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6 DECOMPOSITION OF GLYCOLS FROM NITRATE ESTER PROPELLANTS.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The biodegradability of four glycols derived from the corresponding nitrate esters was investigated using gas chromatography. Propylene glycol was readily degraded under a variety of culture conditions. The initial stage of decomposition of diethylene and triethylene glycol was nonbiological and TMEG was relatively stable under the conditions tested. The GC method was sensitive to direct injections of glycols in aqueous solutions in the low ppm range.		

PREFACE

Propylene glycol dinitrate, diethylene glycol dinitrate, triethylene glycol dinitrate and trimethylolethane trinitrate are used as military propellants and enter waste streams at manufacturing and loading facilities. Current pollution guidelines require an assessment of the environmental fate and hazards associated with these compounds. Previous studies under this work unit have shown these four nitrate esters are biotransformed to glycols. This report describes the degradation of these glycols and assesses their biohazards. We wish to thank Dr. John H. Cornell for his helpful insights.

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TABLE OF CONTENTS

	Page
LIST OF FIGURES	2
INTRODUCTION	3
MATERIALS AND METHODS	6
Media	6
Culture Conditions	6
Gas Chromatography	7
Lyophilization	7
Mutagenicity Testing	8
RESULTS	8
DISCUSSION	11
CONCLUSIONS	13
REFERENCES	14

LIST OF FIGURES

	Page
Figure 1. Biodegradation of nitrate esters to the corresponding glycols.	4
Figure 2. Decomposition of PG under aerobic and anaerobic conditions, filter sterilized (●), nutrient broth (○), basal salts (■) and basal salts with glucose (□).	9
Figure 3. Decomposition of DEG and TEG under filter sterilized conditions (●), in nutrient broth (○) (aerobic) and basal salts (■) and basal salts with glucose (□) (anaerobic).	10

DECOMPOSITION OF GLYCOLS FROM NITRATE ESTER PROPELLANTS

INTRODUCTION

Glycols are used extensively in industry, and their biodegradability is of great importance due to the large quantities entering the environment.

Our interest in this area stems from the fact that four nitrate esters, propylene glycol dinitrate (PGDN), diethylene glycol dinitrate (DEGN), triethylene glycol dinitrate (TEGDN) and trimethylolethane trinitrate (TMETN) are military propellants found as pollutants in waste streams from munition plants and loading operation. Initial studies have shown these esters undergo microbial transformation via successive denitration steps leading to the formation of the corresponding glycols, propylene glycol (PG), diethylene glycol (DEG), triethylene glycol (TEG), and trimethylolethane glycol (TMEG) (Fig. 1).^{1,2}

Cox summarized much of the work on the biodegradation of glycols and emphasized the use of indirect methods including BOD, COD, manometry and turbidity as indices of biodegradability.³ Fincher and Payne studied the degradation of PG, DEG, and TEG as sole carbon-sources using turbidity and manometry as evidence for growth.⁴ Haines and Alexander investigated the bio-

¹ Wiley, B. Ed. 1977. Twenty-sixth Conference on Microbiological Deterioration of Military Materiel. Technical Report NATICK/TR-78/029. Food Sciences Laboratory, US Army Natick Research and Development Command, Natick, MA.

² Wiley, B. Ed. 1979. Twenty-eighth Conference on Microbial Deterioration of Military Materiel. Technical Report NATICK/TR-80/018. Food Sciences Laboratory, US Army Natick Research and Development Command, Natick, MA.

³ Cox, D. P. 1978. The Biodegradation of Polyethylene Glycols. *Adv. Appl. Microbiol.* 23: 173-194.

⁴ Fincher, E. L., and W. J. Payne. 1962. Bacterial Utilization of Ether Glycols. *Appl. Microbiol.* 10: 542-547.

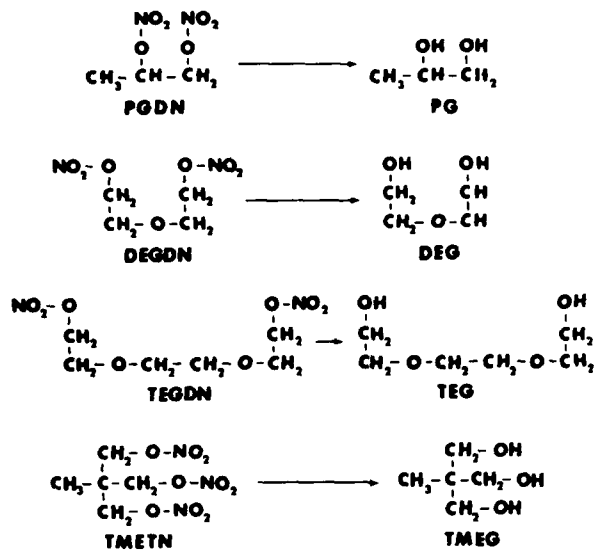


Figure 1. Biodegradation of nitrate esters to the corresponding glycols.

degradation of DEG and TEG using BOD as evidence of decomposition.⁵ Kawai *et al.* reported tetraethylene glycol (10,000 ppm $\mu\text{g/ml}$) to be biodegradable; metabolites were identified by GC/MS after isolation by column chromatography chloroform extraction, and derivatization (silylation).⁶ Jenkins *et al.* showed 1000 ppm solutions of DEG and TEG to be biodegradable using measurements of TOC and turbidity.⁷ These results were confirmed by gas chromatography (GC)

⁵ Haines, J. R., and M. Alexander. 1975. Microbial Degradation of Polyethylene Glycols. *Appl. Microbiol.* 29: 621-625.

⁶ Kawai, F., T. Kimura, M. Fukaya, Y. Tani, K. Ogata, T. Ueno, and H. Fukami. 1978. Bacterial Oxidation of Polyethylene Glycol. *Appl. Environ. Microbiol.* 35: 679-684.

⁷ Jenkins, L. D. L., K. A. Cook, and R. B. Cain. 1979. Microbial Degradation of Polyethylene Glycols. *J. Appl. Bact.* 47: 75-85.

after extraction and derivatization of the residual glycols. Shumilov *et al.* used GC to determine the concentration of DEG and TEG between 600 and 2000 ppm in waste waters.⁸

Alternate methods for analysis of glycols have also received attention. Oxidation of glycols to aldehydes followed by derivatization, using 3-methylbenzothiazol-2-one hydrazone hydrochloride, was developed to analyze for DEG and TEG.⁹ Ponder employed direct injection of hydrolysates which contained DEG down to 500 ppm into a GC equipped with a thermal conductivity detector.¹⁰

It is the purpose of this work to assess the biodegradability of the four glycols derived from the biological transformation of the corresponding nitrate esters. Detailed study of the biodegradation of glycols at low concentrations in aqueous solutions has been limited because of the lack of a suitable analytical method for direct determination of the substrates in the low ppm range. In this connection a GC method for the direct analysis of the four esters of interest has been developed which allows their determination in the low ppm range from aqueous media.

⁸ Shumilov, N. V., A. D. Elizarova, and A. S. Yablokova. 1973. Determination of Glycols in Waste Waters. Mater. Nauchno-Tekh. Konf. Kazan. Khim. Tekhnol. Inst. Kazan. Zavoda Org. Sint. 2nd; 165-8. C.A. 84: 111271T (1976).

⁹ Evans, W. H., and A. Dennis. 1973. Spectrophotometric Determination of Low Levels of Mono-, Di-, and Triethylene Glycols in Surface Waters. Analyst. 98: 782-791.

¹⁰ Ponder, L. H. 1968. Gas Chromatographic Determination of Diethylene Glycol in Poly (ethylene terephthalate). Anal. Chem. 40: 229-231.

PG, DEG and TEG present minimal toxicological problems and no carcinogenicity hazard.¹¹ An estimate of the biohazard caused by the release of residual amounts of TMEG was obtained by determination of its mutagenic properties in the Ames test.

MATERIALS AND METHODS

Media:

Basal salts medium consisted of 3.0 g $\text{NH}_4\text{H}_2\text{PO}_4$, 1.25 g K_2HPO_4 , 0.75 g KH_2PO_4 , 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g CaCl_2 and 0.01 g NaCl per liter of distilled water. Glucose was added at 1.0 g per liter as indicated. The nutrient broth concentration was 4.0 g per liter. The viscous nature of PG, DEG, and TEG required the preparation of initial solutions of 1000 ppm by weight for improved accuracy which was diluted to 100 ppm in the culture medium.

Sterile control flasks with the individual glycols in distilled water were filter-sterilized using a 0.2- μm filter. PG and DEG (Baker grade) and TEG (practical grade) were purchased from J. T. Baker Chemical Co., Phillipsburg, NJ. TMEG (technical grade) was purchased from Aldrich Chemical Co., Milwaukee, WI.

Culture Conditions:

Aerobic batch cultures were incubated in 250-ml Erlenmeyer flasks containing 100 ml of media at 30°C on an orbital shaker at 225 rpm. Anaerobic batch cultures were incubated at 37°C in 250-ml Erlenmeyer flasks filled with media and loosely sealed.

¹¹ Gosselin, R. E., H. C. Hodge, R. P. Smith, and N. N. Gleason. 1976. Chemical Toxicity of Commercial Products, 4th Edition. 145-153. Williams and Wilkens, Baltimore, MD.

Aerobic nutrient broth cultures were inoculated with activated sludge from the Marlboro Easterly sewage treatment plant (Marlboro, MA) and anaerobic broth cultures were inoculated with anaerobic digest from the Nut Island sewage treatment plant (Boston, MA). Individual flasks contained 50 ppm of a glycol and cell growth was harvested after two days. The cell mass was collected at 12,000 rpm on a Sorvall RC-5 centrifuge and washed three times with 0.85% KCl. These cells were used to inoculate corresponding flasks for biodegradation studies.

Gas Chromatography:

Analyses of PG, DEG and TEG were performed on a Perkin Elmer Model 3920 Gas Chromatograph equipped with a flame ionization detector and a Model 1021A electronic noise filter (Spectrum Scientific Corp.). Nitrogen carrier gas flowed at 30 ml per min. through a stainless steel column (46 cm x 0.32 cm) packed with Poropak Q-S, 80-100 mesh. On column injection was used and the detector temperature was 250°C. The column temperatures were 180°C, 220°C, and 250°C and injection volumes were 0.6 µl, 1.0 µl and 3.0 µl for PG, DEG and TEG, respectively. Detection limits were 3 ppm. Analysis of TMEG was performed on a Bendix Model 2500 Gas Chromatograph equipped with a flame ionization detector and a glass column (183 cm x 0.64 cm) packed with Tenax GC, 80-100 mesh. Injection port and column were at 270°C and the detector at 300°C. Injection volumes were 5 µl, and nitrogen carrier gas flowed at 30 ml per min.

Lyophilization:

Lyophilization was performed on 1000 ppm solutions of the four glycols. PG and DEG were resuspended in ether and TEG and TMEG in benzene. Residual glycols were derivatized with N-trimethylsilylimidazole and injected into a

Bendix Model 2500 Gas Chromatograph equipped with a flame ionization detector and a 183 cm x 0.64 cm stainless steel column packed with 5% OV1 on Chromasorb W, 100-120 mesh. Nitrogen carrier gas flowed at 30 ml per min and the detector and injector were at 225°C. The column temperature was 100°C for PG, DEG and TMEG and 125°C for TEG.

Mutagenicity Testing:

The Ames screening test for mutagenicity was performed with TMEG according to standard procedures.^{12 13} Five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) were used to test TMEG at concentrations ranging from 5 to 5000 µg per plate with and without metabolic activation.

RESULTS

Lyophilization of 1000 ppm solutions resulted in the recovery of 0, 4, 24 and 88% of PG, DEG, TEG and TMEG as determined after derivatization and quantitation by GC. These solutions are a tenfold higher concentration than those used in biological studies.

At initial concentrations of 100 ppm, PG rapidly disappears from culture flasks under both aerobic and anaerobic conditions even as the sole carbon-source (Fig. 2). PG disappeared from the sterile controls; 8% under anaerobic and 16% under aerobic conditions during 9 days. PG was not detectable after 2 days in aerobic nutrient broth batch cultures. In basal salts supplemented with glucose, PG was undetectable after 4 days in aerobic and anero-

¹² Ames, B. N. 1979. Supplement to the Methods Paper. Univ. Calif. Berkeley. 1-10.

¹³ Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods for Detecting Carcinogens and Mutagens with the *Salmonella* Mammalian-Microsome Mutagenicity Test. *Mut. Res.* 31: 347-364.

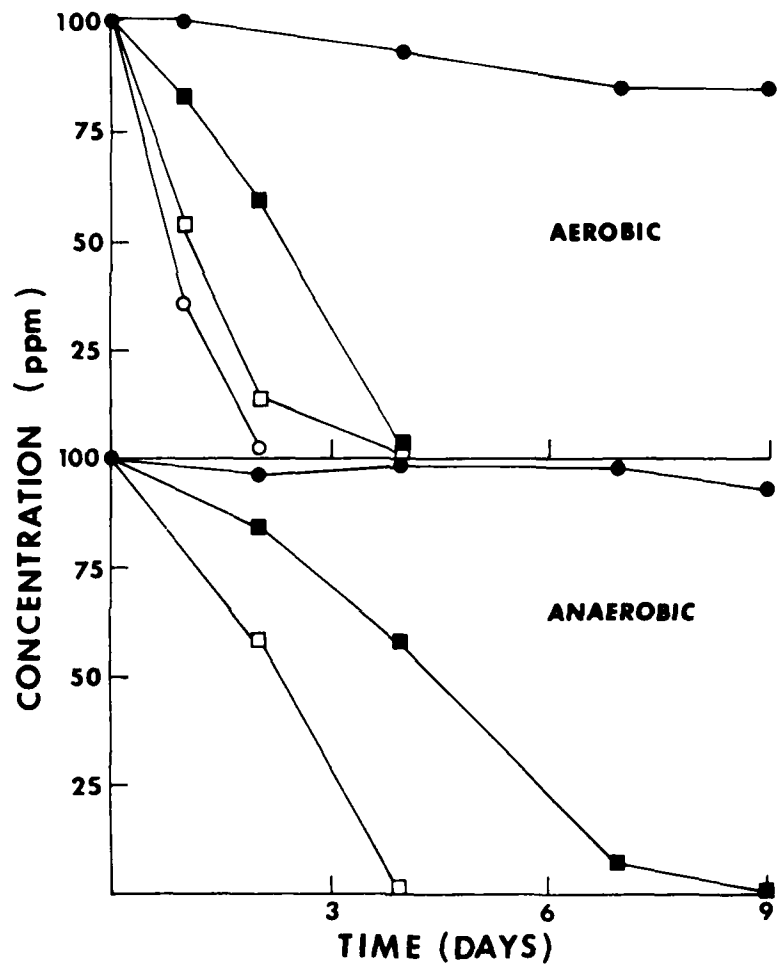


Figure 2. Decomposition of PG under aerobic and anaerobic conditions, filter sterilized (●), nutrient broth (○), basal salts (■), and basal salts with glucose (□).

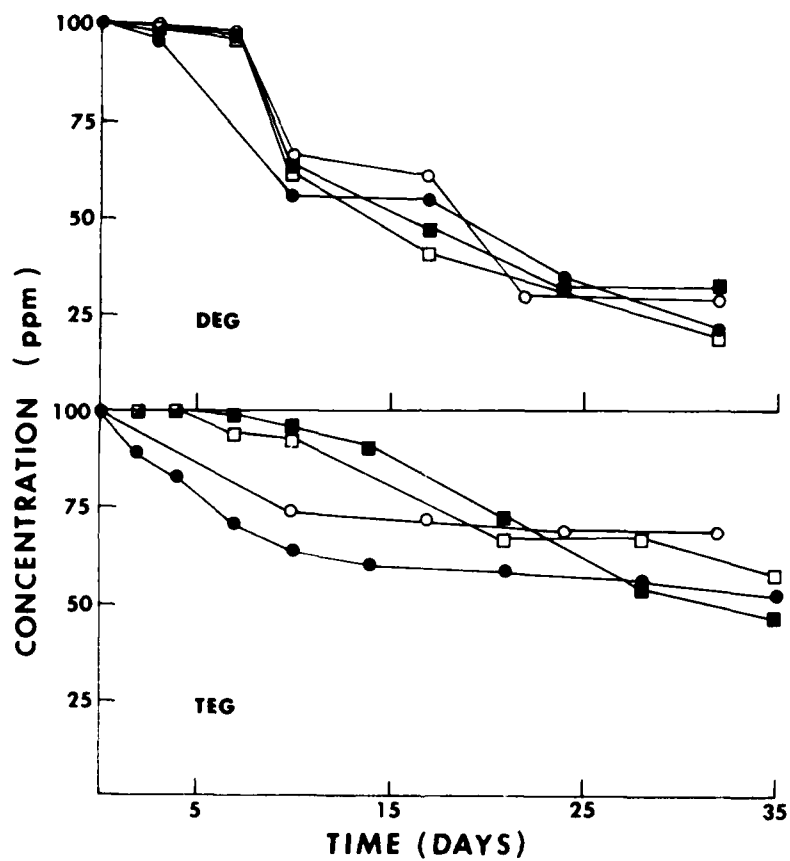


Figure 3. Decomposition of DEG and TEG under filter sterilized conditions (●), in nutrient broth (○) (aerobic) and basal salts (■), and basal salts with glucose (□) (anaerobic).

bic cultures. As the sole carbon-source, PG disappeared after 4 days under aerobic and 9 days under anaerobic conditions.

DEG was degraded under both aerobic and anaerobic conditions but the decomposition appears to be nonbiological. The rate of disappearance of the substrate in the sterile control (70-80%) was equivalent to that of the substrate in the inoculated cultures for a period of 32 days (Fig. 3). The rate of disappearance was independent of culture media, as similar results were obtained whether nutrient broth, basal salts or distilled water were used. Rates were also independent of oxygen as similar results were found under either aerobic or anaerobic conditions.

The pattern of TEG decomposition was similar to that of DEG (Fig. 3), the major difference being the rate of disappearance. TEG was also nonbiologically transformed; after 35 days about 50% of the TEG remained, both in the sterile control and in the inoculated media, whether incubated aerobically or anaerobically.

TMEG was stable under the conditions tested and showed no evidence of nonbiological instability during the 34 days. Microbiological results were variable but indicated at best only very slow rates of decomposition.

TMEG produced no toxic effects in the five *Salmonella* strains up to 5000 μg per plate and was negative as a potential mutagen in the Ames test.

DISCUSSION

The low recovery of three of the four glycols by lyophilization illustrates the difficulty in attempting to extract glycols from aqueous systems for quantitative analysis. The volatility and hydrophilic nature of these compounds preclude efficient extraction from aqueous solutions by standard methods. Jenkins *et al.* recovered 52 and 62% of DEG and TEG by rotary evaporation fol-

lowed by chloroform extraction of residual solids, and much lower (<5%) values by direct extraction with chloroform from solutions saturated with various salts.¹⁴ Similarly, derivatization using silylating agents for GC analysis requires a non-aqueous or nearly water-free preparation.¹⁵ Therefore, quantitative extraction of glycols from aqueous solution is extremely difficult.

We have successfully demonstrated a sensitive quantitative method for direct analysis of glycols at concentrations as low as 3 ppm in aqueous systems. Using this method we have reported on the biodegradability of four glycols. PG is relatively stable to nonbiological forces at low concentrations in aqueous solutions. Microbiological decomposition occurs in rich media and as the sole carbon source. DEG and TEG decompose nonbiologically via mechanisms which presumably produce low molecular weight fragments. No peaks other than the parent peaks were evidenced by GC. Therefore, these fragments were either amenable to microbiological degradation, subject to further nonbiological degradation, or were not detectable by the analytical method used. Cox stressed the potential importance of chemical instability of polyethylene glycols including factors of heat, and peroxide or acid contaminants formed during their production.¹⁶

¹⁴ See reference 7, p. 4.

¹⁵ Esposito, G. G., and M. H. Swann. 1969. Gas Chromatographic Determination of Polyhydric Alcohols in Oils and Alkyd Resins by Formation of Trimethylsilyl Derivatives. *Anal. Chem.* 41: 1118-1120.

¹⁶ See reference 3, p. 3.

Relative rates of disappearance of the parent compounds indicate a sequence of PG>DEG>TEG>TMEG from high to low. This agrees with other reports where rates of biodegradation decrease with increasing degree of polymerization, but the rate difference may be due to chemical/physical factors affecting rates of depolymerization and not due to microbiological factors. Ames testing indicates TMEG may not pose potential mutagenicity problems.

With the exception of TMEG these esters will undergo degradation under conditions suitable to a biological treatment facility. Degradation occurs through a combination of biological and chemical activities which varies with each ester.

CONCLUSIONS

We have demonstrated a sensitive quantitative method for the determination of glycols in aqueous systems in the low ppm range. Propylene glycol, diethylene glycol, triethylene glycol and trimethylolethane glycol, which are produced by the microbiological denitration of the corresponding nitrate ester propellants, are degraded by microbiological and/or chemical/physical factors. Relative rates of disappearance are PG>DEG>TEG>TMEG. There appears to be a minimum of toxicological hazards associated with these compounds.

¹⁶ See reference 3, p. 3.

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