MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES
3 4456 0064263 2

AD
ORNL-6180

ornl

OAK RIDGE
NATIONAL
LABORATORY

MARTIN MARIETTA

Water Quality Criteria for Nitroglycerin

FINAL REPORT

John G. Smith

JULY 1986

OAK RIDGE NATIONAL LABORATORY
CENTRAL RESEARCH LIBRARY
CIRCULATION SECTION
4500N ROOM 175
LIBRARY LOAN COPY
DO NOT TRANSFER TO ANOTHER PERSON
If you wish someone else to see this report, send in name with report and the library will arrange a loan.

SUPPORTED BY
U.S. ARMY MEDICAL RESEARCH AND
DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701-5012
Interagency Agreement No. 84PP4845

Oak Ridge National Laboratory
Oak Ridge, TN 37831

Project Officer
Major David L. Parmer
Health Effects Research Division
U.S. ARMY MEDICAL BIOENGINEERING
RESEARCH AND DEVELOPMENT LABORATORY
Fort Detrick, Frederick, MD 21701-5010

Approved for public release;
distribution unlimited

OPERATED BY
MARTIN MARIETTA ENERGY SYSTEMS, INC.
FOR THE UNITED STATES
DEPARTMENT OF ENERGY

Printed in the United States of America. Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road, Springfield, Virginia 22161
NTIS price codes—Printed Copy: A06 Microfiche A01

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

AD
ORNL-6180

WATER QUALITY CRITERIA FOR NITROGLYCERIN

FINAL REPORT

John G. Smith

Chemical Effects Information Task Group
Information Research and Analysis
Biology Division

JULY 1986

SUPPORTED BY

U.S. ARMY MEDICAL RESEARCH
AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, MD 21701-5012
Interagency Agreement No. 84PP4845

Project Officer
Major David L. Parmer
Health Effects Research Division
U.S. ARMY MEDICAL BIOENGINEERING RESEARCH
AND DEVELOPMENT LABORATORY
Fort Detrick, Frederick, MD 21701-5010

Approved for public release;
distribution unlimited

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

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831
operated by
MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U.S. DEPARTMENT OF ENERGY
Under Contract No. DE-AC05-84OR21400

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES

3 4456 0064263 2

EXECUTIVE SUMMARY

Nitroglycerin is a nitrate ester used primarily in explosives and in medical applications as a vasodilator. It is not very soluble in water but is completely miscible in most organic solvents. Physical decomposition occurs from exposure to increasing temperatures, and explosion may occur from continuous exposure to high temperatures. Under acidic and alkaline conditions, nitroglycerin goes through stepwise denitration to yield glycerol, but under alkaline conditions the glycerol is further hydrolyzed. Nitroglycerin is produced by either a batch or continuous process by adding glycerol to a cooled mixture of nitric and sulfuric acids. The most popular methods of quantitatively analyzing nitroglycerin are those using gas chromatography.

Release of wastewaters from the production and/or use of nitroglycerin can alter habitat by degrading the quality of the receiving waters. Once in the environment, degradation of nitroglycerin is slow and occurs primarily through biodegradation and photolysis.

Nitroglycerin is quite toxic to aquatic organisms. Limited data indicate that some algae may be more sensitive than fish or invertebrates to acute exposures of nitroglycerin. Ninety-six hour LC_{50} values for fish range from 1.67 to 3.2 mg/L, and 48-hr EC_{50} values for invertebrates, based on immobilization, range from 20 to 55 mg/L. The lowest reported concentrations causing significant adverse effects in fish and invertebrates from chronic exposures are 0.22 and 3.1 mg/L, respectively. Seven- to 30-day-old fry appear to be the most sensitive stage of fish. Based on an 8-day bioaccumulation study with fish, bioconcentration factors of 8X to 15X have been calculated; however, because of the short length of this study, these values may not be an accurate indication of this chemical's potential for bioconcentration.

A direct relationship between nitroglycerin and adverse effects to aquatic organisms has not been shown by field studies but wastewaters generated during the production or use of nitroglycerin may severely impact aquatic ecosystems.

Nitroglycerin is rapidly and widely distributed and rapidly absorbed, metabolized, and eliminated in both laboratory animals and humans. Metabolism occurs in both hepatic and extrahepatic tissues via stepwise denitrification; elimination is primarily in the urine and expired air.

The acute toxicity of nitroglycerin to mammals is moderate; it is most toxic when given intravenously and least toxic when given orally. There appear to be minor or no sex and species differences in the acute toxicity. Acute exposure of humans to sublethal concentrations of nitroglycerin causes headache, increased heart rate, and decreased blood pressure.

Subchronic exposure to high concentrations of nitroglycerin causes adverse hematological changes in animals. Chronic exposure of laboratory animals to high concentrations of nitroglycerin also causes

significant adverse hematological changes, as well as adverse changes in the liver and reduction in weight gain. Long-term employment of humans in industries producing nitroglycerin or using nitroglycerin in the production of explosives has frequently been associated with headaches, fatigue, and nausea. There is evidence that long-term exposure to nitroglycerin and/or nitroglycol in the explosives industry may be the cause of some isolated cases of sudden death.

In vivo and in vitro studies have not shown nitroglycerin to be genotoxic. Studies with laboratory animals on developmental toxicity and reproductive effects have failed to demonstrate that nitroglycerin is a teratogen but have shown that exposures to high concentrations cause male infertility and delayed development in offspring, as judged by incomplete ossification of the hyoid bone.

A high incidence of hepatocellular carcinomas, or neoplastic nodules, and interstitial cell tumors of the testes have been observed in rats after two years of exposure to high concentrations of nitroglycerin. Also, an increased incidence in pituitary tumors has been observed in mice exposed for one year to moderate amounts of nitroglycerin.

The final aquatic criterion consists of two concentrations: a Criterion Maximum Concentration and a Criterion Continuous Concentration. A Criterion Maximum Concentration of 0.86 mg/L may be calculated from available data; however, this value is considered tentative because acute toxicity data did not meet all requirements of the USEPA Guidelines. The minimum data base required by the USEPA in calculating a Criterion Continuous Concentration was not available. Calculation of human health criteria for nitroglycerin was based on the finding of its carcinogenicity in rats. Therefore, the recommended criteria which may result in an incremental increase of lifetime cancer risk at levels of 10^{-5}, 10^{-6}, and 10^{-7} are 14.0, 1.40, and 0.140 μg/L, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 231, 23.1, and 2.31 μg/L, respectively.

CONTENTS

EXECUTIVE SUMMARY 1

LIST OF TABLES 5

1. INTRODUCTION 7

1.1 PHYSICAL AND CHEMICAL PROPERTIES 7

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES 11

2. ENVIRONMENTAL EFFECTS AND FATE 12

2.1 ABIOTIC ENVIRONMENTAL EFFECTS 12

2.2 ENVIRONMENTAL FATE 13

2.3 SUMMARY 14

3. AQUATIC TOXICOLOGY 15

3.1 ACUTE TOXICITY TO ANIMALS 15

3.2 CHRONIC TOXICITY TO ANIMALS 19

3.3 TOXICITY TO PLANTS 26

3.4 BIOACCUMULATION 31

3.5 OTHER DATA 33

3.6 SUMMARY 34

4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS 34

4.1 PHARMACOKINETICS 34

4.1.1 Animal Studies 35
4.1.2 Human Studies 38

4.2 ACUTE TOXICITY 40

4.2.1 Animal Studies 40
4.2.2 Human Studies 43

4.3 SUBCHRONIC AND CHRONIC TOXICITY 43

4.3.1 Animal Studies 43
4.3.2 Human Studies 46

4.4 GENOTOXICITY 48

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY 49

4.5.1 Animal Studies 49
4.5.2 Human Studies 51

4.6 ONCOGENICITY 51

4.6.1 Animal Studies 51
4.6.2 Human Studies 53

4.7 SUMMARY 54

5. CRITERION FORMULATION 55

5.1 EXISTING GUIDELINES AND STANDARDS 55

5.2 OCCUPATIONAL EXPOSURE 55

5.3 PREVIOUSLY CALCULATED CRITERIA 56

5.4 AQUATIC CRITERIA 57

5.5 HUMAN HEALTH CRITERIA 60

5.6 RESEARCH RECOMMENDATIONS 60

6. REFERENCES 63

7. GLOSSARY 74

APPENDIX A: SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC LIFE A-1

APPENDIX B: SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH B-1

LIST OF TABLES

1. PHYSICAL AND CHEMICAL PROPERTIES OF NITROGLYCERIN 8

2. SYNONYMS FOR NITROGLYCERIN 10

3. ACUTE TESTS FOR MORTALITY OR IMMOBILIZATION OF AQUATIC SPECIES FOLLOWING EXPOSURE TO NITROGLYCERIN 16

4. ACUTE TOXICITY OF NITROGLYCERIN TO *Lepomis macrochirus* UNDER VARYING CONDITIONS OF WATER QUALITY 18

5. ACUTE TOXICITY OF NITROGLYCERIN TO SELECTED LIFE STAGES OF *Pimephales promelas* 20

6. MEAN PERCENTAGE HATCH OF EGGS, MEAN PERCENTAGE SURVIVAL, AND MEAN TOTAL LENGTH OF *Ictalurus punctatus* FRY CONTINUOUSLY EXPOSED TO NITROGLYCERIN FOR 30 DAYS 21

7. MEAN PERCENTAGE HATCH OF EGGS, MEAN PERCENTAGE SURVIVAL, AND MEAN TOTAL LENGTH OF *Pimephales promelas* FRY CONTINUOUSLY EXPOSED TO NITROGLYCERIN FOR 30 DAYS 22

8. MEAN PERCENTAGE SURVIVAL AND MEAN NUMBER OF YOUNG PRODUCED PER PARTHENOGENETIC FEMALE OF *Daphnia magna* EXPOSED TO NITROGLYCERIN FOR TWO GENERATIONS 24

9. MEAN PERCENTAGE SURVIVAL OF LARVAE, PUPAE, AND ADULTS, PERCENTAGE EMERGENCE OF ADULTS, AND NUMBER OF EGGS PRODUCED PER ADULT OF THE FIRST AND SECOND GENERATIONS OF *Chironomous tetans* DURING CONTINUOUS EXPOSURE TO NITROGLYCERIN 25

10. MEAN PERCENTAGE SURVIVAL AND GROWTH OF *Pimephales promelas* CONTINUOUSLY EXPOSED TO NITROGLCERIN 27

11. SIZE AND REPRODUCTION POTENTIAL OF *Pimephales promelas* CONTINUOUSLY EXPOSED TO NITROGLYCERIN FOR 266 DAYS 28

12. HATCHABILITY OF EGGS OF *Pimephales promelas* EXPOSED CONTINUOUSLY TO NITROGLYCERIN 29

13. GROWTH OF SECOND GENERATION *Pimephales promelas* CONTINUOUSLY EXPOSED TO NITROGLYCERIN FOR 30 DAYS 30

14. ACUTE TOXICITY OF NITROGLYCERIN TO THE FRESHWATER ALGAE *Selenastrum capricornutum*, *Navicula pelliculosa*, *Microcystis aeruginosa*, AND *Anabeana flos-aquae* 32

15. LETHALITY (LD_{50}'S) OF NITROGLYCERIN TO MAMMALS 41

16. CALCULATIONS FOR FINAL ACUTE VALUE (FAV) OF NITROGLYCERIN 58

17. SUMMARY OF PERTINENT DATA FOR CALCULATING THE LIFETIME CARCINOGENIC RISK OF NITROGLYCERIN 62

ACKNOWLEDGMENTS

The author would like to thank Tim Ensminger, head of the Information Research and Analysis Section (IR¢A) of the Biology Division, Oak Ridge National Laboratory (ORNL) for his support during the preparation of this document. Thanks are due to Major David L. Parmer, Jr., Contracting Officer, U.S. Army Medical Bioenginering Research and Development Laboratory, Fort Detrick, Frederick, Maryland, for his advice and technical review. Further review by Glen Suter of Environmental Sciences Division (ORNL), Dr. Lawton Smith of Biology Divison (ORNL), Dr. James Holleman IR¢A (ORNL), Elizabeth Etnier IR¢A (ORNL), and Michael Ryon IR¢A (ORNL) is also appreciated. The author is greatly indebted to Lois Thurston, Carolyn Seaborn, Joe Rich, and Jan Pruett for their assistance in document preparation and collection and organization of reference materials.

1. INTRODUCTION

Nitroglycerin is a nitrate ester that is used as an explosive and, more frequently, with other materials (e.g., nitroglycol) to make explosives such as dynamite, smokeless gunpowders, and blasting gels (ACGIH 1980; Hawley 1981; Stokinger 1982). Nitroglycerin is also used in rocket propellants (ACGIH 1980), as a vasodilator for temporary relaxation of the gastrointestinal and urinary tracts (International Labour Office 1983), and in the treatment of angina pectoris (Ellis et al. 1984). The objectives of this report are to review the available data on the aquatic and human health effects of nitroglycerin and, using the latest USEPA guidelines, to generate water quality criteria values. Appendix A is a summary of the USEPA Guidelines for generating water quality criteria for the protection of aquatic life and its uses (Stephan et al. 1985). Appendix B is a summary of the USEPA Guidelines for generating water quality criteria for the protection of human health (USEPA 1980).

1.1 PHYSICAL AND CHEMICAL PROPERTIES

A summary of some of the physical and chemical properties of nitroglycerin is presented in Table 1, and a list of synonyms is presented in Table 2. Nitroglycerin has a molecular weight of 227.09 and its structural formula is $CH_2NO_3CHNO_3CH_2NO_3$ (ACGIH 1980). In its pure form nitroglycerin is a colorless, transparent, oily liquid, and in its commercial form it is yellowish or pale brown (Urbanski 1983). The compound exists in two isomeric forms which differ in freezing point and crystalline structure (Urbanski 1983). The labile form has a lower freezing point, between 1.9 to 2.8°C (Kast 1906; Hackel 1933, both as reported in Urbanski 1983; Windholz 1983). Estimates of the freezing point for the stable form range from 12.4 to 13.5°C (Nauckhoff 1905, as reported in Urbanski 1983; DiCarlo 1975).

Nitroglycerin is not very soluble in water; at 20°C, estimates range from 1.73 to 2.0 g/L of water (Table 1) (Mark 1965; Lindner 1980). However, nitroglycerin is completely miscible with most organic solvents at room temperature (Table 1) (Urbanski 1983). The solubility of nitroglycerin in ethyl alcohol depends to a large extent on the temperature and the water content of the alcohol. For example, at 20°C about 43, 31.6, and 0.7 g of nitroglycerin will dissolve in 100 cm^3 of 100, 96, and 25 percent ethanol, respectively. At about 50°C nitroglycerin mixes with absolute or 96 percent ethanol in all proportions (Urbanski 1983). The solubility of nitroglycerin in other monohydroxy alcohols such as propyl, isopropyl, and amyl is similar to that of ethanol while being even less soluble in polyhydroxy alcohols (Urbanski 1983). Nitroglycerin is readily soluble in 65 percent acetic acid and concentrated sulfuric acid, but is only slightly soluble in carbon disulfide and in aliphatic hydrocarbons (Urbanski 1983).

At temperatures of 50°C, nitroglycerin begins to decompose (DiCarlo 1975). When heated to 75°C, the compound apparently decomposes within three to four days and is characterized by the generation of acid

TABLE 1. PHYSICAL AND CHEMICAL PROPERTIES OF NITROGLYCERIN

Property	Value	Source
Density (g/cm^3 at 20°C)	1.5931	ACGIH 1980
Specific gravity	d_{15}^{15} 1.599	Windholz 1983
	d_{4}^{4} 1.6144	Windholz 1983
	d_{4}^{15} 1.6009	Windholz 1983
	d_{4}^{25} 1.5918	Windholz 1983
Melting point (°C)	13.2[a]	Lindner 1980
	13 (Stable)	Beard and Noe 1981
	13.5 (Stable)	Windholz 1983
	13.1[a]	ACGIH 1980
	2.8 (Labile)	Windholz 1983
	1.9 (Labile)	Hibbert 1911[b]
	2.1-2.2 (Labile)	Kast 1906[b]
Viscosity (cP)	35.5 at 20°C	Mark 1965
	21.0 at 30°C	Mark 1965
	9.4 at 50°C	Mark 1965
	6.8 at 60°C	Mark 1965
Oils/water partition Coefficient (log P)	2.35	Leo et al. 1971
Hygroscopicity (20°C)	0.06% at 90% rh	Lindner 1980
Solubility in water (g/L)	1.6 at 15°C	Urbanski 1983
	1.73 at 20°C	Mark 1965
	1.8 at 20°C	Urbanski 1983
	2.0 at 20°C	Lindner 1980
	2.46 at 60°C	Mark 1965
	2.5 at 50°C	Urbanski 1983
Solubility in organic solvents	At room temperature, completely miscible in most organic solvents including: methyl alcohol, ethyl acetate, anhydrous acetic acid, benzene, toluene, xylenes, phenol, nitrobenzene, nitrotoluenes, chloroform, dichloroethane, dichloroethylene, ether, ethylene chloride, nitric esters, ethylene bromide, pyridine, acetone	Urbanski 1983; McNiff et al. 1980

TABLE 1. (Continued)

Property	Value	Source
Boiling point (°C)	>180	Urbanski 1983
Vapor pressure (mm Hg)	0.00026 at 20°C	Windholz 1983
	0.0024 at 40°C	Marshall and Peace 1916[b]
	0.0188 at 60°C	Marshall and Peace 1916[b]
	0.098 at 80°C	Marshall and Peace 1916[b]
	0.31 at 93°C	Windholz 1983
Vapor density	7.8	Verschueren 1983
Autoignition point	518°F (270°C)	Stokinger 1982
Heat of evaporation (kcal/mole)	20.64 at 100 K	Roginskii and Sapozhnikov 1931[b,c]
	20.11 at 180 K	
Rate of evaporation[d]	0.2% at 50°C	Naoum 1924[b]
	1.6% at 75°C	Naoum 1924[b]
	10% at 100°C	Naoum 1924[b]
Heat of formation (kJ/g[e])	1.63	Lindner 1980
Heat combustion (kJ/g[e])	6.80	Lindner 1980
Heat of detonation (kJ/g[e])	6.29	Lindner 1980
Activation energy (kJ/mol[e])	169	Lindner 1980
Refractive index (n_N^{20})	1.4732	Urbanski 1983
Dipole moment	3.82 D (pure, liquid)	deKreuk 1942[b]
	2.56 D (in hexane)	deKreuk 1942[b]
	2.88 D (in carbon tetrachloride)	deKreuk 1942[b]
	3.16 D (in benzene)	deKreuk 1942[b]

a. Isomeric form not given.

b. As reported in Urbanski 1983.

c. Authors calculated the value with the vapor pressure data of Marshall and Peace 1916.

d. Based on weight loss of 20-g sample on a 70-mm-diameter watch glass over 24 hr.

e. To convert joules to calories, divide by 4.184.

TABLE 2. SYNONYMS FOR NITROGLYCERIN[a]

Synonym
Angibid; Anginine; Angiolingual; Angorin; Blasting Gelatin (DOT); Blasting Oil; Cardamist; Gilucor nitro; Glonoin; Glycerin trinitrate; Glycerintrinitrate; Glycerol, nitric acid triester; Glycerol trinitrate; Glycerol (trinitrate de); Glyceroltrintraat; Glyceryl nitrate; Glyceryl trinitrate; GTN; Klavikordal; Lenitral; Myoglycerin; NG; NK 843; NTG; Niglin; Niglycon; Nitora; Nitric Acid; Nitrin; Nitrine; Nitrine-TDC; Nitro-Dur; Nitroglicerina; Nitrogliceryna; Nitroglycerine; Nitroglycerol; Nitroglyn; Nitrol; Nitrol (pharmaceutical); Nitrolan; Nitro-lent; Nitroletten; Nitrolinhual; Nitrolowe; Nitromel; Nitrong; Nitrorectal; Nitroretard; Nitro-Span; Nitrostabilin; Nitrostat; Nitrozell retard; Nysconitrine; Perglottal; Propanetriol trinitrate; 1,2,3-Propanetriol, trinitrate; 1,2,3,-Propanetriyl nitrate; S.N.G.; Soup; Temponitrin; Triester of glycerol; Trinalgon; Trinitrin; Trinitroglycerin; Trinitroglycerol; Trinitrol; Vasoglyn

a. MEDLARS (CHEMLINE) 1984; MEDLARS (RTECS) 1984; and MEDLARS (TDB) 1984.

products (Urbanski 1983). When heated to 100°C, measurable volatilization occurs; at temperatures of 135 to 145°C, the compound becomes a reddish color and gives off yellow vapors; at 165°C, considerable denitration occurs and nitric acid and glycerol nitrates are given off; near 185°C, it becomes highly viscous; and near 218°C, detonation occurs (DiCarlo 1975; Snelling and Storm 1913, as reported in Urbanski 1983).

Andreev and Bespalov (1963) studied the effects of varied concentrations of water on the thermal decomposition of nitroglycerin. At temperatures ranging from 100 to 120°C, the rate of decomposition of nitroglycerin decreased as the concentration of water increased. The authors concluded that decomposition of nitroglycerin at elevated temperatures and in the presence of water is initially by hydrolysis, which proceeds slowly in neutral solutions, but is accelerated by acidic decomposition products and their subsequent hydrolysis.

In the presence of neutral solutions, nitroglycerin is relatively stable (DiCarlo 1975; McNiff et al. 1980). Nitroglycerin will degrade under both acidic and alkaline conditions, but the rate of degradation is much more rapid under the latter (Crew and DiCarlo 1968; Fraser 1968; McNiff et al. 1980). Under acidic conditions, nitroglycerin goes through stepwise denitration to yield glycerol (Farmer 1920; Crew and DiCarlo 1968; DiCarlo 1975). Under alkaline conditions, glycerol is not an end product but is further hydrolyzed to mesoxalic acid, oxalic acid, carbon dioxide, and aldehyde resins, and the nitrate is reduced to ammonia (Berl and Delpy 1910; Silberrad and Farmer 1906; Vignon 1903, all as reported in DiCarlo 1975). In the presence of a readily oxidizable compound such as phenylmercaptan, glycerol has been found to be a hydrolysis product of nitroglycerin (Klason and Carlson 1906, as reported in DiCarlo 1975).

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

The traditional means of producing nitroglycerin has been by the batch process, but because of the hazards involved in handling large quantities, continuous processes are now more widely used (Lindner 1980; Urbanski 1983). In both processes, very pure glycerol (qv) and mixed acid (90% nitric acid and 25 to 30% oleum) are used. Theoretically, the yield of nitroglycerin from glycerol is 2.467:1, while the actual yield using concentrated acid is about 2.36:1; the yield is slightly higher in the continuous processes than in the batch process. The final purity of nitroglycerin depends on its final use; smokeless powders and high explosives require a compound of higher purity than do mining explosives (Urbanski 1983). Impurities in nitroglycerin include the spent acids and wash fluids (e.g., sodium carbonate) (Urbanski 1983).

Several methods have been developed and utilized to quantitatively measure the concentration of nitroglycerin and its degradation products in biological media and the environment. The most popular method for measuring nitroglycerin has been gas chromatography (Wu et al. 1982), and, according McNiff et al. (1980), this method is particularly suitable for determination of the compound in biological fluids. A number of variations exist in this assay which differ primarily in the type of

detector used (e.g., DiCarlo 1975). Use of an electron capture detector is reportedly 1000 times more sensitive than a flame ionization detector (Camera and Pravisani 1964, as reported in DiCarlo 1975); their sensitivities have been reported to be in the nanogram and microgram range, respectively (Rosseel and Bogaert 1972).

A number of spectrophotometric methods have been developed for analysis of single dosage units (McNiff et al. 1980; Yacobi et al. 1983). Two commonly used spectrophotometric methods are the Bell (Bell 1964, as reported in Yacobi et al. 1983) and kinetic methods (Fung et al. 1973; Yap et al. 1975, both as reported in Yacobi et al. 1983); both methods are based on alkaline hydrolysis of the nitroglycerin (Yacobi et al. 1983). A spectrophotometric method adapted by the United States Pharmacopeia (1980) involves the separation of nitroglycerin from its degradation products followed by acid hydrolysis to nitrate ion.

Quantitation of nitroglycerin and its degradation products is possible with thin-layer chromatography (DiCarlo 1975; Wu et al. 1982). This method involves the use of ^{14}C-nitroglycerin.

Several high-performance liquid chromatographic (HPLC) procedures have been described (DiCarlo 1975; McNiff et al. 1980). The HPLC method along with ultraviolet detection of the nitrate ester groups has been used for analysis of wastewaters from ammunition plants (Walsh 1976, as reported in Sullivan et al. 1979; Weitzel et al. 1976).

Polarographic methods have been used by Flann (1969, as reported in McNiff et al. 1980) and Woodson and Alber (1969, as reported in McNiff et al. 1980) for analyzing nitroglycerin. This method is based on the reduction of nitrate at the dropping mercury electrode.

In their review of nitroglycerin, Sullivan et al. (1979) listed six methods that they considered not suitable for use in measuring the concentration of this chemical in the environment due to their lack of sensitivity: "(1) the Du Pont nitrometer method which measures nitric oxide gas liberated from the ester by mercury; (2) hydrolysis of the ester followed by reduction of NO_3^- and analysis of NO_2^-; (3) reduction by titanous chloride and back titration of the excess reagent with ferric alum; (4) reduction with ferrous chloride and titration of the ferric iron with titanous chloride; (5) infrared detection of nitrate groups; and (6) the ferrous sulfate-sulfuric acid colorimetric method."

2. ENVIRONMENTAL EFFECTS AND FATE

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

Studies concerning the direct abiotic effects of nitroglycerin were not found; however, studies were available concerning the effects of wastewaters generated from nitroglycerin production and use on the receiving bodies of water. These studies showed that habitat alteration may occur through degradation of water quality. Weitzel et al. (1976)

observed high concentrations of total organic carbon, nitrogen, and chromium in the river receiving wastewater from Radford Army Ammunition Plant (Radford AAP). However, the authors felt that only the elevated levels of nitrogen could be attributed to the waste discharge from nitroglycerin production. In a pond receiving wastes from nitroglycerin production at Badger AAP, Stilwell et al. (1976) observed a high concentration of dissolved solids and low pH values, and, in a pond receiving wastes from the manufacture of rocket paste, they found low oxygen concentrations and anaerobic sediments that had a high chemical oxygen demand and a high Kjeldahl nitrogen concentration.

2.2 ENVIRONMENTAL FATE

Environmental exposure to nitroglycerin occurs following the loss of the chemical into the wastewaters during its production or use. In a U.S. Army Environmental Hygiene Report (1971, as reported in Carnahan and Smith 1977), it was estimated that 21.3 kg/day of nitroglycerin were released into the wastewaters of Radford AAP when the daily production rate of nitroglycerin was 7711.1 kg and the volume of wastewater was 136.3 kL/day. During their 1975 water quality survey of the New River, receiving wastes from Radford AAP nitroglycerin production facility, Weitzel et al. (1976) estimated that a total of about 29.5 g/min of nitroglycerin was discharged in the wastewaters from four discharge sites. They found that the concentration of nitroglycerin in the water generally ranged from 0.01 to 0.02 mg/L. Nitroglycerin was found in only three of 32 sediment cores; the average concentration was slightly greater than 1 mg/kg dry wt.

In a pond receiving nitroglycerin production wastes at Badger AAP, Stilwell et al. (1976) found nitroglycerin in concentrations ranging from <0.6 to 3.4 mg/L in the water, and in the sediment they found the concentration to be 37.5 mg/kg dry wt. In a pond receiving rocket paste wastes at this same plant, Stilwell et al. found nitroglycerin in concentrations ranging from <0.6 to 3.9 mg/L in the water, and in the sediment they found the concentration to be <1.73 mg/kg dry wt.

Once nitroglycerin is in the environment, both abiotic and biotic factors influence its final fate. Physical and chemical degradation of nitroglycerin appears to be slow. The photolytic half-life of nitroglycerin has been estimated to be five days in pure water, thus indicating relatively slow photodegradation (Spanggord et al. 1980a). Spanggord et al. (1980b) estimated that the volatilization half-life of nitroglycerin from water would be about 3000 days. Spanggord et al. (1980b) estimated a sorption coefficient of between 20 and 32 (the lower value was reported in their text and the higher value in a table) and indicated that adsorption of nitroglycerin on sediments may not be a significant environmental fate. The hydrolysis half-life of nitroglycerin at 80°C has been estimated to be 134 days (Svetlov et al. 1976, as reported in Spanggord et al. 1980b); based on this estimate, Spanggord et al. (1980b) concluded that the hydrolysis half-life of nitroglycerin, at temperatures normally observed in the environment, would be in the order of years. This was substantiated by Spanggord et al. (1980b) after they used some alkaline hydrolysis data from Capellos et al. (1978) and

estimated that at 25°C the hydrolysis half-life would be greater than one year at a pH of 3 to 8, less than one year at a pH above 8, and 37 days at a pH of 9.

Nitroglycerin has been reported to be resistant to biodegradation (American Defense Preparedness Association 1975; Smith and Dickinson 1972, both as reported in Wendt et al. 1978). At concentrations of 600 to 900 mg/L the compound has been found to exert toxic effects on mixed microbial populations (American Defense Preparedness Association 1975; U.S. Army Natick Research and Development Command 1973; U.S. Army Natick Research and Development Command 1974, all as reported in Wendt et al. 1978). In contrast, Wendt et al. (1978) observed a 53.6 percent decrease in nitroglycerin after five days in batch shake-flasks containing 67 to 68 mg/L of nitroglycerin plus glucose, mineral salts, and activated sludge. In the absence of glucose, the authors observed only a 3.1 percent decrease in nitroglycerin over the same time period. From this and some unreported data, they concluded that nitroglycerin does not serve as a suitable sole source of carbon or nitrogen for microbial populations. Using a continuous-culture apparatus inoculated with activated sludge, Wendt et al. (1978) detected no nitroglycerin (initial concentration of 30 mg/L) after a residence time of 8 to 15 hr. Examination of the growth media used in their experiments revealed the presence of 1,3- and 1,2-dinitroglycerins and mononitroglycerins. Based on their results, they concluded that under suitable conditions, nitroglycerin may be readily biodegradable and that the pathways of microbial degradation are similar to those observed in mammalian systems (see Figure 1 in Section 4.1).

Spanggord et al. (1980a) studied the biotransformation of nitroglycerin in water and sediment obtained from the New River. They found that 10 ppm of nitroglycerin was biotransformed within 13 days in either the water alone, with sediment added, or with 50 ppm yeast extract, under both aerobic and microaerophilic conditions. Nitroglycerin (10 ppm) was also biotransformed by nitroglycerin-acclimated microogranisms that had been obtained from the aerobic bottle and placed in shaker flasks containing a basal-salts medium. In contrast to Wendt et al. (1978), the authors concluded that nitroglycerin could be used by these organisms as the sole source of carbon. When the concentration of nitroglycerin in the basal-salts medium was increased to 120 ppm, the authors found that the culture could grow. Spanggord et al. (1980a) analyzed the broth from the biotransformation experiments and found nitrite to be the major product; they were unable to detect any dinitro- or mononitroglycols.

2.3 SUMMARY

Nitroglycerin is released into the environment from wastewaters generated during its production and use. Habitat alteration in aquatic systems by nitroglycerin may occur through degradation of water quality. Laboratory data indicate that degradation of nitroglycerin in the environment may be slow, but, under favorable treatment conditions, degradation may be rapid. Environmental degradation of nitroglycerin occurs primarily by biodegradation and photolysis.

3. AQUATIC TOXICOLOGY

3.1 ACUTE TOXICITY TO ANIMALS

Bentley et al. (1978) conducted a series of acute toxicity studies of nitroglycerin with freshwater fish and invertebrates. The acute toxicity was determined under static bioassay conditions with four species of fish (*Lepomis macrochirus*, *Salmo gairdneri*, *Ictalurus punctatus*, and *Pimephales promelas*) and four species of invertebrates (*Daphnia magna*, *Gammarus fasciatus*, *Asellus militaris*, and *Chironomus tentans*); the acute toxicity was also determined under flow-through assay conditions with three species of fish (*L. macrochirus*, *I. punctatus*, and *P. promelas*) and two species of invertebrates (*D. magna* and *C. tentans*). The authors followed the procedures of USEPA (1975, as reported in Bentley et al. 1978). Groups of 30 fish were exposed to each tested concentration (nominal) in both static and flow-through tests, and groups of 15 and 20 invertebrates were used in the static and flow-through tests, respectively. With the exception of *P. promelas*, all organisms were somewhat more sensitive to the compound under flow-through conditions than under static conditions (Table 3). In the static assays, the 96-hr LC_{50} values for fish ranged from 2.5 to 3.2 mg/L, and the 48-hr EC_{50} values (based on immobilization) for invertebrates ranged from 46 to 55 mg/L. In the flow-through assays, the 96-hr LC_{50} values for fish ranged from 1.67 to 3.0 mg/L, and the 48-hr EC_{50} values for invertebrates ranged from 20 to 32 mg/L. The most sensitive fish and invertebrate in the static assays were *P. promelas* (96-hr LC_{50} of 2.5 mg/L) and *D. magna* (48-hr EC_{50} of 46 mg/L), respectively; in the flow-through assays, the most sensitive fish was *L. macrochirus* (96-hr LC_{50} of 1.67 mg/L) and the most sensitive invertebrate was *C. tentans* (48-hr EC_{50} of 20 mg/L).

To determine the effects of water quality on the acute toxicity of nitroglycerin to fish, Bentley et al. (1978) exposed groups of 30 *L. macrochirus* to the compound under various conditions of temperature, hardness, and pH. Using a static assay, fish were exposed to nitroglycerin under the following conditions: (1) temperatures of 15, 20, or 25°C at a pH of 7.1 and hardness of 35 mg/L as $CaCO_3$; (2) hardness values of 35, 100, or 250 mg/L as $CaCO_3$ at a temperature of 20°C and pH of 7.1; (3) pH values of 6.0, 7.0, or 8.0 at a temperature of 20°C and hardness of 35 mg/L as $CaCO_3$. Variations in hardness and pH had no influence on the toxicity, and temperature had only a slight influence, with the compound being less toxic at the lowest temperature [(96-hr LC_{50} values of 3.55, 1.92, and 1.99 mg/L at 15, 20, and 25°C, respectively (Table 4)]. Under all conditions, the 96-hr LC_{50} values ranged from 1.38 to 3.55 mg/L.

To further characterize the toxicity of nitroglycerin to fish, Bentley et al. (1978) conducted an acute toxicity study with *P. promelas* under static bioassay conditions during various stages of development, including eggs, 1-hr-old newly hatched fry, 7-day-old fry, 30-day-old fry, and 60-day-old fry. LC_{50} values were determined for each stage at 24, 48, and 96 hr; a 144-hr LC_{50} was also determined for eggs.

TABLE 3. ACUTE TESTS FOR MORTALITY[a] OR IMMOBILIZATION[b] OF AQUATIC SPECIES FOLLOWING EXPOSURE TO NITROGLYCERIN[c]

Test Species	Test Method[d]	Test Duration	LC_{50} (mg/L)[e]	EC_{50} (mg/L)[e]	Mean Acute Genus Value (mg/L)
Arthropoda					
Crustacea					
Daphnidae					
Daphnia magna	S	24 hr	NA[g]	51 (35-72)[i]	38.3666[h]
	S	48 hr	NA	46 (38-55)	
	F	24 hr	NA	72 (15-350)	
	F	48 hr	NA	32 (21-50)	
Gammaridae					
Gammarus fasciatus	S	24 hr	NA	74 (55-99)	i
	S	48 hr	NA	50 (41-60)	
Asellidae					
Asellus militaris	S	24 hr	NA	57 (46-72)	i
	S	48 hr	NA	50 (38-66)	
Insecta					
Chironomidae					
Chironomus tentans	S	24 hr	NA	76 (57-102)	33.1662
	S	48 hr	NA	55 (47-64)	
	F	24 hr	NA	57 (18-182)	
	F	48 hr	NA	20 (11-37)	
Osteichthyes[j]					
Centrarchidae					
Lepomis macrochirus	S	24 hr	6.4 (4.7-8.8)	NA	1.9423[k]
	S	48 hr	4.3 (2.7-6.8)	NA	
	S	96 hr	2.7 (2.0-3.7)	NA	
	F	24 hr	>1.87	NA	
	F	96 hr	1.67 (0.87-3.25)	NA	

TABLE 3. ACUTE TESTS FOR MORTALITY[a] OR IMMOBILIZATION[b] OF AQUATIC SPECIES FOLLOWING EXPOSURE TO NITROGLYCERIN[c]

Test Species	Test Method[d]	Test Duration	LC_{50} (mg/L)[e]	EC_{50} (mg/L)[e]	Mean Acute Genus Value (mg/L)
Salmonidae					
Salmo gairdneri	S	24 hr	4.8 (3.6-6.6)	NA	2.8000
	S	48 hr	2.9 (2.1-3.9)	NA	
	S	96 hr	2.8 (2.0-3.8)	NA	
Ictaluridae					
Ictalurus punctatus	S	24 hr	6.5 (4.8-8.8)	NA	2.4462
	S	48 hr	4.7 (3.5-6.4)	NA	
	S	96 hr	3.2 (2.6-3.8)	NA	
	F	24 hr	>1.87	NA	
	F	96 hr	>1.87	NA	
Cyprinidae					
Pimephales promelas	S	24 hr	6.3 (4.6-8.6)	NA	2.9189[l]
	S	48 hr	4.1 (3.4-5.0)	NA	
	S	96 hr	2.5 (2.0-3.0)	NA	
	F	24 hr	>6.0<8.0	NA	
	F	96 hr	3.0 (2.2-3.7)	NA	

a. Mortality tests were designed to give LC_{50} values.

b. Immobilization tests were designed to give EC_{50} values.

c. Adapted from Bentley et al. 1978.

d. S = Static; F = Flow-through.

e. Nominal concentrations.

f. Test Animals were 0-24 hr old at start of test.

g. NA = Not applicable.

h. Based on 48-hr values for Arthropoda and 96-hr values for Osteichthyes.

i. 95% confidence interval.

j. 48-hr values not acceptable per guidelines (Stephan et al. 1983).

k. Test animals were in their second or third instar at start of test.

l. Mean Acute Genus Value based on 96-hr values.

m. Value includes 96-hr LC_{50} values in this table and those in Table 4.

n. Value includes all 96-hr LC_{50} values in this table and all of those in Table 5 except the value for eggs.

TABLE 4. ACUTE TOXICITY OF NITROGLYCERIN TO *Lepomis macrochirus* UNDER VARYING CONDITIONS OF WATER QUALITY[a,b]

Water Quality Variable	LC_{50} (mg/L) 96-hr
15°C	3.55 (1.92-6.59)[c]
20°C	1.92 (0.88-4.16)
25°C	1.99 (1.28-3.09)
Hardness, 35 mg/L as $CaCO_3$	1.76 (1.12-2.74)
Hardness, 100 mg/L as $CaCO_3$	1.51 (0.99-2.32)
Hardness, 250 mg/L as $CaCO_3$	1.65 (1.07-2.55)
pH 6.0	1.38 (0.87-2.21)
pH 7.0	1.91 (0.88-4.14)
pH 8.0	2.10 (0.97-4.54)

a. Adapted from Bentley et al. 1978.

b. Under varied conditions of temperature (15, 20, or 25°C) the pH was maintained at 7.1, and the hardness was 35 mg/L as $CaCO_3$. Under varied conditions of hardness (35, 100, or 250 mg/L as $CaCO_3$) the pH was maintained at 7.1, and the temperature was 20°C. Under varied conditions of pH (6.0, 7.0, or 8.0), hardness was maintained at 35 mg/L as $CaCO_3$, and and the temperature was 20°C.

c. 95% confidence interval.

Based on the 96-hr assay, the egg was the least sensitive stage (LC_{50} of >18.0 mg/L), and the 7- and 30-day-old fry the most sensitive stages (LC_{50} of 2.1 mg/L for each) (Table 5). However, the 144-hr LC_{50} (1.2 mg/L) for eggs indicates that this stage may be as sensitive as or more sensitive than the other stages.

Hemphill (1975, as reported in Sullivan et al. 1979) reported 96-hr LC_{50} values of 4.2 ± 0.08 and 23 ± 4.6 mg/L at 20 and 10°C, respectively, for what appears to be P. promelas; however, it was not clear from the presentation of the data if this was the species used.

3.2 CHRONIC TOXICITY TO ANIMALS

Bentley et al. (1978) studied the effects of nitroglycerin on the eggs and fry of I. punctatus and P. promelas. Groups of 50 and 35 eggs of I. punctatus and P. promelas, respectively, were exposed to nitroglycerin in egg cups beginning 48 and 24 hr, respectively, after fertilization and continued through hatching. After hatching, groups of 25 fry were transferred to a growth chamber of a flow-through exposure system and exposed for 30 days. Two control groups of eggs and fry of both species were used, including solvent (acetone) and water controls. The eggs and fry of I. punctatus were exposed to nitroglycerin at concentrations (nominal) of 0.08, 0.15, 0.31, 0.62, and 1.25 mg/L. The eggs and fry of P. promelas were exposed to nitroglycerin at concentrations (measured) of 0.03, 0.06, 0.13, 0.25, and 0.48 mg/L. The authors stated that they had problems with nitroglycerin coming out of solution and forming globules. Although the concentration of the solvent (acetone) was increased, the problem persisted. For I. punctatus, the concentration of acetone was maintained at 125 mg/L from day 1 through 13, and 155 mg/L from day 13 through 30 post-hatch. For P. promelas, the authors changed the stock delivery device, and the concentration of acetone was maintained at 9 µg/L.

Bentley et al. (1978) found that exposure of I. punctatus eggs at a concentration of up to 1.25 mg/L had no significant effect on hatchability (Table 6). Percentage survival of I. punctatus fry was significantly (P = 0.05) reduced after 30 days of exposure to nitroglycerin at concentrations of 0.31, 0.62, and 1.25 mg/L. The total length of I. punctatus fry was significantly reduced after 30 days of exposure to 0.62 and 1.25 mg/L. No significant effects were observed in I. punctatus fry exposed to nitroglycerin at concentrations of 0.15 mg/L or less.

Hatchability of P. promelas eggs was not significantly affected by nitroglycerin at concentrations of up to 0.48 mg/L (Table 7) (Bentley et al. 1978). Survival of fry after 30 days of exposure was significantly (P = 0.05) reduced at concentrations of 0.06 mg/L and higher. The length of fry was significantly (P = 0.05) reduced at a concentration of 0.25 mg/L, but not at 0.48 mg/L. Due to high mortality of fry at these two concentrations, the results on length may not be accurate. No significant effects were observed at a concentration of 0.03 mg/L.

Bentley et al. (1978) conducted a chronic toxicity study of nitroglycerin with D. magna, C. tentans, and P. promelas through their entire

TABLE 5. ACUTE TOXICITY OF NITROGLYCERIN TO SELECTED LIFE STAGES OF *Pimephales promelas*[a]

Life Stage	LC_{50} (mg/L)[b]			
	24-hr	48-hr	96-hr	144-hr
Eggs	>18.0	>18.0	>18.0	1.2 (0.7-2.1)[c]
1-hr Fry	>10.0	8.7<x<10.0	5.5 (4.6-6.6)	-
7-day Fry	5.4 (4.5-6.6)	3.4 (3.0-3.9)	2.1 (1.7-2.6)	-
30-day Fry	6.8 (5.0-9.2)	4.8 (3.9-5.8)	2.1 (1.6-2.6)	-
60-day Fry	5.3 (4.3-6.4)	4.1 (3.4-5.0)	3.4 (2.8-4.2)	-

a. Adapted from Bentley et al. 1978.

b. Nominal concentrations.

c. 95% confidence interval.

TABLE 6. MEAN PERCENTAGE HATCH OF EGGS, MEAN PERCENTAGE SURVIVAL, AND MEAN TOTAL LENGTH OF *Ictalurus punctatus* FRY CONTINUOUSLY EXPOSED TO NITROGLYCERIN FOR 30 DAYS[a]

Nominal Concentration (mg/L)	Hatch (%)	Survival (%)	Length (mm)
Control			
A[b]	90	96	23 ± 2
B[b]	72	92	24 ± 3
Acetone[c]			
A	86	92	24 ± 2
B	91	90	23 ± 2
0.08			
A	86	90	24 ± 3
B	85	94	24 ± 2
0.15			
A	84	84	23 ± 3
B	85	86	23 ± 2
0.31			
A	79	72[d]	23 ± 2
B	74	88	23 ± 2
0.62			
A	100	70[d]	21 ± 2[d]
B	92	74	21 ± 2
1.25			
A	82	26[d]	18 ± 2[d]
B	63	30	18 ± 1

a. Adapted from Bentley et al. 1978.

b. Replicate.

c. Solvent control.

d. P = 0.05.

TABLE 7. MEAN PERCENTAGE HATCH OF EGGS, MEAN PERCENTAGE SURVIVAL, AND MEAN TOTAL LENGTH OF *Pimephales promelas* FRY CONTINUOUSLY EXPOSED TO NITROGLYCERIN FOR 30 DAYS[a]

Mean Measured Concentration (mg/L)	Hatch (%)	Survival (%)	Length (mm)
Control			
A[b]	94	90	25 ± 2
B	90	88	25 ± 2
Acetone[c]			
A	97	18[d]	25 ± 2
B	94	12	26 ± 2
0.03			
A	90	94	23 ± 4
B	93	84	24 ± 3
0.06			
A	94	84[e]	24 ± 3
B	93	74	25 ± 1
0.13			
A	90	62[e]	24 ± 4
B	96	48	22 ± 4
0.25			
A	86	10[e]	19 ± 3[e]
B	94	0	-
0.48			
A	70	6[e]	23 ± 1
B	87	0	-

a. Adapted from Bentley et al. 1978.

b. Replicate.

c. Solvent control.

d. Low survival in the solvent control was due to a system malfunction occurring on day 26 post hatch.

e. P = 0.05.

life cycle. Exposures of D. magna and C. tentans were in aquaria holding 1.75 L of water; 50 mL of test water was delivered every 25 min and 8 min, respectively. P. promelas were exposed in flow-through systems. Groups of 20 D. magna (<24 hr old) were exposed to nitroglycerin at concentrations (nominal) of 0, 1.5, 3.1, 6.2, 12.5, or 25.0 mg/L for two generations. Percentage survival was determined on days 7, 14, and 21 of each generation, and percentage of eggs hatched was determined on days 14 and 21 of each generation. Groups of at least 50 C. tentans (<48 hr old) were also exposed to nitroglycerin at nominal concentrations of 0, 1.5, 3.1, 6.2, 12.5, or 25.0 mg/L for two generations. Percentage survival was determined for the larval (fourth instar), pupal, and adult stages, and percentage emergence and percentage hatching were determined for each generation. Groups of 20 P. promelas, beginning with fry (<24 hr), were exposed for a total of 266 days to nitroglycerin at nominal concentrations of 0, 0.11, 0.22, 0.43, 0.87, or 1.75 mg/L. Percentage survival was determined at days 30, 60, and 174. The effects of nitroglycerin on growth were determined on days 30, 60 (total length), and 266 (total length and wet weight), and on day 266 the effects of nitroglycerin were determined on the following reproductive parameters: total spawns, total eggs, spawns/female, and eggs/spawn. Sexually mature P. promelas were allowed to spawn (usually around day 185 of exposure) in spawning chambers. Eggs were counted, and 50 from each exposure group were returned to their corresponding test water; also, at this time, groups of control eggs were placed in test waters in which little or no spawning had occurred. From these data, the percentage hatchability of the eggs was determined. Upon hatching, groups of 15 fry were exposed for 30 days in their corresponding test water and the effects of nitroglycerin determined on survival and growth.

At a concentration of 12.5 mg/L, nitroglycerin significantly ($P = 0.05$) reduced the number of young produced per female in both generations of D. magna on day 14, and a concentration of 25.0 mg/L completely inhibited reproduction (Table 8) (Bentley et al. 1978). Survival of D. magna was significantly ($P = 0.05$) reduced at a concentration of 25 mg/L (Table 8). Although survival was reduced at a concentration of 12.5 mg/L, the reduction was not significant. These data indicate that the no-observable-effect level of nitroglycerin for D. magna under chronic experimental conditions is 6.2 mg/L; however, based on current guidelines for testing the chronic effects of chemicals on daphnids (USEPA 1985), the results of this study would be considered unacceptable because of the low reproductive success observed. These guidelines state that a chronic toxicity test will be considered unacceptable if "each control daphnid living the full 21 days produces an average of less than 60 young." As can be seen in Table 8, the number of young produced per female in the controls over a 21-day period in each generation is well below 60.

Nitroglycerin significantly ($P = 0.05$) reduced survival of C. tentans larvae at a concentration of 1.5 mg/L in the first generation (Table 9) (Bentley et al. 1978); however, because there was no significant difference between survival of control larvae and larvae exposed to nitroglycerin at concentrations of 3.1 and 6.2 mg/L in the first

TABLE 8. MEAN PERCENTAGE SURVIVAL AND MEAN NUMBER OF YOUNG PRODUCED PER PARTHENOGENETIC FEMALE OF *Daphnia magna* EXPOSED TO NITROGLYCERIN FOR TWO GENERATIONS[a,b]

Nominal Concentration (mg/L)	Generation I Day 7	Generation I Day 14	Generation I Day 21	Generation II Day[b] 7	Generation II Day[b] 14	Generation II Day[b] 21
Controls						
Survival[c]	96 (8)[d]	88 (6)	78 (6)	98 (5)	96 (8)	94 (8)
Young/female[e]		6 (2)	10 (3)		10 (4)	14 (6)
1.5						
Survival	85 (7)	80 (7)	78 (6)	91 (8)	90 (11)	89 (9)
Young/female		9 (6)	14 (6)		4 (1)	16 (6)
3.1						
Survival	89 (8)	89 (8)	88 (6)	86 (9)	86 (9)	84 (11)
Young/female		7 (2)	15 (5)		7 (5)	14 (4)
6.2						
Survival	91 (8)	89 (8)	82 (13)	91 (8)	89 (13)	88 (12)
Young/female		5 (2)	10 (4)		5 (1)	15 (2)
12.5						
Survival	90 (4)	80 (11)	76 (14)	68 (35)	68 (35)	65 (32)
Young/female		1 (2)[f]	7 (4)		1 (1)[f]	9 (2)
25.0						
Survival	58 (30)[f]	26 (13)[f]	4 (8)[f]	-[g]	-	-
Young/female		0 (0)[f]	0 (0)[f]		-	-

a. Adapted from Bentley et al. 1978.

b. Days 7, 14, and 21 of generation II correspond to days 28, 35 and 42 of the total experiment.

c. Survival expressed as a percentage.

d. Values in parentheses are standard deviation.

e. Number of young produced per parthenogenetic female.

f. P = 0.05.

g. No young produced in first generation to initiate second generation.

TABLE 9. MEAN PERCENTAGE SURVIVAL OF LARVAE, PUPAE, AND ADULTS, PERCENTAGE EMERGENCE OF ADULTS, AND NUMBER OF EGGS PRODUCED PER ADULT OF THE FIRST[a] AND SECOND[b] GENERATIONS OF *Chironomous tentans* DURING CONTINUOUS EXPOSURE TO NITROGLYCERIN[c]

Nominal Concentration (mg/L)	Survival (%)			Emergence (%)	Eggs/Adult
	Larvae	Pupae	Adults		
Controls					
First generation	69 (18)[d]	95 (1)	98 (1)	100 (0)	9
Second generation	58 (9)	100 (1)	74 (19)	71 (29)	5
1.5					
First generation	48 (7)[e]	83 (24)	97 (6)	85 (27)	6
Second generation	61 (11)	98 (2)	52 (25)	94 (9)	21
3.1					
First generation	69 (8)	92 (15)	91 (16)	92 (16)	17
Second generation	33 (7)[e]	83 (24)	80 (17)	75 (49)	6
6.2					
First generation	53 (16)	87 (12)	77 (34)	80 (23)	6
Second generation	48 (7)[e]	96 (4)	66 (14)	100 (0)	18
12.5[f]					
First generation	36 (16)[e]	64 (26)	62 (12)[e]	88 (18)	0
25.0[f]					
First generation	28 (31)[e]	83 (15)	16 (31)[e]	43 (42)[e]	0

a. Survival of larvae determined after 10 days exposure, all other measurements recorded after 18 days exposure.

b. Survival of larvae determined after 13 days exposure, all other measurements recorded after 27 days exposure.

c. Adapted from Bentley et al. 1978.

d. Values in parentheses are standard deviation.

e. P = 0.05.

f. Data were not available for the second generation because no eggs were produced at this concentration.

generation, the authors concluded that the reduction was not nitroglycerin related. Survival of both larvae and adults of the first generation was significantly (P = 0.05) reduced at concentrations of 12.5 and 25.0 mg/L, and emergence of adults was significantly (P = 0.05) reduced at a concentration of 25.0 mg/L. (The authors stated in their text that emergence was significantly reduced at a concentration of 12.5 mg/L, but their tabular data are not in agreement with this statement.) At concentrations of 12.5 and 25.0 mg/L, no eggs were produced by adults in the first generation (Table 9). In the second generation, survival of larvae was significantly (P = 0.05) reduced at a concentration of 3.1 and 6.2 mg/L (Table 9). Based on their results, the authors concluded that continuous exposure to 1.5 mg/L does not affect any stage of development of this organism.

Exposure of P. promelas to nitroglycerin at concentrations of up to 1.75 mg/L for 30 days had no significant effect on either survival or total length (Table 10) (Bentley et al. 1978). Exposure for 60 days to concentrations $\geq$0.22 mg/L significantly (P = 0.05) reduced survival, but had no significant effect on total length. After 174 days, survival was significantly (P = 0.05) reduced at concentrations of 0.43 and 0.87 mg/L, and at a concentration of 1.75 mg/L there were no survivors. After 266 days of exposure, there were no survivors in the groups exposed to 0.87 or 1.75 mg/L. There were no significant effects on total length or wet weight of females after exposing them for 266 days to nitroglycerin at concentrations of 0.22 mg/L or less, but at a concentration of 0.43 mg/L, the wet weight of males was significantly (P = 0.05) lower than that of the controls (Table 11). Although not statistically analyzed by the authors, total spawns, total eggs, spawns/female, and eggs/spawn were lower in all exposed groups (Table 11). The hatchability of eggs produced by parental fish exposed to 0.43 mg/L of nitroglycerin and the hatchability of control eggs exposed to 1.75 mg/L nitroglycerin were reduced; however, these data were not statistically analyzed (Table 12). The results of exposing control eggs to 0.87 mg/L were not clear because, in replicate A, 96 percent of the eggs hatched, while in replicate B, only three percent hatched; the authors gave no explanation for this difference. At a concentration of 0.22 mg/L, total length and wet weight of fry from the parental group and control fry exposed to this concentration were significantly (P = 0.05) less after 30 days of exposure (Table 13). Survival of this group was also reduced, but the authors did not indicate that this reduction was significant. At concentrations of 0.43, 0.87, and 1.75 mg/L, which contained only fry transferred from controls, both total length and mean wet weight were significantly (P = 0.05) less than in control groups. No significant effects were observed at a concentration of 0.11 mg/L (Table 13).

3.3 TOXICITY TO PLANTS

Bentley et al. (1978) determined the acute toxicity of nitroglycerin to four species of freshwater algae including two blue-green algae, Microcystis aeruginosa and Anabeana flos-aquae, one green alga, Selenastrum capricornutum, and one diatom, Navicula pelliculosa. Using static bioassay procedures described by USEPA (1971, as reported in

TABLE 10. MEAN PERCENTAGE SURVIVAL AND GROWTH OF *Pimephales promelas* CONTINUOUSLY EXPOSED TO NITROGLYCERIN[a]

Nominal Concentration (mg/L)	30 Days		60 Days		174 Days[b]
	Survival (%)	Total Length (mm)	Survival (%)	Total Length (mm)	Survival (%)
Control					
A[c]	100	18 (2)[d]	100	26 (4)	100
B	98	17 (3)	98	25 (4)	100
Acetone[e]					
A	98	18 (2)	98	27 (3)	100
B	98	18 (3)	93	26 (4)	100
0.11					
A	100	17 (3)	100	26 (5)	93
B	100	18 (3)	90	25 (4)	93
0.22					
A	93	16 (3)	85[f]	26 (4)	93
B	93	18 (4)	83	25 (6)	87
0.43					
A	85	16 (4)	78[f]	25 (5)	60[f]
B	83	16 (4)	73	24 (6)	80
0.87					
A	98	17 (3)	58[f]	27 (4)	13[f]
B	75	17 (3)	58	26 (6)	0
1.75					
A	98	18 (2)	78[f]	28 (4)	0[f]
B	100	17 (3)	85	26 (4)	0

a. Adapted from Bentley et al. 1978.

b. Total lengths were not obtained on day 174.

c. Replicate.

d. Values in parentheses are standard deviation.

e. Solvent control.

f. P = 0.05.

TABLE 11. SIZE AND REPRODUCTION POTENTIAL OF *Pimephales promelas* CONTINUOUSLY EXPOSED TO NITROGLYCERIN FOR 266 DAYS[a]

Nominal Concentration (mg/L)[b]	Total Length (mm)		Wet Weight (g)		Males/ female	Total spawns	Total eggs	Spawns/ female	Eggs/ female	Eggs/ spawn
	Males	Females	Males	Females						
Control										
A[c]	65	53	3.26	1.47	4/3	20	1738	6.6	579	87
B	63	52	3.29	1.37	3/6	27	2067	4.5	345	77
Acetone[d]										
A	74	55	4.66	1.63	3/6	3	457	0.5	76	152
B	66	55	3.60	1.80	3/6	23	2105	3.8	351	73
0.11										
A	63	53	3.97	1.66	3/6	3	163	0.5	33	54
B	65	49	3.78	1.21	3/6	16	1166	2.7	194	73
0.22										
A	64	54	3.62	2.03	2/6	3	190	0.5	31	63
B	66	53	3.60	1.63	3/5	6	493	1.0	82	82
0.43										
A	60	53	1.96[e]	1.48	1/5	0	0	0	0	0
B	57	53	2.14	1.69	3/5	1	131	0.2	26	131

a. Adapted from Bentley et al. 1978.

b. There were no survivors at concentrations of 0.87 and 1.75 mg/mL.

c. Replicate.

d. Solvent control.

e. P = 0.05.

TABLE 12. HATCHABILITY OF EGGS OF *Pimephales promelas* EXPOSED CONTINUOUSLY TO NITROGLYCERIN[a]

Nominal Concentration (mg/L)	Hatchability (%)	
	Eggs from Parents	Eggs Transferred from Control
Control		
A[b]	97 (8)[c]	+[d]
B	94 (13)	+
Acetone[e]		
A	95 (3)	+
B	89 (9)	+
0.11		
A	88 (1)	+
B	92 (5)	+
0.22		
A	82 (2)	+
B	93 (3)	+
0.43		
A	-[f]	96 (2)
B	58 (1)	84 (1)
0.87		
A	-	96 (1)
B	-	3 (3)
1.75		
A	-	2 (3)
B	-	0 (3)

a. Adapted from Bentley et al. 1978.

b. Replicate number.

c. Parentheses show number of egg groups exposed.

d. Control eggs were tested at only those concentrations at which little or no spawning had occurred.

e. Solvent control.

f. No eggs produced by parents.

TABLE 13. GROWTH OF SECOND GENERATION *Pimephales promelas* CONTINUOUSLY EXPOSED TO NITROGLYCERIN FOR 30 DAYS[a]

Nominal Concentration (mg/L)	Survival (%)	Total Length (mm)	Mean Wet Weight (g)
Control			
A[b]	71 (4)[c]	22 (4)	0.10
B	90 (4)	21 (4)	0.09
Acetone[d]			
A	100 (2)	22 (2)	0.10
B	81 (5)	21 (5)	0.10
0.11			
A	80 (1)	22 (4)	0.10
B	58 (3)	22 (5)	0.12
0.22[e]			
A	18 (2)	18 (6)[f]	0.06[f]
B	38 (2)	18 (6)	0.06
0.43[g]			
A	73 (2)	19 (4)[f]	0.07[f]
B	53 (2)	18 (4)	0.05
0.87[g]			
A	70 (2)	22 (2)[f]	0.09[f]
B	90 (2)	18 (3)	0.05
1.75[g]			
A	90 (2)	17 (3)[f]	0.05[f]
B	88 (2)	16 (4)	0.06

a. Adapted from Bentley et al. 1978.

b. Replicate.

c. Values in parentheses are standard deviation.

d. Solvent control.

e. This exposure group consisted of groups of fry from the parental exposure group and from unexposed controls.

f. P = 0.05.

g. Fry transferred from control after hatch.

Bentley et al. 1978), 24-, 48-, and 96-hr EC_{50} values were calculated based on changes in chlorophyll a concentration (all four species), cell numbers (all but A. flos-aquae), or optical density (only A. flos-aquae). Based on the number of cells per milliliter, S. capricornutum was the most sensitive species and was found to have a 96-hr EC_{50} of 0.4 mg/L (Table 14). Based on a decrease in chlorophyll a concentration, S. capricornutum and N. pelliculosa were the most sensitive species, with both having 96-hr EC_{50} values of 1.0 mg/L. M. aeruginosa and A. flos-aquae were the least sensitive of the species, with both having 96-hr EC_{50} values of >10.0 mg/L for both parameters tested.

Sullivan et al. (1979) pointed out that in Bentley et al.'s (1978) algal bioassay with nitroglycerin, the lactose vehicle at a concentration of 10 mg/L caused a 24 percent increase in cell numbers of S. capricornutum. Although lactose had also been used at concentrations of 1 and 100 mg/L, Bentley et al. corrected all cell counts for this species by 24 percent regardless of the lactose concentration; thus they made no allowance for any lessening of this effect at lower vehicle concentrations. Furthermore, the effect of the lactose was apparently not considered in the determinations of chlorophyll a. Based on this, Sullivan et al. felt that the EC_{50} values for chlorophyll a and cell numbers of S. capricornutum were questionable.

Sullivan et al. (1979) also criticized the Bentley et al. (1978) study because they had used a probit analysis to analyze growth response data of algal cultures relative to a control. Sullivan et al. pointed out that a probit analysis requires that the data be binomially distributed, but that percentage growth reduction or amount of growth of a culture are continuous responses and generally normally distributed. They reanalyzed the data and calculated the lowest significant (level of significance not given) response concentrations for the 96-hr cell counts and chlorophyll a values of N. pelliculosa and S. capricornutum. For N. pelliculosa the lowest significant response concentration for both cell counts and chlorophyll a was 0.32 mg/L, and the respective concentrations for S. capricornutum were 1.0 and 0.1 mg/L.

3.4 BIOACCUMULATION

The potential for bioaccumulation of nitroglycerin in fish was studied by Bentley et al. (1978). Twenty each of L. macrochirus, P. promelas, S. gairdneri, and I. punctatus were exposed to ^{14}C-nitroglycerin at an average concentration of 0.42 mg/L in a modified intermittent flow system which delivered the control and test waters at a rate of 5 L/hr. Samples of water and fish were taken on days 1, 2, 4, and 8 from the test aquaria and on day 8 in the control aquarium. Based on the 8-day exposure period the bioaccumulation factor was estimated to be about 8X for S. gairdneri, I. punctatus, and P. promelas and 15X for L. macrochirus. It was not clear from the data if tissue concentrations had attained steady state.

TABLE 14. ACUTE TOXICITY OF NITROGLYCERIN TO THE FRESHWATER ALGAE *Selenastrum capricornutum*, *Navicula pelliculosa*, *Microcystis aeruginosa*, AND *Anabeana flos-aquae*[a]

Species	EC_{50} (mg/L)[b,c]			EC_{50} (mg/L)[c,d]
	24-hr	48-hr	96-hr	96-hr
S. capricornutum	4.0 (0.1-30.4)[e]	1.1 (0.1-22.8)	0.4 (0.1-1.3)	1.0 (0.7-7.0)
N. pelliculosa	>10.0	8.6 (1.2-59.9)	3.3 (0.3-33.0)	1.0
M. aeruginosa	>10.0	>10.0	>10.0	>10.0
A. flos-aquae	>10.0	>10.0	>10.0	>10.0

a. Adapted from Bentley et al. 1978.

b. Based on decrease in number of cells per mL for *S. capricornutum*, *N. pelliculosa*, and *M. aeruginosa*, and decrease in optical density for *A. flos-aquae*.

c. Nominal concentrations.

d. Based on decrease in chlorophyll *a*.

e. 95% confidence interval.

3.5 OTHER DATA

Stilwell et al. (1976) conducted a survey of the algal and benthic macroinvertebrate communities of two on-site ponds that received wastes from the manufacture of nitroglycerin (NG pond) and rocket paste (mixture of nitrocellulose and nitroglycerin) (RP pond) at Badger AAP in Baraboo, Wisconsin. The mean concentration of nitroglycerin in the water of the NG and RP ponds was 7.4 and <1.83 mg/L, respectively, and the mean concentration found in the sediment of the two ponds was 37.5 and <1.73 mg/kg dry wt, respectively. The results of the surveys of the algal and benthic communities were indicative of considerable stress. Benthic macroinvertebrates were found to be absent from both ponds. The algal communities in the NG pond were dominated by pollution tolerant species, the periphyton being dominated by a coccoid myxophycean (blue-green alga) and the phytoplankton being dominated by two species of the genus Scenedesmus (green algae). The number of species identified on the artificial substrates and in the plankton samples in the NG pond was 18 and 6, respectively. The species diversity of the algal community was low on both the artificial (species diversity of 0.63) and natural (species diversity of 1.04) substrates after a 4-week colonization period. The algal community was similarly affected in the RP pond. The periphyton and phytoplankton communities were both dominated by species of blue-green algae considered tolerant or indicative of pollution. The total number of species identified in the periphyton and phytoplankton were 10 and 9, respectively. Species diversity on the artificial and natural substrates after 4 weeks was 0.65 and 0.6, respectively, values similar to those calculated for the NG pond. Although their results were indicative of a severely stressed environment, the authors could not attribute these adverse effects solely to nitroglycerin because other wastes were released during the manufacture of nitroglycerin and rocket paste.

A survey of the water quality, periphyton, and benthic macroinvertabrates was conducted by Weitzel et al. (1976) of the nitroglycerin number two area of the Radford AAP and the segment of New River immediately upstream and downstream from the plant waste discharges. Five discharge sources were sampled for water quality analyses, and six stations were sampled in New River for water quality analyses, periphyton, and benthos, two of these were above the discharge area, one was 900 m downstream from the discharge area, and three were within the discharge area. Samples were collected during a May-June period and an October-November period. Of 32 sediment samples taken, only three contained measurable quantities of nitroglycerin, with a maximum concentration of 1.5 mg/kg occurring in a sample collected 900 m below the discharge area. Nitroglycerin was detected sporadically in the water at all river stations, and, in general, the highest concentrations were found in May. The highest water concentration measured was 0.29 mg/L and was found in a May sample taken 900 m downstream from the discharge area. Average nitroglycerin concentrations in the water were usually around 0.01 mg/L or less. The mean nitroglycerin concentrations in the discharge waters ranged from 42 to 407 mg/L, with means usually less than 100 mg/L. Minor variabilities were observed in the benthos and periphyton, but these were generally not significant. Periphyton production appeared to

be inhibited near two discharge areas, but this inhibition could not be attributed to the waste discharge of the AAP. An increase in periphyton downstream from one discharge area was thought to be due to an increase in nitrogen levels. The authors were unable to correlate these variations with the effluents from the nitroglycerin production areas.

3.6 SUMMARY

Results of acute toxicity studies indicate that nitroglycerin is quite toxic to aquatic organisms. The toxicity of nitroglycerin to algae is highly variable among species. Reported 96-hr EC_{50} values range from 0.4 to >10 mg/L. Reported 96-hr LC_{50} values for fish range from 1.67 to 3.2 mg/L, and 48-hr EC_{50} values for invertebrates range from 20 to 55 mg/L.

Studies on the effects of nitroglycerin to critical life stages of fish (eggs and fry) have shown that the fry stage is the most sensitive stage. Available data indicate that nitroglycerin in concentrations of up to 1.25 mg/L is not toxic to fish eggs. Fry of P. promelas have been found to be more sensitive to nitroglycerin than fry of I. punctatus; the lowest concentrations reported to have significantly affected (based on survival) these two species are 0.06 and 0.31 mg/L, respectively, after a 30-day exposure period. The validity of these data are questionable because problems with the dilution of nitroglycerin occurred during the study.

Available data indicate that fish are more sensitive than invertebrates to nitroglycerin under chronic exposure conditions. The lowest concentration reported to significantly affect fish is 0.22 mg/L; at this concentration, survival of P. promelas was reduced after 60 days of exposure. The lowest concentration reported to significantly affect invertebrates is 3.1 mg/L; at this concentration, survival of second generation larvae of C. tentans was reduced.

Limited data on bioaccumulation indicate that nitroglycerin is not appreciably bioaccumulated in the tissues of fish. Estimates for bioaccumulation factors of nitroglycerin in fish range from 8X to 15X.

A direct relationship between nitroglycerin exposure and adverse effects to aquatic organisms has not been shown by field studies; however, severe effects on biological communities have been observed in ponds receiving waste effluents from the production of nitroglycerin and rocket paste.

4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS

4.1 PHARMACOKINETICS

A tremendous body of literature is available on the physiological and pathological effects of nitroglycerin, particularly on cardiovascular and systemic effects. Therefore, discussions on health effects of

nitroglycerin have been limited to highlights of effects and the exposure levels at which these effects were observed, data which could possibly be used in calculating Human Health Criteria. An extensive review of the mechanisms of action of nitroglycerin may be found in Needleman (1975).

4.1.1 Animal Studies

The pharmacokinetics of nitroglycerin in laboratory animals has been studied extensively, particularly in rats; however, because of the lack of sensitive methodologies, many aspects of the pharmacokinetics of the compound are unclear. Available data on pharmacokinetics indicate that nitroglycerin is rapidly and widely distributed and rapidly absorbed, metabolized, and eliminated (e.g., Fung 1984; McNiff et al. 1980; DiCarlo 1975; DiCarlo et al. 1968; Needleman and Krantz 1965).

DiCarlo et al. (1968) gave rats a single oral dose of ^{14}C-nitroglycerin, and within 30 min radioactivity was observed in the blood, heart, kidneys, liver, lungs, spleen, gastrointestinal (GI) tract, carcass, urine, and feces and in the expired air as carbon dioxide (CO_2); a majority of the activity was found in the liver (7.3 percent) and carcass (33 percent). Four hours after exposure, radioactivity in the liver and carcass had decreased to 2.5 and 16.6 percent, respectively, and the amount of the radioactivity found in the urine and feces and exhaled as CO_2 was 20.95, 2.27, and 19.76 percent, respectively.

Hodgson and Lee (1975) observed patterns of distribution and elimination of nitroglycerin similar to those reported by DiCarlo et al. (1968). Within 4 hr after giving rats ^{14}C-nitroglycerin orally, Hodgson and Lee found a significant amount of the radioactivity in the liver (4.6 percent); this amount changed little after 24 hr (4.3 percent). Significant amounts of radioactivity were also found in the muscle at 4 hr (9.3 percent) and 24 hr (2.8 percent), and from 0.1 to 2.1 percent of the radioactivity was found in each of the other tissues examined at 4 and 24 hr (whole blood, kidneys, brain, and lungs). After 4 hr, 15.6, 7.5, and 3.1 percent of the administered dose was eliminated in the urine, expired air (CO_2), and feces, respectively, and at the end of 24 hr, the amount of radioactivity eliminated via these same routes was 39.8, 25.5, and 6.3 percent, respectively.

Lee et al. (1977) studied the pharmacokinetics of orally administered 1,3-^{14}C-nitroglycerin in mice, rats, rabbits, monkeys, and dogs. Within 24 hr after receiving a single oral dose of the compound, about 50 to 70 percent had been absorbed by mice, whereas 75 to 90 percent had been absorbed by the other species during the same time period. During the first 24 hr, mice and rats excreted considerable quantities of the radioactivity in urine and expired air (19.2 and 18.8 percent, respectively, for mice, and 25.5 and 39.8 percent, respectively, for rats). The other three species excreted a majority of the radioactivity in the urine (45.0 to 72.0 percent) and a much smaller amount in the expired air (2.5 to 7.8 percent) during the first 24 hr. The highest tissue concentrations of radioactivity at the end of 24 hr were generally

observed in the liver of all species (4.3 to 6.8 percent); however, greater amounts of radioactivity were observed in the skeletal muscles of dogs and monkeys, with concentrations of 9.5 and 13.0 percent, respectively, at the end of 24 hr. Small amounts of radioactivity were also found in the kidneys, spleen, lungs, and brain of all species.

Short et al. (1977) performed an in vitro study on the metabolism of nitroglycerin with tissue samples taken from male and female CD rats, Swiss Webster mice, CD-1 mice, New Zealand albino rabbits, beagle dogs, rhesus monkeys, and humans (at autopsy). A sex difference was not observed in the metabolism of this compound by liver homogenates of any species. In all species, nitroglycerin was primarily metabolized to 1,2- and 1,3-dinitroglycerin by the liver; rats and mice produced more 1,3-dinitroglycerin, while rabbits, dogs, monkeys, and humans produced more 1,2-dinitroglycerin. Rats, rabbits, dogs, and monkeys produced greater amounts of dinitroglycerins than humans and mice did, while humans produced greater amounts than mice. Embryos, fetal livers, and carcasses of mice were found to have poor ability to metabolize nitroglycerin. An increased ability to metabolize the compound with increasing age was observed in rat livers.

The dispositon rate of nitroglycerin is influenced by the route of administration (Fung et al. 1984a; Wester et al. 1983; Ioannides et al. 1982). Fung et al. (1984a) estimated plasma half-lives of nitroglycerin in rats following oral and intravenous (iv) administrations. Intravenously administered nitroglycerin had a plasma half-life of about 15 min. The plasma half-life of orally administered nitroglycerin was generally dependent upon the size of the dose and varied from about 30 to 140 min. Wester et al. (1983) estimated a plasma half-life of 18 min for nitroglycerin given iv to monkeys. Following dermal exposure of monkeys to 2 percent nitroglycerin ointment containing 19 mg of ^{14}C-nitroglycerin, a plasma half-life of 4.3 hr was estimated.

Ioannides et al. (1982) studied several factors that might influence the rate of elimination of nitroglycerin from the plasma, including route of administration, age, sex, and species. In rats, the plasma half-lives for iv, sublingual, and oral routes of administration were 6.7, 14.3, and 30.0 min, respectively. The authors indicated that differences in the half-lives were because the estimate of the iv half-life was an elimination half-life, the oral half-life was an absorption half-life, and the sublingual half-life was a mixture of the two. A sex difference was not observed in the plasma half-life of nitroglycerin administered iv to rabbits (12.2 min for males and 11.5 min for females), but a significant sex difference ($P < 0.001$) was observed in rats (4.4 min for males and 7.7 min for females). A difference in the half-life of nitroglycerin given iv was observed in rats at different ages (4.4 min at 8 weeks and 5.5 min at 26 weeks), but there was no indication that this difference was statistically significant. Species differences were observed in the plasma half-life of nitroglycerin given to male animals iv; an increasing half-life was observed with increasing weight. The estimated plasma half-lives (in minutes) for males given nitroglycerin iv were as follows: hamster, 3.7; rat, 4.4; guinea pig, 8.0; ferret, 9.5; and rabbit, 12.2.

The site of nitroglycerin metabolism has been a controversial issue (Needleman et al. 1971; Lang et al. 1972; Blei et al. 1984; Fung 1984). Based on both in vitro and in vivo studies with laboratory animals, it has been thought for a number of years that nitroglycerin was metabolized in the presence of glutathione-organic nitrate reductase in the liver and blood (e.g., Needleman and Hunter 1965; Needleman et al. 1971; Needleman and Harkey 1971; Lang et al. 1972; Lee 1973; Hodgson and Lee 1975; Yacobi et al. 1983). More recent studies with rats, however, have indicated that the liver is not the primary site of metabolism of nitroglycerin (Blei et al. 1984; Fung et al. 1984a; Fung et al. 1984b). Blei et al. (1984) infused rats with nitroglycerin iv following portacaval-shunting and found that the systemic clearance of nitroglycerin was no different in these animals than in animals which had been sham operated. They also observed that systemic plasma clearance of nitroglycerin far exceeded hepatic blood flow in sham and shunt groups. Fung et al. (1984b) injected nitroglycerin into various vessels of Sprague-Dawley rats and found that the highest concentrations of the nitrate were nearest the site of injection; concentrations decreased progressively further from the injection site. Thus, these data indicate that vascular tissues can take up and/or metabolize nitroglycerin. Based on his review of the literature, Fung (1984) provided the following brief scenario on the pharmacokinetics of nitroglycerin: "nitroglycerin is rapidly taken up and metabolized by extrahepatic tissues, including the vasculature. Little, if any, of the distributed drug emanating from the systemic circulation returns to it. This extensive tissue distribution and metabolism contribute to the very low, and often fluctuating, concentration of nitroglycerin observed in the systemic circulation."

Hodgson et al. (1977) studied the metabolic fate and disposition of four radiolabeled metabolites of nitroglycerin in female Charles River CD rats including 1,2- and 1,3-dinitroglycerin and 1- and 2-mononitroglycerin. Fasted rats were given a single oral dose of one of the metabolites and sacrificed after 4 or 24 hours. A similar pattern of absorption was observed for all metabolites except 2-mononitroglycerin; about 59 percent of the radioactivity was absorbed from the GI tract of animals exposed to 2-mononitroglycerin, while from 73 to 82 percent of the radioactivity was absorbed from GI tract of animals exposed to the other metabolites. Most of the 2-mononitroglycerin was excreted in the feces and urine (32.1 and 48.8 percent), whereas most the other three metabolites were excreted in the urine and expired air (20 to 30 percent and 27 to 50 percent, respectively), although relatively large quantities were also excreted in the feces (11 to 14 percent). The concentration of each of the metabolites in the liver at 4 and 24 hours was similar, ranging from about 2 to 9 percent of the administered dose. Distribution of these compounds into other tissues was minor. Analysis of the urine of those animals receiving the dinitroglycerins revealed the presence of mainly free mononitroglycerols; glucuronide conjugates of dinitro- and mononitroglycerols, glycerol, and other polar metabolites; and only small amounts of dinitroglycerin. Urinary analysis of the animals receiving the mononitroglycerins indicated the presence of large amounts of unchanged compounds as well as glycerol and other polar components; however, no glucuronides were detected.

Nitroglycerin is apparently metabolized via stepwise denitrification (Needleman and Hunter 1965; DiCarlo et al. 1968; Hodgson et al. 1977) without any initial preference for either the primary or secondary nitrate group (DiCarlo et al. 1968). The following metabolites have been identified in the urine of rats in studies on the metabolic fate of orally administered ^{14}C-nitroglycerin (DiCarlo et al. 1968; Hodgson and Lee 1975; Hodgson et al. 1977): glyceryl-1,3-dinitrate; glyceryl-1,2-dinitrate; glyceryl-1-nitrate; glyceryl-2-nitrate; glyceryl-1,3-dinitrate glucuronide; glyceryl-1,2-glucuronide; glyceryl mononitrate glucuronides; and glycerol. Based on their review of the literature on the metabolism of nitroglycerin, McNiff et al. (1980) proposed the metabolic fate as presented in Figure 1.

4.1.2 Human Studies

The number of studies concerning the pharmacokinetics of nitroglycerin in humans is limited, but available data indicate that the pharmacokinetics of this compound in humans is similar to that of laboratory animals. Nitroglycerin given buccally or sublingually is rapidly absorbed. Blumenthal et al. (1977) observed peak concentrations of plasma nitroglycerin within about 3 min after sublingually administering a tablet containing 0.3 mg of the compound. Armstrong et al. (1979) observed a peak in nitroglycerin levels in the blood within 2.3 min after administering 0.6 mg of the compound sublingually. A peak nitroglycerin plasma concentration was noted by Wei and Reid (1979) within about 5 min after administering 0.6 mg of the compound sublingually. Bogaert and Rosseel (1972) found that in 3.5 min after giving nitroglycerin buccally, all but 25 to 40 percent of the dose was absorbed.

As has been observed in animal studies, nitroglycerin is widely distributed and rapidly eliminated in humans. Armstrong et al. (1979) estimated that the volume of distribution of nitroglycerin in humans following sublingual administration is 179.6 L (assuming an average body weight of 70 kg, this volume of distribution would be approximately 2.6 L/kg) and the total body clearance is 28.0 L/min. According to the authors, this rate of clearance is much greater than the hepatic blood flow. The authors also estimated that the elimination half-life was 4.4 min. Following iv infusion of nitroglycerin into humans, McNiff et al. (1981) observed a volume of distribution of 3.3 L/kg, a total body clearance of 54.5 L/min, a plasma clearance of 0.72 L/min/kg, and an elimination half-life of 2.8 min. The authors also observed, as Armstrong et al. (1979) had, that the total body clearance of nitroglycerin greatly exceeded the liver plasma flow and also the cardiac output.

The site of nitroglycerin metabolism has not yet been ascertained. The finding that body clearance of nitroglycerin exceeds liver plasma flow and cardiac output indicates that a considerable amount of metabolism occurs in extrahepatic tissues (Armstrong et al. 1979; McNiff et al. 1981). Several in vitro studies have shown that nitroglycerin is also metabolized by whole blood, blood cells, and plasma (e.g., Noonan 1984; Sokoloski et al. 1983).

ORNL–DWG 86-12777

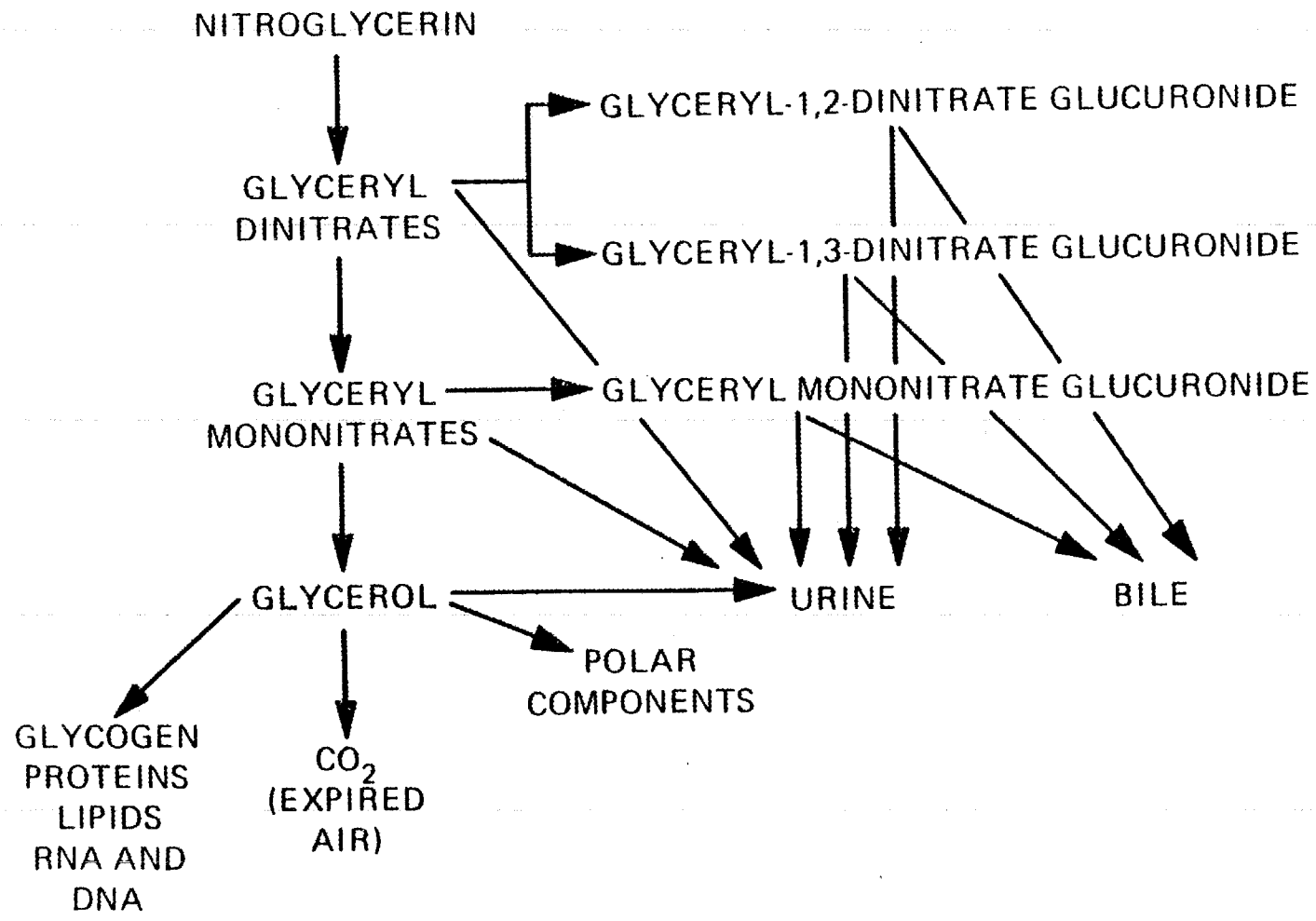

Figure 1. Metabolic fate of nitroglycerin.

The metabolic fate of nitroglycerin appears to be similar between humans and animals. The dinitrates, 1,2- and 1,3-glyceryl dinitrate, have been observed in the saliva of humans within 3.5 min after being given nitroglycerin sublingually (Bogaert and Rosseel 1972). In addition to the two dinitrates, two mononitrates (1- and 2-glyceryl mononitrate) have been identified in vitro in blood and plasma (Noonan and Benet 1982; Noonan 1984).

4.2 ACUTE TOXICITY

4.2.1 Animal Studies

A summary of available acute toxicity values for nitroglycerin is provided in Table 15. These data show that the acute toxicity of nitroglycerin is moderate but that the extent of the toxicity varies with route of exposure; the compound is less toxic when exposure is via oral or subcutaneous routes and most toxic when exposure is iv. Little or no difference exists between sexes or among species in the acute toxicity of this chemical. LD_{50} values for iv and orally administered nitroglycerin range from 10.6 to 32 mg/kg and 500 to 1188 mg/kg, respectively.

Several studies were found concerning the nonlethal effects of nitroglycerin given in a single dose to laboratory mammals. A number of studies have shown that acute exposure to nitroglycerin results in vasodilation. Heinzow and Ziegler (1981) exposed male Sprague-Dawley rats to a single dose of nitroglycerin via the following routes and concentrations: jugular vein, 10 µg/kg; portal vein, 100 µg/kg; jejunum, 200 µg/kg; sublingual, 100 µg/kg; and intraperitoneal, 100 µg/kg. The authors observed a reduction in blood pressure following the administration of nitroglycerin by all routes. The extent of change in blood pressure from greatest to lowest response was as follows: jugular vein, sublingual, intraperitoneal, portal vein, and jejunum.

Oral administration of a single 100-mg/kg dose of nitroglycerin to five male Sprague-Dawley rats resulted in a rapid decrease in systolic blood pressure; baseline pressure values were reestablished within about 10 min (Maier et al. 1980). Flaim (1982) administered nitroglycerin to male Sprague-Dawley rats over three subsequent infusion periods 15 min apart. The infusion dosages were as follows: 15-min point, 2 µg/kg; 30-min point, 8 µg/kg; and 45-min point, 32 µg/kg. The author observed significant changes at all dosage levels in blood flow and vascular resistance. After infusion with 2 µg/kg, significant ($P < 0.05$) reductions were observed in vascular resistance of the kidneys, ileum, and cerebellum, while a signficant ($P < 0.05$) increase in blood flow was observed in the kidneys. After infusion with 8 µg/kg, vascular resistance was significantly reduced in the ileum, jejunum, heart, and brain, and blood flow was increased significantly ($P < 0.05$) in the heart and brain. Significant ($P < 0.05$) decreases were observed in vascular resistance in the kidneys, ileum, jejunum, heart, and brain, and significant ($P < 0.05$) increases were observed in blood flow in the kidneys, heart, and brain after infusion with 32 µg/kg. Also observed was a significant ($P < 0.05$) decrease in CO_2 tension (pCO_2) after infusion with 8 and 32 µg/kg. The author concluded that nitroglycerin was a strong arteriolar vasodilator in rats.

TABLE 15. LETHALITY (LD_{50}'S) OF NITROGLYCERIN TO MAMMALS

Species/Strain	No. per Group	Route[a]/Vehicle	Duration of Observation	LD_{50} (mg/kg)	Comments	Reference
Mouse/Crl:CF_1BR	10	IV/Ethanol	14 days	17.28 (M)[b] 18.22 (F)[b]	All deaths occurred within 5 min; surviving mice normal within 2 hr; no significant pathology	Anderson et al. 1983
Mouse/Albino Swiss	NA[c]	Oral/in lactose (9.72%) added to peanut oil (final solution: 3.41% NG[d], 31.5% lactose, 6.5% peanut oil)	14 days	1188 (M) 1055 (F)	Animals usually died within 5 to 6 hr; survivors recovered within 24 hr; no gross pathology	Lee et al. 1975
Mouse/NA	NA	SC/NA	NA	30 (NA)		Advisory Center on Toxicology 1968[e]
Mouse/NA	NA	IP/NA	NA	205 (NA)		Advisory Center on Toxicology 1968[e]
Mouse/Slc:dd	10	Oral/Propylene glycol	7 days	550 (M) 500 (F)	Deaths occurred within 48 hr; survivors recovered within 48 to 72 hr; no notable findings at autopsy	Oketani et al. 1982c
Mouse/Slc:dd	10	SC/Propylene glycol	7 days	505 (M) 515 (F)	Deaths occurred within 48 hr; survivors recovered within 48 to 72 hr; no notable findings at autopsy	Oketani et al. 1982c
Mouse/Slc:dd	10	IP/Propylene glycol	7 days	110 (M) 104 (F)	Deaths occurred within 10 min; survivors recovered within 48 to 72 hr; no notable findings at autopsy	Oketani et al. 1982c
Mouse/Slc:dd	10	IV/Propylene glycol	7 days	10.6 (M) 10.9 (F)	Deaths occurred within 10 min; survivors recovered within about 2 hr; no notable findings at autopsy	Oketani et al. 1982c
Rat/Crl:CD(SD)BR	10	IV/Ethanol	14 days	24.43 (M) 23.23 (F)	All deaths occurred within 5 min; surviving rats normal within 1 hr; no significant pathology	Anderson et al. 1983

TABLE 15. (Continued)

Species/Strain	No. per Group	Route[a]/Vehicle	Duration of Observation	LD_{50} (mg/kg)	Comments	Reference
Rat/Charles River	NA	Oral/in lactose (9.72%) added to peanut oil (final solution: 3.41% NG, 31.5% lactose, 6.5% peanut oil)	14 days	822 (M) 884 (F)	Animals usually died within 5 to 6 hr; survivors recovered within 24 hr; no gross pathology	Lee et al. 1975
Rat/Slc:SD	10	Oral/Propylene glycol	7 days	525 (M) 540 (F)	Deaths occurred within 48 hr; survivors recovered within 48 to 72 hr; no notable findings at autopsy	Oketani et al. 1982c
Rat/Slc:SD	10	SC/Propylene glycol	7 days	610 (M) 545 (F)	Deaths occurred within 48 hr; survivors recovered within 48 to 72 hr; no notable findings at autopsy	Oketani et al. 1982c
Rat/Slc:SD	10	IP/Propylene glycol	7 days	102 (M) 108 (F)	Deaths occurred within 10 min; survivors recovered within 48 to 72 hr; no notable findings at autopsy	Oketani et al. 1982c
Rat/Slc:SD	10	IV/Propylene glycol	7 days	32 (M) 32 (F)	Deaths occurred within 10 min; survivors recovered within about 2 hr; no notable findings at autopsy	Oketani et al. 1982c
Cat	NA	IM/NA	NA	150 (NA)		Orestano 1937[e]
Dog/Beagle	2 to 4	IV/Propylene glycol	7 days	19.0 (NA)	Death occurred within 30 min; survivors recovered within 2 hr; no notable findings at autopsy	Oketani et al. 1981a

a. IV = Intravenous; SC = Subcutaneous; IP = Intraperitoneal; IM = Intramuscular.

b. M = male; F = female.

c. NA = Not available.

d. NG = nitroglycerin.

e. As reported in Dacre and Tew 1973.

Kypson and Hait (1971) injected groups of seven albino rabbits intraperitoneally with 0, 40, or 100 μg/kg of nitroglycerin. Administration of 40 mg/kg caused a significant increase in plasma lactate ($P < 0.02$), plasma free fatty acids ($P < 0.05$), and plasma glycerol ($P < 0.05$). The same parameters, as well as plasma pyruvate, were significantly increased ($P < 0.005$) after the administration of 100 μg/kg. Based on their results, the authors suggested that nitroglycerin may increase lipolysis and glycolysis.

4.2.2 Human Studies

Changes in the cardiovascular system of humans following acute exposure to nitroglycerin have been reported. As in animal studies, human studies have shown that nitroglycerin is a vasodilator. Blumenthal et al. (1977) observed an increase in the pulse rate and a decrease in the mean arterial pressure after a single administration of nitroglycerin sublingually (0.3 mg), orally (6.5 mg sustained released capsule), and dermally (2 percent ointment) to a healthy human volunteer. The following effects have been observed after a single sublingual administration of 0.6 mg of nitroglycerin to healthy human volunteers: increased heart rate (Armstrong et al. 1979; Brachfeld et al. 1959; Kikendall and Mellow 1980; Wei and Reid 1979); decreased blood pressure (Armstrong et al. 1979; Brachfeld et al. 1959; Wei and Reid 1979); reduction in esophageal sphincter pressure (Kikendall and Mellow 1980); increased myocardial oxygen consumption and increased coronary blood flow mediated by lower vascular resistance (Brachfeld et al. 1959); and fall in myocardial efficiency (Brachfeld et al. 1959). Armstrong et al. (1979) and Wei and Reid (1979) observed falls in systolic blood pressure but not in diastolic blood pressure following sublingual administration of 0.6 mg of nitroglycerin.

Application of 2 percent nitroglycerin to the skin of normal human volunteers has been reported to decrease systolic and diastolic blood pressure and increase heart rate (Magometschnigg et al. 1983) but has no effect on lower esophageal sphincter pressure (Kikendall and Mellow 1980). Trainor and Jones (1966) reported that exposure to nitroglycerin in the atmosphere at a concentration of 0.5 or 0.7 mg/m^3 for 25 min causes headaches and decreases blood pressure.

4.3 SUBCHRONIC AND CHRONIC TOXICITY

4.3.1 Animal Studies

Ellis et al. (1984) gave groups of beagle dogs, consisting of two males and two females each, nitroglycerin in capsules at concentrations of 25, 50, 100, or 200 mg/kg/day for five days; the authors did not indicate that they had used a control group. A dose-response relationship in peak level and duration of methemoglobinemia was observed. In animals given up to 100 mg/kg/day the methemoglobin levels returned to zero within 24 hr. Adverse clinical effects were not observed at the two lowest doses. At the two highest doses, cyanosis was observed within 2 to 3 hr after each dose and lasted for several hours. At the highest concentration the dogs became less active.

Anderson et al. (1983) studied the effects of two weeks of exposure of Crl:CD (SD)BR rats and beagle dogs to nitrostat, a stabilized form of nitroglycerin. [According to the Physicians Desk Reference (1983), each milliliter of nitrostat consists of 0.8 mg nitroglycerin with citric acid and sodium citrate as buffers, and 5 percent alcohol in water.] Groups of ten male and ten female rats were given daily iv injections of nitrostat at concentrations of 2.5, 5, or 10 mg/kg/day, and groups of two male and two female dogs were given 1 or 3 mg/kg/day. Both vehicle (5 percent aqueous ethanol) and untreated control groups were used. At a concentration of 10 mg/kg two rats died for reasons the authors could not determine. A reduction in body weight and food consumption was observed in treated and vehicle control groups, but no dose-response relationship was found. No significant changes were observed in biochemical, hematological, and urinalysis parameters or organ weights and organ weight-to-body weight ratios. The authors also observed no gross or microscopic changes in the tissues.

Oketani et al. (1982b) gave groups of ten male and ten female Slc:SD rats daily ip injections of nitroglycerin at concentrations of 1, 5, 25, or 50 mg/kg for one month. A control group received only the vehicle, propylene glycol. At 50 mg/kg the authors frequently observed tonic convulsions, Straub tail response, and ataxic gait; two of ten males died of dyspnea toward the end of the exposure period. Slight transient convulsions or sedation was observed in some animals exposed to 5 and 25 mg/kg. No adverse effects were observed in animals exposed to 1 mg/kg, and no changes were observed in any group in body weight, food and water consumption, urinalysis, hematology, serum biochemistry, or histopathology.

The subchronic toxicity of nitroglycerin to beagle dogs was studied by Oketani et al. (1982a). Groups of three male and three female dogs were given nitroglycerin iv in concentrations of 1, 2.5, or 5 mg/kg/day for one month, and two control groups received either saline or propylene glycol (vehicle). At the highest concentration, the animals frequently exhibited tonic convulsions, eye hyperemia, lying down, and urinary and rectal incontinence, but these effects lasted no more than 30 min. Slight transient convulsions or tremors were observed in the group that had received 2.5 mg/kg. Adverse effects were not observed in the 1-mg/kg exposure group, and no changes were observed in any group in body weight, food and water consumption, urinalysis, hematology, serum biochemistry, or histopathology.

Ellis et al. (1984) studied the subchronic effects of nitroglycerin to beagle dogs, CD rats, and CD-1 mice. Groups of four male and four female dogs were given nitroglycerin orally at concentrations of 0, 0.01, 0.1, or 1 mg/kg/day in capsules for three weeks; then, because there were no observable adverse effects, the concentrations of nitroglycerin were increased to 0.05, 0.5, or 5 mg/kg/day at the start of the fourth week and were continued through the thirteenth week. Groups of six male and six female rats were given nitroglycerin in their diets at concentrations of 0, 0.001, 0.01, or 0.1 percent for four weeks and then increased to 0.005, 0.05, or 0.5 percent for the remainder of the 13-week exposure period due to the absence of adverse effects. Mice were

treated in the same manner as the rats except that the increased doses were given at the start of the third week. The estimated daily intake of nitroglycerin by rats was 0.8, 6, and 59 mg/kg for males and 0.9, 6.4, and 59 mg/kg for females exposed to 0.001, 0.01, and 0.1 percent, respectively; for exposures to 0.005, 0.05, and 0.5 percent the estimated daily intake was 2.6, 24.5, and 230 mg/kg for males and 3.1, 26.5, and 234 mg/kg for females, respectively. The estimated daily intake of nitroglycerin by mice given 0.001, 0.01, and 0.1 percent was 1.3, 11.5, and 107 mg/kg for males and 1.3, 10.9, and 95 mg/kg for females, respectively; for exposures to 0.005, 0.05, and 0.5 percent the estimated daily intake was 6.4, 60.2, and 608 mg/kg for males and 6.9, 58.7, and 561 mg/kg for females, respectively.

Ellis et al. (1984) observed no adverse effects in dogs at the end of the 13-week exposure period. At the highest concentration, rats were found to have a lower consumption of food and a lower weight gain. Blood tests, necropsy, and histopathology of rats showed no significant difference from controls. Some mice exhibited mild to moderate extramedullary hematopoiesis in the liver and/or spleen, but no dose-response relationship was observed.

In an additional subchronic study, Ellis et al. (1984) exposed a group of three male and three female CD rats to a diet containing 2.5 percent nitroglycerin for 13 weeks. A control group consisting of four male and four female rats was fed the basal diet. The estimated average daily intake of nitroglycerin was 1406 and 1416 mg/kg/day for males and females, respectively. At the end of the exposure period, the authors observed a significant ($P < 0.05$) increase in erythrocytes, reticulocytes, hematocrit, hemoglobin concentration, and alkaline phosphatase, and a decrease in fasting blood glucose of the exposed animals. Histopathological examination revealed the presence of pigment deposits in the liver and spleen and moderate to severe testicular degeneration and/or atrophy with severe to complete aspermatogenesis.

Ellis et al. (1978a, 1984) studied the chronic toxicity of nitroglycerin to CD rats, CD-1 mice, and beagle dogs. Groups of 38 male and 38 female rats and 58 male and 58 female mice were given a diet containing 0, 0.01, 0.1, or 1 percent nitroglycerin for two years. The calculated daily intake of nitroglycerin by male and female rats was 3.04 and 3.99 mg/kg, respectively, for the low dose; 31.5 and 38.1 mg/kg, respectively, for the middle dose; and 363 and 434 mg/kg, respectively, for the high dose. The calculated daily intake of nitroglycerin by male and female mice was 11.1 and 9.72 mg/kg, respectively, for the low dose; 114.6 and 96.4 mg/kg, respectively, for the middle dose; and 1022 and 1058 mg/kg, respectively, for the high dose. Groups of six male and six female beagle dogs were administered nitroglycerin capsules containing 0, 1, 5, or 25 mg/kg daily for one year.

The only effect observed in dogs during the 12-month exposure period was an occasional dose-related occurrence of methemoglobinemia; the concentration of methemoglobin was usually less than 3 percent (Ellis et al. 1978a, 1984).

After two years of exposure, body weight of rats exposed to 1.0 percent nitroglycerin was considerably lower than that of either the controls or the 0.01 and 0.1 percent exposure groups; control weights were about 800 and 500 g for males and females, respectively, and weights of males and females exposed to 1.0 percent nitroglycerin were 600 and 300 g, respectively (Ellis et al. 1978a, 1984). After three months of exposure to 1.0 percent nitroglycerin, the authors observed discoloration of the skin and fur, methemoglobinemia, reticulocytosis, and an increased erythrocyte count, hematocrit, and hemoglobin concentration; total hemoglobin typically consisted of 10 to 30 percent methemoglobin. After 12 months of exposure to 1.0 percent nitroglycerin, erythrocyte counts were normal, but hemoglobinemia persisted. Enlarged livers were also observed at this same time and concentration, and all but two of the rats in this group had cholangiofibrosis, proliferation of bile ducts, and fibrous tissue of the liver. After two years of exposure to 1.0 percent nitroglycerin, methemoglobinemia had disappeared, but elevated levels of serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, and alkaline phosphatase levels were observed in males. In addition, high-dose females were found to live considerably longer than controls (about 25 percent of females treated with 1.0 percent were dead at 24 months versus about 60 percent of the controls). No significant hematological effects were observed in rats exposed for two years to nitroglycerin at concentrations of 0.01 or 0.1 percent.

After 12 months of exposure to 1.0 percent nitroglycerin, mice were found to have compensated anemia and an elevated reticulocyte count accompanied by Heinz bodies (Ellis et al. 1978a, 1984). Methemoglobinemia occurred only in males exposed to 1.0 percent nitroglycerin. Also at 12 months, most animals exposed to 1.0 percent and some exposed to 0.1 percent nitroglycerin had intracellular granules in the liver with lesser amounts in the spleen and/or kidneys. Body weights of mice exposed for two years to 1.0 percent nitroglycerin were considerably lower than those of controls. At the end of the two-year exposure period, no significant increases were observed in unscheduled deaths, although a slight increase was observed in the groups exposed to 1.0 percent nitroglycerin. Hematological parameters after two years were similar to those observed after 12 months.

Suzuki et al. (1975) gave groups of C57BL/6Jms mice, consisting of about 50 animals of each sex, nitroglycerin in their drinking water for 18 months at concentrations estimated to be 0, 1.5, or 6.2 mg/kg/day, and for 12 months at an estimated concentration of 58.1 mg/kg/day. At the end of the exposure periods, body weights of exposure groups did not appear to be substantially different from those of the control group. Nontumorous changes, including inflammations, degenerative changes, and cytological changes in hepatic cells, were similar to those observed in control animals.

4.3.2 Human Studies

Several studies and reviews have been published concerning the chronic toxic effects of nitroglycerin to humans (e.g. J. Am. Med.

Assoc. 1898; Munch et al. 1965; Lund et al. 1968; NIOSH 1978). Exposures to nitroglycerin in industrial situations are frequently associated with exposures to nitroglycol (e.g., such as during the production of explosives), therefore, establishment of effects due directly to exposure to nitroglycerin is difficult (Lund et al. 1968; NIOSH 1978). Headache and fatigue are frequently observed in long-term employees of the explosives industry (Stokinger 1982). Other symptoms observed less frequently include nausea, vomiting, dyspnea, alcohol intolerance, heart palpitation, and angina pectoris (NIOSH 1978; Stokinger 1982). Available information indicates that continued exposure to nitroglycerin or a combination of nitroglycerin and nitroglycol results in increasing tolerance (Lund et al. 1968; Munch et al. 1965; NIOSH 1978). Initial symptoms of exposure may include headaches, nausea, dizziness, heart palpitations, sweating, fatigue, and diffuse pains which tend to eventually become milder or disappear. After several years of exposure, chest pains have been observed within 24 to 72 hr after interruption of exposure but disappear after exposure starts again (Lund et al. 1968). Long-term employment in the explosives industry has been associated with nonatheromatous ischemic heart disease (Lange et al. 1972). Sudden deaths of a few employees of the explosives industry have been observed one to three days after cessation of exposure (Carmichael and Lieben 1963; Hogstedt and Axelson 1977; NIOSH 1978) and have been attributed primarily to nitroglycol (Hogstedt and Davidsson 1980).

Reeve et al. (1983) recently presented the results of a mortality study they conducted on nitroglycerin-exposed workers at a munitions plant in Virginia. They obtained records on 5,668 white male employees who had worked at the plant during the period 1949 through 1977; of this number, 13 percent were dead, 83 percent living, and 4 percent lost to follow-up. Using mortality rates of the general population of the United States, they found no significant increases in deaths due to diseases of the nervous, cardiovascular, respiratory, or digestive systems. More in-depth examination of their data revealed that a slight elevation in the number of deaths due to ischemic heart disease had occurred, but the increase was not significant. When they further broke the records down into age categories of ten-year intervals, an increase in deaths due to ischemic heart disease was observed in four of seven of the categories, but was significant ($P < 0.05$) in only the 40 to 49 age group. The authors indicated that their results were suggestive of an association between nitroglycerin exposure and cardiovascular disease. Additional studies are planned by Stayner et al. (1985) of the same munitions plant to test the hypothesis that exposure to nitroglycerin is associated with an increased risk of dying from ischemic heart disease and to determine whether any observed association exists separately from the risk of dying from acute withdrawal from nitroglycerin exposure and other known medical risk factors. In addition, they will try to determine if exposure to other nitrated compounds used in the manufacture of propellants is associated with an increased risk of ischemic heart disease and whether there is an interaction between these exposures and nitroglycerin.

4.4 GENOTOXICITY

In vivo and in vitro studies using mammalian and bacterial cell systems have failed to show that nitroglycerin is a genotoxin. Lee et al. (1977) studied the cytogenetic effects of nitroglycerin on somatic cell chromosomes from lymphocyte and kidney tissue cultures obtained from beagle dogs and CD rats. Groups of three to five male and female rats were fed an average of 59 to 59.3 mg/kg/day nitroglycerin for 5 weeks and then 229.5 to 233.8 mg/kg/day for an additional 8 weeks. Similarly, groups of one to two male and female dogs were fed an average of 1 mg/kg/day nitroglycerin for 4 weeks and then 5 mg/kg/day for an additional 9 weeks. For both species, lymphocyte and kidney tissues were obtained after 4 and 13 weeks and cultured. Examination of cells from the cultures did not show any apparent changes in the chromosome frequency distribution, number of tetraploids, chromatid breaks, or translocations. Although these results suggest that nitroglycerin is not genotoxic, the use of greater numbers of animals would have improved the validity of the study.

Ellis et al. (1978a) studied the cytogenetic effects of nitroglycerin to bone marrow cells and kidney tissue cultures obtained from CD rats. Groups of four to six animals were given nitroglycerin in their diet for two years at a concentration of 0 or 1 percent. Tissues and cells for the cultures were obtained at the end of the two-year exposure period. The estimated daily intake of nitroglycerin was 363 and 434 mg/kg/day for males and females, respectively. No significant differences were found between the control and exposure groups; however, a slight increase in the number of chromatid breaks and gaps was observed in kidney cultures (1.6 breaks and gaps per 50 cells for controls versus 2.9 per 50 cells for the exposed group).

Lee et al. (1977) studied the mutagenic effects of nitroglycerin on wild type Chinese Hamster Ovary (CHO-K1) cells in vitro. The mutagenicity of nitroglycerin was measured relative to ethyl methanesulfonate, a known mutagen. Nitroglycerin at concentrations of 50 and 144.8 μg/mL resulted in a survival rate of 35 and 1 percent, respectively, and a mutation frequency of zero at both concentrations. In contrast, exposure to ethyl methanesulfonate at a concentration of 124 μg/mL resulted in a survival of 15 percent and a mutation frequency of 28.0×10^{-6}, thus indicating that nitroglycerin was not mutagenic.

Ellis et al. (1978a) also performed a dominant lethal assay with Charles River CD rats. Groups consisting of ten male animals were given nitroglycerin in their food for 13 weeks at concentrations of 0, 0.01, 0.1, or 1 percent (estimated intake of 0, 3.04, 31.5, and 363 mg/kg/day, respectively); each male was then mated with two virgin females. Females were sacrificed at mid-term of pregnancy, and the following data were collected: number of fertile males per number of males treated, number of pregnant females per number of mated females, number of corpora lutea per pregnant female, and the number of total implants, dead implants, and live implants per pregnant female. The authors observed no dominant lethal mutations, no evidence of adverse effects on male fertility, and no preimplantation and postimplantation losses.

Kononova et al. (1972) studied the mutagenic potential of nitroglycerin on the extracellular bacteriophage T4B of Escherichia coli. Treatment of bacteriophages with a 0.084 M solution of nitroglycerin for up to 6 hr did not increase the frequency of mutations in bacteriophages as compared to a control but did affect their survival rate (30 and 16 percent at 3 and 6 hr, respectively, for nitroglycerin versus 100 percent at 3 and 6 hr for the control).

The mutagenic activity of nitroglycerin and several of its metabolites (1,3- and 1,2-dinitroglycerin and 1- and 2-mononitroglycerin) was tested by Ellis et al. (1978b) with the Ames assay at concentrations of 10, 100, 300, and 1000 µg per plate. The following histidine-requiring Salmonella typhimurium tester strains were used: TA-1535, TA-1537, TA-1538, TA-98, and TA-100. No mutagenic activity was observed with 1,3-dinitroglycerin or 1-mononitroglycerin. At concentrations of 10 and 30 µg per plate, 1,2-dinitroglycerin caused a significant (level of significance not given) increase in frame-shift mutations without activation. Weak mutagenic activity was exhibited by both nitroglycerin and 2-mononitroglycerin, both requiring concentrations of 1000 µg per plate and metabolic activation; 2-mononitroglycerin caused only base pair substitution mutations, while nitroglycerin caused both base-pair substitution and frame-shift mutations.

Kaplan et al. (1982) also studied the mutagenic potential of 1-mononitroglycerin under the conditions of the Ames assay, and, like Ellis et al, (1978b), they observed no mutagenic activity.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

4.5.1 Animal Studies

Developmental toxicity studies have failed to show that nitroglycerin is a teratogen (e.g., Ellis et al. 1978a; Oketani et al. 1981b; Oketani et. al. 1981c), and reproductive effects have been observed only at high concentrations (Ellis et al. 1978a, 1984). Ellis et al. (1978a, 1984) fed a group of three male and three female CD rats 2.5 percent nitroglycerin for 13 weeks, and a group consisting of four male and four female rats was fed a standard diet to serve as a control. The estimated average daily consumption of nitroglycerin by males and females was 1406 and 1416 mg/kg, respectively. At the end of the exposure period Ellis et al. observed moderate to severe testicular atrophy and/or degeneration, and severe to complete aspermatogenesis. No effects to the female reproductive system were reported.

Ellis et al. (1978a) conducted a three-generation reproductive study with CD rats. Each generation was given a diet containing 0, 0.01, 0.1, or 1 percent nitroglycerin. The parental generation (F_0) consisted of 10 male and 20 female animals per group, and the later generations consisted of 10 to 12 pairs per group. Ellis et al. observed no significant effects on reproduction at the low and middle concentrations. The highest concentration did not significantly affect fertility of the F_0 generation, but did affect fertility of the succeeding generations; only three litters (F_{2a}) were produced by the F_1 generation.

None of the F_1 dams that littered with the first mating produced a second litter (F_{2b}). Two matings of the F_{2a} litters produced only one litter (F_3). Mating of 14 F_{2a} females with control males resulted in 13 pregnancies, thus showing that the F_{2a} males were infertile. Males in the F_{2a} generation had very small testes, and females exhibited a high incidence of vaginal plugs without sperm. Microscopic examination of the animals showed severe aspermatogenesis and mildly to moderately increased interstitial tissue in the testes. Reproductive parameters, including litter size, live-born index, birth weight, viability and lactation indexes, and weaning weight, were significantly reduced ($P < 0.05$) in the high dose F_{1a} litters, but sex ratios were not significantly affected in this group. In the high dose F_{1b} litters, birth weight, viability index, and weight at weaning were significantly ($P < 0.05$) reduced; the lactation index was also reduced but not significantly. In the high dose F_{2a} litters, litter size and birth weight were significantly ($P < 0.05$) reduced. All other parameters in this group were lower than those observed in controls, but the differences were not statistically significant. The authors thought that the toxic effects seen in the reproductive parameters of the F_1 and F_2 litters were partially due to the poor nutritional status of the dams. The authors indicated that the reduction in litter size of the high-dose F_{1a} group was suggestive of mutagenic and/or teratogenic effects; however, in their cytogenetic and dominant lethal mutation studies [see studies by Ellis et al (1978a) in Section 4.6], they observed no genotoxic effects. Results of their teratogenicity study (Ellis et al. 1978a), which involved a third mating of the females of the F_0 generation, did not show any significant teratogenic effects. However, they observed diaphragmatic hernias in 4 of 19 litters in the high-dose group and none in the other groups. Although the increase in hernias was not found to be statistically significant, the authors suggested that this could have been the cause of the reduced litter sizes found in the study on reproductive effects. Also observed was a significant ($P < 0.05$) increase in the incidence of absent or incompletely ossified hyoid bone; however, the authors pointed out that skeletal anomalies are indicative of delayed development and not the teratogenic potential of a chemical.

Oketani et al. (1981b) studied the teratogenic and embroytoxic effects of nitroglycerin to Japanese albino rabbits. Groups consisting of ten female animals were given nitroglycerin iv in doses of 0, 0.5, 1, or 4 mg/kg/day from day 6 to day 18 of gestation. They observed no difference between the exposed and control groups in the incidence of teratogenic effects based on the incidence of external, visceral, and skeletal abnormalities, nor did they observe any signs of embryotoxicity based on the number of corpora lutea, implantations, dead or live fetuses, sex ratio, body weight of live fetuses, or placental weight.

In a study on the reproductive and teratogenic effects of nitroglycerin to CD-S1c rats, Oketani et al. (1981c) gave groups of 19 to 20 pregnant rats nitroglycerin intraperitoneally in doses of 0, 1, 10, or 20 mg/kg/day from day 7 to day 17 of gestation. Compared to controls, nitroglycerin had no significant effect on delivery, gestation period, lactation, nursing instinct, fetal and postnatal development, or fertility of offspring. Also, evidence of teratogenicity was not found in any of the offspring (F_1 and F_2).

In another study on the reproductive effects of nitroglycerin on SD-Slc rats, Oketani et al. (1981d) administered the chemical on day 17 of gestation to day 21 of lactation. Groups of 17 to 20 rats were given nitroglycerin daily in doses of 0, 1, 10, or 20 mg/kg ip. They observed no significant effects on delivery, gestation period, lactation, nursing instinct, growth, development, or fertility of offspring, nor any evidence of teratogenic effects.

In a study primarily on the effects of nitroglycerin on the fertility of SD-Slc rats, Oketani et al. (1981e) administered the compound iv at doses of 0, 1, 10, or 20 mg/kg/day to groups of 20 male animals for 63 days prior to mating and to groups of 20 females 14 days prior to mating and up to day 7 of gestation. No significant effects were observed on fertility based on the number of male rats copulating with treated and untreated females, percentage of pregnant rats, or the duration of pairing until mating. They also observed no significant effects on indexes of teratogenicity and embryotoxicity.

4.5.2 Human Studies

Data on the effects of nitroglycerin on human reproduction are limited and essentially anecdotal. A 1898 report described the effects of nitroglycerin on the wives of employees of a plant manufacturing the compound (J. Am. Med. Assoc. 1898). These women occupied the same sleeping quarters as their husbands and laundered their husbands' clothes. Symptoms experienced by the wives were uterine malfunctions and increased menstrual bleeding. Fewer children were born, and they were often premature. The surviving children were not as strong as other children, were often cyanotic, and were not as resistant to disease. Laws (1910) described the symptoms experienced by workers engaged in the manufacture of nitroglycerin, including disturbances of the genitourinary system. He attributed marked aphrodisiac effects to the compound and stated that most "nitroglycerin men" had large families.

Mudd (1977) used the vasodilating effects of nitroglycerin to diagnose and treat atherosclerotic impotence. A 56-year old man with known psychogenic impotence was given 0.3 mg of nitroglycerin sublingually and responded quickly and favorably to treatment with associated changes in penile volume, temperature, and sensation.

4.6 ONCOGENICITY

4.6.1 Animal Studies

Three studies were found concerning the oncogenic potential of nitroglycerin in laboratory animals. From the results of their studies, Suzuki et al. (1975) and Takayama (1975) concluded that nitroglycerin is not an oncogen. Ellis et al. (1978a, 1984) reported an increase in the occurrence of hepatocellular carcinomas and interstitial cell tumors of the testis in rats but not in mice or dogs.

In their study, Ellis et al. (1978a, 1984) gave groups of 38 male and 38 female CD rats and 58 male and 58 female CD-1 mice a diet containing 0, 0.01, 0.1, or 1 percent nitroglycerin for two years. Groups of 6 male and 6 female beagle dogs were given capsules orally which contained 0, 1, 5, or 25 mg/kg/day nitroglycerin for one year. The calculated daily intake of nitroglycerin by male and female rats was 3.04 and 3.99 mg/kg, respectively, for the low dose, 31.5 and 38.1 mg/kg, respectively, for the middle dose, and 363 and 434 mg/kg, respectively, for the high dose. The calculated daily intake of nitroglycerin by male and female mice was 11.1 and 9.72 mg/kg, respectively, for the low dose, 114.6 and 96.4 mg/kg, respectively, for the middle dose, and 1022 and 1058 mg/kg, respectively, for the high dose.

Ellis et al. (1978a, 1984) observed no signs of oncogenicity in either mice or dogs at the end of the exposure period; however, results from the one-year exposure study of dogs may not be representative of results from lifetime exposures of this species. In rats, after the first 12 months of exposure to nitroglycerin, the authors observed what they described as progressive signs of hepatocellular carcinomas including areas and foci of hepatocellular alteration in all dose groups; neoplastic nodules in one middle-dose male, three high-dose males, and one high-dose female; and the occurrence of an hepatocellular carcinoma in one high-dose male. At the end of the two-year exposure period, hepatocellular carcinomas were observed in 13 of 21 males and 11 of 25 females of the 1 percent exposure group; 3 of 26 males and 2 of 28 females of the 0.1 percent exposure group; 0 of 28 males and 0 of 32 females of the 0.01 percent exposure group; and 0 of 24 males and 0 of 29 females of the control group. Also at the end of the two-year exposure period, neoplastic nodules were observed in 2 of 21 males and 5 of 25 females of the 1 percent exposure group; 1 of 26 males and 1 of 28 females of the 0.1 percent exposure group; 0 of 28 males and 0 of 32 females of the 0.01 percent exposure group; and 1 of 24 males and 0 of 29 females of the control group. In addition, 11 of 21 males exposed to 1.0 percent nitroglycerin had interstitial cell tumors in one or both testes. The incidence of tumors in the testes of the other two exposure groups was similar to that of the controls (4 to 11 percent). On the other hand, female rats exposed to 1.0 percent nitroglycerin exhibited decreased incidences of mammary gland tumors and pituitary chromophobe adenomas.

Suzuki et al. (1975) studied the oncogenic potential of nitroglycerin in C57BL/6Jms mice exposed to the chemical for up to 18 months. Groups of mice consisting of at least 54 males and 49 females were exposed to nitroglycerin in their drinking water at an average concentration of 0, 1.5, or 6.2 mg/kg/day for 18 months. Another group of mice, consisting of 49 males and 45 females was exposed for 12 months to nitroglycerin in their drinking water at an average concentration of 58.1 mg/kg/day. Based on their results, the authors concluded that the number and incidence of tumor-bearing animals, the number and incidence of histologically defined tumors, the number of tumors per animal, and the pattern of the tumors in the dosed groups were comparable with those of the control group. However, gross examination of the animals revealed that 5 of 34 female mice in the high-dose group had an

unspecified type of pituitary tumor. Histological examination of all groups of animals showed what appeared to be a dose-related increase in the incidence of pituitary adenomas; a total of 6, 3, 1, and 1 pituitary adenomas was observed in the high, middle, low, and control groups, respectively. Using an exact analysis of a contingency table, Ellis et al. (1984) reanalyzed the results that Suzuki et al. had obtained from their gross examination of the pituitary gland and concluded that there was a significant (P = 0.006) increase in the incidence of pituitary tumors at the highest concentration.

Takayama (1975) gave a group of Sprague-Dawley rats consisting of 50 males and 48 females a 0.03 percent concentration of nitroglycerin in their drinking water for 10 months and then gave tap water for an additional 8 months. A control group consisting of 53 male and 49 female rats was given tap water for 18 months. The estimated daily intake of nitroglycerin was 31 mg/kg. At the end of the 18-month period, the authors observed no remarkable histopathological changes and concluded that under their experimental conditions, nitroglycerin was not oncogenic. However, the exposure period used by these authors is not representative of a lifetime exposure for this species (e.g., Ross et al. 1980).

4.6.2 Human Studies

Reeve et al. (1983) conducted a mortality study of workers exposed to nitroglycerin at a munitions plant in Virginia. They examined health records of 5,668 white males who had worked in the nitroglycerin area during some period between 1949 and 1977. They found no increases in deaths due to malignant neoplasms; in fact, they found that the number of deaths due to malignant neoplasms was significantly lower than the expected number in the general United States population.

Hogstedt and Andersson (1979) conducted an epidemiological study of employees of a Swedish dynamite plant and examined the incidence of tumors as a possible cause for excess mortality in the dynamite industry. The primary components of dynamite are nitroglycerin and nitroglycol, and according to the authors they exist in dynamite in a 1:1 ratio. The authors also indicated that between these two chemicals, nitroglycol is the predominant chemical in the air due to its higher volatility. Death certificates were collected on employees who had died between 1951 and 1977, and the official underlying cause of death was used and classified according to the 1965 version of the International Classification of Diseases. The final group of subjects that they chose consisted of 143 male employees who had worked at least one year since 1927 and who had died since 1951. This group consisted of 88 exposed and 55 unexposed employees. The exposed group consisted of those employees with jobs that were somehow directly related to the handling or production of dynamite and/or to the handling of its major components. The unexposed group consisted of those employees that did not handle and/or produce dynamite or handle its major components. The cause of death was broken down into the following categories: all tumors, circulatory diseases, and other causes. The number of observed deaths was then compared with the national average. In both the exposed

and unexposed groups, the number of deaths due to tumors was less than the number expected. The authors also looked at a subgroup of the exposed group that consisted of those employees with at least 10 years of exposure. In those employees who died between 1951 and 1964, the number of deaths due to tumors was one and the expected number was 1.3, while the number of observed and expected deaths between 1965 and 1977 was two and 1.7, respectively. Thus, long-term exposure to nitroglycerin and/or nitroglycol at the levels occurring in this dynamite plant does not appear to significantly increase the incidence of cancer.

4.7 SUMMARY

Studies on the pharmacokinetics of nitroglycerin in laboratory animals and humans indicate that it is rapidly and widely distributed, and rapidly absorbed, metabolized, and eliminated. The disposition rate is influenced by the route of administration, with more rapid disposition occurring following iv and sublingual exposures than after oral or dermal exposures. Metabolism of the compound appears to occur in both hepatic and extrahepatic tissues. Metabolism is via stepwise denitrification with no preference for the primary or secondary nitrate group. Nitroglycerin is eliminated primarily in the urine and expired air.

Laboratory animal data on the acute toxicity of nitroglycerin indicate that it is moderately toxic. The toxicity of nitroglycerin depends upon the route of administration and is most toxic when given iv and least toxic when given orally. Little or no sex and species differences have been observed in the acute toxicity of this compound. Vasodilation and headaches have been observed in humans after sublingual administration.

Short-term exposures (one month) of laboratory animals to nitroglycerin (2.5 mg/kg/day) have been found to cause slight transient toxic effects such as tonic convulsions or tremors. Subchronic exposure (13 weeks) to high concentrations of nitroglycerin has been found to reduce weight gain in rats (230 mg/kg/day), increase erythrocytes, reticulocytes, hematocrit, hemoglobin, and alkaline phosphatase, and decrease fasting blood glucose (1406 mg/kg/day).

Like subchronic exposure, chronic exposure of laboratory animals to nitroglycerin has been associated with hematological changes and reduced weight increases. Methemoglobinemia and reticulocytosis have been observed in mice and rats, increases in erythrocyte counts, hematocrit, and hemoglobin concentrations have been observed in mice, and compensated anemia has been observed in rats after one to two years of dietary exposure to 1.0 percent nitroglycerin (about 1022 mg/kg/day for mice and 363 mg/kg/day in rats). Changes in the liver have also been observed in mice and rats at high concentrations including intracellular granulation in mice and enlarged livers, cholangiofibrosis, proliferation of the bile ducts, and fibrous tissue in the liver of rats. Long-term employment of humans in industries producing nitroglycerin or using nitroglycerin in the production of explosives has been frequently associated with headaches, fatigue, and nausea. There is some evidence that long-term exposure to nitroglycerin and/or nitroglycol in the explosives

industry may cause an increase in death due to cardiovascular disease and also may be the cause of some isolated cases of sudden death.

In vivo genotoxicity studies have failed to demonstrate that nitroglycerin is a genotoxin. Nitroglycerin is reportedly weakly mutagenic under the conditions of the Ames assay. Of the metabolites of nitroglycerin studied for mutagenicity with the Ames assay, 1,2-dinitroglycerin has shown significant mutagenic effects, 2-mononitroglycerin has shown weak mutagenic effects, and 1,3-dinitroglycerin and 1-mononitroglycerin have shown no mutagenic effects. Developmental toxicity and reproductive effects studies with laboratory animals have failed to demonstrate that nitroglycerin is a teratogen, but have shown that exposures to high concentrations (360 mg/kg/day) may cause infertility in male rats, and delayed development, as judged by incomplete ossification of the hyoid bone, in rats exposed prenatally.

A high incidence of hepatocellular carcinomas (52 percent) and interstitial cell tumors of the testes (52 percent) has been observed in rats exposed to 1.0 percent (363 mg/kg/day) nitroglycerin for two years. An increased incidence of hepatocellular carcinomas (13 percent) has also been observed in rats given 0.1 percent nitroglycerin (31.5 mg/kg/day) for two years. An increase in pituitary tumors (14 percent) has been observed in female mice exposed for one year to 58.1 mg/kg/day. Long-term exposure of humans employed in the explosives industry has not been associated with an increased incidence of cancer.

5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

The current Occupational Safety and Health Administration standard for occupational exposure (TWA) to nitroglycerin is 2 mg/m^3 (skin) (0.2 ppm), which is a ceiling limit (USOSHA 1983). The National Institute for Occupational Safety and Health recommended an occupational exposure limit of 0.1 mg/m^3 (0.01 ppm) measured as a ceiling concentration during any 20-minute sampling period (NIOSH 1978). The American Conference of Governmental Industrial Hygenists recommended a threshold limit value (TLV) of 0.5 mg/m^3 (0.05 ppm) and a short-term exposure limit value (STEL) of 1.0 mg/m^3 (0.1 ppm) for skin (ACGIH 1981). An IDLH (concentration immediately dangerous to life or health) of 80 ppm (750 mg/m^3) was given by the International Labour Office (1983).

5.2 OCCUPATIONAL EXPOSURE

Occupational exposure to nitroglycerin has been documented for a number of years. Some of the early reports were of employees in the explosives industry who handled nitroglycerin (J. Am. Med. Assoc. 898; Laws 1910). The employees frequently complained of severe headaches, nausea, and weakness. Headaches have been reported to occur in the explosives industry when air concentrations of nitroglycerin were 0.5 mg/m^3. Long-term employment in the explosives industry, in which

exposures to nitroglycerin and/or nitroglycol have occurred, has been associated with chest pains, nonatheromatous ischemic heart disease, and sudden death two or three days after terminating exposure (Carmichael and Lieben 1963; Hogstedt and Axelson 1977; Hogstedt and Andersson 1979; Lange et al. 1972; Lund et al. 1968). Hogstedt and Andersson (1979) examined the incidence of deaths occurring in employees of a dynamite plant in Sweden from 1951 through 1977. They observed a slightly higher than expected incidence of deaths over the national average, particularly from cardiovascular diseases. From 1965 through 1977 the incidence of death due to cardiovascular disease was significantly higher ($P < 0.05$) for men with at least one year of exposure to dynamite than for the national average. They indicated that exposures in this plant were due primarily to nitroglycerin and nitroglycol. Hogstedt and Davidsson (1980) performed an in-depth analysis of some short-term air samples that had been taken from 1958 through 1978 in two dynamite plants in Sweden. They estimated that the 8-hr time-weighted average concentrations for nitrate esters during this time period ranged from 0.2 to 1.1 mg/m^3.

More recently, Reeve et al. (1983) conducted an epidemiological study on nitroglycerin exposed employees of a munitions plant in Virginia. Based on records obtained on 5,668 white male employees that had worked at the plant at some time between 1949 and 1977, and compared with the general population of the United States, they found no significant increase in deaths due to diseases of the nervous, cardiovascular, respiratory, or digestive systems. When they broke the data down into age groups of ten-year intervals, a significant ($P < 0.05$) increase in the incidence of deaths due to ischemic heart disease was found, thus suggesting an association between nitroglycerin exposure and cardiovascular disease. Additional studies are being conducted by Stayner et al. (1985) on the health effects of exposure to nitroglycerin and other nitrated compounds at this munitions plant.

5.3 PREVIOUSLY CALCULATED CRITERIA

Bentley et al. (1978) conducted an extensive study on both the acute and long-term toxicity of nitroglycerin to a wide variety of freshwater organisms. From an application factor calculated from chronic to acute toxicity ratios and the lowest acute toxicity value from their study, they calculated a water quality criterion of 0.01 mg/L for aquatic organisms. Sullivan et al. (1979) used the data from the study by Bentley et al. (1978) and calculated a water quality criterion for aquatic organisms of 0.0072 mg/L, based on the procedure proposed by the Environmental Protection Agency (USEPA) in 1978 (USEPA 1978, as reported in Sullivan et al. 1979).

Ellis et al. (1978a) conducted a chronic toxicity study of nitroglycerin with mice, rats, and dogs and found the compound to be oncogenic to rats at a 1 percent concentration. Based on the procedure recommended by the EPA (USEPA 1979a, as reported in Ellis et al. 1978a) and the results of their study, Ellis et al. calculated human health criteria for 10^{-5}, 10^{-6}, and 10^{-7} additional lifetime cancer risks of 28.9, 2.8, and 0.29 µg/L, respectively. Using an updated version of

USEPA guidelines (USEPA 1979b, as reported by J. Barkley, pers. comm. 1982) and the data of Ellis et al. (1978a), Dacre (1980) recalculated the human health criteria for 10^{-5}, 10^{-6}, and 10^{-7} additional lifetime cancer risks and obtained estimates of 22.8, 2.28, and 0.23 µg/L, respectively.

5.4 AQUATIC CRITERIA

A summary of the USEPA's proposed methodology for the estimation of water quality criteria suitable for the protection of aquatic life and its uses is presented in Appendix A. The proposed criterion consists of two values, a Criterion Maximum Concentration (CMC) and a Criterion Continuous Concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the Final Acute Value, while the CCC is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value.

Sufficient data were not available to calculate an aquatic criterion on nitroglycerin based on the USEPA guidelines (Stephen et al. 1985); e.g., only six of the required eight families needed in calculating a Final Acute Value were available, and nominal concentrations of nitroglycerin were used in acute flow-through and chronic toxicity tests instead of measured concentrations which are required for calculating a Final Chronic Value. However, because some of the data generated by these tests are uniform in their assessment of the degree of toxicity of nitroglycerin, a tentative Final Acute Value was calculated using the USEPA Guidelines.

A summary of the steps used in calculating the Final Acute Value is presented in Table 16. As previously stated, only six of the required eight families necessary to calculate a Final Acute Value were available; a family from a phylum other than Arthropoda and Chordata and a family in any order of insect or phylum not already represented were not available. However, acute toxicity data were available for eight families of aquatic test animals (see Table 3 in Section 3.1), therefore, the value for N (number of genera used to calculate a Final Acute Value) was taken to equal eight. A Final Acute Value of 1.7258 mg/L is estimated. It is reemphasized that this value is tentative because acute toxicity data did not meet all requirements of the USEPA Guidelines (Stephan et al. 1985).

The Final Chronic Value may be calculated either in the same manner as the Final Acute Value or by dividing the Final Acute Value by the Final Acute-Chronic ratio. In either case, calculation of a Final Chronic Value requires toxicity values from acute and chronic flow-through tests using measured concentrations for three species of organisms; in the case of chronic tests, if results are not available for flow-through tests for daphnids, results from renewal tests are acceptable. Results of life cycle or partial life cycle tests are preferred, but early life stage tests may be used if results of the former tests are not available. Results of both acute and chronic toxicity tests were available for four species; however, a Final Chronic Value was not calculated for the following reasons: (1) Concentrations of nitro-

TABLE 16. CALCULATIONS FOR FINAL ACUTE VALUE (FAV) OF NITROGLYCERIN[a]

Rank (R)	GMAV[b]	log GMAV[c]	$(\log GMAV)^2$	$P=R/(N+1)$[d,e]	$\sqrt{P}$
4	2.9189	0.4652	0.2164	0.4444	0.6667
3	2.8000	0.4472	0.2000	0.3333	0.5774
2	2.4462	0.3885	0.1509	0.2222	0.4714
1	1.9423	0.2883	0.0831	0.1111	0.3333
Sum:		1.5892	0.6504	1.1111	2.0488

a. Based on calculation methods discussed in Stephan et al. (1985).

$$S^2 = \frac{\Sigma((\log GMAV)^2) - ((\Sigma(\log GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4}$$

$$L = (\Sigma(\log GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

$$S^2 = \frac{0.6504 - (1.5892)^2/4}{1.1111 - (2.0488)^2/4} = 0.3081;\ S = 0.5551$$

$$L = (1.5892 - (0.5551)(2.0488))/4 = 0.1129$$

$$A = (0.5551)(\sqrt{0.05}) + (0.1129) = 0.2370$$

$$FAV = e^{0.2370} = 1.7258$$

b. GMAV = genus mean acute value in mg/L.

c. log GMAV = log to the base 10 of GMAV.

d. N = 8.

e. P = probability for each GMAV; R = rank of four highest GMAVs from lowest to highest.

glycerin used in all tests were nominal, and current guidelines (Stephan et al. 1985) specify that concentrations should be measured; (2) Problems were encountered with nitroglycerin coming out of solution and forming globules in the early life stage test for the fish Ictaluras punctatus; 3) Flow-through tests are required for all organisms with the exception of those using Daphnia; a renewal test procedure was used in the chronic toxicity test for Chironomus tentans; and (4) For Daphnia, current procedures for chronic toxicity tests (USEPA 1985) state that if "each control daphnid living 21 days produces an average of less than 60 young," then the test is unacceptable; data in Table 8 clearly indicate that young production of controls is extremely low and thus unacceptable.

A Final Plant Value may be obtained from the lowest reported result of a toxicity test on an important aquatic species; the concentration of the test material should be measured, and the end point should be biologically important (Stephan et al. 1985). Based on a decrease in cell numbers, a 96-hr EC_{50} of 0.4 mg/L has been estimated for the green alga Selenastrum capricornutum (Bentley et al. 1978); however, this value was based on nominal concentrations of nitroglycerin and the guidelines state that concentrations should be measured. Also, this value is a 50 percent effective concentration, and, if the assumption is made that a biologically important end point is that end point at which only 5 percent or even 10 percent of the population is affected, the EC_{50} could greatly exceed a biologically important end point. Sullivan et al. (1979) reanalyzed the data of Bentley et al. and calculated lowest significant response concentrations (level of significance not given) of 0.1 mg/L and 1.0 mg/L based on changes in chlorophyll a concentration and cell numbers, respectively, for S. capricornutum. Because plant data were based on nominal concentrations and the validity of the statistical and experimental procedures is questionable, a Final Plant Value cannot be obtained for nitroglycerin.

A Final Residue Value is obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration factors (Stephan et al. 1985). A maximum permissible tissue concentration is either an FDA action level or a maximum acceptable dietary intake obtained from a chronic wildlife feeding study or a long-term wildlife field study. Bioconcentration factors were available for four fish, and ranged from 8X to 15X; however, it was not clear from the study if tissue concentrations had attained steady state. No maximum permissible tissue concentration was available; thus no Final Residue Value can be calculated for nitroglycerin.

As stated previously, the final aquatic criterion consists of two concentrations: a Criterion Maximum Concentration and a Criterion Continous Concentration (Stephan et al. 1985). A Criterion Maximum Concentration of 0.86 mg/L may be calculated from available data, however, this value is considered tentative because acute toxicity data did not meet all requirements of the USEPA guidelines. Final chronic, plant, and residue values could not be determined for nitroglycerin; thus the minimum data base required by the USEPA in calculating a Criterion Continous Concentration was not available.

5.5 HUMAN HEALTH CRITERIA

Based on the results of a study conducted by Ellis et al. (1978a, 1984), nitroglycerin is suspected of being a human carcinogen. In this study, 14/21 male and 16/25 female CD rats had hepatocellular carcinomas or hepatic neoplastic nodules after two years of oral exposure to nitroglycerin at calculated dose levels of 363 and 434 mg/kg/day, respectively. There are currently no suitable methods to determine a threshold for carcinogenic effects (USEPA 1980); therefore, the recommended concentration for maximum protection of human health is zero. However, because attainment of this concentration in some cases may not be feasible, USEPA (1980) has presented a range of concentrations corresponding to incremental cancer risks of 10^{-7} to 10^{-5}. For example, a risk of 10^{-5} indicates that one additional case of cancer may occur for every 100,000 people exposed. The USEPA (1980) has adopted the model of Crump (1980, as reported in USEPA 1980), GLOBAL 79, to estimate the water concentration that would cause a lifetime carcinogenic risk of 10^{-5}; this model is a linearized, multistaged model. The water concentration of nitroglycerin which is estimated to cause a lifetime carcinogenic risk at 10^{-5}, 10^{-6}, and 10^{-7} has been calculated with the GLOBAL 79 model using the GLOBAL 82 program of Howe and Crump (1982). A summary of the pertinent data and formulae used in calculating risk for nitroglycerin is presented in Table 17. The corresponding recommended criteria for nitroglycerin are 14.0, 1.40, and 0.14 µg/L. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 231, 23.1, and 2.31 µg/L, respectively. Based on the estimated Final Acute Value for aquatic life (1.7258 mg/L), maintainance of concentrations at the levels recommended for the human health criteria would more than adequately protect aquatic life.

5.6 RESEARCH RECOMMENDATIONS

As has been pointed out, not enough data were available to calculate aquatic criteria for nitroglycerin. The following research recommendations are given so that sufficient data will be available to meet the USEPA Guideline requirements (USEPA 1980; Stephan et al. 1985) for generating water quality criteria.

1. Acute toxicity tests are needed for a genus from a phylum other than Arthropoda and Chordata (e.g., Annelida, Mollusca, Rotifera) and for a genus in any order of insect or phylum not already represented.

2. Chronic flow-through tests using measured concentrations are needed for three different aquatic genera where at least one is a fish, one is an invertebrate, and one is a sensitive freshwater species.

3. Acute flow-through tests using measured concentrations are needed for species of organisms for which acceptable chronic toxicity tests have been performed.

4. A toxicity test on an important aquatic plant species is needed in which measured concentrations were used and the end point is biologically important.

5. A maximum permissible tissue concentration and a definitive steady-state or 28-day bioaccumulation study for nitroglycerin are needed.

TABLE 17. SUMMARY OF PERTINENT DATA FOR CALCULATING THE LIFETIME CARCINOGENIC RISK OF NITROGLYCERIN[a]

Dose to male rats (mg/kg/day)	Hepatocellular carcinomas (No. responding/no. tested)
0.0	1/24
3.04	0/28
31.5	4/26
363.0	14/21

a. In order to present a conservative estimate of carcinogenic risk, the water quality criterion for nitroglycerin is based on the induction of hepatocellular carcinomas in male rather than female rats (Ellis et al. 1978a, 1984).

$$\log BCF = (0.85 \log P) - 0.70$$

$$q_1^*(A) = \frac{\text{95\% upper confidence interval}}{MLE}$$

$$q_1^*(H) = q_1^*(A) \times \left(\frac{W_H}{W_A}\right)^{0.33} \times \frac{L}{l_e}$$

$$C_A = \frac{70 \times 10^{-5}}{q_1^*(H)(2 + 0.0065\ BCF)}$$

$$C_O = \frac{70 \times 10^{-5}}{q_1^*(H)(0.0065\ BCF)}$$

Where:

BCF = Bioconcentration factor = 19.8381 L/kg

2 = human water consumption, L/day

0.0065 = human fish consumption, kg/day

Log P = 2.35

95% Upper confidence interval = 1.50499×10^{-5}

MLE = Maximum likelihood estimate = 3.211326×10^{-3} mg/kg/day

$q_1^*(A)$ = Carcinogenic potency factor for animals = 4.6865×10^{-3} $(mg/kg/day)^{-1}$

$q_1^*(H)$ = Carcinogenic potency factor for humans = 2.34812×10^{-2} $(mg/kg/day)^{-1}$

W_H = Average weight of humans = 70 kg

W_A = Average weight of experimental animals = 530 gm

l_e = Duration of exposure = 730 days

L = Lifespan of test animal = 730 days

C_A = Concentration of nitroglycerin in water, calculated to keep the lifetime cancer risk below 10^{-5} = 14.0 μg/L

C_O = Concentration in organisms only, calculated to keep the lifetime cancer risk below 10^{-5} = 231 μg/L

6. REFERENCES

ACGIH. 1980. American Conference of Governmental Industrial Hygienists. Documentation of the Threshold Limit Values, 4th ed., pp. 307-308. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

ACGIH. 1981. American Conference of Governmental Industrial Hygienists. Documentation of the Threshold Limit Values, 4th ed., pp. 307-308. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

Advisory Center on Toxicology. 1968. NAS/NRC, Washington, DC, Toxicological Reports (supplied as privileged information), p. 120. (As reported in Dacre and Tew 1973).

American Defense Preparedness Association. 1975. Wastewater Treatment in the Military Explosives and Propellants Production Industry, Vol. II. Technical Report. Project No. 802872. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC. (As reported in Wendt et al. 1978).

Anderson, J.A., E.J. McGuire, J.R. Watkins, J.E. Fitzgerald, and F.A. de la Iglesia. 1983. Toxicology studies with a stable intravenous formulation of nitroglycerin. J. Appl. Toxicol. 3:161-165.

Andreev, K.K. and G.N. Bespalov. 1963. The effect of water on the nitroglycerin at elevated temperatures. Vzryvchatykh Veshehestv, Sb. Statei. pp. 131-171. (Chem. Abs. 59:11177b).

Armstrong, P.W., J.A. Armstrong, and G.S. Marks. 1979. Blood levels after sublingual nitroglycerin. Circulation 59:585-588.

Barkley, J.S., Jr. 1982. Personal communication.

Beard, R.R. and J.T. Noe. 1981. Aromatic nitro and amino compounds. In G.D. Clayton and F.E. Clayton, eds. Vol. 2A. Patty's Industrial Hygiene and Toxicology. pp. 2470-2471. John Wiley & Sons, New York.

Bell, F.K. 1964. Spectrophotometric method for the assay of individual nitroglycerin tablets. J. Pharm. Sci. 53:752-755. (As reported in Yacobi et al. 1983).

Bentley, R.E., J.W. Dean, S.J. Ells, G.A. LeBlanc, S. Sauter, K.S. Buxton, and B.H. Sleight III. 1978. Laboratory Evaluation of the Toxicity of Nitroglycerine to Aquatic Organisms. Final Report, AD A061739. EG&G Bionomics, Wareham, MA. DAMD 17-74-C-4101.

Berl, E. and I. Delpy. 1910. (Title not given). Chem. Ber. 43:1421. (As reported in DiCarlo 1975).

Blei, A.T., J. Gottstein, and H.-L. Fung. 1984. Role of the liver in the disposition of intravenous nitroglycerin in the rat. Biochem. Pharm. 33:2681-2686.

Blumenthal, H.P., H.-L. Fung, E.F. McNiff, and S.K. Yap. 1977. Plasma nitroglycerin levels after sublingual, oral and topical administration. Br. J. Clin. Pharmacol. 4:241-242.

Bogaert, M.G. and M.-T. Rosseel. 1972. Plasma levels in man of nitroglycerin after buccal administration. J. Pharm. Pharmacol. 24:737-738.

Brachfeld, N., J. Bozer, and R. Gorlin. 1959. Action of nitroglycerin on the coronary circulation in normal and in mild cardiac subjects. Circulation 19:697-704.

Camera, E. and D. Pravisani. 1964. (Title not given). Anal. Chem. 36:2108. (As reported DiCarlo 1975).

Capellos, C., W.J. Fisco, C. Ribaudo, V.O. Hogan, J. Campisi, F.X. Murphy, T.C. Castorina, and D.H. Rosenblatt. 1978. Kinetic Studies and Product Characterization During the Basic Hydrolysis of Glyceryl Nitrate Esters. Technical Report ARLCD-TR-79022, AD E400341. Large Caliber Weapon Systems Laboratory, Dover, NJ.

Carmichael, P. and J. Leiben. 1963. Sudden death in explosives workers. Arch. Environ. Health 7:424-439.

Carnahan, R.P. and L. Smith. 1977. Treatment of wastewater containing nitroglycerin and nitrated esters. Proc. 32nd Ind. Waste Conf. Lafayette, IN, May 10-12, 1977. Ann Arbor Science, Ann Arbor, MI.

Crew, M.C. and F.J. DiCarlo. 1968. Identification and assay of isomeric ^{14}C-glyceryl nitrates. J. Chromatogr. 35:506-512.

Crump, K.S. 1980. An improved procedure for low-dose carcinogenic resk assessment from animal data. J. Environ. Path. Toxicol. (In press). (As reported in USEPA 1980).

Dacre, J.C. 1980. Calculation of Interim Environmental Criterion for Trinitroglycerin (TNG). Memorandum for the Record, June 16. (As reported by J. Barkley 1982).

Dacre, J.C. and R.W. Tew. 1973. Mammalian Toxicology and Toxicity to Aquatic Organisms of Nitroglycerin, a Waterborne Munitions Waste Pollutant - A Literature Evaluation. AD 777902. Tulane University Medical School, New Orleans, LA. DADA 17-73-C-3152.

deKreuk, L.J. 1942. (Title not given). Rec. Trav. Chim. 61:819. (As reported in Urbanski 1983)

DiCarlo, F.J. 1975. Nitroglycerin revisited: Chemistry, biochemistry, interactions. Drug Metab. Rev. 4:1-38.

DiCarlo, F.J., M.C. Crew, L.J. Haynes, M.D. Melgar, and R.L. Gala. 1968. The absorption and biotransformation of glyceryl trinitrate-1,-^{14}C by rats. Biochem. Pharmacol. 17:2179-2183.

Ellis, H.V., J.H. Hagensen, J.R. Hodgson, J.L. Minor, C.-B. Hong, E.R. Ellis, J.D. Girvin, D.O. Helton, B.L. Herndon, and C.-C. Lee. 1978a. Mammalian Toxicity of Munitions Compounds. Phase III: Effects of Life-time Exposure. Part II: Trinitroglycerin. Progress Report No. 8, AD A078746/5. Midwest Research Institute, Kansas City, MO. DAMD-17-74-C-4073.

Ellis, H.V., III, J.R. Hodgson, S.W. Hwang, L.M. Halpap, D.O. Helton, B.S. Andersen, D.L. Van Goethem, and C.-C. Lee. 1978b. Mammalian Toxicity of Munitions Compounds. Phase I: Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization, Disposition and Metabolism, and Ames Tests of Additional Compounds. Report No. 6. AD A069333. Midwest Research Institute, Kansas City, MO. DAMD-17-74-C-4073.

Ellis, H.V., III, C.B. Hong, C.C. Lee, J.C. Dacre, and J.P. Glennon. 1984. Subacute and chronic toxicity studies of trinitroglycerin in dogs, rats, and mice. Fundam. Appl. Toxicol. 4:248-260.

Farmer, R.C. 1920. The decomposition of nitric esters. J. Chem. Soc. 117:806-818.

Flaim, S.F. 1982. Peripheral vascular effects of nitroglycerin in a conscious rat model of heart failure. Am. J. Physiol. 243:H974-H981.

Flann, B.C. 1969. (Title not given). J. Pharm. Sci. 58:122. (As reported in McNiff et al. 1980).

Fraser, R.T.M. 1968. Stability of nitrate esters. Chem. Ind. 1117-1118.

Fung, H.-L. 1984. Pharmacokinetic determinants of nitrate action. Am. J. Med. 76:22-26.

Fung, H.-L., P. Dalecki, E. Tse, and C.T. Rhodes. 1973. Kinetic assay of single nitroglycerin tablets. J. Pharm. Sci. 62:696-697. (As reported in Yacobi et al. 1983).

Fung, H.-L., H. Ogata, A. Kamiya, and G.A. Maier. 1984a. Pharmaco kinetics of nitroglycerin after parenteral and oral dosing in the rat. J. Pharm. Sci. 73:873-879.

Fung, H.-L, S.J.C. Sutton, and AJ. Kamiya. 1984b. Blood vessel uptake and metabolism of organic nitrates in the rat. J. Pharmacol. Exp. Ther. 228:334-341.

Hackel, J. 1933. (Title not given). Roczniki Chem. 16:213. (As reported in Urbanski 1983).

Hawley, G.G. 1981. The Condensed Chemical Dictionary. p. 734. Van Nostrand Reinhold Co., New York.

Heinzow, B. and A. Ziegler. 1981. Comparison of the effects of nitroglycerin administered to rats by different routes. J. Cardiovasc. Pharmacol. 3:573-580.

Hemphill, L. 1975. TL_m study of Atlas Powder Company nitroglycerin storehouse wastes. Unpublished. Joplin, Missouri. 47 pp. (As reported in Sullivan et al. 1979).

Hibbert, H. 1911. U.S. Pat. 994841, 994842; VII Intern. Congress of Appl. Chemistry, Vol. 4, p. 37, New York, 1912; Z. ges. Schiess-u Sprengstoffw. 9:126 (1914). (As reported in Urbanski 1983).

Hodgson, J.R. and C.-C. Lee. 1975. Trinitroglycerol metabolism: Denitration and glucuronide formation in the rat. Toxicol. Appl. Pharmacol. 34:449-455.

Hodgson, J.R., J.P. Glennon, J.C. Dacre, and C.-C. Lee. 1977. Metabolism and disposition of isomers of dinitro- and mononitroglycerol. Toxicol. Appl. Pharmacol. 40:65-70.

Hogstedt, C. and K. Andersson. 1979. A cohort study on mortality among dynamite workers. J. Occup. Med. 21:553-556.

Hogstedt, C. and O. Axelson. 1977. Nitroglycerine-nitroglycol exposure and the mortality in cardio-cerebrovascular diseases among dynamite workers. J. Occup. Med. 19:675-678.

Hogstedt, C. and B. Davidsson. 1980. Nitroglycol and nitroglycerine exposure in a dynamite industry 1958-1978. Am. Ind. Hyg. Assoc. J. 41:373-375.

Howe, R.B. and K.S. Crump. 1982. GLOBAL 82. A Computer Program to Extrapolate Quantal Animal Toxicity Data to Low Doses. Science Research Systems, Ruston, LA. 13 pp.

International Labour Office. 1983. Encyclopaedia of Occupational Health and Safety. pp. 1459-1461. International Labour Office, Geneva, Switzerland.

Ioannides, C., D.V. Parke, and I.W. Taylor. 1982. Elimination of glyceryl trinitrate: Effects of sex, age, species and route of administration. Br. J. Pharmacol. 77:83-88.

J. Am. Med. Assoc. 1898. The effects of nitroglycerin upon those who manufacture it. J. Am. Med. Assoc. 31:793-794.

Kaplan, D.L., J.H. Cornell, and A.M. Kaplan. 1982. Biodegradation of glycidol and glycidyl nitrate. Appl. Environ. Microbiol. 43:144-150.

Kast, H. 1906. (Title not given). Z. Ges. Schiess-U. Sprengstoffw. 1:225. (As reported in Urbanski 1983).

Kikendall, J.W. and M.H. Mellow. 1980. Effect of sublingual nitroglycerin and long-acting nitrate preparations on esophageal motility. Gastroenterology 79:703-706.

Klason, P. and T. Carlson. 1906. (Title not given). Chem. Ber. 39:2753. (As reported in DiCarlo 1975).

Kononova, S.D., A.M. Korolev, L.T. Eremenko, and L.L. Gumanov. 1972. The mutagenic effect of some esters of nitric acid on bacteriophage T4B. Sov. Genet. 8:635-640.

Kypson, J. and G. Hait. 1971. Metabolic effects of nitroglycerin and tranyleypromine in unanesthetized rabbits. Proc. Soc. Exp. Biol. Med. 136:285-289.

Lang, S., E.M. Johnson, Jr., and P. Needleman. 1972. Metabolism of and vascular responses to glyceryl trinitrate in the eviscerated rat. Biochem. Pharmacol. 21:422-424.

Lange, R.L., M.S. Reid, D.D. Tresch, M.H. Keelan, V.M. Bernhard, and G. Coolidge. 1972. Nonatheromatous ischemic heart disease following withdrawal from chronic industrial nitroglycerin exposure. Circulation 46:666-678.

Laws, C.E. 1910. Nitroglycerin head. J. Am. Med. Assoc. 54:793.

Lee, C.-C., J.V. Dilley, J.R. Hodgson, D.O. Helton, W.J. Wiegand, D.N. Roberts, B.S. Andersen, L.M. Halfpap, L.D. Kurtz, and N. West. 1975. Mammalian Toxicity of Munition Compounds. Phase I. Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization, and Disposition and Metabolism. Report No. 1. AD B011150. Midwest Research Institute, Kansas City, MO. DAMD-17-74-C-4073.

Lee, C.-C., H.V. Ellis, III, J.J. Kowalski, J.R. Hodgson, S.W. Hwang, R.D. Short, J.C. Bhandari, J.L. Sanyer, T.W. Reddig, J.L. Minor, and D.O. Helton. 1977. Mammalian Toxicity of Munition Compounds. Phase II. Effects of Multiple Doses. Part I. Trinitroglycerin. Progress No. 2. AD A047067. Midwest Research Institute, Kansas City, MO. DAMD17-74-C-4073.

Lee, N.H. 1973. The metabolism of glyceryl trinitrate by liver and blood from different species. Biochem. Pharmacol. 22:3122-3124.

Leo, A., C. Hansch, and D. Elkins. 1971. Partition coefficients and Their Uses. Chem. Rev. 71:525-616.

Lindner V. 1980. Explosives. In Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., Vol. 9, pp. 561-620. John Wiley & Sons, New York.

Lund, R.P., J. Haggendal, and G. Johnsson. 1968. Withdrawal symptoms in workers exposed to nitroglycerin. Br. J. Ind. Med. 25:136-138.

Magometschnigg, D., H. Horwatitsch, and M. Pichler. 1983. Plasma concentration of cutaneously applied trinitroglycerin. Int. J. Clin. Pharmacol. Ther. Toxicol. 21:178-182.

Maier, G.A., C. Arena, and H.-L. Fung. 1980. Relationship between in vivo organic nitrate reductase activity in rats. Biochem. Pharamcol. 29:646-648.

Mark, H.F. 1965. Explosives. In Kirk-Othmer Encyclopedia of Chemical Technology, 2nd ed., Vol. 8. pp. 602-604. Interscience Publishers.

Marshall, A. and G. Peace. 1916. (Title not given). J. Soc. Chem. Ind. 109:298. (As reported in Urbanski 1983).

McNiff, E.F., A. Yacobi, F.M. Young-Chang, L.H. Golden, A. Goldfarb, and H.-L. Fung. 1981. Nitroglycerin pharmacokinetics after intravenous infusion in normal subjects. J. Pharm. Sci. 70:1054-1058.

McNiff, E.F., P.S.K. Yap, and H.-L. Fung. 1980. Nitroglycerin. Anal. Profiles Drug Subst. 9:519-541.

MEDLARS (CHEMLINE). 1984. National Library of Medicine. Computer printout retrieved October 12.

MEDLARS (RTECS). 1984. National Library of Medicine. Registry of Toxic Substances file. Computer printout retrieved October 12.

MEDLARS (TDB). 1984. National Library of Medicine. Toxicology Data Bank file. Computer printout retrieved October 12.

Mudd, J.W. 1977. Impotence responsive to glyceryl trinitrate. Am. J. Psychiatry 134:922-925.

Munch, J.C., B. Friedland, and M. Shepard. 1965. Glyceryl trinitrate. II. Chronic toxicity. Ind. Med. Surg. 34:940-943.

Naoum, P. 1924. Nitroglycerin u. Nitroglycerinspregstoffe. Springer, Berlin. (As reported in Urbanski 1983).

Nauckhoff, S. 1905. (Title not given). Z. Angew. Chem. 11:53. (As reported in Urbanski 1983).

Needleman, P., ed. 1975. Organic Nitrates. Springer-Verlag, New York.

Needleman, P., D.J. Blehm, A.B. Harkey, E.M. Johnson, Jr., and S. Lang. 1971. The metabolic pathway in the degradation of glyceryl trinitrate. J. Pharmacol. Exp. Ther. 179:347-353.

Needleman, P. and A.B. Harkey. 1971. Role of endogenous glutathione in the metabolism of glyceryl trinitrate by isolated perfused rat liver. Biochem. Pharmacol. 20:1867-1876.

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

Needleman, P. and J.C. Krantz Jr. 1965. The biotransformation of nitrolgycerin. Biochem. Pharmacol. 14:1225-1230.

NIOSH. 1978. Occupational Exposure to Nitroglycerin and Ethylene Glycol Dinitrate. DHEW (NIOSH) Pub. No. 78-167. U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health, Morgantown, WV.

Noonan, P.K. 1984. Nitroglycerin pharmacokinetics using specific and sensitive analytical procedures. Diss. Abstr. Int. B 44:3090.

Noonan, P.K. and L.Z. Benet. 1982. Formation of mono- and dinitrate metabolites of nitroglycerin following incubation with human blood. Int. J. Pharm. 12:331-340.

Oketani, Y., T. Mitsuzono, K. Ichikawa, Y. Itono, T. Gojo, M. Gofuku, and N. Konoha. 1981a. Toxicological studies on nitroglycerin (NK-843). 4. Acute toxicity in dogs. Oyo Yakuri 22:629-632.

Oketani, Y., T. Mitsuzono, K. Ichikawa, Y. Itono, T. Gojo, M. Gofuku, and N. Konoha. 1981b. Toxicological studies on nitroglycerin (NK-843). 6. Teratological study in rabbits. Pharmacometrics 22:633-638.

Oketani, Y., T. Mitsuzono, K. Ichikawa, Y. Itono, T. Gojo, M. Gofuku, and N. Konoha. 1982a. Toxicological studies on nitroglycerin (NK-843). 5. Intravenous subacute toxicity in dog. Iyakuhin Kenkyu 13:125-144.

Oketani, Y., T. Mitsuzono, K. Ichikawa, Y. Itono, T. Gojo, M. Gofuku, and N. Konoha. 1981c. Toxicological studies on nitroglycerin (NK-843). 8. Teratological study in rats. Pharmacometrics 22:737-751.

Oketani, Y., T. Mitsuzono, K. Ichikawa, Y. Itono, T. Gojo, M. Gofuku, S. Nagayoshi, and N. Konoha. 1982b. Toxicological studies on nitroglycerin (NK-843). 2. Intraperitoneal subacute toxicity in rat. Iyakuhin Kenkyu 13:95-108.

Oketani, Y., T. Mitsuzono, K. Ichikawa, Y. Itono, T. Gojo, S. Nagayoshi, and N. Konoha. 1982c. Toxicological studies on nitroglycerin (NK-843). 1. Acute toxicity in mouse and rat. Iyakuhin Kenkyu 13:90-94.

Oketani, Y., T. Mitsuzono, K. Ichikawa, Y. Itono, T. Gojo, C. Tateda, M. Gofuku, and N. Konoha. 1981d. Toxicological studies on nitroglycerin (NK-843). 9. Perinatal and postnatal study in rats. Pharmacometrics 22:753-763.

Oketani, Y., T. Mitsuzono, Y. Itono, K. Ichikawa, T. Gojo, M. Gofuku, and N. Konoha. 1981e. Toxicological studies on nitroglycerin (NK-843). 7. Fertility studies in rats. Pharmacometrics 22:639-648.

Orestano, G. 1937. Pharmacological action of nitroglycerin. I. Arch. Ital. Sci. Farmakol. 6:153-172. (As reported in Dacre and Tew 1973).

Physicians' Desk Reference. 1983. Physicians' Desk Reference. Medical Economics Co., Oradell, NJ. p. 1518.

Reeve, G., T. Bloom, R. Rinsky, and A. Smith. 1983. Cardiovascular disease mortality among nitroglycerin workers. Paper presented at the 1983 Meeting of the Society of Epidemiologic Research.

Roginskii, S.Z. and L.M. Sapozhnikov. 1931. (Title not given). Zh. fiz. khim. 2:80. (As reported in Urbanski 1983).

Ross, R.H., M.G. Ryon, M.W. Daugherty, J.S. Drury, J.T. Ensminger, and M.V. Cone. 1980. Scientific Rationale for the Selection of Toxicity Testing Methods: Human Health Assessment. Oak Ridge National Laboratory, Oak Ridge, Tennessee. ORNL/EIS-151.

Rosseel, M.-T. and M.G. Bogaert. 1972. Gas chromatography of the nitrate esters of glycerol, isosorbide and isomannide. J. Chromatogr. 64:364.

Short, R.D., J.C. Dacre, and C.-C. Lee. 1977. A species and developmental comparison of trinitroglycerin metabolism in vitro. Biochem. Pharmacol. 26:162-163.

Silberrad, O. and R.C. Farmer. 1906. (Title not given). J. Chem. Soc. 89:1759. (As reported in DiCarlo 1975).

Smith, L.L. and R.L. Dickinson (eds.). 1972. Propellant Plant Pollution Abatement. Biological and Engineering Investigation to Develop Optimum Control Measures to Prevent Water Pollution. Final Engineering Report. Production Project :E-249 (Phase I). Hercules Inc., Radford Army Ammunition Plant, Radford, VA. (As reported in Wendt et al. 1978).

Snelling, W. and C. Storm. 1913. (Title not given). Z. Ges. Schiess-u. Sprengstoffw. 8:1. (As reported in Urbanski 1983).

Sokoloski, T.D., C.C. Wu, L.S. Wu, and A.M. Burkman. 1983. Interaction of nitroglycerin with human blood components. J. Pharm. Sci. 72:335-338.

Spanggord, R.J., T. Mill, T.-W. Chou, W.R. Mabey, J.H. Smith, and S. Lee. 1980a. Envirnomental Fate Studies on Certain Munition Wastewater Constituents. Final Report, Phase II - Laboratory Studies. AD A099256. SRI International, Menlo Park, CA. DMAD 17-78-C-8081.

Spanggord, R.J., T. Mill, T.-W. Chou, W.R. Mabey, J.H. Smith, and S. Lee. 1980b. Envirnomental Fate Studies on Certain Munition Wastewater Constituents. Final Report, Phase I - Literature Review. AD A082372. SRI International, Menlo Park, CA. DMAD 17-78-C-8081.

Stayner, L.T., A. Dannenberg, M. Thun, G. Reeve, T. F. Bloom, and W. Halperin. 1985. Cohort and Case-Control Study of Persons Occupationally Exposed to Nitroglycerin. Protocol of a Proposed Study by the National Institute for Occupational Safety and Health. July 25.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. Final Report PB85-227049. US Environmental Protection Agency, Office of Research and Development, Washington, DC.

Stilwell, J. M., D.C. Cooper, M.A. Eischen, M.C. Matthews, B.E. Sherwood, and T.B. Stanford. 1976. Aquatic Life Field Studies at Badger Army Ammunition Plant. Final Phase II Report, Volume I. AD A033547. Battelle Columbus Laboratories, Columbus, OH. DAMD 17-74-C-4123.

Stokinger, H.E. 1982. Aliphatic Nitro Compounds, Nitrates, Nitrites. Chapter 53. In G.D. Clayton and F.E. Clayton, eds., Patty's Industrial Hygiene and Toxicology, Vol. 2C. pp. 4186-4188. John Wiley & Sons, New York.

Sullivan, J.H., Jr., H.D. Putnam, M.A. Keirn, B.C. Pruitt, Jr., J.C. Nichols, and J.T. McClave. 1979. A Summary and Evaluation of Aquatic Environmental Data in Relation to Establishing Water Quality Criteria for Munitions-Unique Compounds. AD A06067. Water and Air Research, Inc., Gainesville, FL. DAMD 17-77-C-7027.

Suzuki, K., K. Sudo, T. Yamamoto, and K. Hashimoto. 1975. The carcinogenicity of N-ethoxycarbonyl-3-morpholinosydnonimine (molsidomine) in comparison with nitroglycerin in C57BL/6Jms mice. Pharmacometrics 9:229-242.

Svetlov, B.S., V.P. Shelaputina, and E.P. Malyutina. 1976. Neutral hydrolysis of polynitrates of polyhydric alcohols. Kinet. Katal. 17:508-511. (As reported in Spanggord et al. 1980b).

Takayama, S. 1975. Carcinogenicity of molsidomine and nitroglycerin in rats. Oyo Yakuri 9:217-228.

Trainor, D.C. and R.C. Jones. 1966. Headaches in explosive magazine workers. Arch. Environ. Health 12:231-234.

United States Pharmacopeia. 1980. The United States Pharmacopeia. pp. 552-553. United States Pharmacopeial Convention, Inc., Rockville, MD.

Urbanski, T. 1983. Chemistry and Technology of Explosives. Pergamon Press, New York.

U.S. Army Environmental Hygiene Report. 1971. Water Quality Engineering Special Study No. 24-001-72. October. (As reported in Carnahan and Smith 1977)

U.S. Army Natick Research and Development Command. 1973. Twenty-second Conference on Microbiological Deterioration of Military Material. Technical Report 75-2-FSL. Food Sciences Laboratory, U.S. Army Natick Research and Development Command, Natick, MA. (As reported in Wendt et al. 1978).

U.S. Army Natick Research and Development Command. 1974. Twenty-third Conference on Microbiological Deterioration of Military Material. Technical Report 75-87-FSL. Food Sciences Laboratory, U.S. Army Natick Research and Development Command, Natick, MA. (As reported in Wendt et al. 1978).

USEPA. 1971. U.S. Environmental Protection Agency. Algal Assay Procedure: Bottle Test. National Euthrophication Research Program, Pacific Northwest Water Laboratory, Corvallis, OR. 82 pp. (As reported in Bentley et al. 1978).

USEPA. 1978. U.S. Environmental Protection Agency. Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life. T.C. Joring, ed. Fed. Reg. 43:21506-21518. (As reported in Sullivan et al. 1979).

USEPA. 1980. U.S. Environmental Protection Agency. Guidelines and Methodology Used in the Preparation of Health Effects Assessment Chapters of the Consent Decree Water Quality Criteria. Fed. Reg. 45:79347-79357.

USEPA. 1975. U.S. Environmental Protection Agency. Methods for Acute Toxicity Tests With Fish, Macroinvertebrates, and Amphibians. Ecological Research Series, 600/3-75-009. (As reported in Bentley et al. 1978).

USEPA. 1979a. U.S. Environmental Protection Agency. Water Quality Criteria. Fed. Reg. 44:15926-15981. (15 March 1979) (As reported in Ellis et al. 1978a).

USEPA. 1979b. U.S. Environmental Protection Agency. Water Quality Criteria. Fed. Reg. 44:43671-43673. (25 July 1979) (As reported by J. Barkley pers. comm. 1982).

USEPA. 1985. U.S. Environmental Protection Agency. Toxic Substances Control Act Test Guidelines; Final Rules. Part 797-Environmental Effects Testing Guidelines. Fed. Guidelines 50(188):39321-39397.

USOSHA. 1983. U.S. Occupational Safety and Health Administration. Toxic and Hazardous Substances. (29 CFR 1910.441).

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals. p. 916. Van Nostrand Reinhold Co., New York.

Vignon, L. 1903. (Title not given). Bull. Soc. Chim. Fr. 29:26. (As reported in DiCarlo 1975).

Walsh, J.T. 1976. Chemical Characterization of Nitroglycerin Biodegradation Products. U.S. Army Natick Research and Development Laboratory. Unpublished manuscript. Natick, MA. 21 pp. (As reported in Sullivan et al. 1979)

Wei, J.Y. and P.R. Reid. 1979. Quantitative determination of trinitroglycerin in human plasma. Circulation 59:588-592.

Weitzel, R.L., R.C. Eisenman, and J.E. Schenk. 1976. Aquatic Field Surveys at Iowa, Radford and Joliet Army Ammunition Plants. Final Report. Volume II - Radford Army Ammunition Plant. AD A036777. Environmental Control Technology Corp., Ann Arbor, MI. DAMD 17-75-C-5046.

Wendt, T.M., J.H. Cornell, and A.M. Kaplan. 1978. Microbial degradation of glycerol nitrates. Appl. Environ. Microbiol. 36:693-699.

Wester, R.C., P.K. Noonan, S. Smeach, and L. Kosobud. 1983. Pharmacokinetics and bioavailability of intravenous and topical nitroglycerin in the Rhesus monkey: Estimate of percutaneous first-pass metabolism. J. Pharm. Sci. 72:745-748.

Windholz, M., ed. 1983. The Merck Index. p. 948. Merck & Co., Inc., Rahway, NJ.

Woodson, A.L. and L.C. Allen. 1969. (Title not given). J.A.O.A.C. 52:847. (As reported in McNiff et al. 1980).

Wu, C.C., T.D. Sokoloski, A.M. Burkman, M.F. Blanford, and L.S. Wu. 1982. Methods for the quantitation of nitroglycerin and its metabolites in human plasma. J. Chromat. 228:333-339.

Yacobi, A., A.H. Amann, and D.M. Baaske. 1983. Pharmaceutical considerations of nitroglycerin. Drug Intell. Clin. Pharm. 17:255-263.

Yap, S.K., C.T. Rhodes, and H-L. Fung. 1975. Factors affecting the kinetic assay of nitroglycerin in dosage forms. Am. J. Hosp. Pharm. 32:1039-1042. (As reported in Yacobi et al. 1983).

7. GLOSSARY

AAP	Army Ammunition Plant
ACGIH	American Conference of Governmental Industrial Hygienists
ADI	Acceptable daily intake
BAF	Bioaccumulation factor
CCC	Criterion continuous concentration
CHO	Chinese hamster ovary
CMC	Criterion maximum concentration
EC_{50}	Effective concentration causing 50 percent death (based on immobilization)
FAV	Final acute value
FCV	Final chronic value
FEL	Frank effect level
FPV	Final plant value
FRV	Final residue value
GMAV	Genus mean acute value
GI	Gastrointestinal
HPLC	High pressure liquid chromatography
LC_{50}	Lethal concentration causing 50 percent death
LD_{50}	Lethal dose causing 50 percent death
LOAEL	Lowest observed adverse effect level
NOAEL	No observed adverse effect level
NOEL	No observed effect level
ppm	Parts per million
SMAV	Species mean acute value

SMCV	Species mean chronic value
STEL	Short-term exposure limit
TLV	Threshold limit value
USAMBRDL	U.S. Army Medical Bioengineering Research and Development Laboratory
USEPA	U.S. Environmental Protection Agency

APPENDIX A:

SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final US Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect aquatic life and is slanted towards the specific regulatory needs of the US Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C.E. Stephan, D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species all of the time was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species was very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from acute and chronic toxicity to animals, toxicity to plants, and

bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived; however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative; much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

3. Definition of the material should include an operational analytical component. It is also necessary to reference or

describe analytical methods that the term is intended to denote. Primary requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants; (b) FDA action levels (FDA Guidelines Manual); and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.

2. All data used should be available in typed, dated and signed hardcopy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.

3. Questionable data, whether published or not, should not be used.

4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.

5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentration of test material in test solutions were measured using acceptable analytical methods.

6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:

   a. the family Salmonidae in the class Osteichthyes;

   b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish);

   c. a third family in the phylum Chordata (e.g, fish or amphibian);

   d. a planktonic crustacean (e.g, cladoceran or copepod);

   e. a benthic crustacean (e.g, ostracod, isopod, or amphipod);

   f. an insect (e.g., mayfly, midge, stonefly);

   g. a family in a phylum other than Arthropoda or Chordata (e.g, Annelida or Mollusca); and

   h. a family in any order of insect or any phylum not represented.

2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.

3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.

4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it

may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).

3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L) should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.

5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:

   a. Tests with daphnids and other cladocerans should be started with organisms <24 hr old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} is not available from a test, the 48-hr LC_{50} should be used in place of the desired 48-hr EC_{50}. An EC_{50} or LC_{50} of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

b. The result of tests with all other aquatic animal species should be the 96-hr EC_{50} value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} value is not available from a test, the 96-hr LC_{50} should be used in place of the desired EC_{50}.

c. Tests with single-cell organisms are not considered acute tests, even if the duration was $\leq$96 hr.

d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.

6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate a that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV because a species can only be considered protected from acute toxicity if all life stages are protected.

8. Consider the agreement of the data within and between species. Questionable results in comparison to other acute and chronic data for the species and other species in the same genus probably should not be used.

9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentration of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

   NOTE: Data reported by original investigators should not be rounded off and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

12. Calculate the cumulative probability (P) for each GMAV as R/(N+1).

13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).

14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.

16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.

4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0

5. Similarly normalize the values of the water quality characteristic for each species individually.

6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).

9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

10. For each species calculate the SMAV using: $SMAV = e^{Y}$.

11. Obtain the FAV at Z by using the procedure described in Section 5. (No. 10-14).

12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.

13. The Final Acute Equation is written as: FAV = $e^{(V[\ln(\text{water quality characteristic}) + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

   NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.

3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.

5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young <48 hr old, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young <24 hr old and last for not <21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.

b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

   NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.

7. If chronic toxicity of material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.

8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (No. 10-14). Then go to Section 7 (No. 13).

9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used. If acute tests were not conducted as part of the same study, acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.

10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

    a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.

    b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and saltwater species.

    c. If the most appropriate species mean acute-chronic ratios are <2.0, and especially if they are <1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

    If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.

13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.

14. Go to Section 9.

8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.

    a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality

characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z by the Final Acute-Chronic Ratio.

c. Use V = pooled acute slope as L = pooled chronic slope.

d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.

a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.

c. Decide whether data for each species is useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.

d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species.

This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

e. Similarly normalize the values of the water quality characteristic for each species individually.

f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.

i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^{Q}$).

k. Obtain the FCV at Z by using the procedure described in Section 5 (No. 10-14).

l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.

m. The Final Chronic Equation is written as: $FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$, where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.

2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.

3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) a FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11., because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from

water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.

3. If a maximum permissible tissue concentration is available for a substance (e.g, parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

   a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.

   b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.

   c. A BCF obtained from an exposure that adversely effected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.

   d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

   e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, then the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

   a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.

   b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.

   a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.

   b. Calculate the geometric mean normalized BCF.

   c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.

      - For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.

      - For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).

- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.

3. The Criterion Continuous Concentration (CCC) is equal to the lower of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.

4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) µg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) µg/L more than once every three years on the average.

Where

(1) = insert freshwater or saltwater,

(2) = name of material,

(3) = insert the Criterion Continuous Concentration, and

(4) = insert the Criterion Maximum Concentration.

13. REFERENCES

ASTM Standards E 729. Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.

ASTM Standards E 724. Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs.

Dixon, W.J. and M.B. Brown, eds. 1979. BMDP Biomedical Computer Programs, P-Series. University of California, Berkeley. pp. 521-539.

Erickson, R.J. and C.E. Stephan. In Preparation. Calculation of the Final Acute Value for Water Quality Criteria for Aquatic Life. US Environmental Protection Agency, Duluth, MN.

FDA Administrative Guidelines Manual. Food and Drug Administration.

Hamelink, J.L., et al. 1971. A Proposal: Exchange Equilibria Control the Degree Chlorinated Hydrocarbons are Biologically Magnified in Lentic Environments. Trans. Am. Fish. Soc. 100:207-214.

Huth, E.J., et al. 1978. Council of Biological Editors Style Manual, 4th ed., p. 117.

Lunsford, C.A. and C.R. Blem. 1982. Annual Cycle of Kepone Residue in Lipid Content of the Estuarine Clam, Rangia cuneata. Estuaries 5:121-130.

Schnoor, J.L. 1982. Field Validation of Water Quality Criteria for Hydrophobic Pollutants. In: Aquatic Toxicology and Hazard Assessment: Fifth Conference, ASTM STP 766, J.G. Pearson, R.B. Foster, and W.E. Bishop, eds., American Society for Testing and Methods, pp. 302-315.

Sidwell, V.D. 1981. Chemical and Nutritional Composition of Finfishes, Whales, Crustaceans, Mollusks, and Their Products. NOAA Technical Memorandum NMFS F/SEC-11, National Marine Fisheries Service.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. Final Report, PB85-227049. US Environmental Protection Agency, Office of Research and Development, Washington DC. 97 pp.

US Environmental Protection Agency (USEPA). 1982. Fed. Reg. 47:49234-49252, October 29, 1982.

APPENDIX B:
SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final US Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect human health and is slanted towards the specific regulatory needs of the US Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

a) Carcinogenicity, b) Toxicity, and c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the criteria values represent either estimations of the maximum allowable ambient water concentrations of a pollutant which would prevent adverse health effects or, for suspect and proven carcinogens, estimations of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAN HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes including ingestion of contaminated water and edible aquatic and nonaquatic organisms, as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish.

Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to be insignificant; however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).

2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.

3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used depending upon lipid solubility and availability of bioconcentration data:

(1) For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980)

Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}},$$

where BCF_{sp} is the bioconcentration factor for an aquatic species and PL_{sp} is the percent lipids in the edible portions of that species.

(2) Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent

lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

(3) For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to

10^{-5} (one additional case of cancer in populations ranging from ten million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065BCF)}$$

where,
C = ambient water concentration;
PR = the probable risk (e.g., 10^{-5}; equivalent to one case in 100,000);
BCF = the bioconcentration factor; and
q_1^* = a coefficient (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = q_1^* \times \frac{C\,(2 + 0.0065\ BCF)}{70}$$

or

$$PR = q_1^* \times \frac{2C + (0.0065\ BCF \times C)}{70},$$

where, 2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 x BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* \ X,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X},$$

showing that the coefficient q_1^* is the ratio of risk to dose; an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there

is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5}, which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared to the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then RR(X) = 5). In such cases the "excess" relative cancer risk is expressed as RR(X) - 1, and the actual numeric, or proportional excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(control).$$

Using the standard risk/dose equation:

$$PR(X) = b \times X$$

and substituting for PR(X):

$$[RR(X) - 1] \times PR(control) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(control)}{X},$$

where b is equal to the carcinogenic potency or q_1^*.

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.

2. The data set giving the highest estimate of carcinogenic lifetime risk (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.

4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the 2/3 power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}.$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W},$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3}.$$

3. The dose must also be normalized to a lifetime average exposure. For an carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is l_e, and the total length of the experiment is L_e, then the lifetime average exposure (X_m) is

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}}.$$

4. If the duration of the experiment (L_e) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of $(L/L_e)^3$ to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = ppm \times F \times r,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man; 0.05 for a 0.35-kg rat; and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}.$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol, and is absorbed proportionally to to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption; which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp\{-g(d)H(t)\},$$

where P(t;d) is the probable response for dose d and

time t; g(d) is the polynomial function defining the effect of dose level, and H(t) the effect of time:

$$g(d) = \sum_{i=0}^{a} \alpha_i d^i,$$

$$H(t) = \sum_{i=1}^{b} \beta_i t^i,$$

(with α and $\beta \geq 0$, and $\sum \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each α as a multiplicative constant (Crump 1980):

$$P(d/t) = 1 - \exp\{ - \sum_{i=0}^{a} \alpha d^i\},$$

or as given in the EPA guidelines (USEPA 1980):

$$P(d) = 1 - \exp[-(q_o + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where P(d) is the lifetime risk (probability) of cancer at dose d.

For a given dose the excess cancer risk A(d) above the background rate P(o) is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)},$$

where

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

Point estimates of the coefficients $q_1 \ldots q_k$ and consequently the extra risk function A(d) at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function A(d) has approximately the form:

$$A(d) = q_1 \times d.$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R. Thus A(d) and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1. This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^*, the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_o - L_1) = 2.70554,$$

where L_o is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$\chi^2 = \sum_{i=1}^{h} \frac{(X_i - N_iP_i)^2}{N_iP_i \times (1 - P_i)},$$

where N_i is the number of animals in the ith dose group, X_i is the number of animals in the ith dose group with a tumor response, P_i is the probability of a response in the ith dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (X^2) is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at, and below which, the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065\ kg \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criteria.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

(1) NOEL = No-Observed-Effect-Level,
(2) LOEL = Lowest-Observed-Effect-Level,
(3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
(4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.

2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.

3. If a LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to a NOAEL or an LOAEL.

4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only a NOEL and a LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, NOAEL, LOAEL, and clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the

toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.

2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported, but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criteria (using the uncertainty factor approach). Also the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = TLV \times BR \times DE \times d \times A_A/(A_O \times SF),$$

where,

BR = daily air intake (assume 10 m^3),
DE = duration of exposure in hours per day,
d = 5 days/7 days,
A_A = efficiency of absorption from air,
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = C_A \times DE \times d \times A_A \times BR \times 70\ kg/(BWA \times A_O \times SF),$$

where,

C_A = concentration in air (mg/m^3),
DE = duration of exposure (hr/day),
d = number of days exposed/number of days observed,
A_A = efficiency of absorption from air,
BR = volume of air breathed (m^3/day),
70 kg = standard human body weight,
BWA = body weight of experimental animals (kg),
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may,

under some circumstances, result in depressed fluid intake which, in turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant; however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

6. REFERENCES

Crump, K.S. 1979. Dose-response problems in carcinogenesis. Biometrics 35:157.

Crump, K.S., and W.W. Watson. 1979. GLOBAL,79. A FORTRAN program to extrapolate dichotomous animal carcinogenicity data to low dose. National Institute of Health Science Contract No. 1-ES-2123.

International Commission on Radiological Protection. 1977. Recommendation of the ICRP, Publication No. 26. Pergammon Press, Oxford, England.

Krewski, D., K.S. Crump, J. Farmer, D.W. Gaylor, R. Howe, C. Portier, D. Salsburg, R.L. Sielken, and J. Vanryzin. 1983. A comparison of statistical methods for low-dose extrapolation utilizing time-to-tumor data. Fund. Appl. Toxicol. 3:140-160.

NAS. 1977. Drinking water and health. Safe Drinking Water Committee, Advisory Center on Toxicology, National Research Council, National Academy of Science, Washington, DC. 939 pp.

Stephan, C.E. 1980. July 3 memorandum to J. Stara, US Environmental Protection Agency. (as cited in USEPA 1980).

Stokinger, M.E. and R.L. Woodward. 1958. Toxicological methods for establishing drinking water standards. J. Am. Water Works Assoc. 50:517.

USEPA. 1980. Water Quality Criteria Documents, Environmental Protection Agency. Fed. Regist. 45:79318-79357.

Veith, G.D., et al. 1980. Measuring and estimating the bioconcentration factors of chemicals in fish. J. Fish. Res. Bd. Can. 36:1040. (as cited in USEPA 1980).

DISTRIBUTION LIST

No. of Copies	
25	Commander US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBG-M Fort Detrick, Frederick, MD 21701
12	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
5	US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, MD 21701
2	Commander US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBZ-IL Fort Detrick, Frederick, MD 21701
1	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234
1	Chief USAEHA Regional Division, West Fitzsimmons AMC Aurora, CO 80045
1	Chief USAEHA Regional Division, North Fort George G. Meade, MD 20755
1	Chief USAEHA Regional Division, South Bldg. 180 Fort McPherson, GA 30330
1	Commander USA Health Services Command ATTN: HSPA-P Fort Sam Houston, TX 78234

1 Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234

1 Commander
US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

1 Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

1 Dean
School of Medicine
Uniformed Services University of
the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

1 Commander
U.S. Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

1 HQDA
ATTN: DASG-PSP-E
5111 Leesburg Pike
Falls Church, VA 22041-3258

1 HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314