1988). An assessment of the mutagenic potential of DEGDN using the mouse lymphoma cell, a forward mutation assay, showed that DEGDN is a weak mutagen (Kawakami *et al.*, 1988).

High performance liquid chromatography (HPLC) appears to be the method of choice for analysis of DEGDN. High performance liquid chromatography is preferred over gas chromatography because it avoids the destruction of nitro compounds resulting from the temperature programming of gas chromatography (Holleman *et al.*, 1983).

Biotransformation and photolysis are the only methodologies found in the literature for the treatment of DEGDN in water or sludge (Spanggord *et al.*, 1985; Cornell *et al.*, 1981). Studies have shown that hydrolysis with lime or sodium sulfide does not decompose DEGDN effectively in wastewater (Smith *et al.*, 1983).

III. DISCUSSION

Although there is valuable information available with which to analyze the environmental fate, lethality, skin, and eye effects of DEGDN, only one oral study in rats (Krasovsky *et al.*, 1973) provides information that could be used potentially to derive a drinking water health advisory (HA) value, and it is less than ideal. There are currently no studies on the absorption, distribution, metabolism, or excretion of DEGDN in test animals or humans. In addition, evaluation of the mutagenic potential of DEGDN is limited with the available genotoxicity studies.

Three lethality studies were considered for HA derivation, but none were acceptable. The well designed acute oral LD_{50} studies of DEGDN in rats and mice did not establish a NOAEL or LOAEL, and usually are not used to derive a One-day HA value. Typically, LD_{50} studies do not provide detailed toxicity information about a compound and are not useful in establishing a dose-response relationship, necessary for identifying a NOAEL or LOAEL. The Krasovsky *et al.* (1973) LD_{50} study with rats, mice, and guinea pigs could not be considered for potential use in calculating HA values because the authors did not provide any data or experimental information with which to evaluate the results.

A subacute study of sufficient quality is not available for derivation of a Ten-day HA value. The 20-day, cumulative toxicity study by Krasovsky *et al.* (1973) lacked experimental detail and data, and the single-dose dermal developmental study with pregnant rats did not produce a dose-response relationship. Further, no toxicokinetic data to support the use of the dermal exposure pathway are available on DEGDN.

The only study considered minimally adequate for calculating Longer-term and Lifetime HA values is the Krasovsky *et al.* (1973) 6-month oral toxicity study with male rats. The authors apparently evaluated animal body weight, several clinical chemistry parameters, blood pressure, reflexive behavior, and at necropsy, gross pathology and organ weight. Dose-response information and a NOAEL and LOAEL for DEGDN were reported. However, the report lacked details of the experimental design and results. From the information reported, the study was limited by the use of only one sex of a single species, and a small number of animals per dose group (eight/dose). Thus, this study is not sufficient to derive a HA.

The primary irritation, dermal sensitization, and ophthalmologic effects of DEGDN have been studied extensively and the data appear to be of good quality. The mutagenic potential of DEGDN has been studied in both microbial and nonmicrobial cell systems. Although only one study in each cell system has been published, they appear to be good quality studies. There is, however, a need for additional genotoxicity studies in view of the observed weak mutagenic potential of DEGDN in a forward mutation assay with mouse lymphoma cells (Kawakami *et al.*, 1988). Lacking are *in vivo* lifetime/carcinogenicity data, which are needed to determine a Lifetime HA and to assess carcinogenic potential. Reproductive and developmental studies of DEGDN also are not available.

Spanggord *et al.* (1985, 1987) have published extensive information on DEGDN chemical and biological transformation processes, which is useful for estimating the persistence and ultimate fate of DEGDN after it has entered water, soil, and sediment. Because DEGDN is highly water soluble (0.4 g/100g at 20-25°C), has a low (<100) octanol-water partition coefficient (9.6), slowly volatilizes from aquatic media, and does not bind readily to soil, the potential exists for DEGDN to enter drinking water supplies through a variety of media. Yet, no studies are available of DEGDN levels in the environment, particularly the aquatic environment.

IV. CONCLUSIONS AND RECOMMENDATIONS

- An acute (≤ 10 day), dose-response study of DEGDN toxicity in rodents using oral exposure is not available and is recommended to establish a NOAEL for a One-day HA.

- Because an adequate subacute study is not available and is needed to calculate a Ten-day HA value, it is recommended that subacute oral toxicity studies of less than 30 days' duration be completed in both sexes of a rodent and one other mammalian specie. The studies should demonstrate clear dose-response relationships and NOAELs and include, among other indices, pathological examinations of test and control animals.

- Adequately designed subchronic and chronic studies are needed for the derivation of Longer-term and Lifetime HAs. These should be conducted in both sexes of at least two mammalian species.

- A lifetime cancer bioassay in rats and mice (both sexes) at three to five dose levels is needed for calculating a Lifetime HA and for assessing the carcinogenic potential of DEGDN. The bioassay also should evaluate systemic, noncarcinogenic effects from chronic and lifetime exposure.

- Studies are required to assess the reproductive and developmental effects of DEGDN. Thus, a reproductive toxicity study should be performed in at least one mammalian species, and developmental toxicity studies in at least two species.

- Additional genotoxicological information is needed to further clarify the issue regarding the weak mutagenicity of DEGDN observed in a lymphoma cell line.

- Further dermal sensitization and ophthalmologic studies do not appear necessary.

- Although data are available on the toxicokinetics of other nitric esters of glycerin, studies are needed on DEGDN absorption, distribution, metabolism, and excretion in humans and test animals. Toxicokinetic studies support the use of certain toxicity data in calculating HA values and are especially important when exposure pathways other than oral must be considered.

- To determine potential sources of exposure to DEGDN, studies of DEGDN levels in the environment are recommended.

V. REFERENCES

Brown LD, Korte Jr. DW. 1988a. Primary Dermal Irritation Potential of Diethyleneglycol Dinitrate (DEGDN) in Rabbits. Toxicology Series 154. Institute Report No. 304. Letterman Army Institute of Research, San Francisco, CA. Available from the National Technical Information Service (NTIS), Springfield, VA. Order No. ADA201960.

Brown LD, Korte Jr. DW. 1988b. Acute Dermal Toxicity of Diethyleneglycol Dinitrate in Rabbits. Toxicology Series 155. Institute Report No. 291. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA201956.

Brown LD, Ryabik JRG, Wheeler CR, Korte Jr. DW. 1989. Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in Rats. Toxicology Series 136. Institute Report No. 335. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA207233.

Burrows EP, Rosenblatt DH, Mitchell WR, Parmer DL. 1989. Organic Explosives and Related Compounds: Environmental and Health Considerations. Technical Report No. 8901. U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD.

Cornell JH, Wendt TM, McCormick NG, Kaplan DL, Kaplan AM. 1981. Biodegradation of Nitrate Esters Used as Military Propellants—A Status Report. Toxicological Report NATICK/TR-81/029. U.S. Army Natick Research and Development Laboratories, Natick, MA. Available from NTIS, Springfield, VA. Order No. ADA149665.

Fisher DJ, Burton DT, Paulson RL. 1987. Toxicity of DEGDN, Synthetic-HC Smoke Combustion Products, Solvent Yellow 33 and Solvent Green 3 to Freshwater Aquatic Organisms. Final Report for Phase II. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADA196906.

Hiatt GFS, Korte Jr. DW. 1988. Primary Eye Irritation Potential of Diethyleneglycol Dinitrate (DEGDN) in Rabbits. Toxicology Series 153. Institute Report No. 303. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA201961.

Hiatt GFS, Ryabik JRG, Korte Jr. DW. 1988. Dermal Sensitization Potential of Diethyleneglycol Dinitrate (DEGDN) in Guinea Pigs. Toxicology Series 143. Institute Report No. 307. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA201959.

Holleman JW, Ross RH, Carroll JW. 1983. Problem Definition Study on the Health Effects of Diethyleneglycol Dinitrate, Triethyleneglycol dinitrate, and Trimethylolethane Trinitrate and Their Respective Combustion Products. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADA127846.

Kawakami TG, Aotaki-Keen A, Rosenblatt LS, Goldman M. 1988. Evaluation of Diethyleneglycol Dinitrate (DEGDN) and Two DEGDN Containing Compounds. Final Report. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADA202289.

Kirk-Othmer. 1980. Encyclopedia of Chemical Technology, Vol. 9. Explosives and Propellants. pp. 561, 572-577, 608.

Krasovsky GN, Korolev AA, Shigan SA. 1973. Toxicological and hygienic evaluation of diethylene glycol dinitrate in connection with its standardization in water reservoirs. J. Hygiene Epidem. Microb. 17:114-119.

Mitala JJ, Boardman JP. 1981. Dermal Teratology Study on DEGDN, MTN, and DEGDN/MTN Mixture in Rats. Project 5-084. Adria Laboratories, Plain City, Ohio. Sponsor: Hercules, Inc. EPA/OTS Doc. No. 88-8100006.

Prerovska I, Teisinger J. 1965. Clinical picture of chronic poisoning with dinitrodiglycol. Pracovni Lekarstvi 17(2):41-43. (In Czechoslovakian; translation).

Rinkenbach WH. 1927. Preparation and properties of diethyleneglycol dinitrate. Industrial and Engineering Chemistry 19:925-927.

Ryabik JRG, Brown LD, Wheeler CR, Korte Jr. DW. 1989. Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in ICR Mice. Toxicology Series 137. Institute Report No. 336. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA134154.

Sano SK, Korte Jr. DW. 1988. Mutagenic Potential of Diethyleneglycol Dinitrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Toxicology Series 147. Institute Report No. 292. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA201795.

Smith LL, Carrazza J, and Wong K. 1983. Treatment of wastewaters containing propellants and explosives. J. Hazardous Materials 7:303-316.

Spanggord RJ, Chou T-W, Mill T, Podoll RT, Harper JC, Tse DS. 1985. Environmental fate of nitroguanidine, diethyleneglycol dinitrate, and hexachloroethane smoke. Final Report, Phase I. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADB114553.

Spanggord RJ, Chou T-W, Mill T, Haag W, Lau W. 1987. Environmental fate of nitroguanidine, diethyleneglycol dinitrate, and hexachloroethane smoke. Final Report, Phase II. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADB114553.

APPENDIX A

REVIEW OF HEALTH EFFECTS AND OTHER DATA: DIETHYLENE GLYCOL DINITRATE

I. GENERAL INFORMATION

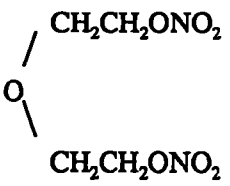
Diethylene glycol dinitrate (DEGDN), a nitrate ester, is a highly water soluble, clear to pale-yellow liquid at room temperatures. Other than nitroglycerin, DEGDN is the most widely used military explosive plasticizer (Kirk-Othmer, 1980). The Germans during World War II were the first to use DEGDN to any extent. It has been replacing nitroglycerin, an explosive plasticizer, in many military gun propellants including those used in the 120 mm shells for the M1 Abrams tank because it is more stable and less shock-sensitive than nitroglycerin (Holleman *et al.*, 1983; Burrows *et al.*, 1989).

Diethylene glycol dinitrate may be produced by nitrating diethylene glycol with a mixture of nitric and sulfuric acids plus water (Rinkenbach, 1927). Complete nitration occurred within 30 minutes at temperatures of 5-10°C. The yield of DEGDN from nitrating diethylene glycol with mixed acid is approximately 85% of the theoretical value (Kirk-Othmer, 1980). After nitration, the compound can be purified by washing (Burrows *et al.*, 1989).

In the past, DEGDN was manufactured and processed at the Radford Army Ammunition Plant, Radford, VA, which discharged its wastewaters through underground pipelines to a biological treatment facility prior to discharge into the New River (Spanggord *et al.*, 1987). It is presently manufactured at the Naval Ordinance Station, Indian Head, MD, where effluent wastes ultimately enter the Potomac River (Fischer *et al.*, 1987).

General chemical and physical properties of DEGDN are presented in Table I-1.

Table I-1. General Chemical and Physical Properties of Diethylene Glycol Dinitrate (DEGDN)

Property	Value
CAS No.	693-21-0
Synonyms	Dinitrodiglycol; Ethanol, 2,2'-oxybisdinitrate; Di(hydroxyethyl) ether dinitrate; Bis(hydroxyaethyl)-aether-dinitrat (German); Diglykoldinitrat (German); Diethylene glycol dinitrate (U.S.); Diethylenglykoldinitrate (Czech); Dinitrodiglykol (Czech); Diglycoldinitraat (Dutch); Diglycol (dinitrate DE) (French); Dinitrate de diethylene-glycol (French); Dinitrodigliocol (Italian)
Molecular weight	196.14
Empirical formula	$C_4H_8N_2O_7$
Structure	
Physical state	Colorless to pale yellow liquid at room temperature (21°C)
Melting point	-11.3°C
Boiling point	160°C (when heated rapidly)
Heat of combustion	11.68 kJ/g (2.79 kcal/g)
Heat of formation	2.17 kJ/g (0.52 kcal/g)
Density	1.38 g/cm^3
Vapor pressure	5.9 µm Hg; 0.0036 torr; 0.00593 torr at 25°C
Octanol-water partition coefficient (K_{ow})	9.6
Stability characteristics	Stable at room temperature in ethanol, acetone (a desensitizer), or freshwater (28.6 mg DEGDN/L at 22°C for 48 hours); explosion temperature 240°C
Solubility characteristics	Water: 0.4 g/100 g at 20-25°C; 0.46 g/100 g at 60°C; 3,900 mg/L Ethanol: Soluble

SOURCE: Adapted from Holleman *et al.*, 1983; Spanggord *et al.*, 1985; Brown *et al.*, 1989; Kirk-Othmer, 1980; Fisher *et al.*, 1985; Sax, 1984; Clayton and Clayton, 1982; U.S. DOT.

II. SOURCES OF EXPOSURE

No data are available regarding actual or potential sources of exposure to DEGDN, although DEGDN may enter the aquatic environment from pretreatment plants associated with its production. Based on DEGDN's water soluble properties, stability, and low rate of evaporation from water, the potential exists for DEGDN to occur in drinking water derived from surface or ground-water supplies.

III. ENVIRONMENTAL FATE

Photolysis is the major chemical transformation process for DEGDN loss from water, with a half-life less than 35 days. Microbial biotransformation is also an important fate process in water containing organic nutrients. Nevertheless, volatilization and hydrolysis are slow under most environmental conditions; consequently, DEGDN is considered stable in aquatic environments. Soil sorption is a relatively unimportant fate, and biotransformation in soil appears to be slow. Irreversible binding (physical process) of DEGDN to sediment seems to dominate DEGDN fate and movement in that medium.

A. PHOTOLYSIS

Spanggord *et al.* (1985, 1987) identified sunlight photolysis as the dominant fate process for DEGDN in the aquatic environment. Although DEGDN photolysis occurs at an efficiency of only 18% at a concentration of 61 µM, its half-life ranges from 15 days in summer to 59 days in winter based on first-order photolysis rate constants. An initial DEGDN concentration of 3.13×10^{-5} M was reported to have a half-life of 27 days in a sample of Kansas River water and 35 days in distilled water exposed to sunlight. Major photolytic transformation products were 2-hydroxyethyl-nitratoacetate, nitrate, glycolic acid, and formic acid.

B. BIOTRANSFORMATION

Aerobic and anaerobic microbial biotransformations are important fate processes for DEGDN in waters containing other organic substrates (Spanggord *et al.*, 1985, 1987). Ethanol, which is a major organic chemical component in the Radford Army Ammunition Plant (RAAP) bioreactor effluent, was determined to be an effective co-metabolic substrate for microorganisms present in the RAAP wastewater and in the New River (Spanggord *et al.*, 1985, 1987). The RAAP bioreactor's aerated lagoon water and rotating bioreactor effluent aerobically reduced DEGDN from 10 ppm to <0.45 ppm in 5 days with or without added organic nutrient. In New River water, DEGDN loss under aerobic conditions was only 14% after 50 days, but in the presence of 180 ppm ethanol, DEGDN transformation was complete in 5 days. Glucose plus yeast extract (100 ppm each) were not as effective as ethanol in the aerobic degradation of DEGDN. With glucose plus yeast extract, there was a 70% loss in 20 days and a 90% loss after 34 days. Under anaerobic conditions, New

River water plus 1% bottom sediment and 180 ppm ethanol completely degraded DEGDN in 12 days. It took 16 days to transform 50% of DEGDN in New River water plus 1% bottom sediment and glucose and yeast extract (DEGDN was not detected after 26 days). Without added organic substrates, DEGDN loss in New River water was 15% after 16 days and 95% after 41 days. Spanggord *et al.* (1987) found that the biotransformation of DEGDN proceeds with a second-order biotransformation rate constant of 3.9×10^{-11} mL/organism/hour. Thus, in water, such as the New River, with a microbial population of 1×10^{6} organisms/mL, the half-life of DEGDN was projected to be 740 days. Biotransformation products do not build up in aquatic media indicating that the products are metabolized as carbon and energy sources. In soil and soil-water mixtures under aerobic conditions, biotransformation is very slow, slower than in sediment-water mixtures (Spanggord *et al.*, 1985).

C. HYDROLYSIS

Spanggord *et al.* (1985) observed very slow hydrolytic rates for DEGDN in water at 25°C. At pH 7.0 the hydrolytic half-life was >800 days. Half-life decreased with increasing pH.

D. SORPTION ON SEDIMENT AND SOIL

Experimental studies of the adsorptive properties of DEGDN in sterile and non-sterile sediments suggest that DEGDN is non-biologically lost in sediment probably through irreversible, physical binding to the sediment (Spanggord *et al.*, 1985). In contrast, the relatively low soil sorption partition coefficients for DEGDN, yielding K_{oc} values of 100 and 108 on U.S. EPA standardized soils, indicate that sorption on soils is not significant (Spanggord *et al.*, 1985).

IV. TOXICOKINETICS

A. ABSORPTION

No quantitative data on the absorption of DEGDN from oral, inhalation, or dermal exposures were found in the available literature.

B. DISTRIBUTION

No studies on the distribution of DEGDN were located in the literature.

C. METABOLISM

No studies on the metabolism of DEGDN in the body were found in the literature.

D. EXCRETION

No data on the excretion of DEGDN from the body were found in the literature.

V. HEALTH EFFECTS

A. HUMANS

Information on the human health effects of nitric esters of glycerine, including DEGDN, comes from reports of chronic occupational exposures to these compounds in Czechoslovakia where their toxicity to humans has been known since about 1952 (Styblova, 1966). However, none of these reports clearly distinguish between the effects of DEGDN and those of other compounds produced at the munitions factories.

In a plant producing dinitroglycol (DNG) and nitroglycerine, Prerovska and Teisinger (1965) observed the effects of DNG and dinitrodiglycol (DEGDN) on workers exposed to these compounds for 5 to 7 years. Due to changes in working conditions, the investigators could not differentiate between the effects of each substance produced at the plant. Also, exposure concentrations were not reported. Autopsies of four employees who died suddenly between 1958 and 1961 revealed slight to significant signs of coronary sclerosis without any signs of coronary artery blockage. Among 45 surviving employees at the plant, 37 reported precordial pain, headaches, and more rarely, collapse with loss of consciousness. Three of the 37 employees also showed obvious signs of coronary sclerosis, eight had intermediary coronary syndrome, and one had a myocardial infarction. Almost all subjects had blood cholesterol levels around 220 mg%, and in some it reached 300 mg%. After measures were taken to remove from the high-risk areas those workers with cardiovascular, liver, or kidney diseases as well as those with neural disorders, ulcers, or any disease causing general weakness, only specific subjective difficulties such as headaches after a holiday and intolerances for alcohol were reported. No cardiogram changes or deviations in serum lipids were found. Styblova (1966) studied the nervous system effects in 38 employees from the Prerovska and Teisinger (1965) group. In about a third of these employees, they found intense, long-lasting, throbbing headaches that ceased after several days at work, but returned in full intensity on nonworking days, even of one day's duration. Some employees also experienced hyperemia of the face, bloodshot eyes, depression, and sleep disorders. Intense shaking of the upper limbs was reported in five workers aged 20, 30, 34, 35, and 40 years. For three of these workers, this ailment continued for 1-2 years after stopping work.

Kuzelova and Cermakova (1974) studied six cases of sudden death among employees at two explosives plants including those deaths described by Prerovska and Teisinger (1965). The employees had been exposed to nitric esters of glycerin including DEGDN from 4-13 years (average 8 years). All deaths occurred in the morning. Five victims died after 1-3 days away from the workplace. The average age at death was 37 years, though ages ranged from 29-46 years. Anginal pain, a typical sign of chronic exposure to nitric esters of glycerine, had occurred in the victims during days away from work or before arrival to work. Cause of death based on the post-mortem findings was unclear.

B. ANIMAL EXPERIMENTS

1. Short-term Exposure

a. Acute

Single-dose, acute oral toxicity of DEGDN was studied in male and female Sprague-Dawley rats and ICR mice (Table V-1) (Brown *et al.*, 1989; Ryabik *et al.*, 1989). For rats, the median lethal dose (LD_{50}) ± S.E. (at the 95% confidence limit) was 990.4±30.0 mg/kg for males and 753.1±35.9 mg/kg for females. For mice, the LD_{50} ± S.E. (at the 95% confidence limit) was 1,394.7±59.3 mg/kg for males and 1,320.7±73.5 mg/kg for females. Diethylene glycol dinitrate produced signs of neurotoxicity in rats and mice characterized primarily by behavioral and reflexive clinical signs. Krasovsky *et al.* (1973) also determined oral LD_{50} values of 1,180 mg/kg for white rats, 1,250 mg/kg for white mice, and 1,250 mg/kg for guinea pigs (strain not specified), but did not provide experimental detail or data.

Brown *et al.* (1989) administered DEGDN suspended in corn oil to male and female Sprague-Dawley rats (ten/sex/dose) by a single gavage treatment at a volume of 10 mL/kg using the following doses: 794, 891, 1,000, 1,120, or 1,260 mg DEGDN/kg. The animals were observed for mortality and signs of acute toxicity at 1, 2, 4, and 6 hours after dosing and daily for the remainder of the 14-day study. The percent mortalities corresponding to the above DEGDN dose groups were 0, 25, 42.8, 87.5, and 100, respectively, for males, and for females, 11.1, 70, 87.5, 85.7, and 100, respectively. Dose levels and DEGDN mortality data are presented in Table V-2. The majority of

Table V-1. Oral Median Lethal Dose (LD_{50}) for DEGDN

Species	Median Lethal Dose (LD_{50}) mg/kg		Source
Sprague-Dawley Rats	990.4±30.0 male	753.1±35.9 female	Brown *et al.* (1989)
White Rats	1,180		Krasovsky *et al.* (1973)
ICR Mice	1,394.7±59.3 male	1,320.7±73.5 female	Ryabik *et al.* (1989)
White Mice	1,250		Krasovsky *et al.* (1973)
Guinea Pigs	1,250		Krasovsky *et al.* (1973)

SOURCE: Adapted from Brown *et al.* (1989), Krasovsky *et al.* (1973), Ryabik *et al.* (1989)

Table V-2. Mortality of Sprague-Dawley Rats Dosed by Gavage with DEGDN[a]

Dose Level (mg/kg)	Number of Deaths/Group[b]	Died Within 24 Hours	Died Between 24-48 Hours	Percent Mortality
		Male		
794	0/7	0	0	0
891	2/8	0	0	25.0
1000	3/7	2	1	42.8
1120	7/8	3	4	87.5
1260	8/8	7	1	100.0
Vehicle[c]	0/5	0	0	0
		Female[d]		
631	1/9	0	0	11.1
794	7/10	0	2	70.0
891	7/8	3	4	87.5
1000	6/7	3	3	85.7
1260	7/7	7	0	100.0

[a]LD_{50}s are 990.4 mg/kg for males and 753.1 mg/kg for females.
[b]Number remaining after misdoses removed from study (initially 10 animals/group, except female group at the 794 mg/kg dose which had 16 and the male control group which had 5 animals assigned).
[c]Corn oil 2.47-2.67 mL.
[d]No vehicle control group.

SOURCE: Adapted from Brown *et al.* (1989)

deaths occurred within 24 hours of dosing. The clinical signs most frequently observed in all dose groups (but females more than males) were behavioral and neurological disturbances including inactivity, twitching, tremors, hypertonia, jumping, and ataxia. These clinical signs began 1-3 hours after dosing and with the exception of inactivity, persisted for no more than 72 hours. Other dose-related clinical signs included prostration, moribund condition, squinting, lacrimation, and chromodacryorrhea, depressed grasp or righting reflexes, increased startle reflex, and cyanosis. Signs of irritability, hunched posture, diarrhea, and discolored perianal region observed in both treatment and vehicle control group animals were attributed to the administration of corn oil but also could have been indicators of general ill health. Weight gain of survivors was not affected by DEGDN. Treatment-related multifocal necrohemorrhagic gastritis was observed at necropsy in the 794 mg/kg and 1,000 mg/kg treatment groups of both males and females, which indicated an effect level at the lowest administered dose.

Ryabik *et al.* (1989) administered DEGDN suspended in corn oil to ICR mice (ten/sex/dose) by single gavage treatment at volumes ranging from 0.31 to 0.39 mL for males and 0.23 to 0.31 mL for females and at doses of 1,000, 1,180, 1,390, 1,640, or 1,930 mg DEGDN/kg. The animals were observed for mortality and signs of acute toxicity at 1, 2, and 4 hours after dosing and daily for the remainder of the 14-day study. The percent mortalities corresponding to the above DEGDN dose groups were 0, 20, 70, 60, and 100, respectively, for males, and for females, they were 10, 30, 70, 90, and 80, respectively. Dose levels and DEGDN-related mortality data are presented in Table V-3. The majority of deaths occurred within 4 to 27 hours after dosing. Nearly all of the remaining deaths occurred between 27 and 45 hours after dosing. Clinical signs of DEGDN toxicity, which are similar to those observed for rats, (Brown *et al.*, 1989) began 2 hours after dosing in all dose groups, and most did not persist beyond 72 hours post-dosing. The clinical signs most frequently observed at all dose levels were behavioral and neurological disturbances including inactivity, twitching, tremors, hypertonia, and hyperactivity. Other dose-related clinical signs included prostration, moribund condition, depressed grasp and righting reflexes, and increased startle reflex. The additional signs of hunched posture, squinting, rough coat, and perianal discoloration were not considered direct manifestations of DEGDN toxicity, but rather indicators of general ill health. Weight gain of DEGDN survivors was not significantly affected, and no gross pathological lesions were found. The lowest tested dose caused adverse effects.

Table V-3. Mortality of ICR Mice Dosed by Gavage with DEGDN[a]

Dose Level (mg/kg)	Number of Compound Related Deaths/Number in Group		Percent Mortality	
	Male	Female	Male	Female
1000	0/10	1/10	0	10
1180	2/10	3/10	20	30
1390	7/10	7/10	70	70
1640	6/10	9/10	60	90
1930	10/10	8/10	100	80
Vehicle Control[b]	0/5	0/5	0	0

[a]LD_{50}s are 1,394.7 mg/kg for males and 1,320.7 mg/kg for females.
[b]Corn oil 10 mL/kg.

SOURCE: Adapted from Ryabik *et al.* (1989)

Krasovsky *et al.* (1973) reported oral LD_{50}s of 1,250 mg/kg for white mice, 1,180 mg/kg for white rats, and 1,250 mg/kg for guinea pigs (strains were not specified). Clinical signs in these species included symptoms typical of central nervous system damage and acute cyanosis. The authors did not provide any further experimental detail or data.

In an acute dermal toxicity study, a DEGDN (100% concentration) dose of 2.0 g/kg was applied in a gauze dressing with semi-occlusive wrap for a 24-hour period to the clipped back skin of ten New Zealand white rabbits (five/sex) (Brown and Korte, 1988b). The authors observed no systemic signs clearly attributable to dose and no gross or microscopic pathological changes in the treated animals during a 14-day observation period. Thus, the compound was considered to have minimal potential for acute dermal toxicity. However, dermal applications of DEGDN to pregnant rats on gestation days 6-15, caused an aberrant right subclavian artery in one fetus, considered by the authors to be treatment related (Mitala and Boardman, 1981) (See V.B.4 of this review).

Krasovsky *et al.* (1973) intravenously administered an acute, single dose of 0.4 mg/kg DEGDN in water to rabbits (species and number not specified) and observed a pronounced and prolonged hypotensive effect without electrocardiographic (EKG) changes, indicating that the compound impaired vascular tone without influencing myocardial activity. Although the Krasovsky *et al.* (1973) study lacks experimental data and detail, the results are consistent with those of Valachovic (1965) in dogs.

b. Primary Irritation, Dermal Sensitization, and Ophthalmologic Effects

In studies of primary dermal irritation potential with New Zealand white rabbits, DEGDN produced only a slight erythema, persisting for 1-3 days, in two males and one female after removal of the dressings and was classified as a nonirritant (Brown and Korte, 1988b) (experimental details in V.B.1.a of this review). No evidence of dermal sensitization to DEGDN occurred in guinea pigs. Diethylene glycol dinitrate produced no primary eye irritation in New Zealand white rabbits.

Brown and Korte (1988a) used a modified Draize procedure to determine the primary dermal irritation potential of DEGDN in five males and one female New Zealand white rabbits. DEGDN was applied (0.5 mL on a gauze patch) to close-clipped backs of the animals for 4 hours, and dermal reactions were scored and graded at 1, 24, 48, and 72 hours after removal of the patches. Neither

edema, erythema, nor any other recognizable skin reaction were produced during the 72-hour observation period.

The dermal sensitization potential of DEGDN on thirty male Hartley guinea pigs was determined using a modified Buehler closed patch, dermal sensitization procedure (Hiatt *et al.*, 1988). During the induction phase of the test, a patch containing 0.5 mL DEGDN at 100% concentration was applied on the clipped and shaved skin of the test animals for 3 consecutive weeks. Two weeks after the 3-week induction phase, the clipped and shaved patch sites on the animals were challenged with an additional 0.5 mL DEGDN. No skin responses were observed following each induction dose or after the challenge dose, indicating that DEGDN has no potential for causing dermal sensitization in guinea pigs.

Hiatt and Korte (1988) evaluated the potential for DEGDN to produce primary eye irritation in six male New Zealand white rabbits using a modified Draize method. Application of 0.1 mL DEGDN at 100% concentration to the inside of the lower eye lid produced no corneal opacity and no changes in lens clarity or surface morphology. Slight iridial vasodilation was observed in one rabbit but did not persist, and slight conjunctival redness and swelling occurred within 1-4 hours in three rabbits but cleared by 24 hours. Although DEGDN produced some eye irritation, the authors did not consider the changes sufficient to classify DEGDN as an ocular irritant.

c. Subacute

The only subacute DEGDN study located in the literature is that by Krasovsky *et al.* (1973). These authors evaluated the functional condition of white male rats (number not specified) and the dynamics of recovery on several blood parameters following successive oral exposures to DEGDN at 1/5, 1/25, or 1/125 of the LD_{50} (1,180 mg/kg) over a 20-day period. Blood samples were taken from the test animals before the compound was administered and at 30, 90, and 310 minutes after dosing on days 1, 5, 10, 15, and 20 of the experiment. The authors apparently measured the blood levels of methemoglobin, erythrocytes, hemoglobin, and glutathione before and after treatment. However, neither experimental detail nor results were presented. This study cannot be considered conclusive because it was not substantiated with appropriate data.

2. Longer-term Exposure

No 90-day studies of DEGDN toxicity were available in the literature. However, Krasovsky *et al.* (1973) reported on the effects of DEGDN on white male rats (eight/dose; strain not specified) given oral doses of vegetable oil solutions of 0.05, 0.5, or 5.0 mg DEGDN/kg six days per week for 6 months. An additional group of eight rats served as the controls. Although the experimental design and results were not given in detail, the authors apparently kept track of animal body weight, a number of clinical chemistry parameters, reflex behavior, blood pressure, and at necropsy gross pathology and organ weight. In the mid- and high-dose groups, changes in conditioned reflex activity and immunobiological status (not specified) were reported. The earliest and most substantial effect was that on the conditioned reflex activity. The high dose also provoked some decrease in blood pressure by the 5th and 6th months and a change in the mitotic activity of bone marrow, but no further details were presented. The authors reported no significant differences from controls in blood levels of cholinesterase activity, erythrocytes, leukocytes, reticulocytes, hemoglobin, and methemoglobin; in the diameter of erythrocytes; in urinary 17-ketosteroid levels; in bromsulphalein load; in organ weights and their ascorbic acid contents; and in the level of thiol groups in liver homogenates. The study suggested a No-Observed-Adverse-Effect level (NOAEL) of 0.05 mg/kg/day and a Lowest-Observed-Adverse-Effect Level (LOAEL) of 0.5 mg/kg/day. This study was not sufficient for Health Advisory development because of the small number of animals used, and details of the experiment and results were not reported.

No chronic (18-24 month, rodent) toxicity studies using DEGDN were found in the available literature.

3. Reproductive Effects

No studies on the reproductive effects of DEGDN were found in the available literature.

4. Developmental Toxicity

Mitala and Boardman (1981) dermally applied 11 µL DEGDN/rat/day to the closely-clipped scapular area of 25 pregnant Sprague-Dawley HAP (SD) BR rats on gestation days 6 through 15. The authors extracted DEGDN (10%) through repeated washings from solutions of diethylene glycol

but did not report the purity of the test substance applied to the rats. Assuming 100% purity and a reported body weight of 0.22 kg, the quantity of DEGDN applied to the rats would have corresponded to a calculated dose of 0.07 mg/kg/day. All rats were weighed on days 0, 6, 12, 16, and 20 of gestation and were sacrificed on gestation day 20. Minimum body weight at the beginning of mating was 0.22 kg. The dams were subjected to post-mortem abdominal, thoracic, and cesarean section examinations. The fetuses were weighed individually and subjected to gross examinations and soft tissue or skeletal examinations. No treatment-related effects on maternal body weight, excised uterine weights, number of corpora lutea, total implantations, live fetuses, or fetal sex ratio were observed in the dams. There were no significant differences in fetal body weights or crown-rump lengths. The only fetal observation, considered by the investigators to be biologically important, though not statistically significant, was the occurrence of an aberrant right subclavian artery in one fetus (1 of 254 fetuses; 24 litters) of a treated dam. This anomaly was deemed meaningful because a greater incidence had been observed by these same authors in a previous study of the toxicity of a mixture of metriol trinitrate ($C_5H_9N_3O_9$) and DEGDN. Thus, while DEGDN was not found to be toxic *in utero* when dermally administered to pregnant rats on days 6-15 of gestation, the aberrant right subclavian artery seen in one fetus of the DEGDN group was judged by the authors to be compound related.

5. Carcinogenicity

No *in vivo* studies on the carcinogenicity of DEGDN were located in the available literature.

The results of a short-term mammalian cell transformation assay for the detection of potential chemical carcinogens *in vitro* demonstrated that DEGDN did not cause cell transformation (Kawakami *et al.*, 1988). The *in vitro* carcinogenic potential of DEGDN was determined with and without the addition of the chemical promoter, 12-0-tetradecanoyl phorbol 13-acetate (TPA), by the Rauscher leukemia virus-infected rat embryo cell (RLV-RE) transformation assay. Diethylene glycol dinitrate, with and without TPA, failed to transform RLV-RE cells.

6. Genotoxicity

DEGDN was not mutagenic in the Ames assay. *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 were exposed to DEGDN over a 1,000-fold range of concentrations (5, 1, 0.2,

0.04, 0.008, 0.0016 µL/plate) in the presence and absence of exogenous S9 metabolic activation (Sano and Korte, 1988). These dose levels represent a concentration range that decreases from the minimum toxic level (the maximum or limit dose) by a dilution factor of five. A range finding toxicity test was conducted to determine the sublethal concentrations of DEGDN. Under the conditions of the Ames assay, DEGDN did not induce an increase in revertant colony counts necessary for a positive response or a dose-response effect.

In a forward mutation assay, Kawakami *et al.* (1988) assessed the mutagenic potential of DEGDN using the point mutation at the thymidine kinase locus in the mouse lymphoma cell (L5178Y TK=/-). The results indicated that DEGDN is a weak but direct mutagen because it increased mutagenic activity without exogenous S9 metabolic activation and the mutagenic activity was not enhanced in the presence of S9 activation. The mammalian metabolic activator, S9, used in this study was derived from Aroclor 1254 induced rat liver. A quantity of 65 µg DEGDN/mL or 117 µM DEGDN was necessary to induce one mutant.

7. Other Effects

Fisher *et al.* (1987) determined that the acute toxicity of DEGDN to freshwater aquatic fish, invertebrates, and algae was relatively low compared to other nitrate esters, especially nitroglycerin and ethyleneglycol dinitrate. Nine aquatic species were tested including fish (fathead minnow, channel catfish, bluegill, and rainbow trout), invertebrates (water flea, midge larva, mayfly larva, and amphipod), and algae (*Selenastrum capricornutum*). The invertebrate 48-hour LC_{50}s ranged from 90.1 to 355.3 mg/L, with the water flea (*Daphnia magna*) being most sensitive. Three of the fish species displayed sensitivities similar to the invertebrates, with a mean 96-hour LC_{50} of 273.5 mg/L. Only the fathead minnow (*Pimephales promelas*) was more tolerant with a 96-hour LC_{50} of 491.4 mg/L. The most sensitive fish was the bluegill (*Lepomis macrochirus*) with a 96-hour LC_{50} of 258.0 mg/L. The alga, *Selenastrum capricornutum*, was the most sensitive of all the species tested with DEGDN in this study. The exposure concentration that produced 50% algistatic effect (EC_{50}) in 5 days for this alga was 39.1 mg/L, based on dry weight.

C. CARCINOGENIC POTENTIAL

No *in vivo* studies on the potential carcinogenicity of DEGDN were found in the literature. Therefore, no calculation of excess cancer risk has been made. Diethylene glycol dinitrate is classified in Group D; not classifiable as to human carcinogenicity (U.S. EPA, 1986).

VI. OTHER CRITERIA, GUIDANCE, AND STANDARDS

Neither the American Conference of Governmental Industrial Hygienists nor the Occupational Safety and Health Administration have determined limits for DEGDN. A search of published literature and government documents produced no information on existing standards, criteria, or guidance on DEGDN.

VII. ANALYTICAL METHODS

High performance liquid chromatography (HPLC) appears to be the method of choice for analysis of DEGDN and other nitrate esters. HPLC is preferred over gas chromatography because it avoids the destruction of nitro compounds resulting from the temperature programming of gas chromatography (Holleman *et al.*, 1983). Fisher *et al.* (1985) developed an HPLC method for separating DEGDN utilizing a Waters Associates HPLC with a variable wavelength ultraviolet (UV) detector (215 and 254 nm) and a Varian Techtron Model 635 spectrophotometer (215 and 254 nm). Details of the final HPLC conditions are presented in Table VII-1. The lowest concentration of DEGDN detected by the HPLC method using 100 µL injections of 0.45 µ filtered diluent was 0.286 mg/L (the detection limit for this method without sample concentration or cleanup). Water samples filtered immediately before being injected into the HPLC caused a loss of 1% of the original DEGDN as determined by HPLC. The HPLC retention time for DEGDN in diluent water ranged from 5.814 to 5.857 minutes (N=8) with a 30% deionized/glass distilled (DI) H_2O:70% methanol mobile phase.

Yinon and Hwang (1983) developed an HPLC-mass spectrometry method suitable for the analysis of thermally sensitive and involatile explosives including DEGDN. Other published methods for analyzing DEGDN include gas chromatography (Camera and Pravisani, 1964) and gas-liquid chromatography (Alley and Dykes, 1972). ^{13}C nuclear magnetic resonance spectra are being developed for a number of nitrate esters of aliphatic alcohols (Narasimhan *et al.*, 1987).

A rapid, quantitative method for estimating DEGDN in explosive nitrate mixtures is available (Parihar *et al.*, 1967). Separation of individual compounds containing DEGDN is accomplished with thin-layer chromatography utilizing as absorbent alumina neutral (200 mesh) with 20% $CaSO_4$. Nonpolar solvents were more preferable to polar ones, which produce tailing. After extraction from the plates, quantities of the nitrate compounds are estimated colorimetrically to 2-4 µg with Griess-Romijin reagent.

Table VII-1. HPLC Conditions for Quantification of DEGDN in Water

Parameter	Value
HPLC	Waters Associates HPLC, dual M45 pumps with Model 680 gradient controller, Model 780 Data Module (integrator), U6K injector, Model 481 variable wavelength UV detector and Z-Module Radial Compression Column System
Column	Waters Radial-PAK, μBONDAPAK C_{18}
Standard Solvent	Diluent freshwater
Mobile Phase	30% DI* H_2O:70% CH_3OH
Method	Isocratic
Flow Rate	1 mL/min
Detector	UV 215 nm
Injection Volume	100 μL

*Deionized/glass distilled (DI)

SOURCE: Adapted from Fisher *et al.* (1985).

VIII. TREATMENT TECHNOLOGIES

Biotransformation and photolysis are the only methodologies found in the literature for the treatment of DEGDN in water or sludge (Spanggord *et al.*, 1985; Cornell *et al.*, 1981). Studies have shown that hydrolysis using lime or sodium sulfide is not effective for the decomposition of DEGDN in wastewater (Smith *et al.*, 1983).

Waters from a local pond in California and from various surface waters near the Radford Army Ammunition Plant (RAAP), Radford, VA, were used to investigate the aqueous biotransformation of DEGDN (Spanggord *et al.*, 1985). Aerobic and anaerobic biodegradation of DEGDN were insignificant in waters from the California pond to which DEGDN alone had been added. In samples to which DEGDN plus organic nutrients (glucose and yeast extract) were added, HPLC analysis showed a 50% loss of DEGDN in 19 days and a 94% loss after 40 days. In water samples obtained from a lagoon and from the New River near the RAAP, well developed aerobic and anaerobic DEGDN biotransformation microbes were found. However, in the absence of organic nutrients, these organisms were slow to transform DEGDN. In New River water under aerobic conditions, loss of 10 ppm DEGDN was only 14% after 50 days, but complete biotransformation occurred in 5 days after 180 ppm ethanol was added to the sample. Ethanol (a solvent used at the Radford Plant) proved to be a better metabolic substrate than glucose plus yeast extract. In other experiments with microorganisms obtained from the RAAP Bioreactor Plant effluent, ethanol was shown to influence the growth of the DEGDN biotransformation organisms but did not appear to be necessary as an energy source to promote DEGDN biotransformation (Spanggord *et al.*, 1987). In the Bioreactor Plant with a microbial population of 10^{10} organisms/mL, the first-order rate constant for biotransformation of DEGDN was estimated to be 3.9×10^{-11} mL/organism/hour (Spanggord *et al.*, 1987).

Cornell *et al.* (1981) studied the aerobic microbial degradation of DEGDN using bacterial cultures obtained by inoculating nutrient broth with freshly activated sludge from a domestic sewage treatment plant. Microbial biotransformation of DEGDN occurred in both batch and continuous cultures under aerobic conditions. Tentative identification of the DEGDN metabolites was established with thin-layer chromatography (TLC). Initially, DEGDN underwent biologically mediated denitrification producing diethylene glycol mononitrate. After subsequent microbial action, all nitrate esters disappeared. The identities of the initial intermediates of metabolism were

confirmed by comparison with synthesized standards. The authors suggested that the mononitrate esters were ultimately transformed to a glycol, but this was not confirmed experimentally.

Photolysis studies show that 3.13×10^{-5} M DEGDN in aqueous solution can be degraded (optical absorbance of 313 nm) with half-lives ranging from 35 days in pure water to 27 days in Kansas River water (Spanggord *et al.*, 1985). Under environmental conditions, photolytic half-lives for DEGDN in water range from 15 days in summer to 59 days in winter (Spanggord *et al.*, 1987). The major photochemical transformation products of DEGDN include 2-hydroxyethylnitratoacetate, nitrate, glycolic acid, and formic acid. Nitrogen balance studies indicate that all of the nitrogen is ultimately converted to nitrate (Spanggord *et al.*, 1987).

IX. REFERENCES

Alley BJ, Dykes HWH. 1972. Gas-liquid chromatographic determination of nitrate esters, stabilizers and plasticizers in nitrocellulose-base propellants. J. Chromatog. 71:23-37.

Brown LD, Korte Jr. DW. 1988a. Primary Dermal Irritation Potential of Diethyleneglycol Dinitrate (DEGDN) in Rabbits. Toxicology Series 154. Institute Report No. 304. Letterman Army Institute of Research, San Francisco, CA. Available from the National Technical Information Service (NTIS), Springfield, VA. Order No. ADA201960.

Brown LD, Korte Jr. DW. 1988b. Acute Dermal Toxicity of Diethyleneglycol Dinitrate in Rabbits. Toxicology Series 155. Institute Report No. 291. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA201956.

Brown LD, Ryabik JRG, Wheeler CR, Korte Jr. DW. 1989. Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in Rats. Toxicology Series 136. Institute Report No. 335. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA207233.

Burrows EP, Rosenblatt DH, Mitchell WR, Parmer DL. 1989. Organic Explosives and Related Compounds: Environmental and Health Considerations. Technical Report No. 8901. U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD.

Camera E, Pravisani D. 1964. Separation and analysis of alkylpolynitrates by gas chromatography. Analytical Chemistry 36(1):2108-2109.

Clayton GD, Clayton FE, eds. 1982. Patty's Industrial Hygiene and Toxicology, 3rd ed., vol 2C. New York: John Wiley & Sons, pp. 4031, 4039-4040.

Cornell JH, Wendt TM, McCormick NG, Kaplan DL, Kaplan AM. 1981. Biodegradation of Nitrate Esters Used as Military Propellants—A Status Report. Toxicological Report NATICK/TR-81/029. U.S. Army Natick Research and Development Laboratories, Natick, MA. Available from NTIS, Springfield, VA. Order No. ADA149665.

Fisher DJ, Burton DT, Lenkevich MJ, Cooper KR. 1985. Analytical Methods for Determining the Concentration of Solvent Yellow 33, Solvent Green 3, Synthetic-HC Smoke Combustion Products and DEGDN in Freshwater. Final Report for Phase I. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADA176875.

Fisher DJ, Burton DT, Paulson RL. 1987. Toxicity of DEGDN, Synthetic-HC Smoke Combustion Products, Solvent Yellow 33 and Solvent Green 3 to Freshwater Aquatic Organisms. Final Report for Phase II. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADA196906.

Hiatt GFS, Korte Jr. DW. 1988. Primary Eye Irritation Potential of Diethyleneglycol Dinitrate (DEGDN) in Rabbits. Toxicology Series 153. Institute Report No. 303. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA201961.

Hiatt GFS, Ryabik JRG, Korte Jr. DW. 1988. Dermal Sensitization Potential of Diethyleneglycol Dinitrate (DEGDN) in Guinea Pigs. Toxicology Series 143. Institute Report No. 307. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA201959.

Holleman JW, Ross RH, Carroll JW. 1983. Problem Definition Study on the Health Effects of Diethyleneglycol Dinitrate, Triethyleneglycol dinitrate, and Trimethylolethane Trinitrate and Their Respective Combustion Products. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADA127846.

Kawakami TG, Aotaki-Keen A, Rosenblatt LS, Goldman M. 1988. Evaluation of Diethyleneglycol Dinitrate (DEGDN) and Two DEGDN Containing Compounds. Final Report. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADA202289.

Kirk-Othmer. 1980. Encyclopedia of Chemical Technology, Vol. 9. Explosives and Propellants. pp. 561, 572-577, 608.

Krasovsky GN, Korolev AA, Shigan SA. 1973. Toxicological and hygienic evaluation of diethylene glycol dinitrate in connection with its standardization in water reservoirs. J. Hygiene Epidem. Microb. 17:114-119.

Kuzelova M, Cermakova B. 1974. Cases of sudden death as a consequence of long-term work with nitric esters of glycerin. Vnitrni Lekarstvi 20:447-451. (In Czechoslovakian; translation).

Mitala JJ, Boardman JP. 1981. Dermal Teratology Study on DEGDN, MTN, and DEGDN/MTN Mixture in Rats. Project 5-084. Adria Laboratories, Plain City, Ohio. Sponsor: Hercules, Inc. EPA/OTS Doc. No. 88-8100006.

Narasimhan S, Srinivasan SK, Venkatasubramanian N. 1987. ^{13}C NMR spectra of alcohols and corresponding nitrate esters. Magn. Reson. Chem. 25(1):91-92.

Parihar DB, Sharma SP, Verma KK. 1967. Rapid estimation of explosive nitrates J. Chromatog. 31:551-556.

Prerovska I, Teisinger J. 1965. Clinical picture of chronic poisoning with dinitrodiglycol. Pracovni Lekarstvi 17(2):41-43. (In Czechoslovakian; translation).

Rinkenbach WH. 1927. Preparation and properties of diethyleneglycol dinitrate. Industrial and Engineering Chemistry 19:925-927.

Ryabik JRG, Brown LD, Wheeler CR, Korte Jr. DW. 1989. Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in ICR Mice. Toxicology Series 137. Institute Report No. 336. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA134154.

Sano SK, Korte Jr. DW. 1988. Mutagenic Potential of Diethyleneglycol Dinitrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Toxicology Series 147. Institute Report No. 292. Letterman Army Institute of Research, San Francisco, CA. Available from NTIS, Springfield, VA. Order No. ADA201795.

Sax, NI. 1984. Dangerous Properties of Industrial Materials, 6th ed. New York: Van Nostrand Reinhold Co., pp. 1011-1012.

Smith LL, Carrazza J, and Wong K. 1983. Treatment of wastewaters containing propellants and explosives. J. Hazardous Materials 7:303-316.

Spanggord RJ, Chou T-W, Mill T, Podoll RT, Harper JC, Tse DS. 1985. Environmental fate of nitroguanidine, diethyleneglycol dinitrate, and hexachloroethane smoke. Final Report, Phase I. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADB114553.

Spanggord RJ, Chou T-W, Mill T, Haag W, Lau W. 1987. Environmental fate of nitroguanidine, diethyleneglycol dinitrate, and hexachloroethane smoke. Final Report, Phase II. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD. Available from NTIS, Springfield, VA. Order No. ADB114553.

Styblova V. 1966. The neurotoxic effects of explosives. Ceskoslovenska Neurologie 29(6):378-381. (In Czechoslovakian; translation).

U.S. EPA. 1986. Guidelines for Carcinogen Risk Assessment. Fed. Reg. 51(185):33992-34003. September 24.

Valachovic A. 1965. Effect of dinitroglycol on the output of the heart muscle in dogs as established in an acute experiment. Pracovni Lekarstvi 17(2):1-7. (In Czechoslovakian; translation).

Yinon J, Hwang D-G. 1983. High-performance liquid chromatography—mass spectrometry of explosives. J. Chromatog. 268:45-53.