ER ID# 14703

AD
UCRL-21109

Conventional Weapons Demilitarization: A Health and Environmental Effects Data-Base Assessment

Explosives and Their Co-Contaminants Final Report, Phase II

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December 1987

Supported by
U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21701-5012

Project Order 83PP3818

Project Officer: Mitchell J. Small

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UCRL--21109

DE90 009440

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No 0704-0188

1a REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS -----
2a SECURITY CLASSIFICATION AUTHORITY -----	3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.
2b DECLASSIFICATION/DOWNGRADING SCHEDULE -----	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) UCRL - 21109	5 MONITORING ORGANIZATION REPORT NUMBER(S) -----
6a NAME OF PERFORMING ORGANIZATION Lawrence Livermore National Laboratory 6b OFFICE SYMBOL (If applicable)	7a NAME OF MONITORING ORGANIZATION U.S. Army Biomedical Research and Development Laboratory
6c ADDRESS (City, State, and ZIP Code) Environmental Sciences Division P.O. Box 5507, L-453 Livermore, CA 94550	7b ADDRESS (City, State, and ZIP Code) Attn: SGRD-UBZ-C Fort Detrick Frederick, MD 21701-5010
8a NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research & Development Command 8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Army Project Order 83PP3818
8c ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 62720A PROJECT NO: 3E1-62720A835 TASK NO: AA WORK UNIT ACCESSION NO: 006

11 TITLE (Include Security Classification)
Conventional Weapons Demilitarization: A Health and Environmental Effects Data-Base Assessment

12 PERSONAL AUTHOR(S) D. Layton, B. Mallon, W. Mitchell, L. Hall, R. Fish, L. Perry, G. Snyder, K. Bogen, W. Malloch, C. Ham, and P. Dowd

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Final, Phase II	FROM 6/86 TO 12/87	December, 1987	427

16 SUPPLEMENTARY NOTATION
Subtitle: Explosives and their co-contaminants

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Chemical hazards, demilitarizations; health effects; environmental effects; contaminant transport, contaminant transformation, RA3
06	06		
06	09		

19 ABSTRACT (Continue on reverse if necessary and identify by block number) The demilitarization of conventional explosives by open burning and open detonation results in the deposition of residues in soils. Co-contaminants, consisting of impurities present in explosives along with environmental degradation products of the explosives, are also found in soils where operations have occurred. To support studies of the health and environmental risks of such by-products, this report presents assessments of data available on parameters that affect the risks posed by explosives and their principal co-contaminants. Specifically, data-base assessments cover factors that influence the transport and fate of the contaminants in environmental media (e.g., soils, water, etc.) and subsequently, human exposures via different pathways (e.g., inhalation, ingestion, and skin absorption). Importantly, information on dose-response relationships for various toxic effects in humans and laboratory animals is evaluated so that acceptable daily intakes for noncarcinogenic substances and virtually safe dose rates for carcinogenic substances can be defined. Toxic effects on

(continued on next page)

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian	22b TELEPHONE (Include Area Code) 301-663-7325 22c OFFICE SYMBOL SCRD-RMI-S

DD Form 1473, JUN 86 Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

plant and aquatic species are also addressed. The explosives and co-contaminants considered include TNT (co-contaminants: 2,4-dinitrotoluene; 2,6-dinitrotoluene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2-amino-4,6-dinitrotoluene, and 4-amino-2,6-dinitrotoluene); RDX (co-contaminant: HMX); tetryl (co-contaminant: picric acid); PETN; and ammonium picrate (co-contaminant: picric acid).

FOREWORD

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David W. Layton 1/19/90

PI Signature Date

CONTENTS

Preface xxi
Abstract 1
1. Introduction 3
Explosives in the Demilitarization Inventory 4
Demilitarization Technologies and Environmental Residuals 4
Overview of Data-Base Assessments 7
2. Identification of the Primary Co-Contaminants Associated with the Open Burning and Open Detonation of Military Explosives 13
Co-Contaminants of TNT 13
Production and Impurities 15
Photolysis Products 15
Microbial Products 18
Plant and Human Metabolites 21
Hydrolysis Products 21
Complex and Compound Formation 21
Field Measurements of TNT and Its Degradation Products . . . 22
Summary 23
Co-Contaminants of RDX 26
Production and Impurities 27
Photolysis Products 28
Microbial Products 29
Animal Metabolites 31
Hydrolysis Products 31
Field Measurements of RDX Contamination 31
Summary 32
Co-Contaminants of HMX 35
Production and Impurities 35
Photolysis Products 36
Microbial Products 36
Hydrolysis Products 37
Field Measurements of HMX Contamination 37
Summary 37
Co-Contaminants of Tetryl 37
Production and Impurities 39
Photolysis Products 39
Microbial Products 40
Hydrolysis Products 40
Complex and Compound Formation 40
Field Measurements of Tetryl Contamination 41
Summary 41
Co-Contaminants of PETN 41
Production and Impurities 43
Photolysis Products 43
Microbial Products 43
Human Metabolites 44
Hydrolysis Products 44
Summary 44

Co-Contaminants of Ammonium Picrate (Explosive D) 44
Production and Impurities 46
Photolysis Products 46
Microbial Products 46
Animal Metabolites 47
Hydrolysis Products 47
Complex and Compound Formation 47
Field Measurements of Ammonium Picrate and Its Degradation Products 48
Summary 48
Overview of Co-Contaminants 49
3. Data-Base Assessment for 2,4,6-Trinitrotoluene 51
Environmental Partitioning and Transport Properties 51
Soil Transport and Adsorption 51
Partition Coefficients: Plant/Soil, Beef-Fat/Diet, and Fish/Water 55
Equilibrium Distribution in Reference Landscapes 56
Environmental Transformation Processes 57
Photolysis 58
Biotransformation 62
Compound and Complex Formation 69
Hydrolysis 71
Toxic Effects on Animals and Humans 71
Toxicokinetics 71
Toxic Effects on Organs and Systems 74
Teratogenicity 78
Genotoxicity 78
Carcinogenicity in Animals 81
Carcinogenicity in Humans 85
Estimation of Intake Limits to Protect Public Health . . . 85
Toxic Effects on Plants 89
Toxic Effects on Aquatic Species 89
4. Data-Base Assessment for 2,4-Dinitrotoluene 93
Environmental Partitioning and Transport 93
Soil Transport and Adsorption 93
Partition Coefficients: Plant/Soil: Beef-Fat/Diet, and Fish/Water 94
Equilibrium Distribution in Reference Landscapes 94
Environmental Transformation Processes 97
Photolysis 97
Biotransformation 98
Hydrolysis 102
Toxic Effects on Animals and Humans 102
Toxicokinetics 102
Toxic Effects on Organs and Systems 108
Teratogenicity 111
Genotoxicity 111
Carcinogenicity 116
Estimation of Intake Limits to Protect Public Health . . . 118
Toxic Effects on Aquatic Species 119

5. Data-Base Assessment for 2,6-Dinitrotoluene 123
Environmental Partitioning and Transport 123
Environmental Transformation Processes 123
Photolysis 123
Hydrolysis 127
Biotransformation 127
Toxic Effects on Animals and Humans 129
Toxic Effects on Organs and Systems 132
Genotoxicity 134
Carcinogenicity 134
Estimation of Intake Limits to Protect Public Health . . . 139
Toxic Effects on Aquatic Species 140
6. Data-Base Assessment for 1,3-Dinitrobenzene 145
Environmental Partitioning and Transport 145
Environmental Transformation Processes 145
Photolysis 145
Biotransformation 146
Hydrolysis 150
Toxic Effects on Animals and Humans 150
Toxicokinetics 151
Toxic Effects on Organs and Systems 152
Genotoxicity 155
Estimation of Intake Limits to Protect Public Health . . . 156
Toxic Effects on Aquatic Species 156
7. Data-Base Assessment for 1,3,5-Trinitrobenzene 161
Environmental Partitioning and Transport 161
Environmental Transformation Processes 161
Photolysis 161
Biotransformation 161
Hydrolysis 165
Toxic Effects on Animals and Humans 165
Toxicokinetics 166
Toxic Effects on Organs and Systems 166
Teratogenicity 167
Genotoxicity 168
Carcinogenicity 168
Estimation of Intake Limits to Protect Public Health . . . 169
Toxic Effects on Aquatic Species 169
8. Data-Base Assessment for 2-Amino-4,6-Dinitrotoluene and 4-Amino-2,6-Dinitrotoluene 171
Environmental Partitioning and Transport 171
Environmental Transformation Processes 171
Photolysis 171
Biotransformation 172
Complex Formation 178
Toxic Effects on Animals and Humans 179
Toxicokinetics 179
Toxic Effects on Organs and Systems 180
Teratogenicity 180
Genotoxicity 180
Carcinogenicity 181
Estimation of Intake Limits to Protect Public Health. . . . 181
Toxic Effects on Aquatic Species 182

9. Data-Base Assessment for RDX 183
Environmental Partitioning and Transport 183
Soil Transport and Adsorption 183
Partition Coefficients: Plant/Soil, Beef-Fat/Diet, and Fish/Water 184
Equilibrium Distribution in Reference Landscapes 186
Environmental Transformation Processes 188
Photolysis 188
Biotransformation 193
Hydrolysis 194
Toxic Effects on Animals and Humans 194
Toxicokinetics 194
Toxic Effects on Organs and Systems 197
Teratogenicity 199
Genotoxicity 199
Carcinogenicity 200
Estimation of Intake Limics to Protect Public Health . . . 201
Toxic Effects on Aquatic Species 201
10. Data-Base Assessment for HMX 203
Environmental Partitioning and Transport 203
Environmental Transformation Processes 204
Photolysis 204
Biotransformation 209
Toxic Effects on Animals and Humans 211
Toxicokinetics 211
Toxic Effects on Organs and Systems 213
Teratogenicity 216
Genotoxicity 216
Carcinogenicity 217
Estimation of Intake Limits to Protect Public Health . . . 217
Toxic Effects on Aquatic Species 218
11. Data-Base Assessment for Pentaerythritol Tetranitrate 221
Environmental Partitioning and Transport 221
Environmental Transformation Processes 221
Photolysis 221
Biotransformation 221
Hydrolysis 222
Toxic Effects on Animals and Humans 222
Toxicokinetics 222
Toxic Effects on Organs and Systems 229
Teratogenicity 231
Genotoxicity 231
Carcinogenicity 231
Estimation of Intake Limits to Protect Public Health . . . 231
Toxic Effects on Aquatic Species 232
12. Data-Base Assessment for Tetryl 235
Environmental Partitioning and Transport Properties 235
Environmental Transformation Processes 236
Photolysis 239
Hydrolysis 239
Biotransformation 240
Toxic Effects on Animals and Humans 241
Toxicokinetics 241
Toxic Effects on Organs and Systems 242

Teratogenicity 245
Genotoxicity 245
Carcinogenicity 246
Estimation of Intake Limits to Protect Public Health . . . 246
Toxic Effects to Aquatic Species 247
13. Data-Base Assessment for Ammonium Picrate and Picric Acid 249
Environmental Partitioning and Transport Properties 249
Soil Transport and Adsorption 249
Partition Coefficients: Plant/Soil, Beef-Fat/Diet, and Fish/Water 255
Equilibrium Distribution in Reference Landscapes 255
Environmental Transformation Processes 255
Photolysis 256
Biotransformation 256
Hydrolysis 258
Toxic Effects on Animals and Humans 258
Toxicokinetics 258
Toxic Effects on Organs and Systems 260
Teratogenicity 264
Genotoxicity 264
Carcinogenicity 268
Estimation of Intake Limits to Protect Public Health . . . 269
Toxic Effects on Aquatic Species 272
14. Health and Environmental Data Available on Explosives and Associated Co-Contaminants 273
Data-Base Deficiencies Applicable to Most Compounds 273
Data-Base Requirements for Specific Explosives and Co-Contaminants 275
TNT 275
2,4-DNT 275
2,6-DNT 276
DNB 276
TNB 276
2-A-4,6-DNT and 4-A-2,6-DNT 277
RDX 277
HMX 277
PETN 277
Tetryl 278
Ammonium Picrate and Picric Acid 278

Appendix A. Chemical/Physical Properties and Environmental Partition Coefficients: Calculations and Supporting Data 279

Appendix B. Concentrations of Explosives in Environmental Media: Modeling, Detection Methods, and Measured Levels 309

Appendix C. Carcinogenic Potency Assessment for 2,6-DNT 335

Appendix D. Estimation of Daily Intakes for Toxic Susbstances Lacking Chronic Toxicity Data 347

References 349

LIST OF TABLES

Ch. 1. INTRODUCTION

1. Composition of the major high-explosive compounds in the conventional munitions demilitarization inventory (from Layton et al., 1986, based on data in Department of the Army, 1967a) 5

2. Topics covered in the data-base assessments on individual substances 10

3. Summary of the explosives and co-contaminants addressed in the data-base assessments 11

Ch. 2. IDENTIFICATION OF THE PRIMARY CO-CONTAMINANTS ASSOCIATED WITH THE OPEN BURNING AND OPEN DETONATION OF MILITARY EXPLOSIVES

1. Impurities present in military-grade TNT (from Kaye, 1980, p. T244) 16

2. Photochemical degradation products of TNT that have been identified or measured in distilled water 17

3. Biotransformation products of TNT that have been identified by various investigators 19

4. Co-contaminants and degradation products of TNT in the environment 24

5. Co-contaminants and degradation products of RDX 33

6. Co-contaminants and degradation products of HMX 38

7. Co-contaminants and degradation products of tetryl 42

8. Co-contaminants and degradation products of PETN 45

9. Co-contaminants and degradation products of ammonium picrate 49

10. Explosive degradation products of primary and secondary concern 50

Ch. 3. DATA-BASE ASSESSMENT FOR 2,4,6-TRINITROTOLUENE (TNT)

1. Chemical identifiers of trinitrotoluene 52

2. Chemical and physical properties of 2,4,6-trinitrotoluene. Properties are for an ambient temperature of 20°C unless otherwise noted 53

3. Partition coefficients for use in estimating contaminant movement in environmental media and human exposures to TNT 54

4. Fractional distributions of TNT among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of TNT to the upper soil layer. For details of the compartments, see Appendix B. 57

5. Summary of TNT photolysis rates derived from experiments conducted by Mabey et al. (1981). 60

6. Biotransformation products of TNT that have been identified by various researchers. 65

7. Biotransformation of 10 mg/L TNT in different sources of water (Spanggord et al., 1980b). 66

8. Overview of studies dealing with the genotoxicity of TNT. 79

9. Incidence of tumors in female rats and mice after two years of consuming feed containing TNT(m). Data are from Furedi et al. (1984a, 1984b). 83

10. TNT(m) cancer potencies based on dose-response data from the chronic bioassay conducted by Furedi et al. (1984a, 1984b). 87

11. Acceptable dose rates for TNT(m) calculated from the potencies presented in Table 3-10 88

12. Acute toxicity of TNT on aquatic organisms. 90

Ch. 4. DATA-BASE ASSESSMENT FOR 2,4-DINITROTOLUENE

1. Chemical identifiers of 2,4-dinitrotoluene. 94

2. Chemical and physical properties of 2,4-DNT. Properties are for an ambient temperature of 20°C. 95

3. Partition coefficients for use in estimating human exposures to 2,4-DNT. 96

4. Fractional distributions of 2,4-DNT among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of 2,4-DNT to the upper-soil layer. For details of the compartment, see Appendix B. 97

5. Photolysis rates of 2,4-DNT in various waters. 99

6. Photoproducts of 2,4-DNT identified by Burlinson and Glover (1977a) 100

7. Overview of studies completed on the genotoxicity of 2,4-DNT 112

8. Acute toxicity of 2,4-DNT to aquatic organisms under static conditions. 120

9. Acute toxicity of 2,4-DNT to aquatic organisms under flow-through tests (from Liu et al., 1983c). 121

Ch. 5. DATA-BASE ASSESSMENT FOR 2,6-DINITROTOLUENE

1. Chemical identifiers of 2,6-dinitrotoluene 124

2. Chemical and physical properties of 2,6-DNT. Properties are for an ambient temperature of 20°C 125

3. Partition coefficients for use in estimating human exposures to 2,6-DNT 126

4. Fractional distributions of 2,6-DNT among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of 2,6-DNT to the upper-soil layer. For details of the compartments, see Appendix B 127

5. Rates of photolytic degradation of 2,6-DNT in water. 128

6. Overview of studies completed on the genotoxicity of 2,6-DNT 135

7. Dose rates for 2,6-DNT corresponding to different lifetime cancer risks. Dose rates are calculated from the potencies presented in Table C-1 of Appendix C 141

8. Acute toxicity of 2,6-DNT to aquatic organisms 143

Ch. 6. DATA-BASE ASSESSMENT FOR 1,3-DINITROBENZENE

1. Chemical identifiers of 1,3-dinitrobenzene 146

2. Chemical and physical properties of DNB. Properties are for an ambient temperature of 20°C 147

3. Partition coefficients for use in estimating human exposures to DNB 148

4. Fractional distributions of DNB among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady- state addition of DNB to the upper-soil layer. For details of the compartments, see Appendix B. 149

5. Photolytic degradation of DNB (from Simmons and Zepp, 1986). 150

6. Dose-response relationships for DNB from subchronic toxicity studies by Cody et al. (1981) and Linder et al. (1986) 157

7. Acute toxicity of DNB to aquatic organisms 159

Ch. 7. DATA-BASE ASSESSMENT FOR 1,3,5-TRINITROBENZENE (TNB)

1. Chemical identifiers of TNB. 162

2. Chemical and physical properties of TNB. Properties are for an ambient temperature of 20°C 163

3. Partition coefficients for use in estimating human exposures to TNB 164

4. Fractional distributions of TNB among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of TNB to the upper-soil layer. For details of the compartments, see Appendix B 165

5. Acute toxicity of TNB to aquatic organisms 170

Ch. 8. DATA-BASE ASSESSMENT FOR 2-AMINO-4,6-DINITROTOLUENE AND 4-AMINO-2,6-DINITROTOLUENE

1. Chemical identifiers of 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene 172

2. Chemical and physical properties of 2-amino-4,6-dinitrotoluene. Properties are for an ambient temperature of 20°C. 173

3. Chemical and physical properties of 4-amino-2,6-dinitrotoluene. Properties are for and ambient temperature of 20°C 174

4. Partition coefficients for use in estimating human exposures to 2-amino-4,6-dinitrotoluene 175

5. Partition coefficients for use in estimating human exposures to 4-amino-2,6-dinitrotoluene 176

6. Fractional distributions of 2-amino-4,6-dinitrotoluene among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of 2-amino-4,6-dinitrotoluene to the upper-soil layer. Not shown are fractional distributions for 4-amino-2,6-dinitrotoluene, which were almost identical. For details of the compartments, see Appendix B 177

7. Oral LD50's of 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene for rats and mice (data from Ellis et al., 1978) 181

Ch. 9. DATA-BASE ASSESSMENT FOR RDX

1. Chemical identifiers of RDX. 184

2. Chemical and physical properties of RDX. Properties are for an ambient temperature of 20°C 185

3. Summary of measured K_d values for RDX. 186

4. Partition coefficients for use in estimating human exposures to RDX 187

5. Fractional distributions of RDX among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of RDX to the upper-soil layer. For details of the compartments, see Appendix B. 188

6. Summary of data on RDX photolysis rates. 190

7. Acute toxicity of RDX to aquatic organisms from Bentley et al., 1977a) 202

Ch. 10. DATA-BASE ASSESSMENT FOR HMX

1. Chemical identifiers of HMX. 204

2. Chemical and physical properties of HMX. Properties are for an ambient temperature of 20°C unless otherwise noted. 205

3. Partition coefficients for use in estimating human exposures to HMX 206

4. Fractional distributions of HMX among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of HMX to the upper-soil layer. For details of the compartments, see Appendix B. 207

5. Rates of photolytic degradation for HMX (derived from data presented in Spanggord et al., 1983b). 208

6. Excretion of ^{14}C-HMX after oral or intravenous administration (data are from Wilson, 1985) 213

7. Acute toxicity of HMX to aquatic organisms (Bentley et al. 1977b) 219

Ch. 11. DATA-BASE ASSESSMENT FOR PENTAERYTHRITOL TETRANITRATE (PETN)

1. Chemical identifiers of PETN 222

2. Chemical and physical properties of PETN. Properties are for an ambient temperature of 20°C 223

3. Partition coefficients for use in estimating human exposures to PETN. 224

4. Fractional distributions of PETN among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of PETN to the upper-soil layer. For details of the compartments, see Appendix B. 225

Ch. 12. DATA-BASE ASSESSMENT FOR TETRYL

1. Chemical identifiers of tetryl 236

2. Chemical and physical properties of tetryl. Properties are for an ambient temperature of 20°C 237

3. Partition coefficients for use in estimating human exposures to tetryl. 238

4. Partitioning of tetryl between water and soils (data from Hale et al., 1979). 239

5. Fractional distributions of tetryl among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of tetryl to the upper-soil layer. For details of the compartments, see Appendix B 240

6. Hydrolysis half-lives of tetryl. 241

Ch. 13. DATA-BASE ASSESSMENT FOR AMMONIUM PICRATE AND PICRIC ACID (PiOH)

1. Chemical identifiers of ammonium picrate 250

2. Chemical identifiers of PiOH 250

3. Chemical and physical properties of ammonium picrate. Properties are for an ambient temperature of 20°C. 251

4. Chemical and physical properties of PiOH. Properties are for an ambient temperature of 20°C 252

5. Partition coefficients for use in estimating human exposures to ammonium picrate. 253

6. Partition coefficients for use in estimating human exposures to PiOH. 254

7. Fractional distributions of PiOH among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of PiOH to the upper-soil layer. For details of the compartments, see Appendix B 256

APPENDIX A

1. Vapor pressure/temperature equations for selected military explosives and co-contaminants. 281

2. Measured vapor pressures for selected military explosives and co-contaminants 282

3. Summary of vapor-pressure values at 20°C for selected military explosives and their co-contaminants. 285

4. Measured log K_{ow} and retention time (RT) on reverse phase HPLC for energetic materials. Compounds of unknown log K_{ow} placed by RT order 287

5. Summary of log K_{ow} values for selected military explosives and associated co-contaminants. 293

6. Aqueous solubilities of explosives and co-contaminants. In cases where there are two values listed, the preferred value is listed first. 295

7. Diffusion coefficients in air and water for explosives and co-contaminants. 299

8. Calculated values of K_{oc} from Eq. A-14 or A-15 302

9. Estimated barley uptake factors (K_{sp}) for explosives and their co-contaminants. K_{sp} values predicted from Eq. A-17. 304

10. Estimates of K_{fw} for military explosives and related co-contaminants. K_{fw} values predicted from $\log K_{fw} = 0.79 \log K_{ow} - 0.40$. 306

11. Estimates of the beef fat/diet partition coefficient, K_{fd}, for explosives and their co-contamintants using the two regression equations from Kenega (1980). 307

APPENDIX B

1. Parameter values used in GEOTOX for the Western and Southeastern ecoregions. 311

2. Sensitivity of lifetime average exposure (mg/kg-d) to environmental transport model inputs for TNT. 314

3. Detection limits of unltraviolet (UV) and electrochemical reduction (ED) detectors (Bratin et al., 1981) 322

4. Concentration of TNT and associated co-contaminants found in soils and waters by various investigators 323

5. Amounts of RDX, HMX, tetryl, PETN, ammonium picrate, and co-contaminants found in soils and waters. 330

APPENDIX C

1. Carcinogenic potencies calculated from 2,6-DNT components of DNT dose 337

2. Carcinogenic potencies for 2,6-DNT in TNT. 340

LIST OF FIGURES

Ch. 1. INTRODUCTION

1. Schematic diagram of some commonly used technologies for demilitarizing conventional ordnance containing propellants and explosives (from Layton et al , 1986). 6

2. Overview of the relationships between contaminated environmental media and exposure pathways. 8

Ch. 2. IDENTIFICATION OF THE PRIMARY CO-CONTAMINANTS ASSOCIATED WITH THE OPEN BURNING AND OPEN DETONATION OF MILITARY EXPLOSIVES

1. Chemical structures of the six explosives addressed in Section 2 14

2. An example of photolytic oxidation followed by microbial reduction of TNT 18

3. Formation of 2,4'-Azoxy from microbial reduction 20

4. Proposed microbial degradation pathway for RDX (McCormick et al., (1981). 30

Ch. 3. DATA-BASE ASSESSMENT FOR 2,4,6-TRINITROTOLUENE (TNT)

1. Proposed photolytic degradation mechanisms (Burlinson et al., 1979b). 61

2. Possible Meisenheimer compound formed by irradiation of TNT. 63

3. Biotransformation pathways of TNT. 64

4. Reduction of a nitro group to an amine (adapted from Spanggord, 1980a). 69

Ch. 9. DATA-BASE ASSESSMENT FOR RDX

1. Photolytic decomposition of RDX (Glover and Hoffsommer, 1979a, 1979b; Kubose, 1977). 192

Ch. 10. DATA-BASE ASSESSMENT FOR HMX

1. Photolytic decomposition mechanism for HMX proposed by Spanggord et al. (1983b). 210

APPENDIX B

1. Diagram of the eight-compartment structure of GEOTOX 310

2. Gas Chromatography with electron capture detection (GC-ECD) of ground water from Hawthorne Naval Ammunition Depot, Nevada (adapted from Pereira et al., 1979) 319

3. Electrochemical detection (ED) contrasted with ultraviolet (UV) detection of HPLC of explosive mixture after sorption and desorption on Porapak-R resin. (a) ED (20 L injected). (b) UV absorption at 210 nm (50 L injected) (from Maskarinec et al., 1984) 321

GLOSSARY OF ACRONYMS

2-amino-4,6-DNT	2-A-dinitrotoluene
4-amino-2,6-DNT	4-A-dinitrotoluene
Ammonium Picrate	Explosive D
CH_3OH	methanol
CO	carbon monoxide
CO_2	carbon dioxide
2,6-diamino-4-NT	2,6-diamino-4-nitrotoluene
2,4-diamino-6-NT	2,4-diamino-6-nitrotoluene
dinitroso-RDX	1,3-dinitroso-5-nitro-1,3,5-triazacyclohexane
3,5-DNA	3,5-dinitroaniline
DNB	1,3-dinitrobenzene
DNP	dinitrophenol
2,4-DNT	2,4-dinitrotoluene
2,6-DNT	2,6-dinitrotoluene
HCHO	formaldehyde
HCN	hydrogen cyanide
HCO_3^-	bicarbonate ion
$HCONH_2$	formamide
$HCOO^-$	formate ion
HCOOH	formic acid
2-hydroxyamino-2,4-DNT	2-hydroxyamino-4,6-dinitrotoluene
4-hydroxyamino-2,6-DNT	4-hydroxyamino-2,6-dinitrotoluene
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine
mononitroso-RDX	1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane
N_2	nitrogen gas
NB	nitrobenzene
N-methylpicramide	N-methyl-2,4,6-trinitroaniline
NO	nitric oxide
N_2O	nitrous oxide
NO_2	nitrogen dioxide
OB	open burning
OD	open detonation

PE	pentaerythritol
PEDN	pentaerythritoldinitrate
PETN	pentaerythritoltetranitrate
PiOH	picric acid or 2,4,6-trinitrophenol
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
RT	retention time
tetryl	N-methyl-N,2,4,6-tetranitrobenzenamine
TNB	1,3,5-trinitrobenzene
TNBA	2,4,6-trinitrobenzoic acid
TNBAH	2,4,6-trinitrobenzyl alcohol
TNBOH	2,4,6-trinitrobenzaldehyide
TNG	trinitroglycerine
TNT	2,4,6-trinitrotoluene
trinitroso-RDX	1,3,5-trinitroso-1,3,5-triazacyclohexane

PREFACE

The U.S. Army Biomedical Research and Development Laboratory (USABRDL) has been charged with the responsibility of developing environmental criteria for military materials such as propellants, pyrotechnics, and explosives, as well as by-products associated with their demilitarization. USABRDL has funded Lawrence Livermore National Laboratory (LLNL) to prepare a health and environmental effects data-base assessment on technologies for demilitarizing conventional ordnance and the environmental by-products of those technologies. The study has been conducted in two phases. In Phase I, demilitarization technologies were examined, along with their solid, liquid, and gaseous effluents. The by-products were then ranked according to their potential health and environmental risks. In Phase II, data-base assessments were completed on the environmental chemistry, toxicology, and effects of the high-priority substances. This report contains data-base assessments on explosives and associated co-contaminants (i.e., impurities in explosives and environmental degradation products) that are likely to be found at open-burning and open-detonation grounds. The assessments focus on those parameters that are needed to carry out analyses of the health and environmental risks of explosive residues. A companion report includes data-base assessments of propellants and their co-contaminants.

ABSTRACT

The demilitarization of conventional explosives by open burning (OB) and open detonation (OD) results in the deposition of residues in soils. Co-contaminants, consisting of impurities present in explosives along with environmental degradation products of the explosives, are also found in soils where OB/OD operations have occurred. To support studies of the health and environmental risks of such by-products, this report presents assessments of data available on parameters that affect the risks posed by explosives and their principal co-contaminants. Specifically, data-base assessments cover factors that influence the transport and fate of the contaminants in environmental media (e.g., soils, water, etc.) and subsequently, human exposures via different pathways (e.g., inhalation, ingestion, and skin absorption). Importantly, information on dose-response relationships for various toxic effects in humans and laboratory animals is evaluated so that acceptable daily intakes for noncarcinogenic substances and virtually safe dose rates for carcinogenic substances can be defined. Toxic effects on plant and aquatic species are also addressed. The explosives and co-contaminants considered include TNT (co-contaminants: 2,4-dinitrotoluene; 2,6-dinitrotoluene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2-amino-4,6-dinitrotoluene, and 4-amino-2,6-dinitrotoluene); RDX (co-contaminant: HMX); tetryl (co-contaminant: picric acid); PETN; and ammonium picrate (co-contaminant: picric acid).

1. INTRODUCTION

Demilitarization of conventional explosives results in the release of various liquid, solid, and gaseous contaminants into the environment. The process of assessing the health and environmental risks of such contaminants begins with the identification of possible hazards, which is essentially a judgment of the potential effects of a substance, based on available information. The next step, assuming that a potential hazard exists, is to estimate the concentrations that would occur in different media following release(s) into the environment. Concentrations are determined directly by measuring levels in soils, water, etc. and by estimation techniques; for example, models that simulate contaminant transport in air, water, and soils. An analysis is then conducted to define the human exposures that could result from the contaminant's occurrence in soils, water, etc. Based on the results of that analysis, a dose assessment is conducted to determine the absorbed doses from inhalation, ingestion, or skin absorption.

To relate the absorbed doses to potential effects, dose-response relationships must be defined, if possible, for pertinent toxic effects. Risk assessments require data on the site-specific characteristics of the environmental media (e.g., soil, ground water, surface water, etc.) receiving the contaminants, the behavior of the contaminants in the various media, the transfer of the contaminants to man as well as target plant and animal species, and importantly, the toxicity of the substances. The specific parameters and supporting data required in chemical risk analyses depend largely on the methodologies and models used. Generally, such analyses utilize a mixture of measured and estimated parameters involving the properties of the contaminants, environmental media, and the plant and animal species potentially at risk. The basic objectives of this report are to assess the available data to quantify the health and environmental risks of explosives and their demilitarization by-products; to provide estimates of selected parameters required in risk analyses; and to identify relevant data gaps and key uncertainties.

EXPLOSIVES IN THE DEMILITARIZATION INVENTORY

Conventional munitions are subject to demilitarization for several reasons, including obsolescence of weapons, deterioration of chemical components and poor serviceability. Munitions that are scheduled for demilitarization are identified in a computerized listing that is compiled on a quarterly basis by the Demilitarization and Disposal Task Group of the Joint Conventional Ammunition Program. The munitions in the demilitarization inventory are distributed among a number of depots and ammunition plants across the continental U.S. (see Layton et al., 1986). Table 1-1 contains a listing of the composition of the most important explosives in the demilitarization inventory. The explosives include 2,4,6-trinitrotoluene (TNT); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX); ammonium picrate (Explosive D); N-methyl-N,2,4,6-tetranitrobenzenamine (tetryl); and pentaerythritoltetranitrate (PETN). TNT, RDX, and Explosive D are used primarily as the main charges in munitions, whereas tetryl and PETN are typically used as booster explosives. Composition B and TNT are the dominant fillers in the demilitarization inventories, accounting for 75% of the 1.1×10^4 tonnes of explosives. On an individual basis, TNT constitutes about 60 wt% of the total, followed by RDX (30 wt%), Explosive D (8 wt%), tetryl (2 wt%), and PETN (<0.1 wt%). Minor constituents that are not included here are isomers of dinitrotoluene (DNT) in TNT, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine (HMX) as a constituent of RDX.

DEMILITARIZATION TECHNOLOGIES AND ENVIRONMENTAL RESIDUALS

The technologies and procedures for demilitarizing conventional ordnance can be classified as destructive or nondestructive. Historically, the primary methods for destroying explosives have consisted of incineration, open burning, and open detonation. Various kinds of furnaces have been used to incinerate energetic materials, but rotary-kiln systems (notably the APE 1236 deactivation furnace) have become the most widely used incineration technology. The burning and detonation of ordnance on the ground surface or in pits are probably the oldest methods of demilitarization, and they are used at many different facilities. Nondestructive methods typically involve the

Table 1-1. Composition of the major high-explosive compounds in the conventional munitions demilitarization inventory (from Layton et al., 1986, based on data in Department of the Army, 1967a).

Composition	Explosive ingredient							
	TNT	RDX	Ammonium picrate	Tetryl	PETN	Aluminum	Other	Total
	wt%							
Composition A-3		91					9	100
Composition B	40	60						100
Cyclotol	25	75						100
Explosive D			100					100
H-6	30	45				20	5	100
HBX	38	40				17	5	100
PETN					100			100
Tetryl				100				100
Tetrytol	20			80				100
TNT	100							100
Tritonol	80					20		100

extraction of explosive fillers for sale or reuse. Figure 1-1 is a schematic representation of the destructive and nondestructive technologies. Except for the mechanical processing of munitions to remove fuses, booster charges, etc., each of the demilitarization methods creates environmental residuals. Furnaces, for example, release gases and particles to the atmosphere and produce solid wastes from particulate-control devices. Open burning and open detonation result in atmospheric releases of gases and particles as well as residues in soils. Explosive recovery systems, such as steamout and washout systems, have both liquid and solid wastes, with some limited atmospheric emissions.

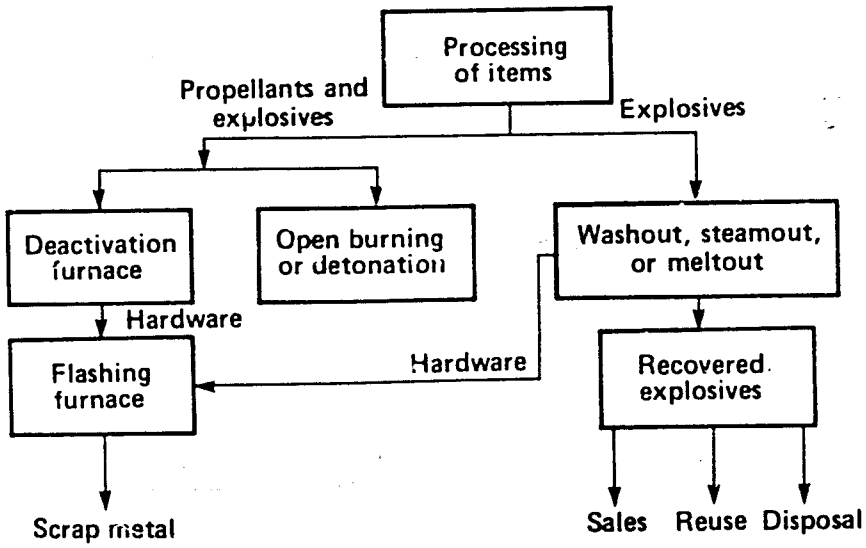

Figure 1-1. Schematic diagram of some commonly used technologies for demilitarizing conventional ordnance containing propellants and explosives (from Layton et al., 1986).

Our earlier review of the demilitarization capabilities at the 16 Depot System Command and Armament, Munitions, and Chemical Command installations shows that the most prevalent demilitarization technologies are deactivation furnaces, open burning, and open detonation (see Layton et al., 1986). A potentially important source of health and environmental risks from current and past operations, therefore, is the presence of residues in soils and associated ground and surface waters (most of the washout facilities are no longer in use). Consequently, the data-base assessments presented in this study will focus on the data required to assess the health and environmental risks associated with contaminated soils.

OVERVIEW OF DATA-BASE ASSESSMENTS

According to regulations proposed by the U.S. Environmental Protection Agency (U.S. EPA, 1987) pursuant to the Resource Conservation and Recovery Act, operators seeking permits for open-burning and open-detonation operations will have to perform analyses that show such operations will not adversely affect human health or the environment. Accordingly, a fundamental goal of the data-base assessments contained in this report is to provide data on the parameters needed to carry out facility-specific risk analyses. The parameters and data assessed generally fall into two categories: (1) those that contribute to the estimation of contaminant exposures and absorbed doses and (2) those that contribute to estimation of potential effects. The exposure pathways of potential concern for OB/OD operations are shown in Fig. 1-2. In this schematic diagram, exposure pathways are linked to several environmental compartments where residual contamination can occur. Soil-based exposures consist of the inhalation of gases emitted from surface soils; ingestion and dermal absorption of contaminated soils; and the ingestion of animals and crops that have bioaccumulated contaminants. Water-based exposures occur via both ground and surface waters. Ground-water contamination can result from the migration of explosives from surface soils through the vadose zone to an unconfined aquifer. Subsequent exposures result from water consumption and the consumption of crops that have been irrigated with the contaminated ground water or perhaps even livestock that have consumed it. Surface-water contamination results from runoff from explosives-contaminated soils and/or seepage from contaminated ground water. Exposures can occur from direct consumption of water and aquatic species, animals, or crops that have accumulated an explosive or co-contaminant from surface water or sediments. Water-based exposures can also occur via dermal absorption and inhalation during showers.

Some of the parameters needed to conduct definitive exposure and dose assessments include contaminant-specific properties such as aqueous solubility and vapor pressure, etc.; partition coefficients (e.g., soil/water, air/water, etc.); and degradation rates for various processes, including biodegradation, photolysis, and hydrolysis. Data are also needed on site-specific parameters describing pertinent characteristics of the environmental media transporting contaminants; the population at risk; and potential food-chain pathways.

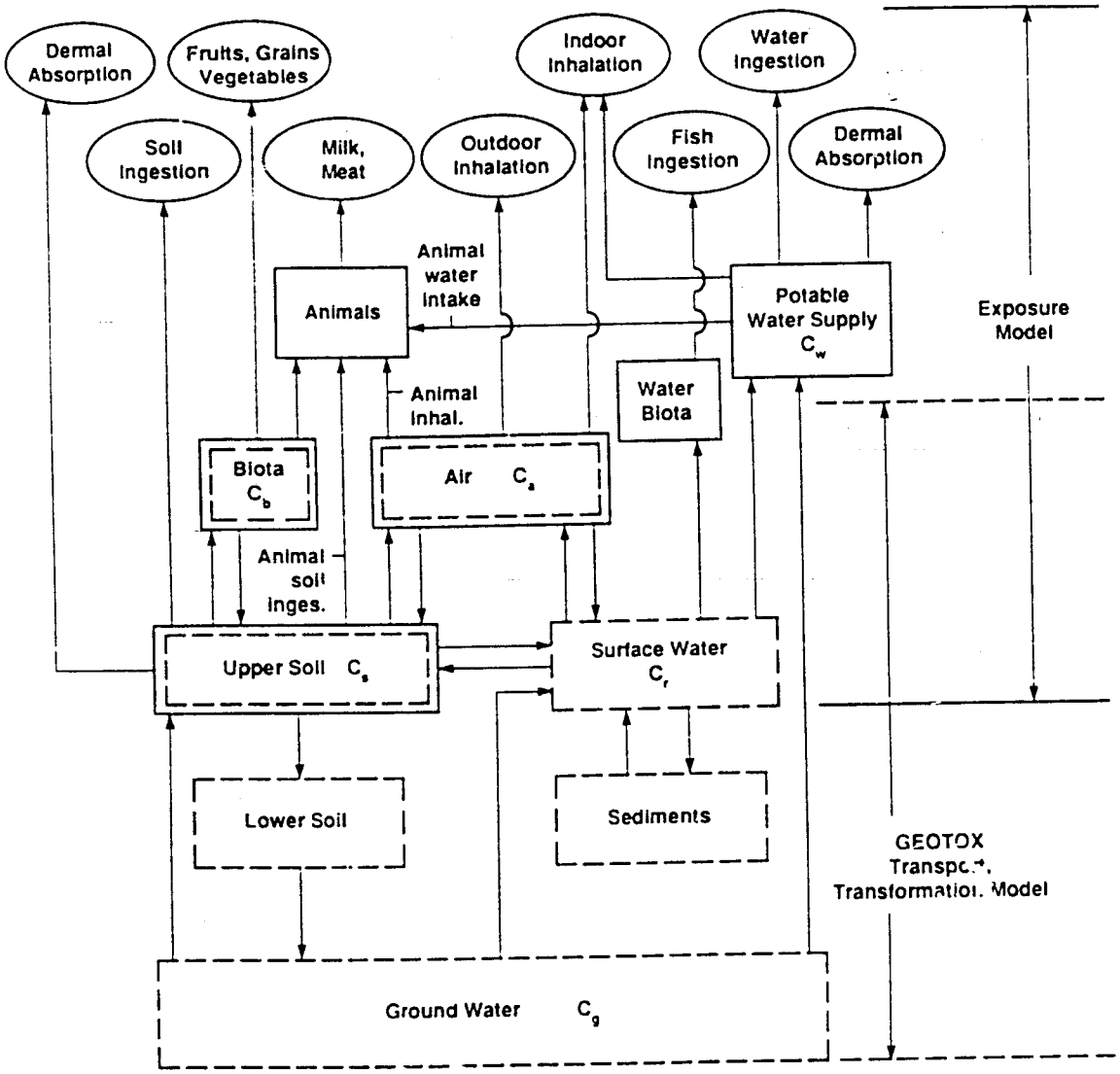

Figure 1-2. Overview of the relationships between contaminated environmental media and exposure pathways. Environmental media are outlined with dashed lines and exposure pathways are outlined with solid lines.

The second key area that the data-base assessments address is the potential health effects of the explosive residues and associated co-contaminants. We examine the nature of toxic (i.e., carcinogenic and noncarcinogenic) effects produced in man and laboratory animals, together with the dose-response relationships for different toxic endpoints. In this regard, an important focus of our assessments is the derivation of safe intakes for noncarcinogenic contaminants and acceptable dose rates[a] for carcinogenic substances, based on potency assessments of animal bioassay data. The final topics addressed are effects on aquatic species and plants. Although effects on aquatic species are of potential importance in some cases (e.g., direct discharges of ammunition production waste waters to a receiving stream), CB/OD operations do not normally pose a significant threat to aquatic systems because residues largely reside in the subsurface environment. Consequently, our emphasis on aquatic effects is restricted primarily to overviews of the available literature related to toxic effects. In Table 1-2 we list the primary topics covered in the data-base assessments on individual contaminants.

The explosives and associated co-contaminants that are considered in the data-base assessments are listed in Table 1-3. Co-contaminants are either impurities in the explosives or degradation products that are likely to be found along with the parent explosives in environmental media. Section 2 of the report reviews the primary co-contaminants. Subsequent sections contain data-base assessments on each explosive and its co-contaminants. A concluding section reviews deficiencies in the data bases on the various explosives and co-contaminants and recommends areas of future research. Supporting appendices deal with chemical and physical properties (Appendix A); modeling and measurements of explosives and co-contaminants in environmental media (Appendix B); cancer potency assessment of 2,6-DNT (Appendix C); and estimation of safe intakes for contaminants lacking chronic toxicity data (Appendix D).

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[a] An acceptable dose rate is a chronic intake of a carcinogen that corresponds to a specific lifetime risk of cancer deemed acceptable by a regulator. Based on the circumstances involved, such risk can range from 10^{-7} to 10^{-4}.

Table 1-2. Topics covered in the data-base assessments on individual substances.

Environmental Partitioning and Transport Properties
Chemical and physical properties
Partition coefficients
Equilibrium distribution in reference environments
Environmental Transformation Processes
Photolysis
Hydrolysis
Biotransformation
Toxic Effects on Animals and Humans
Toxicokinetics
Toxic effects on organs and systems
Teratogenicity
Genotoxicity
Carcinogenicity
Estimation of intakes to protect human health
Toxic Effects on Aquatic Species
Acute toxicity
Subchronic and chronic toxicity
Toxic Effects on Plants

Table 1-3. Summary of the explosives and co-contaminants addressed in the data-base assessments.

Explosive	Co-contaminant	Source(s)[a]
TNT	1,3,5-Trinitrobenzene (TNB)	I,P
	1,3-Dinitrobenzene (DNB)	I
	2,4-Dinitrotoluene (2,4-DNT)	I
	2,6-Dinitrotoluene (2,6-DNT)	I
	2-Amino-4,6-dinitrotoluene (2-amino-4,6-DNT)	B
	4-Amino-2,6-dinitrotoluene (4-amino-2,6-DNT)	B
RDX	HMX	I
Tetryl	Picric acid (PiOH)	H
PETN		
Ammonium picrate	Picric acid (PiOH)	H

[a] I = impurity in parent explosive.
P = photolysis product of explosive.
B = biodegradation product of explosive.
H = hydrolysis.

2. IDENTIFICATION OF THE PRIMARY CO-CONTAMINANTS ASSOCIATED WITH THE OPEN BURNING AND OPEN DETONATION OF MILITARY EXPLOSIVES

Residues of explosive compounds in soils are often accompanied by co-contaminants consisting of manufacture impurities in the explosives, as well as degradation products resulting from the hydrolysis, photolysis, or biotransformation. The main objective of this section is to identify the co-contaminants that are likely to be found at detectable levels at OB/OD sites. Subsequent sections on the individual explosives and co-contaminants deal with the quantitative aspects of transformation processes (e.g., estimates of degradation half-lives). In order to determine which of the contaminants are of potential importance, we review data on the levels of impurities in explosives, the degradation products resulting from the dominant transformation processes, and the persistence of the various substances in environmental media. Data are derived from laboratory studies dealing with transformation processes and field studies in which soil and water samples were analyzed for the presence of explosive-related residues. Our analyses focus on the six explosives (i.e., TNT, RDX, HMX, tetryl, PETN, and ammonium picrate) shown in Fig. 2-1.

CO-CONTAMINANTS OF TNT

TNT is the most widely used military explosive, and it is also present in the largest quantity in the demilitarization inventory (see Layton et al., 1986). In addition, this explosive has the greatest number of co-contaminants of potential concern. Impurities include 2,4-DNT and 2,6-DNT, DNB, and TNB. Co-contaminants also result from photolytic and microbial degradation processes. TNB is a photolytic product of concern as well as an impurity. The important biotransformation products are the aminodinitrotoluenes. Hydrolysis is not an important source of contaminants.

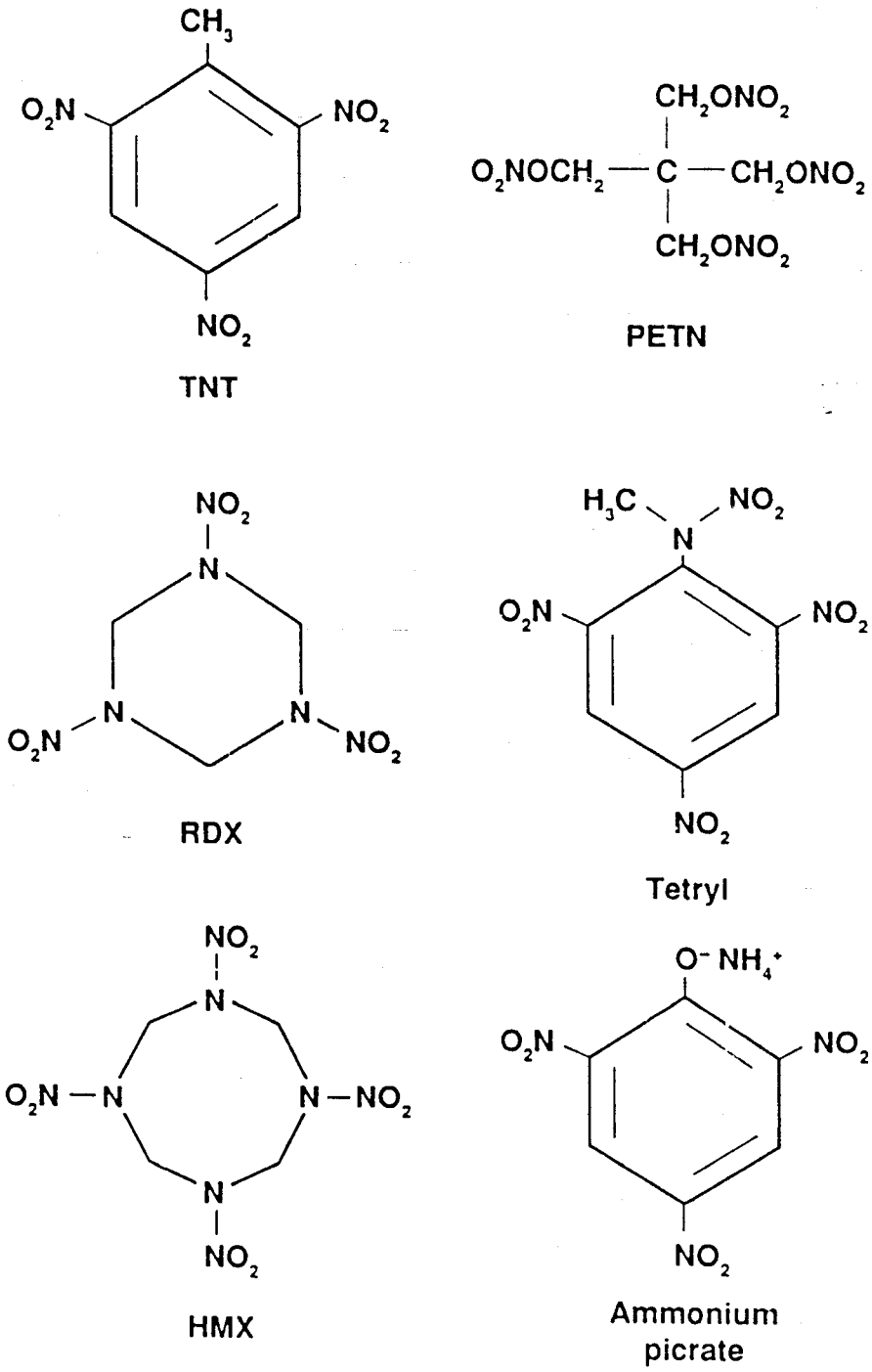


Figure 2-1. Chemical structures of the six explosives addressed in Section 2.

Production and Impurities

TNT is produced in the U.S. by the progressive nitration of toluene in a multistage process that uses mixtures of nitric and sulfuric acid. The primary impurities in TNT result from the incomplete nitration of toluene, oxidation (e.g., methyl-group oxidation), and the production of TNT isomers (see Rosenblatt et al., 1971). Aqueous sodium sulfite solution (sellite) treatment removes the undesirable unsymmetrical TNT isomers by a chemical reaction that converts those isomers to water-soluble salts. The treatment also forms complexes with the preferred isomer. The aqueous extract is called "red water." Impurities reported in military-grade TNT are listed in Table 2-1. Several of the impurities are TNT isomers that were not completely removed by the sellite treatment. The dinitrotoluenes (DNT) listed in Table 2-1 occur because nitration conditions are not sufficient to convert all the DNT to TNT. The benzenes result from either the decarboxylation of benzoic acid derivatives or from nitration of benzene, a contaminant of the toluene feedstock (Spanggord et al., 1982a). Oxidation of the methyl group of TNT accounts for the formation of the alcohol, aldehyde, and acid, while the last four compounds listed in Table 2-1 are condensation products of transient intermediates derived from TNT.

Photolysis Products

The photolytic products of TNT in distilled and natural waters have been studied by several investigators. For example, Burlinson et al. (1973, 1979a) and Kaplan et al. (1975) have studied the photolysis of TNT in distilled water at room temperature with a mercury lamp or the sun as light sources. Table 2-2 lists the photolysis products identified by those researchers. It should be noted that not all of the photolytic products were identified in the above studies. Kaplan et al. (1975), for instance, could not identify about 60 wt% of the products present.

In natural waters and surface soils, transformation of TNT can result from a combination of photolytic and microbial processes. With ample sunlight, photolytic decomposition predominates, but when light is absent, microbial degradation is the sole transformation pathway. Often there is a combination of degradation pathways; for example, TNT can degrade

Table 2-1. Impurities present in military-grade TNT (from Kaye, 1980, p. T244).

Co-contaminant	Maximum wt%
TNT isomers	
2,4,5-TNT	0.30
2,3,4-TNT	0.20
2,3,6-TNT	0.05
2,3,5-TNT	0.05
DNT isomers	
2,6-DNT	0.25
2,4-DNT	0.50
2,3-DNT	0.05
2,5-DNT	0.10
3,4-DNT	0.10
3,5-DNT	0.01
Nitrobenzenes	
DNB	0.02
TNB	0.10
TNT oxidation products	
2,4,6-Trinitrobenzyl alcohol (TNBOH)	0.25
2,4,6-Trinitrobenzaldehyde (TNBAL)	0.20
2,4,6-Trinitrobenzoic acid (TNBA)	0.05
TNT condensation products	
2,2',4,4',6,6'-Hexanitrobibenzyl (HNBB)	0.40
3-Methyl-2,4,4',6,6'-pentanitrodiphenylmethane (MPDM)	0.40
2,2'-Dicarboxy-3,3',5,5'-tetranitro-azoxybenzene (white compound)	0.05
3,3'5,5'-Tetranitroazoxybenzene	0.01

photolytically to trinitrobenzene, which then degrades microbially to nitroanilines, as shown in Fig. 2-2.

Burlinson (1980) placed samples of "raw" Potomac River water spiked with TNT in sunlight, shade, and darkness. A fourth sample of river water was prepared by adding sediment and a nutrient (ethanol). TNT was also added to distilled water. The latter two samples were exposed to sunlight. The degradation products in all the crocks containing the TNT solutions were

Table 2-2. Photochemical degradation products of TNT that have been identified or measured in distilled water.

Product	Light Source	
	Mercury lamp	Sunlight
	References[a]	
TNB	2	1,2,3
TNBOH	2	1,2
TNBAL	2	1,2
TNBA	2	
4,6-Dinitroanthranil	2	
2,4,6-Trinitrobenzonitrile	2	1,2
3,5-Dinitrophenol	2	
2-Amino-4,6-dinitrobenzoic acid	2	
syn-2,4,6-Trinitrobenzaldoxime	2	
N-(2-carboxy-3,5-dinitrophenyl)-2,4,6-trinitrobenzamide	2	3
Four isomers of tetranitroazoxytoluene (see Table 2-3)		1
2,2'-Dicarboxy-3,3',5,5'-tetranitroazoxybenzene (white compound)	2	
2,2'-Dicarboxy-3,3',5,5'-tetranitrodesoxybenzene (desoxy white)	2	
2-Carboxy-3,3',5,5'-tetranitroazoxybenzene (monocarboxy white)	2	
1,3,7,9-Tetranitroindazolo-2,1-α-indazol-6-ol-12-one	2	3

[a] 1 = Burlinson et al., 1973.

2 = Kaplan et al., 1975.

3 = Burlinson et al., 1979a (only products at environmentally encountered pH's are listed in Table 2-2)

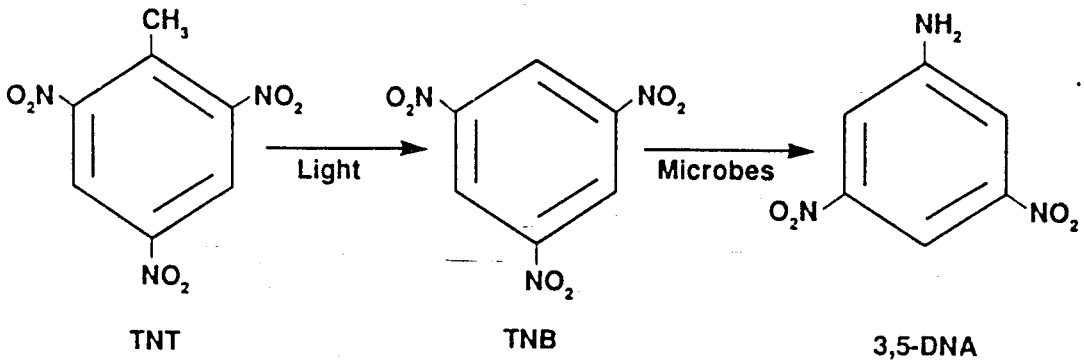


Figure 2-2. An example of photolytic oxidation followed by microbial reduction of TNT.

observed for about 38 d. The substances present in the distilled water that was exposed to sunlight were all photolytic products, such as TNBAL, while the river-water sample kept in the dark produced substances that were all of microbial origin (e.g., aminodinitrotoluenes). The other samples contained a range of products resulting from photolytic (sunlight exposures) and/or microbial (dark) degradation processes. Burlinson proposed that the initial photolytic product is TNBAL, because his distilled water sample exposed to sunlight produced only TNBAL from TNT. In the river-water samples, the TNBAL was quickly converted to TNB, which then degraded microbially to 3,5-dinitroaniline (3,5-DNA). In actual field measurements, Spanggord et al. (1983a) identified many of the same products in analyses of active lagoon waters.

Microbial Products

Many bacteria, fungi, yeasts, and other microorganisms reduce the TNT nitro groups to amines (Spanggord et al. 1980a; McCormick et al., 1976; Parrish, 1977). The biotransformation products that have been identified in various studies are summarized in Table 2-3. Most ^{14}C-ring-labeled-TNT (denoted ^{14}C-TNT) experiments have demonstrated that microbial transformation does not proceed to ring cleavage. One study did, however, report ring

Table 2-3. Biotransformation products of TNT that have been identified by various investigators.

Product	Reference[a]
2-Amino-4,6-dinitrotoluene (2-amino-4,6-DNT)	1,2,3,4,5
4-Amino-2,6-dinitrotoluene (4-amino-2,6-DNT)	1,3,4,5
2,6-Diamino-4-nitrotoluene (2,6-diamino-4-NT)	1,3,5
2,4-Diamino-6-nitrotoluene (2,4-diamino-6-NT)	1,2,3,5
2,4,6-Triaminotoluene (TAT)	2
4-Hydroxylamino-2,6-dinitrotoluene (4-hydroxyamino-2,6-DNT)	2,3
2-Hydroxylamino-4,6-dinitrotoluene (2-hydroxyamino-4,6-DNT)	2
2,2',6,6'-Tetranitro-4,4'-azoxytoluene (4,4'-Azoxy)	1,2,3,5
4,4',6,6'-Tetranitro-2,2'-azoxytoluene (2,2'-Azoxy)	1,2,3,5
4,2',6,6'-Tetranitro-2,4'-azoxytoluene (2,4'-Azoxy)	1,2
2,4',6,6'-Tetranitro-4,2'-azoxytoluene (4.2'-Azoxy)	1,2,5
Product binding with humic acids, lipids, and proteins	6
Nitrate ion	1
Nitrite ion	1

[a] 1 = Kayser *et al.*, 1977.
2 = McCormick *et al.*, 1976.
3 = Spanggord *et al.*, 1980b.
4 = Burlinson, 1980.
5 = Kaplan and Kaplan, 1982a.
6 = Kaplan and Kaplan, 1983.

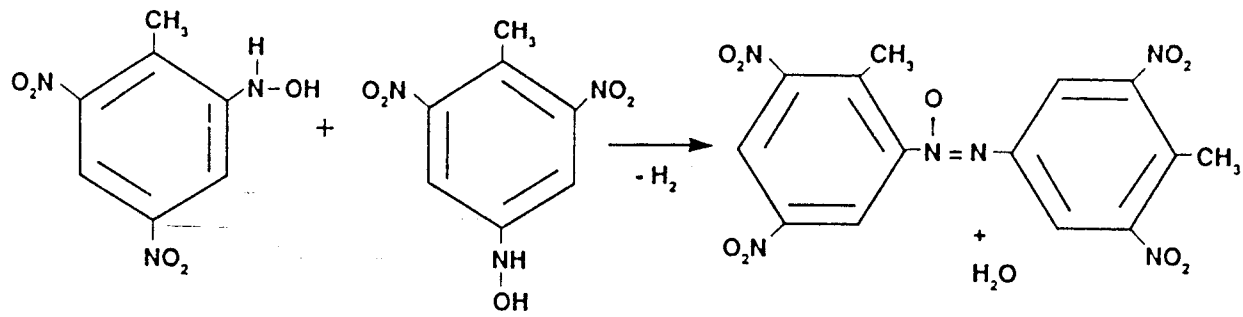


Figure 2-3. Formation of 2,4'-Azoxy from microbial reduction.

cleavage by gram-negative bacteria (Traxler, 1976). In Fig. 2-3 we show dimerization of the reduction intermediates, which produces an azoxy product. The reactive groups can be in either the 2- or the 4- position, making four possible isomers (McCormick et al., 1976).

Microbes reduce TNT faster under aerobic conditions than they do under anaerobic conditions. However, the aerobic products are mostly mono- and diamines, whereas the anaerobic products are mostly di- and triamines. In addition, pH has a strong effect on the extent of the reduction. In a 24-h experiment, Kayser et al. (1977) discovered that TNT was only 45% converted at pH 6-6.2, while it was 99% reduced at pH 7.4-7.8. A high transformation rate continued through pH 9.1; at this point there was an accumulation of amine products. Corn-steep liquor and other nutrients enhance the efficiency of microorganisms in TNT reduction. For some microorganisms, added nutrients are essential for reduction.

Hoffsommer et al. (1978) summarized previous studies conducted at the Naval Ammunition Depot (NAD), McAlester, Oklahoma, that showed that plants grew better in streams contaminated with TNT than in uncontaminated streams. They suggested that several strains of bacteria biotransform TNT into aminonitrotoluenes, thus supplying additional nutrients for plant life. The TNT-reduction products, 2-amino-4,6-DNT and 4-amino-2,6-DNT, were detected in TNT-contaminated streams at NAD, McAlester, Oklahoma. In addition, three species of microorganisms (pseudomonads) that were especially adapted for TNT reduction were isolated from the waters.

Plant and Human Metabolites

Palazzo and Leggett (1986) studied the effect of TNT on yellow nutsedge (*Cyperus esculentus*) grown in hydroponic aquaria. After 42 d in 5 to 20 mg/L solutions, the nutsedge had absorbed TNT and produced two metabolites: 2-amino-4,6-DNT and 4-amino-2,6-DNT. These metabolites were present in the plant roots at up to 18 times the concentration of TNT. The tubers also showed the same pattern of absorption and metabolism, but to a smaller extent. Other products were not identified.

Metabolism in humans produces most of the same products as photolysis and microbial degradation: the methyl group is progressively oxidized, and the nitro group is reduced. Azoxy compounds result from coupling. Yinon and Hwang (1985) list the following metabolic products of TNT: 2-amino-4,6-DNT, 4-amino-2,6-DNT, 4-hydroxyamino-2,6-DNT, 2,4-diamino-6-NT, 2,6-diamino-4-NT, 1NBA, TNBOH, 4,4'-azoxy, and 2,2'-azoxy.

Hydrolysis Products

TNT reacts with bases, especially at high temperature (Kaye, 1980, pp. T251-T253). However, no hydrolysis of TNT was observed at a pH of approximately 8, after 108 d in seawater (Hoffsommer and Rosen, 1973). Therefore we do not expect hydrolysis to be a significant source of degradation products.

Complex and Compound Formation

Less TNT and its biotransformation products can be extracted from organic-rich soils and sediments than would be expected on the basis of calculated and measured partition coefficients (Kaplan and Kaplan, 1982b; Hoffsommer *et al.*, 1978; Carpenter *et al.*, 1978; Isbister *et al.*, 1984; and Spanggord *et al.*, 1980b). McCormick (1986) suggested that microbial reduction of TNT produces highly substituted anilines or their precursors, which react immediately with carboxyl groups of humic acids, lipids, or proteins to form insoluble precipitates. In research that supports this hypothesis, Kaplan and Kaplan (1983) performed tests with humic acids, and observed binding with TNT and the two amino reduction products, 2,4-diaminonitrotoluene and

2,6-diaminonitrotoluene. They concluded that TNT itself did not bind to the humic acids, but the two amines bound readily. The binding reactions were enhanced at increased acidity (pH 4), where the amines were more reactive. The fate or toxicity of the complexes is unknown. Similarly, Spanggord et al. (1980b) recovered much less than the expected amount of TNT from unsterilized sediment when measuring sediment/water partition coefficients. They suggested that either biodegradation, irreversible sorption, or reactions with the sediment may account for the unrecovered TNT.

In the above experiments, TNT probably remained in organic matter and in the bacterial floc at higher levels than those expected on the basis of the low soil/water partition coefficient measured in the sterilized soil. Other factors (e.g., chemical reactions, complex formation, etc.) need to be studied to define the extent of the interactions of TNT and its degradation products, with naturally occurring organic substances.

Field Measurements of TNT and Its Degradation Products

The results of field measurements of TNT and associated compounds in soils and ground and surface waters are consistent with the above laboratory studies. Spanggord et al. (1983a) studied the disposition of TNT and RDX in waste disposal lagoon waters at the Louisiana Army Ammunition Plant. They found a combination of photolytic transformation products and biotransformation products similar to those found by Burlinson (1980) in experiments with Potomac River water. The products were TNB; 3,5-DNA; the amino-DNT isomers; TNBOH; 3,5-dinitrophenol; 2-hydroxy-4,6-dinitrobenzoic acid; 2-amino-4,6-dinitrobenzoic acid; and 2,4-dinitrobenzoic acid. The major product was 2-amino-4,6-dinitrobenzoic acid.

Some of these products appear repeatedly in analyses of samples of soil and natural waters. Mizell et al. (1982) reported concentrations of TNT, 2,4-DNT, 2,6-DNT, DNB, and TNB in ground and surface waters and soils associated with former TNT washout lagoons and open burning/open detonation sites at the Blue Grass facility in Kentucky. Many other investigators (e.g., Rosenblatt, 1981; Newell, 1984; Swisdak and Young, 1977) report concentrations of TNT and the DNT's in soils and in ground water. Co-contaminants of TNT in ground water were TNB (Rosenblatt, 1981), 2,4-DNT, 2-amino-4,6-DNT and

4-amino-2,6-DNT (Swisdak and Young, 1977). The latter three compounds were in shallow ground water, beneath and downgradient from a TNT disposal site.

The above information indicates a gradual movement through soils and ground water of TNT, its photolysis product, TNB; impurities--the DNT's; and its biodegradation products, aminonitrotoluenes and aminotoluene. The other compounds that may have been formed have not been reported in the literature.

Summary

The large array of potential TNT co-contaminants and conversion products that we have identified are presented in Table 2-4. The compounds are arranged by functional groups: nitroaromatic isomers of TNT and DNT; methyl-group oxidation products; nitro-group reduction products; and dimers of some of the above compounds. The most prevalent of the nitroaromatic impurities from the manufacturing process are 2,4-DNT and 2,6-DNT. The other compounds are impurities that were incompletely removed by the sellite treatment.

The co-contaminant TNB is both a manufacturing impurity and the result of photolytic oxidation and decarboxylation of the methyl group of TNT. Likewise, TNBOH, TNBAL, and TNBA are the result of photolytic oxidation of the same methyl group, but to a lesser extent. The anthranils, nitriles, and oxime in Table 2-4 are also of photolytic origin, possibly by a different mechanism. Finally, the degradation products detected in lagoons, 3,5-dinitrophenol and 2-amino-4,6-dinitrobenzoic acid, are a combination of photooxidation of the methyl group and reduction and/or substitution of the nitro group. Amines result from the metabolic reduction of the nitrotoluenes.

The remaining compounds in Table 2-4 are condensation products of the compounds listed previously. They result from all sources: impurities, photolytic products, and microbial products. They may represent final products or they too may condense or complex with other TNT transformation products or humic materials, proteins, and other natural compounds. The compounds in Table 2-4 that have been detected in the environment were isolated from field samples. Excluded from the review were substances from sources such as holding ponds, lagoons, and waste-water discharges. In our

Table 2-4. Co-contaminants and degradation products of TNT in the environment.

Source	Compound	Detected in environment?[a]
Parent	TNT	Yes
Impurity	2,4,5-Trinitrotoluene	
Impurity	2,3,4-Trinitrotoluene	
Impurity	2,3,6-Trinitrotoluene	
Impurity	2,3,5-Trinitrotoluene	
Impurity	2,4-DNT	Yes
Impurity	2,6-DNT	Yes
Impurity	2,3-DNT	
Impurity	2,5-DNT	
Impurity	3,4-DNT	
Impurity	3,5-DNT	
Impurity Photolysis	TNB	Yes
Impurity Photolysis	DNB	Yes
Impurity Photolysis Thermal	2,4,6-TNBOH	
Impurity Photolysis Thermal	TNBAL	
Impurity Photolysis Metabolism	TNBA	
Metabolism Photolysis	3,5-Dinitrophenol	
Photolysis Thermal	4,6-Dinitroanthranil	
Photolysis	2,4,6-Trinitrobenzonitrile	

Table 2-4. (Continued)

Source	Compound	Detected in environment?[a]
Photolysis	syn-2,4,6-Trinitrobenzaldoxime	
Photolysis	2-Amino-4,6-dinitrobenzoic acid	
Microbial	4-Hydroxylamino-2,6-DNT	
Microbial	2-Hydroxylamino-4,6-DNT	
Microbial	2-Amino-4,6-DNT	Yes
Microbial	4-Amino-2,6-DNT	Yes
Microbial	2,4-Diamino-6-NT	
Microbial	2,6-Diamino-4-NT	
Microbial	2,4,6-Triaminotoluene	
Microbial	3,5-DNA (from TNB)	
Impurity	HNBB	
Impurity	MPDM	
Impurity Photolysis	White compound	
Impurity	3,3',5,5'-Tetranitroazoxybenzene	
Photolysis Microbial	4,4'-Azoxy 2,2'-Azoxy 2,4'-Azoxy 4,2'-Azoxy (See Table 2-3)	

Table 2-4. (Continued)

Source	Compound	Detected in environment?[a]
Photolysis	Desoxy white compound	
Photolysis	Monocarboxy white	
Photolysis	N-(2-Carboxy-3,5-dinitrophenyl)-2,4,6-trinitrobenzamide	
Photolysis	1,3,7,9-Tetranitroindazolo-2,1-α-indazol-6-ol-12-one	
Microbial	Nitrate ion	
Microbial	Nitrite ion	

[a] Compounds with a "yes" appearing after them have been measured in environmental media; the other compounds in this table may also occur, but have not been reported.

data-base assessments we will examine DNB, TNB, 2,4-DNT, 2,6-DNT, and the two aminodinitrotoluenes, as these co-contaminants have been frequently detected in environmental media.

Although humic or plant-tissue complexes with TNT degradation products have not been isolated from field samples, laboratory evidence for them is strong. Little is known of their structures, fates, or toxicity. Laboratory experiments also show that plant tissues accumulate TNT and, more extensively, the 2-amino-4,6-DNT and 4-amino-2,6-DNT metabolites. It is not known if these compounds are free or are bound in some way. We consider the complexes of TNT, 2-amino-4,6-DNT and 4-amino-2,6-DNT with humic and plant materials of secondary concern from an environmental standpoint, only because they have not been found in the field. Clearly, more research needs to be conducted in this area.

CO-CONTAMINANTS OF RDX

The second most important explosive in the demilitarization inventory is RDX. Large-scale usage of RDX began during WW II. One common explosive

formulation containing RDX is Composition B, which is 60 wt% RDX and 40 wt% TNT. RDX does not have the stable aromatic ring of TNT; therefore, it degrades to low-molecular-weight compounds, and ultimately to carbon dioxide and water. Its photolytic decomposition is slow, and its microbial degradation is anaerobic, with few exceptions. The primary impurity in RDX is HMX, which is itself a high explosive with different applications.

Production and Impurities

Two classes of RDX are used in the U.S. They differ in melting point and amount of HMX. Both types have equal sensitivity to impact, friction, and heat initiation. Type A RDX is produced by direct nitration of hexamine with nitric acid. This process produces pure RDX (melting point of 205°C); the military specification is for a melting point >200°C. Type B (military specification is melting point >190°C) can be produced by the Bachmann process: hexamine, nitric acid, sulfuric acid, ammonium nitrate, and acetic anhydride are combined at 68°C, yielding approximately 80 to 84 wt% RDX (Department of the Army, 1967b). The RDX is subsequently recrystallized from cyclohexanone (Lindner, 1980). Because numerous aliphatic and cyclic nitro compounds are produced with Type B RDX, some of these compounds are present in the final product. The major impurity, HMX, constitutes 8 to 13 wt% of production-grade (Type B) RDX. Other identified by-products that may be formed are (Kaye, 1980, p. C612-C613)

1,5-Endomethylene-3,7-dinitro-1,3,5,7-tetrazacyclooctane,
1,7-Diacetoxy-2,4,6-trinitro-2,4,6-triazaheptane,
1,5-Endoethylene-3,7-dinitro-1,3,5,7-tetrazacyclooctane,
1,5-Endoethylidine-3,7-dinitro-1,3,5,7-tetrazacyclooctane,
1-Nitro-3,5-dicyclohexyl-1,3,5-triazacyclohexane,
Dinitroxydimethylnitramide,
Diacetoxydimethylnitramide,
N,N'-Dimethylol-N,N'-dicyclohexyldiaminoethane,
1,3,5-Trinitroso-1,3,5-triazacyclohexane, and
3,7-Dinitro-3,7-diaza-1,5-dioxacyclooctane.

The high melting point of RDX prevents its use in cast loading of projectiles. However, it can be blended with substances that have lower melting points such as TNT or mononitronaphthalene (Kaye, 1980).

Photolysis Products

Photolysis is an important degradation pathway for RDX. In aqueous solution, the RDX molecule mineralizes on exposure to UV and short-wavelength visible light. Smetana and Bulusu (1977) reported that RDX in distilled water degrades photolytically to different products and at different reaction rates at 254, 310 to 330, and 350 nm. At 254 nm (below the natural sunlight spectrum), the rate was fastest, and HCN and HCHO were produced. Irradiation at the shorter wavelength end (310 and 330 nm) of the sun's spectrum at the earth's surface produced NO, N_2, N_2O, CO, CO_2, H_2O, and H_2. They found that RDX is very stable after exposure to UV light at 366 nm and produces no gas during 10 h of irradiation.

Glover and Hoffsommer (1979a) exposed aqueous solutions of RDX to UV light and found nitrate, nitrite, ammonia, formaldehyde, and organic nitro compounds. Sikka et al. (1980) reported "nitrosamine" at about 5% of the initial RDX concentration in an aqueous solution, after photolysis with 300-nm light for 35 min (this is the only mention of this product in the literature reviewed).

Kubose and Hoffsommer (1977) reported that RDX was "rapidly destroyed" in a laminar-flow tray system under a medium-pressure mercury lamp (220 nm to 1367 nm). RDX decomposed through ring cleavage in distilled water to the following products: NO_3^-, NO_2^-, CH_2O, NH_3, N_2O, and N_2. Approximately half of the initial nitrogen was accounted for in the products. An unidentified gaseous product was thought to be CO_2. Mono-nitroso-RDX was produced by irradiation of distilled-water solutions of RDX with visible light. Tap water RDX solutions did not produce measurable mono-nitroso-RDX under the same conditions, probably because the distilled-water solutions became acidic during photolysis, going from a pH of 7 to 3.5 or 4. On the other hand, the tap water solutions had a natural buffering capacity that maintained the pH at 6.5 to 7.

Stals et al. (1971) irradiated RDX in the solid phase with 254 nm light. They identified the principal products as N_2, NO_2, NO, CO_2, CO, and HCN, with

traces of the following compounds identified by their mass spectra: HCHO, NH_3, H_2O, O_2, C_3H_6, C_2H_4, $CH_3N_2O_2$, $(CN)_2$, C_2H_6NO, and either $C_2H_2N_2O$, $C_2H_4N_3$, or C_2NO_2. They also reported possible traces of HNO, HNO_2, and H_2. After irradiation, the remaining RDX crystals were held together by a tacky polymeric material.

Microbial Products

Most researchers have found that RDX degrades by microbial action primarily under anaerobic conditions. McCormick et al. (1981) reported the products of anaerobic microbial degradation in a nutrient broth to be mono-, di-, and trinitroso-RDX analogues; hydrazine; 1,1-dimethylhydrazine; 1,2-dimethylhydrazine; formaldehyde; and methanol. They proposed a degradation pathway initially involving reduction of the nitro groups to the mono-, di-, and trinitroso-RDX compounds. These intermediates were then further reduced to hydroxylamines that broke into fragments and ultimately degraded to hydrazines and oxidized methanes. Their proposed reaction pathway is shown in Fig. 2-4.

Kayser and Burlinson (1982) analyzed the effluent from soil-column experiments conducted by Hale et al. (1979), who had studied the migration of ^{14}C-RDX and its degradation products through 24-in. soil columns over a 6-month period. The soils represented a range of pH, texture, and organic carbon content. At the end of the 6-month period, a sandy-loam soil proved to be the least retentive of RDX and its decomposition products: 29% of the ^{14}C activity in the effluent was RDX that passed through the column intact, and an additional 40% of the radiolabel was volatile decomposition products. These products were not completely characterized. Formic acid and formamide were suspected to be part of the solid residue. Less RDX was found in the effluent from the more organic-rich soils.

In an aerobic environment with Composition B, TNT degraded, but RDX remained unchanged (Hoffsommer et al., 1978). Several studies found aerobic microbial degradation of RDX only with added nutrients or specialized conditions. Isbister et al. (1984) examined an aerobic compost composed of alfalfa and Purina Sweetena horse feed. When they innoculated this nutrient-rich compost with ^{14}C-RDX, they recovered 54% of the ^{14}C as CO_2 in 6 wk. They suggested that the remainder of the ^{14}C activity in the solids

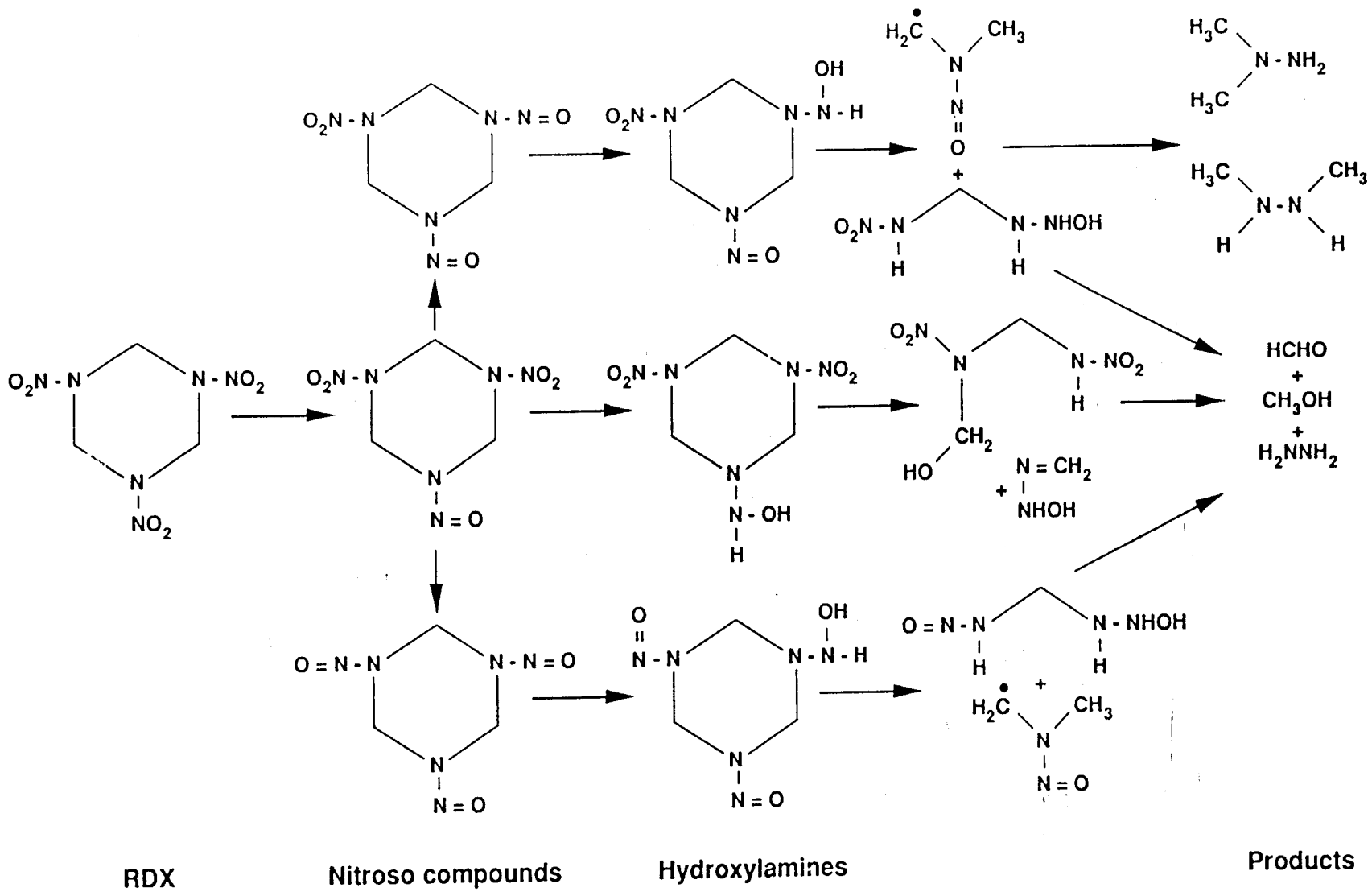


Figure 2-4. Proposed microbial degradation pathway for RDX (McCormick et al., (1981).

could be adsorbed RDX or breakdown products. Similarly, Spanggord et al. (1983a) found that RDX underwent aerobic microbial degradation only under very specialized conditions: in Holston River water or effluent water from the Holston Army Ammunition Plant to which yeast had been added. They could not culture an organism from the water, nor could they find a chemical contaminant that would bring about this transformation. Sikka et al. (1980) also studied the microbial degradation of RDX in Holston River water. Their experimental conditions did not, however, specify the degree of oxygenation. RDX concentrations in samples of Holston River water with and without added yeast remained constant for one month. However, when Sikka et al. added 1% Holston River sediment obtained downstream from the RDX waste outfalls from the Holston Army Ammunition Plant, biodegradation began after a lag time. When they repeated these experiments with ^{14}C-RDX, they were unable to isolate the products. Although aerobic conditions in flowing streams are not expected to favor microbial degradation, anaerobic degradation could take place in sediments and in lakes that are rich in organic nutrients.

Animal Metabolites

Metabolic products of RDX administered to rats and swine include several one-carbon fragments: CO_2, bicarbonate ion, and formic acid (Ryon et al., 1984 citing Schneider et al., 1977, 1978).

Hydrolysis Products

Hydrolysis would be a major transformation process only in very alkaline waters. Hoffsommer and Rosen (1973) measured the hydrolysis of RDX in seawater (pH of approximately 8) and found only 12% hydrolysis after 112 d. Hoffsommer et al. (1977) reported the alkaline (pH>9) hydrolysis products to be NO_2^-, N_2O, NH_3, N_2, CH_2O, and $HCOO^-$, with relative amounts dependent on hydroxyl ion concentration. In natural waters, the pH is usually too low for hydrolysis to be an important source of degradation products.

Field Measurements of RDX Contamination

Newell (1984) reported the results of a major survey of explosives in soils at OB/OD grounds. RDX and HMX soil contamination was found at many

sites. In addition, persistent concentrations of RDX in leaching pond and lagoon water has been reported by several authors (e.g., Rosenblatt, 1981, 1986; Mizell et al., 1982; Phung and Bulot, 1981; Spanggord et al., 1983a). Other reports detail RDX concentrations in surface and ground waters in proximity to ammunition plants and OB/OD areas (e.g., Mizell et al., 1982; Clear and Collins, 1982).

Phung and Bulot (1981) measured the concentration of two RDX degradation products in soil and wells under a settling pond used by the Lone Star Ammunition Plant in Texarkana, Texas from 1942 to 1978. The highest NO_3^- concentration was only slightly above drinking-water standards and CN^- was either at background levels or undetectable.

Summary

Photolysis and anaerobic microbial degradation with subsequent ring cleavage degrade the RDX molecule to low-molecular-weight compounds (see Table 2-5). The products from these two processes, although similar, are not the same. Potentially hazardous hydrazines are produced by microbial degradation. Hydrazine itself will sorb to and degrade on clay surfaces (Hayes et al., 1984). It has a half-life of 7 to 9 h in organic-rich water due to reaction with humic materials and 5 d in hard water (Slonim and Gisclard, 1976), which is shorter than the half-life of RDX (Spanggord et al., 1980b). In addition, hydrazines are extremely volatile. As secondary transformation products of RDX, the concentrations of the hydrazines in environmental media are expected to be quite low; indeed, we were unable to find concentration data in the literature. Consequently, the hydrazines are not likely to pose an environmental hazard.

RDX hydrolyzes slowly in alkaline water. It also appears that RDX slowly moves through the soil and degrades to volatile products in the presence of anaerobic microorganisms. Because RDX degrades to low-molecular-weight volatile products, very few co-contaminants have been found in environmental media.

Table 2-5. Co-contaminants and degradation products of RDX.

Source	Compound	Detected in environment?[a]
Parent	RDX	Yes
Impurity	HMX	Yes
Hydrolysis Photolysis	Nitrogen	
Photolysis Hydrolysis	Ammonia	
Photolysis Hydrolysis Thermal	Nitrous oxide	
Thermal Photolysis	Nitric oxide	
Photolysis	Nitrogen dioxide	
Photolysis Hydrolysis	Nitrite ion	
Photolysis	Nitrate ion	Yes
Photolysis Microbial	Formamide	
Microbial	Hydrazine	
Microbial	1,1-Dimethylhydrazine	
Microbial	1,2-Dimethylhydrazine	
Photolysis	Hydrogen cyanide	
Photolysis	Nitrosamine	
Microbial	Methanol	
Photolysis Microbial Hydrolysis	Formaldehyde	

Table 2-5. (Continued)

Source	Compound	Detected in environment?[a]
Hydrolysis Metabolism	Formate ion Formic acid	
Hydrolysis Metabolism	Bicarbonate ion	
Photolysis Microbial Metabolism	Carbon dioxide	
Photolysis	Carbon monoxide	
Microbial Photolysis	1-Nitroso-3,5-dinitro-1,3,5-triazacyclohexane	
Microbial	1,3-Dinitroso-5-nitro-1,3,5-triazacyclohexane	
Microbial Impurity	1,3,5-Trinitroso-1,3,5-triazacyclohexane	
Impurity	1,5-Endomethylene-3,7-dinitro-1,3,5,7-tetrazacyclooctane	
Impurity	1,7-Diacetoxy-2,4,6-trinitro-2,4,6-triazaheptane	
Impurity	1,5-Endoethylene-3,7-dinitro-1,3,5,7-tetrazacyclooctane	
Impurity	1,5-Endoethylidine-3,7-dinitro-1,3,5,7-tetrazacyclooctane	
Impurity	1-Nitro-3,5-dicyclohexyl-1,3,5-triazacyclohexane	
Impurity	Dinitroxydimethylnitramide	
Impurity	Diacetoxydimethylnitramide	
Impurity	N,N'-Dimethylol-N,N'-dicyclohexyldiaminoethane	
Impurity	3,7-Dinitro-3,7-diaza-1,5-dioxacyclooctane	

Table 2-5. (Continued)

Source	Compound	Detected in environment?[a]
Photolysis	Propene	
Photolysis	Ethene	
Photolysis	Polymer	
Photolysis	Cyanogen	

[a] Compounds with a "yes" after them have been measured in environmental media; the other compounds in this table may also occur, but have not been detected and reported.

CO-CONTAMINANTS OF HMX

Although we have considered HMX as an impurity of RDX for the purposes of identifying potentially important contaminants at OB/OD areas, HMX is also a powerful explosive in its own right and has found increasing uses in different munitions. Consequently, we have addressed HMX here as a separate explosive. In the subsections below, we identify the co-contaminants that result from its synthesis and environmental transformations. HMX is an eight-membered ring with the same functional groups as RDX. It is also similar to RDX in explosive properties; that is, sensitivity to impact and friction, but it is less sensitive to initiation. Therefore, the contamination of RDX with HMX has little effect on the explosive properties of the RDX. In military explosives, HMX is rarely used by itself and is often mixed with TNT. The Army Technical Manual, TM9-1300-214 (Department of the Army, 1967b), for example, lists two octol formulations that consist of 75 wt% TNT and 25 wt% HMX, and 70 wt% TNT and 30 wt% HMX, respectively.

Production and Impurities

The synthesis of HMX is essentially a modification of the procedure used to produce Type B RDX. In this process, hexamine in glacial acetic acid, a solution of ammonium nitrate in nitric acid, and acetic anhydride are added in

stages to acetic acid, rather than in one step as for RDX. The temperature is also lower, 44°C versus 68°C. Yields of 55 to 60% are obtained and the filtered product is nearly 99% pure HMX. It is then recrystallized from acetone and/or cyclohexanone (see Department of the Army, 1967b; Lindner, 1980).

Foster and Demerson (1982) reported the effects of thermal aging of three HMX-based explosives, LX-10, PBX-9501, and HMX/kraton. At 40°C, 0.01 to 0.05 mL of CO_2 and 0.06 to 0.16 mL of N_2O per 40-g sample were collected over an 18-month period. Small amounts of O_2 and N_2 were also collected from some formulations.

Photolysis Products

Spanggord et al. (1983b) reported the presence of nitrite, nitrate, and formaldehyde, but no evidence of N-nitroso derivatives when distilled water solutions of HMX were illuminated by sunlight. Similarly, Bulusu (1975) found the same products from photolytic and thermolytic decomposition. Andrews and Osmon (1977) studied a saturated aqueous solution of HMX in distilled water irradiated by a germicidal UV lamp (wavelengths primarily 240 to 260 nm) in a continuous-flow system. They report that HMX was completely eliminated in 2 h with a drop in pH from 6.9 to 3.6. However, they used an acetone solution of HMX to prepare the solution; with TNT they found that acetone accelerated photolytic degradation. Torbit (1970) studied the decomposition products of HMX in acetone and in the solid state. In acetone, the products were N_2, C_2H_2, O_2, CO_2, and N_2O. In a solid in vacuo, the products were N_2, C_2H_4, and O_2. Solid HMX in a helium atmosphere yielded N_2, C_2H_4, HCHO, and N_2O. Stals et al. (1971) state that the solid-state photolytic decomposition products and mechanisms of HMX are "similar" to those of RDX.

Microbial Products

Spanggord et al. (1983b) reported that microbial degradation of HMX took place only with the addition of nutrients. With nutrients in an anaerobic medium, they found products corresponding to the N-nitroso intermediates of RDX microbial degradation. They also isolated 1,1-dimethylhydrazine. Only in

one instance did aerobic microbial degradation occur--in Holston River water or effluent from the Holston Army Ammunition Plant to which yeast was added.

Hydrolysis Products

Little research has been done on the hydrolysis of HMX. Croce and Okamoto (1979) measured an alkaline hydrolysis rate constant for HMX that was an order of magnitude lower than for RDX. No mention was made of hydrolysis products, but they are probably analogous to those of RDX; at pH 13, RDX was hydrolyzed to gaseous products and formate ion (Hoffsommer *et al.*, 1977).

Field Measurements of HMX Contamination

Newell (1984) reported the concentration of HMX in soils at open-burning sites at depths of 15 to 45 cm to be slightly higher than at the surface or in "residue" samples at the same site.

Summary

Photolysis is the major degradation route for HMX in flowing streams and well-lighted waters. During photolytic degradation, HMX is transformed to low-molecular-weight compounds. Photolysis of solid HMX results in the formation of some polymeric products, probably due to the confinement of the intermediates. Only with added nutrients and anaerobic conditions will HMX degrade microbially. Rarely does aerobic decomposition take place. Table 2-6 lists the co-contaminants of HMX discussed in this section.

CO-CONTAMINANTS OF TETRYL

Tetryl has been used as a booster explosive and as a main charge in the form of a mixture with TNT (e.g., tetrytol). Tetryl is structurally similar to TNT, with a methylnitramine group replacing the methyl group of TNT. This substitution facilitates the hydrolysis of tetryl to picrate ion and methylnitramine. Photolysis produces N-methylpicramide. Tetryl has a stable aromatic ring that remains intact during environmental transformation processes.

Table 2-6. Co-contaminants and degradation products of HMX.

Source	Compound	Detected in environment?[a]
Parent	HMX	Yes
Impurity	RDX	Yes
Photolysis Thermal	Nitrogen	
Photolysis Thermal	Nitrous oxide	
Photolysis	Nitrite ion	
Photolysis	Nitrate ion	
Thermal	Hydroxymethylformamide	
Microbial	1,1-Dimethylhydrazine	
Photolysis	Formaldehyde	
Photolysis Thermal	Carbon dioxide	
Photolysis	Ethyne	
Photolysis	Ethene	
Microbial	1-Nitroso-3,5,7-trinitro-1,3,5,7-tetraazocine	
Microbial	1,3-Dinitroso-5,7-dinitro-1,3,5,7-tetraazocine	
Microbial	1,3,5-Trinitroso-7-nitro-1,3,5,7-tetraazocine	
Microbial	1,5-Dinitroso-3,7-dinitro-1,3,5,7-tetraazocine	
Microbial	1,3,5,7-Tetranitroso-1,3,5,7-tetraazocine	

[a] Compounds with a "yes" after them have been measured in environmental media; the other compounds may also occur, but have not been reported.

Production and Impurities

Tetryl has been produced by two processes. One process involves the addition of N,N-dimethylaniline in concentrated sulfuric acid to fuming nitric acid. One methyl group of N,N-dimethylaniline is oxidized to volatile CO_2 as the aromatic ring and amine of the compound are progressively nitrated. The other method consists of forming dinitromethylaniline from dinitrochlorobenzene and monomethylamine. The dinitromethylaniline is subsequently dissolved in sulfuric acid, and the addition of nitric acid forms tetryl (Urbanski, 1986b).

Tetryl from the above processes is subsequently purified to remove residual acid and byproducts such as compounds with an additional nitro group on the aromatic ring (Urbanski, 1986b). These impurities would have adverse effects on the storage stability of tetryl. The explosive can be used with a variety of epoxies, rubbers, silicones, and adhesives. It is compatible with aluminum, tin, copper, nickel, lead, titanium, silver, cadmium, bronze, and copper-plated steel. It reacts slightly with steel, zinc, and zinc-plated steel, and is incompatible with iron (Department of the Army, 1967b).

On the basis of vacuum stability tests, Farey and Wilson (1975) infer that tetryl can be stored at "normal" temperatures for many years without detectable decomposition. Tetryl's stability is due to the lack of impurities that might cause lower-melting-point eutectics and thus chemical decomposition. Furthermore, its melting point (i.e., 130 to 132°C) is well above stockpile temperatures. When heated alone for 4 wk at 80°C, tetryl degrades to 0.42% N-methylpicramide and 0.16% picric acid. Under the same conditions with lead azide, N-methylpicramide production increased to 2.41%; picric acid to 1.32%; and additionally, picramide, 6.70%, and 4-nitroaniline, 0.47%, were produced. At 120°C, Dubovitskii et al. (1961) isolated thermal decomposition products consisting of 2,4,6-trinitroanisole, picric acid, N-methyl-picramide, CO_2, CO, NO, NO_2, and N_2. They also found that picric acid accelerates the decomposition.

Photolysis Products

Kayser et al. (1984) reported that N-methylpicramide was the major detectable photoproduct of tetryl in distilled water exposed to laboratory

"room light." They stated that the photolysis rate is at least an order of magnitude greater than the hydrolysis rate.

Microbial Products

We could find no data on the microbial transformation of tetryl. However, the work of McCormick et al. (1976) showing reduction of many aromatic nitrocompounds to their amine counterparts, suggests, by analogy, that the nitro groups of tetryl may also be reduced to amines. Another microbial interaction might be the reduction of hydrolysis or photolysis products, for example, picric acid to picramic acid.

Hydrolysis Products

Kayser et al. (1984) measured the hydrolysis of tetryl both in laboratory light and in the dark. A solution of 12 mg/L of tetryl in distilled water produced different products at different rates depending on light. Because the rate was an order of magnitude slower in the dark, a borax buffer was added and the pH adjusted to 9. In the buffered solution in the dark, tetryl hydrolyzed to methylnitramine as the primary product (66%), picrate ion (28%); NO_2^- (4.1%), NO_3^- (3.1%) and N-methylpicramide (4.1%). On the other hand, the distilled water in laboratory room light hydrolyzed or photolyzed to N-methylpicramide (41.0%) as the major product. These investigators also report increasing hydrolysis rates with both pH and temperature. Urbanski (1986b) states that tetryl reacts with weak bases and strong acids, but not with dilute mineral acids. When tetryl is boiled with dilute alkali (or concentrated acid), the nitroamino group is hydrolyzed.

Complex and Compound Formation

Kayser and Burlinson (1982) analyzed water samples derived from a soil-leaching experiment conducted by Hale et al. (1979). Leachates had passed through 15 cm of soil samples spiked with ^{14}C-labeled tetryl. After 6 mo of irrigation, they found 5 to 14% of the radioactivity in the leachate to be picric acid. Further extraction studies showed many water-soluble and polar products, but no volatile products or tetryl. Lakings and Gan (1981)

found that tetryl was very difficult to recover from plant and animal tissue. They concluded that it was "adsorbed" by the protein or other macromolecules in the tissue matrices. The amine in tetryl or a reduced nitro group apparently reacts in a fashion similar to the amino reduction products of TNT, and, like them, may bind with humic acids, proteins, and lipids.

Field Measurements of Tetryl Contamination

The study by Newell (1984) of the explosives' contamination of soils at OB/OD sites showed that tetryl was present at concentrations as high as 1000 μg/g at several sites.

Summary

Existing studies seem to indicate that the co-contaminants of tetryl (see Table 2-7) will have aromatic structures. Unlike TNT, tetryl undergoes hydrolysis in varying degrees, yielding picric acid or picrate ion and methylnitramine. Photolysis of tetryl produces N-methylpicramide as the major product. This compound has not been identified in environmental media. Tetryl and its amine biotransformation products are likely to complex with humic acids, lipids, and proteins, based on the difficulty in extracting tetryl from soils, plants and animal tissue. Because picric acid is formed by tetryl transformation and occurs as ammonium picrate (Explosive D), it as a compound of primary concern. Methylnitramine and N-methylpicramide are of secondary concern because they have not been found in environmental media.

CO-CONTAMINANTS OF PETN

PETN is a nonaromatic explosive whose production began after WW I. It has been used extensively as a mixture with TNT in small caliber projectiles and grenades. To a lesser extent, it has been used in detonating fuses, boosters, and detonators (Department of the Army, 1967b). Recently, PETN has been incorporated in a rubber-like matrix ("sheet explosive") and used for forming, cladding and hardening metals (Kaye, 1978). It is also used medically as a drug in the treatment of high blood pressure.

Table 2-7. Co-contaminants and degradation products of tetryl.

Source	Compound	Detected in environment?[a]
Parent	Tetryl	Yes
Co-contaminant	TNT	
Thermal	Nitric oxide	
Thermal	Nitrogen dioxide	
Hydrolysis	Nitrate ion	
Hydrolysis	Nitrite ion	
Thermal	Nitrogen	
Thermal	Carbon monoxide	
Thermal	Carbon dioxide	
Hydrolysis	Methylnitramine	
Thermal	Picramide	
Photolysis Hydrolysis Thermal	N-Methylpicramide	
Thermal	4-Nitroaniline	
Thermal	2,4,6-Trinitroanisole	
Thermal	PiOH	
Hydrolysis	Picrate ion	

[a] Tetryl has been measured in environmental media, the other potential co-contaminants may also occur, but have not been reported.

Production and Impurities

PETN can be made by either a batch or continuous process. In the batch process, pentaerythritol (PE) is treated with concentrated nitric acid at 15 to 25°C. The PETN produced by that reaction is precipitated by dilution with water. The PETN is purified by dissolving it in acetone and sodium carbonate at 50°C, and reprecipitating with added water. The Biazzi continuous process uses the same chemicals except that the sodium carbonate used for pH adjustment is replaced by gaseous ammonia (Lindner, 1980). Impurities from the batch process for making PETN include pentaerythritol trinitrate (PE-tri-N), dipentaerythritol hexanitrate, and tripentaerythritol acetonitrate (Lindner, 1980). PETN is used alone and molded with plastics. "Sheet explosive" is made with PETN in a rubber-like matrix. Epoxy resins, silicone rubbers, polyurethane, organic rubber, terpene resin, Teflon, and polyethylacrylate rubber are some of the plastics used with PETN (Kaye, 1978).

Occluded acid reduces the stability of PETN. Specification-grade PETN has been stored for as long as 18 months at 65°C without "significant" deterioration (Department of the Army, 1967b). The decomposition products of PETN at 210°C are NO, CO, NO_2, N_2O, CO_2, H_2, and N_2 (Lindner, 1980). The addition of other nitro explosives lowers the melting point of the explosive and decreases its stability (Kaye, 1980).

Photolysis Products

No information was found on the photolysis of PETN, which is reasonable because PETN absorbs very little UV or visible light above 280 nm (Deb, 1957). In fact, with HPLC analysis, UV detection of PETN is usually done at 210 nm because PETN absorption is so weak at the more typical 254 nm (Maskarinec et al., 1984; Bowermaster and McNair, 1983).

Microbial Products

We could not identify any studies that dealt specifically with the biotransformation of PETN in environmental media.

Human Metabolites

Davidson et al. (1970) found that the principal metabolites of PETN in man are PE and pentaerythritol mononitrate (PEMN) in man. PE is not mineralized. Some pentaerythritol dinitrate (PEDN) was found in the blood.

Hydrolysis Products

PETN hydrolyzed in boiling water and produced PEDN as the major product (Aubertein and Rehling, 1953). Dilute acid or base accelerated hydrolysis, with base being more effective. In environmental waters, hydrolysis will likely be very slow.

Summary

PETN constitutes only a very small portion of the explosives in the demilitarization inventory, and therefore it is not surprising that it has not been identified in environmental surveys. Moreover, PETN probably degrades very slowly to PE, progressing through the trinitro-, dinitro- and mononitroesters by hydrolytic and microbial means (Table 2-8). No information was found on photolysis. Because PE is formed by different mechanisms and appears to be very stable, we did a preliminary investigation on its environmental and toxicological effects. PE is very soluble in water (Verschueren, 1983), very stable in sunlight (Lund et al., 1986), and resistant to microbial degradation (Ilic, 1978). Oral LD_{50} values are high, with reported values for mice of 25.5 g/kg and for guinea pigs, 11.3 g/kg (Keplinger, 1964). No effects for rats were seen in diets of 1 wt% PE for 90 d (Keplinger, 1964). PE is not metabolized (DiCarlo et al., 1965a). Therefore, we do not believe that PE constitutes an environmental hazard, given its low order of toxicity and the lack of observed concentrations of its parent explosive, PETN.

CO-CONTAMINANTS OF AMMONIUM PICRATE (EXPLOSIVE D)

Ammonium picrate is the ammonium salt of picric acid. The U.S. added ammonium picrate to its arsenal after the turn of the century. Because it is less sensitive to impact and friction than TNT, although inferior to it in

Table 2-8. Co-contaminants and degradation products of PETN.[a]

Source	Compound
Parent	PETN
Impurity Microbial	PE-tri-N
Impurity	Dipentaerythritol hexanitrate
Impurity	Tripentaerythritol acetonitrate
Impurity	Plastics and rubbers
Thermal	Nitric oxide
Thermal	Nitrous oxide
Thermal	Nitrogen dioxide
Thermal	Nitrogen
Thermal	Carbon monoxide
Thermal	Carbon dioxide
Thermal	Hydrogen
Metabolism	PE
Metabolism Microbial	PEMN
Microbial Hydrolysis Metabolism	PEDN

[a] We were unable to find reports containing concentration data on PETN in environmental media.

explosive power, Explosive D has been used in Naval armor-piercing projectiles (Urbanski, 1986b). Between the World Wars, it was used in many projectiles, rocket warheads, and bombs. During WW II, usage was highest, increasing from 1.5 million lb/month in 1942 to 6.5 million lb/month in 1944 (Kaye, 1978). Ammonium picrate is less sensitive to shock than picric acid and does not form

shock-sensitive salts on contact with metals. However, its usage has declined because of the development of other explosives of greater strength (Kaye, 1978; Department of the Army, 1967b).

Production and Impurities

Ammonium picrate is formed by gradually neutralizing a hot-water suspension of picric acid with ammonia. The ammonium picrate salt goes into solution as it is formed in the exothermic reaction. The salt crystallizes on cooling. It is washed with cold water, and then dried (Department of the Army, 1967b). Impurities allowed in the military-grade explosive include traces of PiOH, ammonia, chlorine, sulfates, and ash (Kaye, 1978). Ammonium picrate can withstand storage at ordinary temperatures for 20 yr without apparent deterioration. After 2 yr at 50°C, it becomes more sensitive to mechanical friction, but returns to its previous insensitivity after storage at ambient temperature (Department of the Army, 1967b).

Photolysis Products

Ruchhoft and Norris (1946) observed that sunlight has no effect on ammonium picrate solutions. Unfortunately, they did not describe the details of their experiment. Andrews and Osmon (1977) exposed a solution of 500 ppm ammonium picrate in distilled water to a 30-W UV lamp with a 240- to 260-nm bandwidth. After 1 h with 1000 ppm hydrogen peroxide, Explosive D was no longer detectable. Unfortunately, they did not attempt to photolyze ammonium picrate without hydrogen peroxide.

Microbial Products

Only one study was found on microbial action on ammonium picrate. Ruchhoft and Norris (1946) found that in 1% sewage sludge, there was no attack on ammonium picrate at any concentration from 0 to 1000 ppm during a 5-day incubation period. Concentrations above 100 ppm inhibited the biochemical oxygen demand (BOD) of the medium. Using fresh activated sewage sludge and 300 ppm ammonium picrate, they observed (1) an immediate loss of 100 ppm

ammonium picrate, but no further loss during aeration; (2) loss of BOD after 2 h aeration; and (3) no change in suspended solids. When they added ammonium picrate to sewage sludge, they observed a "reduction." No mention of products was given, but hydrolysis to PiOH and reduction of a nitro group to form picramic acid are possible transformation processes.

Animal Metabolites

Ammonium picrate is rapidly transformed to PiOH in rabbits (Weeks et al., 1983).

Hydrolysis Products

According to Kaye (1978), Explosive D is hydrolyzed by strong alkalies to ammonia and PiOH. There is evidence for hydrolytic action also at neutral pH's. Andrews and Osmon (1977) had to reduce the pH of their solutions to pH 2 to eliminate PiOH interference with their measurements of Explosive D concentrations. Ammonium picrate dissociates at neutral pH and exists as a solvated ion pair of picrate and ammonium ions.

Complex and Compound Formation

Because ammonium picrate dissociates to picrate ion (PiO^-) in water, the products and complexes formed by the latter are possible aqueous products of Explosive D. The bond between the oxygen of the picrate ion and the nitrogen of the ammonium ion can be thought of as an ion pair in aqueous solution. PiOH forms reversible ion pairs with free amino groups, and this reaction is used to monitor the extent of protein synthesis (Hancock et al., 1975). Also, ion pairs of quaternary ammonium salts with picric acid can be used to separate the ammonium salts with ion pair chromatographs (Eksborg and Schill, 1973). PiOH is used to stain proteins yellow for medicinal purposes and for decorative purposes in silk dyes. The reaction of the acid with proteins in living animals also forms a stable ion pair complex, as demonstrated by Burton et al. (1984) in their study of the uptake and depuration of PiOH in oysters. They found that some acid was eliminated readily and some retained.

PiOH forms complexes readily with aromatic compounds and forms complexes or ion pairs with proteins (Fieser and Fieser, 1962). The complexes are formed between picric acid and the ring (pi) electrons of aromatic compounds, including naphthalene and larger polynuclear aromatic hydrocarbons (PAH). The complexes are called pi-complexes and produce colors that are specific for each PAH. PAH's in the water and sediment could remove PiOH from solution.

Because humic substances are mainly polyphenols (Stevenson, 1972) and probably incorporate phenols of pesticide origin into their structure (Bollag, 1981), we suspect that PiOH will become incorporated in or bound by humic acids, too. This could be a significant environmental fate in organic-rich streams. Just as we expect the picrate ion to form less soluble ion pairs as more highly substituted amines become available to it, the picrate ion will form insoluble precipitates with the heavier metal cations (Selig, 1986). The ready substitution of metal for ammonium ions in wet ammonium picrate has been demonstrated (Kaye, 1980).

Field Measurements of Ammonium Picrate and Its Degradation Products

Concentration data on ammonium picrate in environmental media are very sparse. Ruchhoft and Norris (1946) made a brief reference to ammonium picrate concentrations in wash waters and evaporation ponds that varied from 100 to 300 ppm. Van Denburgh et al. (1980) measured ammonium picrate (reported as picric acid) in ground waters in the vicinity of disposal beds at an ammunition facility.

Summary

The most likely environmental transformation processes of ammonium picrate are the hydrolysis to PiOH or cation exchange with heavy metals. The PiOH may undergo some additional microbial transformation to picramic acid or form complexes with humic acids. Co-contaminants from these processes are listed in Table 2-9. Because much of this is deduced from studies of PiOH and not directly related to the disposal of ammonium picrate, more information is needed.

Table 2-9. Co-contaminants and degradation products of ammonium picrate.

Source	Compound
Parent	Ammonium picrate
Impurity Hydrolysis	PiOH
Hydrolysis Metabolism	Picrate ion
Impurity Hydrolysis	Ammonia
Impurity	Chloride
Impurity	Sulfates
Impurity	Ash

[a] We were unable to find reports containing concentration data on ammonium picrate in environmental media.

OVERVIEW OF CO-CONTAMINANTS

In summary, we have identified a number of compounds resulting from the environmental transformation of the six military explosives to be of primary concern. These compounds are listed in Table 2-10. It should be noted that little information is available on the fate of humic acid complexes, lipid-type compounds, or complexes with the aromatic transformation products of some of the explosives. Although we have listed them under secondary concern, they could be important.

Table 2-10. Explosive degradation products of primary and secondary concern.

Compound	Primary Concern	Secondary Concern
TNT	TNT TNB DNB 2,4-DNT 2,6-DNT 2-amino-4,6-DNT 4-amino-2,6-DNT	Complexes or compounds formed from primary-concern compounds and humic acids, lipids, proteins, etc.
RDX	RDX HMX	Hydrazine 1,1-Dimethylhydrazine 1,2-Dimethylhydrazine
Tetryl	Tetryl PiOH, PiO$^-$ Picramic acid	Complexes or compounds formed from primary-concern compounds and humic acids, lipids, proteins, etc. N-methylpicramide Methylnitramine
PETN		PE PE-tri-N PEDN PEMN
Ammonium picrate	Ammonium picrate Picric acid and picramic acid	Complexes or compounds formed from primary-concern compounds and humic acids, lipids, proteins, etc.

3. DATA-BASE ASSESSMENT FOR 2,4,6-TRINITROTOLUENE (TNT)

The demilitarization of conventional ordnance containing TNT, the primary explosive filler in the demilitarization inventory, produces several by-products, including residual TNT and related co-contaminants (see Section 2). Considerable environmental and toxicological research has been completed on TNT as well as on 2,4-DNT and 2,6-DNT; less work has been carried out on the other co-contaminants, namely: DNB; TNB; 2-amino-4,6-DNT; and 4-amino-2,6-DNT. Although TNT is subject to photolytic degradation and to biotransformation, it still persists in environmental media for many years, and has been detected in soils and ground waters at many different demilitarization sites across the continental U.S. Potentially important exposure pathways for TNT are the consumption of contaminated ground water and ingestion of crops grown on soils containing TNT. We begin this section with a review of TNT's environmental chemistry, followed by a review of data on the toxic effects of TNT on animals and humans, in order to provide estimates of safe intake rates to protect public health. We conclude the data-base assessment of TNT with discussions of its toxicity to aquatic species and plants.

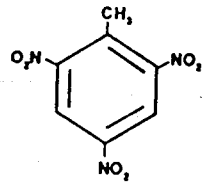
ENVIRONMENTAL PARTITIONING AND TRANSPORT PROPERTIES

Table 3-1 contains information related to the identification of TNT, and Table 3-2 presents information on chemical and physical properties selected because of their potential use in performing environmental assessments. The values contained in Table 3-2 represent actual measurements of properties (e.g., vapor pressure, solubility in water, etc.) or estimates based on the calculations and supporting data described in Appendix A.

Soil Transport and Adsorption

The behavior of TNT in soil environments has been investigated through the use of soil-column studies and measurements of soil/water partitioning. Two groups of researchers have used soil columns to study the movement of TNT

Table 3-1. Chemical identifiers of trinitrotoluene.

		Structural formula:
Empirical formula:	$C_7H_5N_3O_6$	
Molecular weight:	227.13	
CAS reg. no.:	118-96-7	
RTECS no.:	XU0175000	

in soils. Kaplan and Kaplan (1982b) added 300 mL of distilled water per day to a 25-cm-high column (21 cm in diameter) containing soil with 1.34 wt% organic matter and 600 mg of TNT that had been mixed in to the top 2.5 cm of soil and backfilled with 4 cm of clean soil. They detected TNT in column leachate after 14 d of water treatments. In addition, they detected 2-amino-4,6-DNT and 4-amino-2,6-DNT at maximum concentrations of 2 and 3 mg/L, respectively, compared with a maximum concentration of 15 mg/L for TNT. At day 100, the concentrations of the two amino compounds ranged from 200 to 800 μg/L.

Hale et al. (1979) studied the movement of radiolabeled (^{14}C label) TNT through soil lysimeters. Their lysimeter columns were 91 cm in diameter and 152 cm long. Soils of various organic content were irrigated with 1.25 to 2.5 cm/wk of deionized water to assure proper moisture content. The columns initially contained 908 g of TNT (i.e., 20,000 ppm in the top 7.62 cm). After 26 wk, the concentration in the top 10 cm of the fastest draining soil labeled "Princeton" (a fine sandy loam), irrigated at 2.5 cm/wk, had decreased to about 6000 ppm. The concentration in the top 30 cm was approximately 6500 ppm. The second fastest draining soil, "Bennington" (a silty loam, acidic and medium textured), irrigated at 1.25 cm/wk, retained 93% of the initial TNT within the top 30 cm of soil after 26 wk. In the two other soils with higher organic carbon content or higher alkalinity, little migration of TNT occurred in 26 weeks.

Table 3-2. Chemical and physical properties of 2,4,6-trinitrotoluene. Properties are for an ambient temperature of 20°C unless otherwise noted.

Property	Units	Value	Source
Boiling point	°C	240 (explodes)	Verschueren, 1983
Freezing/melting point	°C	80.1	Windholz *et al.*, 1983
Density	g/mL	1.65	Windholz *et al.*, 1983
Vapor pressure	torr	1.1×10^{-6}	Measured value[a] from Leggett, 1977
Aqueous solubility	mol/L mg/L	5.4×10^{-4} 123	Average of 2 measured values reported in Spanggord *et al.*, 1980a.
Henry's law constant	(L-torr)/mol	2.0×10^{-3}	Calculated as the ratio of vapor pressure to aqueous solubility
Diffusion coefficients: Air Water	m^2/s m^2/s	5.9×10^{-6} 5.8×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition coefficient (K_{ow})	log K_{ow}	1.60 1.84	Measured, Gould and Hansch, 1986 Estimated, Rosenblatt, 1981

[a] See Appendix A.

Kayser and Burlinson (1982) analyzed water and soil samples from the experiments by Hale et al. (1979) to determine the presence of TNT and associated biodegradation products. Neither TNT nor its microbial degradation products were detected in samples of water that had leached through the soil columns. Thin-layer chromatography of one of the water samples with the highest level of ^{14}C activity showed that the degradation products present were polar and non volatile. Some of the soil extracts, on the other hand, did contain TNT, 2-amino-4,6-DNT, and 4-amino-2,6-DNT. Each soil type examined contained differing amounts of the amino degradation products.

The soil organic carbon (SOC)/water partition coefficient (K_{OC}) has been measured by Spanggord et al. (1980b) and Tucker et al. (1985) (see Table 3-3). Spanggord et al. carried out a series of sorption-desorption experiments and measured K_{OC} values ranging from 910 to 2500, with a mean of 1600 (n = 6, standard deviation of 600). Tucker et al. measured the soil/soil-water partition coefficient (K_d) of TNT for 12 different soil types. For each soil type they measured SOC content (in wt%), clay content (wt%), pH, cation-ion-exchange-capacity (CEC) (in meq/100 g soil), and silt content (wt%). The organic carbon content of seven of the soils was below 1 wt%. A regression equation of SOC against the K_d values for TNT showed that this parameter accounted for 64% of the variance in K_d values; however, a regression equation with CEC as the sole independent value accounted for 78% of the variance of K_d.

Based on their experimental results, Tucker et al. calculated the K_{OC} for TNT to be 534 (standard deviation not given). The discrepancy between their K_{OC} value and the average K_{OC} value from Spanggord et al. could be due to differences in soil characteristics, such as the CEC and the pH of the soils tested. Tucker et al. indicate that adsorption to soil particles will be the primary factor affecting the migration of TNT in soils with a CEC level greater than 10 meq/100 g. Vapor-phase diffusion may only become important in soils in arid areas where water infiltration is low (i.e., less than 0.25 cm/yr).

Partition Coefficients: Plant/Soil, Beef-Fat/Diet, and Fish/Water

Data on the movement and uptake of TNT in environmental media, including biota, are needed to understand its behavior in the environment and to

Table 3-3. Partition coefficients for use in estimating contaminant movement in environmental media and human exposures to TNT.

Partition coefficient	Parameter	Units	Value	Source
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	470	Calculated [a] from water solubility and melting point
			1600 ± 600	Mean of six measured values, Spanggord *et al.*, 1980b
			534	Derived from experimental results, Tucker *et al.*, 1985
Plant/soil	K_{sp}	(mg/kg plant)[b]/(mg/kg soil)	2	Calculated[a] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	2.6×10^{-3}	Calculated[a] from water solubility and log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[b]/(mg/kg water)	7	Calculated[a] from log K_{ow}
			9.5	Based on a short-term uptake-study with bluegills by Liu *et al.*, 1983a

[a] See Appendix A.

[b] Wet-weights for plant and fish.

estimate potential exposure pathways to humans via plants, crops, fish, etc. Unfortunately, data for calculating partition coefficients for TNT as well as the other explosives are limited. Therefore, we have used regression equations to estimate partition coefficients from other properties (see Appendix A). Table 3-3 contains estimated and/or measured values of the partitioning coefficients for TNT between plant and soil (K_{sp}); beef-fat and diet (K_{fd}) and fish and water (K_{fw}). Although we were unable to find measured values for K_{sp} and K_{fd}, Liu et al. (1983a) did perform exploratory bioconcentration tests (4-day exposures) using bluegill sunfish, water fleas, worms, and algae. TNT did not bioaccumulate extensively. The 4-day bioconcentration factor for the bluegill fish was 9.5. The range of factors for the other species was 1.6 for water fleas, 3.0 for worms, and 453.0 for algae. Liu et al. (1983a) recommended that a full-scale (42-day) bioaccumulation test be performed using the bluegill fish.

Equilibrium Distribution in Reference Landscapes

To gain insight as to the potential behavior of TNT in environmental media, we have used the computer model termed GEOTOX (see Layton et al., 1986; McKone and Layton, 1986) to estimate the equilibrium distribution of TNT among environmental compartments of landscapes that are representative of areas in the Southeastern and Western U.S. where demilitarization operations occur. For the purposes of this analysis, we do not consider degradation processess. Each landscape is defined by a system of eight compartments consisting of the atmosphere, air particles, biota, upper soil (i.e., "A" horizon), lower soil (i.e., "B" horizon), ground water, surface water, and sediments. We have estimated the fractional distribution of TNT in compartments based on a steady-state input of that explosive to the upper (surface) soil layer. Input data consist of TNT properties (i.e., Henry's law constant, K_{oc}, air and water diffusion coefficients, K_{sp}, K_{fd}, and K_{fw}) and properties of the reference landscapes (see Appendix B for information on the compartmental characteristics of the two landscapes). Appendix B includes a brief discussion of the GEOTOX model as well as an overview of data on measured concentrations of TNT in environmental media and detection methods. Table 3-4 shows the predicted fractional distribution of TNT among the eight compartments for the two landscapes. Most of the TNT should reside in the

Table 3-4. Fractional distributions of TNT among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of TNT to the upper soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction of total inventory present[a]	
Air	1.9×10^{-8}	9.9×10^{-9}
Air particles	9.0×10^{-9}	1.9×10^{-8}
Biota	1.7×10^{-4}	0.015
Upper soil	0.037	0.12
Lower soil	0.49	0.098
Ground water	0.47	0.77
Surface water	0.0014	0.0045
Sediments	1.6×10^{-4}	5.3×10^{-4}

[a] Values do not add to one because of rounding.

subsurface environments of the two landscapes (i.e., soils and ground water). Differences in the distributional pattern in the subsurface zones would be due to compartmental properties such as soil organic carbon, soil mass, etc. For example, the upper soil zone in the Western ecoregion should contain a third of the amount of organic carbon content as the same zone in the Southeastern ecoregion (see Appendix B). The predicted preferential movement of TNT toward the subsurface compartments indicates that the most important transformation processes should be those that can be sustained in subsurface environments (e.g., biotransformation rather than photolysis).

ENVIRONMENTAL TRANSFORMATION PROCESSES

In Section 2 we identified many of the products associated with the degradation of TNT in environmental media. The emphasis of this subsection is on the transformation mechanisms and on the rates of photolysis and biotransformation.

Photolysis

Previous research has shown that the rate of TNT photolysis depends in part on the concentration of photoproducts. Spanggord et al. (1980b), for example, showed that photolysis of a 0.11-mg/L aqueous solution of TNT resulted in disappearance of the dissolved TNT at a linear rate for approximately 100 minutes; then concentrations decreased at a faster rate. In contrast, photolysis of 0.022 mg/L TNT resulted in linear disappearance after 350 minutes. Interestingly, because of the rate acceleration induced by decomposition products, there was less TNT remaining in the more concentrated solution at 250 minutes than in the less concentrated one. Mabey et al. (1983), referring to the above study, noted that the photolysis rate after 33% TNT loss from the initial 0.11-mg/L solution above was 20 times greater than the initial rate. Spanggord et al. (1980b) examined the photolytic degradation of 1.1 mg/L TNT in samples of natural waters exposed to sunlight in borosilicate tubes. Photolysis rate constants were $120 \times 10^{-5}\ s^{-1}$ for Holston River water, $15 \times 10^{-5}\ s^{-1}$ for Waconda Bay water, and $58 \times 10^{-5}\ s^{-1}$ for Searsville Pond water. Photolysis of TNT in distilled water, by comparison, proceeded with a rate constant of $1.7 \times 10^{-5}\ s^{-1}$. In other work, Spanggord et al. (1983a) estimated the half-life of TNT in lagoon water from the Louisiana Army Ammunition Plant at a depth of 50 cm to be 8 d in the middle of March, based on a depth-independent rate constant of 4.3 cm/d. Mabey et al. (1983) estimated the photolytic half-life of TNT in pure water at 40°N latitude using the calculational procedure of Zepp and Cline (1977). In summer the half-life was estimated to be 14 h and in winter, 45 h, based on a quantum yield of 2.7×10^{-3} and absorption coefficient data for TNT.

Mabey et al. (1981) completed two experiments to study the enhancement of photolysis rates by humic substances in natural waters. In the first experiment, aqueous samples (filtered through a 0.2-μm filter) contained in borosilicate reaction tubes were illuminated by single wavelengths from a mercury lamp; in the second experiment, samples in either the borosilicate tubes or in dishes were illuminated by sunlight (May to June in Menlo Park, Ca.). The photolysis half-lives corresponding to the rate constants obtained from the experiments conducted with a mercury lamp (see Table 3-5) and natural waters ranged from about an hour to 5.9 h. The TNT in a pure-water sample, in contrast, had a half-life of about 27 h. When sunlight was used, the photolysis rates of TNT in pure water were also slower than the rates for the

natural waters. In addition, they found that the geometry of the solution vessel influenced the rates of photolysis. For example, a tube receives sunlight from more directions than a dish and consequently, photolytic rate constants for tube experiments were faster than the dish equivalent. Mabey et al. (1981) suggested that photolysis of TNT in natural waters proceeds through an excited triplet state of TNT and that acetone or humic materials act as sensitizers for the production of the excited state, which hastens photolysis. This is supported by the fact that TNT degrades rapidly in natural, sunlit waters in spite of the fact that TNT does not absorb light wavelengths above 400 nm (Spanggord, 1980b). Mabey et al. (1981) noted that the ten-fold difference in photolysis rate constants for pure and natural waters is consistent with photolytic enhancement by humic substances. Additional confirmation of the action of humic acids as triplet sensitizers was provided by comparing the rate constants and quantum yields of TNT photolysis with humic acids present with rate constants of a known triplet sensitizer (see Mabey et al., 1983). On the other hand, Burlinson (1980) found that the rates of disappearance of TNT dissolved in Potomac River water and in distilled water (samples were in dishes exposed to sunlight) were not greatly different.

Photolysis rates are also dependent on pH, according to studies by Burlinson et al. (1979a, 1979b). Burlinson et al. (1979b) proposed that in the photolytic degradation of TNT, one of the adjacent nitro group attracts a hydrogen from the methyl group (I) (see Fig. 3-1), which then transfers the H^+ to form the 2,4,6-trinitrobenzyl anion (II). The course of the reaction depends on the pH of the solution; that is, with an excess of protons (acid), the ion (II) is returned to TNT and with an absence of protons (base), the ion (II) is oxidized to an alcohol, aldehyde, or carboxylic acid, or converted to a nitrile. Some of these products react further or combine to form larger molecules. The carboxylic acid can decarboxylate to form TNB.

Burlinson et al. (1979a) measured the disappearance of TNT in aqueous solutions under varying pH's and found that the half-life of disappearance was inversely proportional to pH. A mercury lamp was used with a Pyrex filter to screen out UV light with wavelengths shorter than 280 nm. At pH 7.4 the half-life of TNT was about an hour, but at pH 3.6 the half-life was about 2 h. Mabey et al. (1983) measured the photolysis rate of 1.1 mg/L TNT in

Table 3-5. Summary of TNT photolysis rates derived from experiments conducted by Mabey et al. (1981).

Light source[a]	TNT concentration (mg/L)	Water sample	Reaction vessel	Rate constant for photolysis ($\times 10^5$ s^{-1})
313 nm[b]	0.63	Holston R., South Fork	Tube	6.29
313 nm[b]	0.63	Searsville Pond	Tube	10.4
366 nm[c]	0.63	Searsville Pond	Tube	18.8
313 nm[b]	0.63	Searsville Pond[d]	Tube	3.27
313 nm[e]	0.28	Searsville Pond[d]	Tube	9.66
313 nm[e]	0.57	Searsville Pond[d]	Tube	8.32
313 nm[e]	1.01	Searsville Pond[d]	Tube	7.16
313 nm[b]	0.63	Pure water	Tube	0.71
Sunlight[f]	Unspecified	Holston R., North Fork	Tube	49.7
Sunlight[f]	Unspecified	Holston R., South Fork	Tube	48.8
Sunlight[f]	Unspecified	Pure water	Tube	4.62
Sunlight[g]	Unspecified	Holston R., South Fork	Tube	103
Sunlight[g]	Unspecified	Holston R., South Fork	Dish	29.7
Sunlight[g]	Unspecified	Holston R. with sediment	Dish	20.9
Sunlight[g]	Unspecified	Searsville Pond	Tube	167
Sunlight[g]	Unspecified	Searsville Pond	Dish	50
Sunlight[g]	Unspecified	Searsville Pond	Flask	84
Sunlight[g]	Unspecified	Pure water	Tube	19

[a] Mercury lamps were used for the single-wavelength light sources.

[b] Light intensity of 1.37×10^{-6} $einstein^{-1}$ cm-s.

[c] Light intensity of 9.85×10^{-6} $einstein^{-1}$ cm-s.

[d] One-to-one dilution with pure water.

[e] Light intensity of 7.67×10^{-7} $einstein^{-1}$ cm-s.

[f] Afternoon sun in May, Menlo Park, Ca.

[g] Midday sun in July, Menlo Park, Ca.

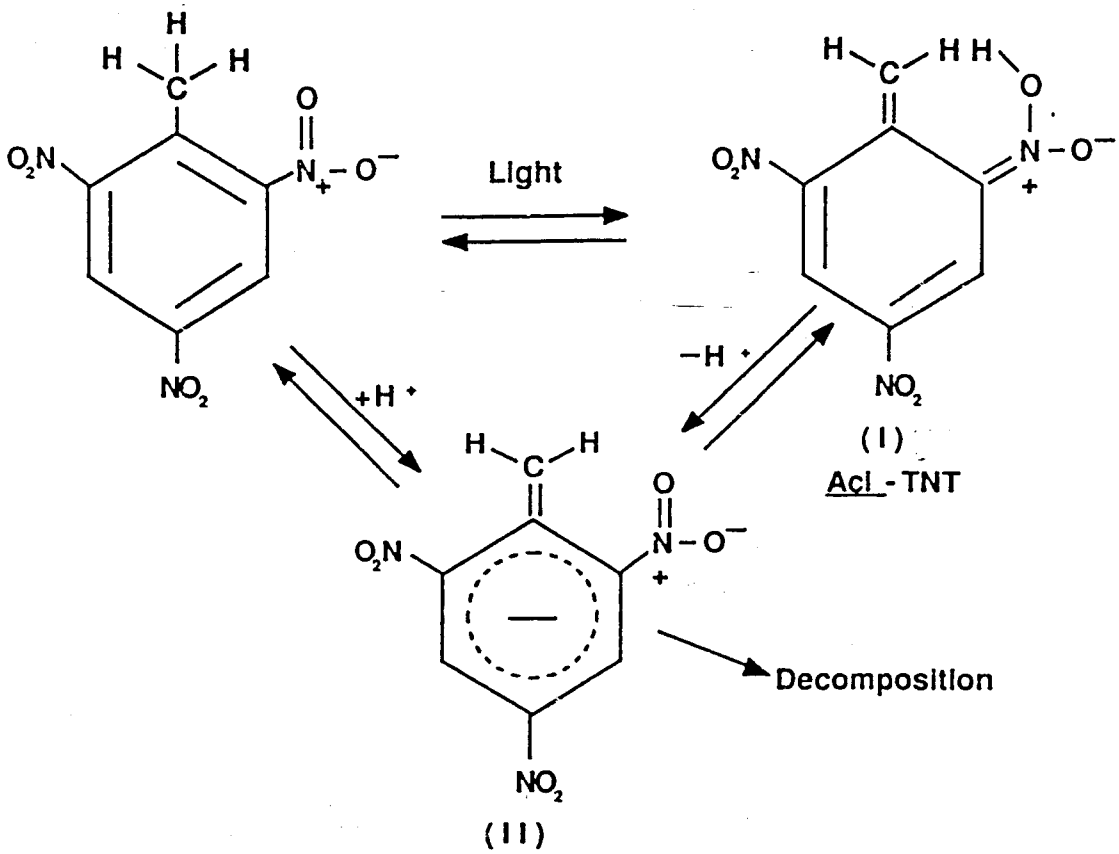


Figure 3-1. Proposed photolytic degradation mechanisms (Burlinson et al., 1979b).

humic-rich Searsville Pond water and pure water at pH 4 and 8. They found only a small effect of pH on TNT photolysis in natural waters; however, in pure water the photolysis half-lives were 12 and 3.7 h, respectively, for pH 4 and 8.

Burlinson et al. (1979a) also showed that the composition of photoproducts varies with pH. For example, between pH 3.6 and 7.4 the major products were 2-amino-4,6-dinitrobenzoic acid, TNBAL, and 4,6-dinitroanthranil. At pH 8.0 to 8.5, the major photoproducts were TNB, TNBAL, and a trace of TNBOH. At pH 11.1 only TNB was present. Also, the photoproducts were the same regardless of whether sunlight or a mercury lamp was used.

The TNT photolysis products listed above are those isolated from irradiated solutions and are not necessarily the final products. Some products of TNT photolysis are also photo-sensitive. Kaplan et al. (1975) and Burlinson et al. (1979a) studied the reactions and products resulting from photolysis of TNBAL, TNBOH, and 4,6-dinitroanthranil. Irradiation of TNBAL initially yields 2-nitroso-4,6-dinitrobenzoic acid and finally a dimer that decarboxylates to the monocarboxy white compound. The alcohol reacts in an analogous way, ultimately forming TNBAL and dimers, desoxy white compound, and the monocarboxy white compound. Kaplan et al. (1975) suggest that the dimers react further to form three- to four-ringed "insoluble" compounds.

Photolysis of TNT in an aqueous solution also produced an unidentified pink intermediate that slowly reverted to TNT in the dark (Mabey et al., 1983). This product did not promote photolysis of TNT as other products did. Also, Mabey et al. did not consider this intermediate to be the aci-TNT shown in Fig. 3-1 as structure I. It could, however, be a Meisenheimer compound (see Fig. 3-2).

The mechanism of TNT photolysis has not been fully established. Possible mechanisms consists of the 2,4,6-trinitrobenzyl anion pathway proposed by Burlinson et al. (1979b) or the triplet state proposed by Mabey et al. (1981). The photolysis products are both photosensitive themselves and act as catalysts for further TNT photolysis. Humic substances accelerate TNT photolysis, while oxygen retards TNT photolysis (Mabey et al., 1981). The photolysis results in the oxidation of the methyl group and finally decarboxylation.

Biotransformation

Many bacteria, yeasts, and fungi reduce the nitro groups in TNT to amines or azoxy dimers. The degree and rate of reduction vary with the types of organisms and environmental conditions. With appropriate conditions, all nitro groups can be converted, but reduction stops without mineralization of the aromatic ring (see Fig. 3-3.) Table 3-6 lists the biotransformation products that have been identified by various researchers.

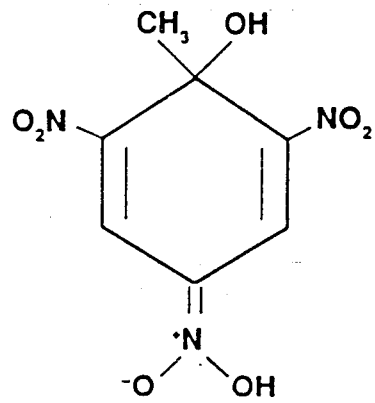


Fig. 3-2. Possible Meisenheimer compound formed by irradiation of TNT.

Microbes that have or can develop the ability to reduce TNT are common. Of the 190 fungi studied by Parrish (1977) for their ability to reduce TNT, only six could not. Spanggord et al. (1980b) studied organisms isolated from several types of water, and all reduced TNT. Osmon and Klausmeier (1973) found that many soils and water samples had microorganisms with TNT-reducing ability.

Biotransformation experiments using natural waters have been completed by Burlinson (1980) and Spanggord et al. (1980b). In the study by Burlinson (1980) 20 mg/L of TNT in raw Potomac River water was reduced by half over a period of 7.5 d. An adaptation period or lag period was not observed. Experiments conducted by Spanggord et al. (1980b) using natural waters (see Table 3-7) required small amounts of organic nutrient for cell growth (not for TNT biotransformation). They found that TNT biotransformation had a lag time of 13 to 40 d and a half-life of 8 to 25 d, depending on the water source.

Many researchers have studied microbial degradation of TNT in the presence of added nutrients and/or concentrated microbial populations. Nutrients increase the rates of the reactions and in some cases, enable recalcitrant reactions (i.e., mineralization) to proceed. Won et al. (1974) studied the influence of glucose and yeast on the biotransformation of TNT by a specially adapted pseudomonad organism. Although the rate of TNT loss in cultures without added nutrient was reported only on thin layer chromatograms, it was much obviously slower than the rate with 0.5% added glucose or yeast extract, based on a comparison of the chromatograms of the products at 24-h

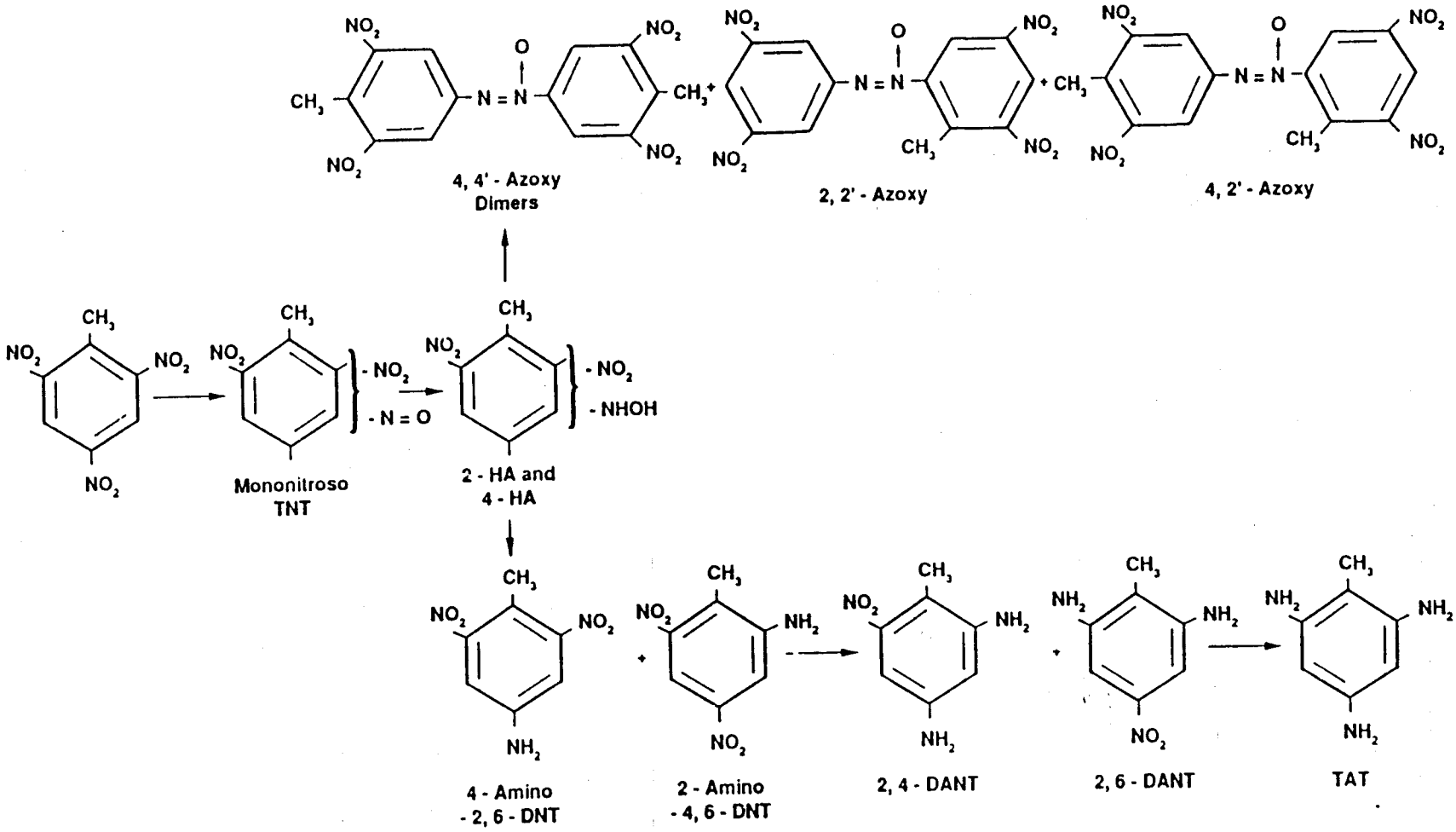


Figure 3-3. Biotransformation pathways of TNT.

Table 3-6. Biotransformation products of TNT that have been identified by various researchers.

Compound	References
4-amino-2,6-DNT	a,b,c,d,e,f,g
4-hydroxyamino-2,6-DNT	d,g
2-amino-4,6-DNT	a,b,c,e,f,g
2,4-DANT	b,c,d,f,g
2,6-DANT	b,c
TAT	d
4,4'-azoxy	c,d,f,g
2,2'-azoxy	d,g
2,4'-azoxy	c

[a] Burlinson, 1980.
[b] Hoffsommer et al., 1978.
[c] Kaplan and Kaplan, 1982a.
[d] McCormick et al., 1976.
[e] Naumova et al., 1983.
[f] Spanggord et al., 1980b.
[g] Won et al., 1974.

intervals. Whereas a substantial amount of the initial TNT persisted in the solution without added nutrient after 96 h, TNT decreased to 1% of the original level within 24 h when 0.5% yeast was added to cultures. Also, these additional products were isolated when nutrients were present: 4-amino-DNT, 4-hydroxyamino-2,6-DNT and diamino-NT.

Yeast extract added to a basal mineral-salts medium enabled a variety of microorganisms to transform TNT in an aerobic environment. Osmon and Klausmeier (1973) tested microorganisms from sewage effluent; an effluent from a TNT loading facility; aquaria water; and soil for their ability to

Table 3-7. Biotransformation of 10 mg/L TNT in different sources of water (Spanggord et al., 1980b).

Water sample	Dissolved in DMSO[a]	Lag time (d)	Half-life (d)
Waconda Bay[b] water 1	*	20	25
Waconda Bay[b] water 2	*	13	19
Waconda Bay[b] water 3 with 1 wt% sediment	*	--	18
Searsville Pond[c] water	*	33	16
Searsville Pond[c] water		40	8

[a] TNT in solutions marked with an asterisk were dissolved in dimethylsulfoxide (DMSO) before addition to the water.

[b] Waconda Bay organisms have been previously exposed to TNT.

[c] Searsville Pond organisms have not been previously exposed to TNT.

biodegrade 100 mg/L TNT in a mineral-salts medium. In all cases, biotransformation was complete within 6 d. When the TNT-reducing organisms were isolated, the majority were "pseudomonas-like". Likewise, Kayser et al. (1977) found that organisms in local sewage sludge and "a pseudomonas strain isolated from the TNT contaminated streams at NAD, McAlester" were equivalent in their ability to reduce TNT in aqueous solution when supplemented with corn-steep nutrient.

Biotransformation products and rates are also affected by the presence of oxygen in the medium. Kayser et al. (1977) measured the loss of TNT in aerobic and anaerobic media supplemented with "corn-steep liquor". After 24 h, the aerobic sample lost 99.5% of the TNT. In the anaerobic medium, only 69% of the TNT disappeared. The anaerobic products contained three times as many amines as the aerobic products. McCormick et al. (1976) used five bacterial strains to study the effects of aerobic vs. anaerobic conditions, as well as cell growth phase, on biotransformation. A cell-free extract of three

anaerobic bacteria (C. pasteurianum, V. alkalescens, and E. coli (anaerobic)) reduced all three nitro groups of TNT to amines in a hydrogen atmosphere. When resting cells were used under the same conditions, C. pasteurianum and V. alkalescens again reduced TNT to its triamino analogue, whereas E. coli (anaerobic) reduced TNT only to the diamino compound. On the other hand, the two aerobic bacteria (E. coli (aerobic) and Pseudomonad FR2) reduced TNT no further than the diaminonitrotoluenes. They also observed that the dimer, 4,4'-azoxy was produced more frequently by the aerobic bacteria, even when grown in a hydrogen atmosphere.

The microbial degradation of TNT is directly affected by the pH of the medium. Kayser et al. (1977) found 99% of the TNT in batch-type experiments (using sewage sludge supplemented with nutrients) disappeared at pH 7.4 to 7.8, but only 45% at pH 6.0 to 6.2. Naumova et al. (1983) found a larger percentage of 2-amino-4,6-DNT in cell cultures that had been treated with TNT at a pH of 5.5 than at a pH of 7.8. With Ps. denitrificans, however, the effect of a pH increase from 5.5 to 7.8 was slight; the ratio of 2-amino-4,6-DNT to 4-amino-2,6-DNT changed from 3.9 to 3.2; whereas with E. coli, the ratio changed from 4.4 to 1.9.

Other researchers have reported different ratios of the bioreduction products. Parrish (1977) studied 190 fungal organisms for their ability to transform TNT and observed reduction solely in the 4-position of the benzene ring. Products included 4-amino-2,6-DNT, 4-hydroxyamino-2,6-DNT, and 4,4'-azoxy. Kayser et al. (1977) found unequal amounts of the amino-DNT isomers in their oxidation ditch facility using sludge microorganisms and corn-steep liquor (the 4-amino-2,6-DNT to 2-amino-4,6-DNT ratio was 8.3). On the other hand, Won et al. (1974) did not detect 4-amino-2,6-DNT in cultures of "pseudomonas-like organisms" used to degrade TNT. They did detect the 4,4'-azoxy dimer, presumably formed from the precursor to 4-amino-2,6-DNT. Naumova et al. (1983) did not measure the 4,4'-azoxy dimer in their cell cultures, but found that the ratio of the 2-amino-4,6-DNT to 4-amino-2,6-DNT varied from 5 to 1 in the exponential growth phase to nearly 2 to 1 in the stationary and drying-off phases. Because the 2- and 6- positions of TNT are equivalent, a random reduction would result in production of twice as much 2-amino-4,6-DNT as 4-amino-2,6-DNT (see Fig. 3-3). Their measurements were

made after 2 h growth with large bacterial populations of Pseudomonas denitrificans and Escherichia coli. In an earlier study, Naumova et al. (1982) found that the 2-amino isomer predominates in the bioconversion of TNT by fungi and yeasts as well.

Kaplan and Kaplan (1982a) investigated the bioreduction of TNT in compost at 55°C by thermophilic bacteria, Bacillus stearothermophilus Donle, B. subtilis Cohn, and B. coagulans Hammer, and a fungus, hyphomycete, Thermomyces languinosa Tsiklinslaya. Thermophilic biotransformation of TNT produced 2-amino-4,6-DNT, 4-amino-2,6-DNT, and 2,4'-azoxy compound. Five mesophilic bacteria used in a study by McCormick et al. (1976) produced the 4,4'-azoxy and 2,2'-azoxy isomers as well as 4-amino-2,6-DNT and 2,4-DANT.

The basic biotransformation pathway for TNT includes the reduction of a nitro group to a hydroxylamine, followed by either dimerization or further reduction. McCormick et al. (1976) measured the uptake of three molecules of hydrogen for the reduction of a nitro group to an amine as shown in Fig. 3-4. The complete sequence is shown in Fig. 3-3.

Under environmental conditions, mineralization of TNT is not expected to occur. Microbes degrade products in which the aromatic ring has intermediates containing hydroxyl groups ortho or para to each other (Dagley, 1975). In TNT, a dihydroxy intermediate could be produced by reduction of two nitro groups to amines, with subsequent substitution of the amines by hydroxyl ions. However, the dihydroxybenzene produced would have the hydroxy groups meta to each other instead of ortho or para, and hence would be unreactive.

Only very slight mineralization of TNT (0.4%) occurred when Kayser et al. (1977) measured ^{14}C-CO_2 produced in a composting experiment with corn-steep nutrients and sludge microorganisms. Likewise, Carpenter et al. (1978) found less than 0.5% ^{14}C-CO_2 in an activated sludge system after 3 to 5 d in an aerated reactor. Isbister et al. (1984) reported no CO_2 from TNT degradation in compost. Even when 1000 ppm yeast extract was grown with 10 ppm of radiolabeled TNT, less than 0.5% of the initial ^{14}C was present in CO_2 (Spanggord et al. (1980b). Only one study, Traxler (1976), reported that large inocula of bacterial cells from two unspecified sources metabolized ^{14}C-TNT to CO_2.

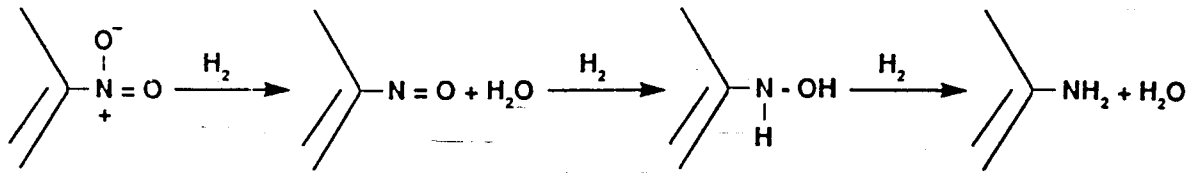


Figure 3-4. Reduction of a nitro group to an amine (adapted from Spanggord, 1980a).

In summary, many yeasts, fungi, and bacteria reduce the nitro groups of TNT. Products include dimers of reduction intermediates and mono-, di- and triamines. It appears that the 4-nitro group is attacked most readily, but it dimerizes in preference to reduction to the amine. Aerobic conditions produce mono- and diamines, whereas an anaerobic medium may produce triamines as well. At low pH's some bacteria produce more 2-amino-4,6-DNT than the 4-amino isomer. No cleavage of the aromatic ring is expected from microbial action. The maximum influence on reaction rates was observed by adding nutrients so that co-metabolism could result. The presence of oxygen and alkalinity increase the microbial reduction rate only slightly. Environmental half-lives for microbial reduction of TNT in surface water and possibly moist soils are expected to be from one week to a month with a possible lag time of up to a month (see Table 3-7).

Compound and Complex Formation

Although the microbial transformation products of TNT are not mineralized in natural waters, they do appear to degrade. McCormick (private communication, 1986) suggests that microbial reduction of TNT produces highly substituted anilines or their precursors, which subsequently react with carboxyl groups of humic acids, lipids, or proteins to form insoluble precipitates. In research that supports this process, Kaplan and Kaplan (1983) performed tests of the binding of humic acids with TNT and two amino

reduction products, namely, 2,4-dinitroaniline (2,4-DNA) and 2,6-dinitroaniline (2,6-DNA). They concluded that TNT itself did not bind to the humic acids, but the two amines bound readily. The binding reactions were enhanced at increased acidity (i.e., pH 4), where the amines were also less stable. The fate or toxicity of the complexes is unknown.

Carpenter et al. (1978) provided additional evidence for complex formation in their study of the microbial degradation of ^{14}C-TNT in an activated-sludge system. They found that the ^{14}C activity in the lipid and protein fractions of the product was associated with insoluble precipitates. Infrared spectra indicated that the precipitates were polyamide-type macromolecules that were resistant to further biodegradation. Virtually all of the radioactivity was contained in the sludge pellet after centrifuging. Several other composting experiments have shown that the TNT microbial products are nonextractable from the organic compost material. For example, Kaplan and Kaplan (1982a) studied TNT degradation in compost and found progressively more TNT degradation products were bound to the organic matter after the compost had been cured and stabilized. Likewise, Isbister et al. (1984) found that ^{14}C-TNT was rapidly transformed into humus-like materials (1/2 of the ^{14}C-TNT in compost was not extractable after 3 wk). They found increasing concentrations of [^{14}C] in the compost solids. After 6 weeks, they could extract only an average of 19.7% of the label with benzene/methanol and benzene; in contrast, 66.5% and the [^{14}C] was in the compost solids (only 86.9% of the label was recovered). Hoffsommer et al. (1978) performed experiments in an "oxidation ditch facility" to "find an efficient biological process for destroying TNT in waste water." They suggested that TNT is absorbed into the bacterial floc, where it is reduced to its amino analogues. The bacteria subsequently die and the amines are slowly extracted, or the remaining nitro groups are reduced further by other microorganisms. Their paper describes other experiments that implied that the amines continued to degrade to polar compounds. Using a closed system, they added ^{14}C-TNT to nutrient- and microorganism-rich water. After three days, aminodinitrotoluenes accounted for 26% of the ^{14}C activity in the aqueous phase and 8% of the activity in or on the bacterial floc. After 30 d, amines only accounted for 0.28% of the activity in the supernatant and 2.8% in the floc. When both the floc and the supernatant were extracted with benzene after 3 and 30 d, the total nonextractable ^{14}C increased from 50.5 to 90.8% (clearly some complexes or compounds were formed). The latter figure included 33.2% in the

supernatant (probably polar substances) and 57.6% in or on the floc. Similarly, Spanggord et al. (1980b) recovered only 30 to 40% of ^{14}C-TNT after 240 h from unsterilized, unpreserved sediment, while measuring sediment/water partition coefficients. They suggested that their low recovery might result from biodegradation, irreversible sorption, or reactions with the sediment. When the sediment was autoclaved and $HgCl_2$ was added, ^{14}C-TNT recovery was increased to 90%.

Hydrolysis

Hydrolysis is not expected to be an environmental degradation pathway of TNT. Although hydrolysis of TNT does occur at high pH (Urbanski, 1986a), it does not occur in seawater that has a pH of approximately 8 (Hoffsommer and Rosen, 1973).

TOXIC EFFECTS ON ANIMALS AND HUMANS

Several literature reviews have dealt with the toxic effects of TNT. Included among these reviews are Hathaway (1977), Zakhari et al. (1978), and Ryon et al. (1984). The primary intent of this assessment is to discuss the salient toxicological properties of TNT and to assess dose-response data available for determining dose rates that would safeguard the health of individuals exposed to contaminated foods, soils, etc. In the first part of our assessment we examine pertinent studies dealing with toxicokinetics, followed by a review of toxic effects to major organs and systems. Subsequent sections discuss the teratogenicity, genotoxicity, and carcinogenicity of TNT. The final subsection includes an analysis of dose-response data for quantifying safe intakes.

Toxicokinetics

TNT metabolism has been studied by a number of investigators including Putnam and Herman (1919), Voegtlin et al. (1921a, 1921b), Lee et al. (1975), Hodgson et al. (1977), and El-hawari et al. (1978). Information is available on its absorption, rate of excretion and pathways of metabolism. In addition, many of the TNT metabolites have been identified.

Absorption. TNT, like other nitroaromatics, can be absorbed through the skin, lungs, and gastrointestinal tract (Zakhari et al., 1978). While many studies have been performed to elucidate the toxic characteristics of TNT in humans and animals, few studies have quantified the rate or extent of absorption. Of the studies completed, the work of Neal et al. (1944a, 1944b), Haythorn (1920) and El-hawari et al. (1978) give the most detailed information on absorption of TNT following oral or dermal exposure. A number of occupational health researchers (Stewart et al., 1945; El Ghawabi et al., 1974; Morton et al., 1976; Buck and Wilson, 1975) have studied the absorption of TNT vapor and dust.

Dermal absorption has been identified as an important pathway of TNT uptake (Putnam and Herman, 1919; Neal et al., 1944a, 1944b). In an early study, Neal et al. (1944b) examined the dermal absorption of TNT by pigs. A sample of the TNT (suspended in glycerol) was applied to the backs of two animals. Small quantities of TNT metabolites (e.g., 4-amino-2,6-DNT) were detected in the urine for up to eight days following exposure. The amount of 4-amino-2,6-DNT ranged from 0.002 to 0.014 mg per 80 mL of urine. El-hawari et al. (1978) studied the absorption of TNT in rats after dermal or oral administration of ^{14}C-TNT. After the dermal application, 21.8% of the applied radiolabel was recovered from the urine, feces, and gastrointestinal tract of the treated rats within 24 h. In contrast, approximately 80% of the radiolabel was recovered after oral administration. Neal et al. (1944a) rubbed 500 mg of TNT into the palms of two human subjects, which were subsequently covered for eight hours. Urine samples from these two individuals contained 4-amino-2,6-DNT up to 15 h after the exposure to TNT.

TNT is soluble in saliva and gastric juices (Haythorn, 1920), and TNT dust that has collected in the posterior nasopharynx can be swallowed. Zakhari et al. (1978) reviewed data on occupational exposures to TNT and noted that TNT workers have complained of a bitter taste in their mouths. Horecker and Snyder (1944) administered oral doses of TNT at 1 mg/kg-d to two human subjects on four successive days. Blood samples were collected by venipuncture daily just before ingestion of TNT so that each blood sample was taken 24 hours after the last TNT dose. Each blood sample was analyzed for 4-amino-2,6-DNT and 2,4-diamino-6-nitrotoluene (2,4-amino-6-NT). These metabolites amounted to about 3% of the TNT ingested.

Metabolism. TNT is metabolized by a complex sequence of oxidative and reductive reactions. The metabolic reduction of TNT entails a series of steps in which one or more of the nitro groups are first reduced to hydroxylamines (such as 2-hydroxylamino-4,6-DNT and 4-hydroxylamino-2,6-DNT) and eventually to amino compounds (2,6-diamino-4-NT and 2,4-diamino-6-NT). Alternatively, the methyl group of TNT can be oxidized to an aminodinitrobenzyl alcohol (4-amino-2,6-dinitrobenzyl alcohol, and 2-amino-4,6-dinitrobenzyl alcohol) or to an aminodinitrobenzoic acid (2-amino- 4,6 dinitrobenzoic acid, and 4-amino-2,6-dinitrobenzoic acid) (see Channon et al., 1944; Yinon and Hwang, 1984). Approximately half of the metabolized TNT is excreted in the urine as glucuronides (Channon et al., 1944). El-Hawari et al. (1978) have also identified glucuronide conjugates in the urine of rats exposed to TNT. These glucuronides have not been characterized. However, since glucuronic acid typically reacts with an alcohol or carboxyl moiety, it has been suggested that glucuronides are formed after TNT has been oxidized to trinitrobenzyl alcohol or aminodinitrobenzyl alcohol (Dacre and Rosenblatt, 1974).

Two azoxy compounds produced in the metabolic reduction of TNT have been identified as 4,4'-azoxy and 2,2'-azoxy (Channon et al., 1944; Lemberg and Callaghan, 1945a). The isolation of 1,3-diamino-5-nitrobenzene (1,3-diamino-5-NB) as a metabolite of TNT has generated considerable interest, as it represents one of the few instances in which a methyl group is known to be removed from a benzene ring by metabolism. It has been proposed that this metabolite is formed by the oxidation of trinitrobenzyl alcohol to trinitrobenzoic acid. The acid is thought to undergo decarboxylation and reduction to form 1,3-diamino-5-NB (Lemberg and Callaghan, 1944, 1945a).

TNT metabolites have been identified in urine from humans as well as from monkeys, rats, rabbits and dogs (Lemberg and Callaghan, 1944, 1945a; Channon et al., 1944; Snyder, 1946; El-hawari et al., 1978; Yinon and Hwang, 1985). The data of Lemberg and Callaghan (1944, 1945a) indicate that the relative importance of a specific metabolic pathway may differ between species. They identified 4-amino-2,6-DNT, 2-amino-4,6-DNT, and 4-hydroxylamino-2,6-DNT as the major urinary metabolites of TNT in humans. Minor metabolites were 2,4-diamino-6-NT, 1,3-diamino-5-NB, and 4-amino-3-hydroxyl-2,6-DNT. In contrast, the major metabolites of TNT recovered from the urine of rats were 2,4-diamino-6-NT and 1,3-diamino-5-NB. However, Channon et al. (1944)

isolated 4-DHA-2,6-DNT, 4-amino-2,6-DNT, and 2-amino-4,6-DNT as the sole metabolites of TNT from the urine of rabbits.

Distribution and excretion. No appreciable accumulation of TNT has been observed in the organs of laboratory animals. Hodgson et al. (1977) studied the distribution of radioactivity after ^{14}C-TNT was administered to rats. The liver, kidney, and blood all contained only small amounts of radioactivity. Most of the radiolabel was excreted in the urine, although 19 to 27% of the radiolabel was recovered in the bile. Expired air did not contain any radioactivity. El-hawari et al. (1978) reported large amounts of glucuronides in the urine and bile of TNT-dosed rats. Rats without a bile cannula excreted most of the dose in urine, which indicates that TNT metabolism involves enterohepatic circulation. Lemberg and Callaghan (1945b) found that 20% of a single oral dose of TNT was excreted in the urine of rats as azo and azoxy compounds. They also found that human subjects excreted an average of 40% of small oral doses of TNT (10 to 30 mg) as aromatic amino compounds in the urine. Lee et al. (1975) studied the distribution and excretion of radiolabel after oral administration of ^{14}C-TNT to rats. They found that 26% of the radioactivity was present in the gastrointestinal tract 24 h after dosing, while 53.3% of the administered dose was excreted in the urine. Small amounts of radioactivity were found in blood, liver, and skeletal muscle. Twenty four hours after dosing, the tissue/plasma ratios of radioactivity were 18.1 in the liver and 7.4 for the kidneys. After 5 d the tissue/plasma ratios of radioactivity increased to 30.3 for the liver and 19.9 for the kidneys. El-hawari et al. (1978) studied the excretion of radioactivity in rats after they were given a single oral or dermal dose of 50 mg/kg TNT. Although the total amount of radiolabel recovered as excreta depended on the route of administration (and on differences in the total amount of TNT absorbed), the majority of radioactivity was excreted in the urine (17 to 60%). Between 1 and 11% of the radiolabel was excreted in the feces.

Toxic Effects on Organs and Systems

TNT causes hepatotoxic and hematotoxic responses in man and laboratory animals. The toxic effects of TNT in humans have been documented since large-scale production of TNT began during WW I (McConnell et al. 1946a),

although most of the reports lack quantitative exposure and dose-response data. No attempts were made in early occupational health studies to account for the effects of other toxic substances that may have been present in the workplace. Many TNT-worker deaths occurred during WW I due to toxic hepatitis and aplastic anemia. Additionally, McConnell et al. (1946b) compiled summary listings of thousands of cases of occupational TNT intoxication. Most of these non-fatal poisonings occurred during WW I and WW II. Numerous symptoms of toxicity have resulted from occupational exposures at atmospheric concentrations that were measured at less than 1.5 mg/m^3 (see El-Ghawabi et al., 1974, and Hathaway, 1977). Mild irritative symptoms included nasal discomfort, sneezing, sore throat, epistaxis, coryza, erythema, rashes, skin eruptions, gastritis, nausea, and vomiting. Symptoms of severe toxicity that have been attributed to TNT exposure include cyanosis, toxic jaundice, aplastic anemia, cataracts, menstrual disorders and numerous neurological manifestations.

Hematological effects. Acute TNT intoxication is generally characterized by methemoglobinemia, red blood cell (RBC) hemolysis, a decrease in the RBC count, and a decrease in the hemoglobin content of RBCs. Zakhari et al. (1978) suggested that these toxic effects may be caused by hydroxylamine metabolites of TNT. Channon et al. (1944) stated that it is difficult to quantify the extent of methemoglobin formation and hemolysis because hematological damage is often masked by the compensatory response of the hematopoietic system. However, aplastic anemia has been documented by Crawford (1954) in a number of fatal TNT intoxications.

El Ghawabi et al. (1974) studied TNT toxicity in 38 exposed munitions workers and 20 unexposed controls. Complete blood and liver examinations were performed. Because the mean exposure levels varied from 0.2 to 7.5 mg/m^3, depending on the work area, and because toxicity data were not separated on the basis of exposure concentration, it is difficult to use these data to assess minimum toxicity levels. The exposed group had a higher number of upper respiratory complaints (sneezing, sore throat, and coughing) and gastrointestinal ailments (stomach ache, anorexia, constipation, flatulence, nausea and vomiting) than unexposed workers. Although the data are suggestive of TNT-related toxicity, the workers were also exposed to dust and other throat irritants. Exposed workers had significantly lower ($p < 0.05$) hemoglobin levels than controls.

Hamilton (1946) (as cited in Zakhari et al. 1978) noted that the earliest clinical evidence of TNT intoxication is usually an increase in the mononuclear leukocyte count. However, El Ghawabi et al. (1974) found no consistent effects on white blood cell counts from occupationally exposed individuals. Cone (1944) stated that about 60% of the TNT workers he studied had shown an early transitory leukocytosis and/or erythrocytosis that was probably due to initial stimulation of the hematopoietic system.

Morton et al. (1976) conducted a study to assess the effectiveness of controls to reduce TNT exposures at an ammunition plant. Unfortunately, engineering efforts to decrease exposure were implemented at the ammunition plant just before the study began. The only pre-study data showed an eight-hour, time-weighted average atmospheric concentration of 0.35 mg/m^3 TNT. At this level, several employees complained of altered taste perception, respiratory difficulties, dermatitis, and anemia. Testing was carried out on 43 employees who worked eight-hour shifts at the TNT fill and pack line. The hemoglobin levels of exposed workers were lower than preemployment levels.

Buck and Wilson (1975) evaluated the effects of TNT exposures by comparing blood-chemistry data on 533 employees exposed to TNT with similar data on 865 workers who were not exposed. At levels well below the threshold limit value of 1.5 mg/m^3, significant effects on the hematological system were documented. For example, abnormal hemoglobin levels ($p < 0.0005$) and reticulocyte counts ($p < 0.05$) were noted in workers exposed to less than 0.5 mg/m^3 TNT concentration.

Stewart et al. (1945) found slight decreases in the RBC count, hemoglobin level, and hematocrit, in workers at munitions plants who were exposed to TNT. No statistical analysis of data was reported. They also found an elevation in indirect-reacting bilirubin in groups of workers exposed to concentrations of TNT between 0.3 to 1.3 mg/m^3. Additionally, a strong correlation between reticulocytosis and TNT exposure was reported. The marked increases in bilirubin concentration and reticulocytosis are indicative of hemolytic anemia.

Adverse hematologic effects have also been documented in laboratory animals exposed to TNT. For example, Furedi et al. (1984a) examined the chronic toxicity of TNT to Fischer-344 rats. Animals were fed TNT in the diet at 0.4, 2.0, 10.0, or 50.0 mg/kg-d for two years. At the 10.0 mg/kg-d dose level, anemia and methemoglobinemia were observed. Levine et al. (1984) detected dose-related splenomegaly with congestion during a 13-week subchronic

TNT feeding study in rats. Dose rates of 125 and 300 mg/kg-d TNT caused a significant ($p < 0.05$) increase in the incidence of splenomegaly compared with controls. Anemia was also observed in treated animals. Splenomegaly has been reported in dogs given 32 mg/kg-d of TNT for 26 wk (Levine et al., 1983a).

Hepatotoxic effects. There are many laboratory tests that have been used to detect the hepatic effects of TNT. Analyses of serum glutamic-oxaloacetic transaminase (SGOT) and lactate dehydrogenase (LDH) levels have been widely used, although there are no tests that specifically measure TNT-induced hepatic toxicity. El Ghawabi et al. (1974) reported that hepatic toxicity appears to be due to a combination of toxic metabolites and red blood cell debris, which makes quantification of toxic effects difficult. In fact, El-Ghawabi et al. (1974) did not find any significant differences in the results of liver-function tests among workers exposed to TNT compared to unexposed controls. Buck and Wilson (1975) found no abnormalities in liver function, renal function, carbohydrate metabolism, or in the immune systems of 626 workers exposed to TNT. Morton et al. (1976) found a direct correlation between an increase in exposure to TNT and an increase in LDH and SGOT levels among munitions workers. Elevated levels of LDH and SGOT were observed at concentrations of TNT between 0.3 and 0.8 mg/mg^3.

Levine et al. (1984) reported dose-dependent hepatomegaly and hepatocytomegaly in rats that were fed 125 or 300 mg/kg-d TNT in their diet over a 13-wk period. Furedi et al. (1984a) found significant (p value not specified) increases in the liver weight of male rats after 24 months of dietary dosing with 10 mg/kg-d of TNT. Dietary administration of TNT (0.5 or 2.0 mg/kg-d) has been associated with the development of hepatic hypertrophy in dogs (Levine et al., 1983a).

Ocular effects. Zakhari et al. (1978) cited several studies that implicated TNT in the development of cataracts among exposed individuals. However, El-Ghawabi et al. (1974) did not observe any cataracts among a group of 38 workers exposed to TNT.

Dermal effects. TNT-induced dermatitis has been documented in munitions workers involved in all aspects of TNT production (Schwartz, 1944; Zakhari et al., 1978). Dermatitis most commonly develops on areas of exposed skin

such as the hands, wrists, and forearms. Although dermatitis usually remains localized, Schwartz (1941) noted that generalized dermatitis occasionally develops.

Reproductive effects. Testicular atrophy with degeneration of the seminiferous tubular epithelium was seen in rats who were fed TNT in the diet at 125 or 300 mg/kg-d for 13 weeks (Levine et al., 1981).

Teratogenicity

No studies have been reported in the literature on the teratogenicity of TNT.

Genotoxicity

TNT has been studied for its ability to induce reverse mutations in bacteria (Won et al., 1976; Ellis et al., 1978; Spanggord et al., 1982b; Kaplan and Kaplan, 1982b; Whong and Edwards, 1984), forward mutations in mouse lymphoma cells (Styles and Cross, 1983), DNA damage in rat hepatocytes (Ashby et al., 1985), micronuclei in mouse erythrocytes (Ashby et al., 1985), and chromosomal aberrations in rat bone marrow cells (Geshev and Kincheva, 1974 as cited in Zakhari et al., 1978). Table 3-8 summarizes the principal findings of these studies.

TNT has given consistently positive results in the Ames assay with Salmonella typhimurium, strains TA98, TA100, TA1537, and TA1538. TNT has been inactive in strain TA1535. In general, TNT has not been mutagenic to S. typhimurium in the presence of the exogenous metabolic activator S9 (Won et al., 1976; Kaplan and Kaplan, 1982b; Whong and Edwards, 1984). However, Ellis et al. (1978) and Spanggord et al. (1982b) reported that TNT was mutagenic to S. typhimurium regardless of the presence or absence of S9.

Geshev (1974, as cited in Zakhari et al., 1978) applied a 30% solution of TNT to the skin of rats 5 d/wk for six months. Bone marrow cells isolated from approximately 50% of these animals displayed chromosomal abnormalities. These abnormalities included chromosomal breaks, translocations, and exchanges of chromatin. TNT induced forward mutations in mouse lymphoma cells in the

Table 3-8. Overview of studies dealing with the genotoxicity of TNT.

Test system	Dose or concentration	Results	Reference
S. typhimurium plate test +/-S9	110-880 nmol/plate	Positive in TA100, TA1537, TA1538, TA98 (-S9)	Whong and Edwards, 1984
S. typhimurium plate test +/-S9	0.5-10 µg/mL	Positive with TA98 (-S9)	Won et al., 1976
S. typhimurium plate test	10-5000 µg/plate	Positive in TA1537, TA1538, TA98, TA100, (+/-S9)	Spanggord et al., 1982b
S. typhimurium plate test +/-S9	0.5-500 µg/plate	Positive with strains TA1538, TA98, TA100 (-S9)	Kaplan and Kaplan, 1982b
S. typhimurium plate test +/-S9	10-1000 µg/plate	Positive with strains TA1538, TA1537, TA98, TA100 (+/-S9)	Ellis et al., 1978

Table 3-8. (Continued)

Test system	Dose or concentration	Results	Reference
Mouse lymphoma cells TK +/-S9	1.6-200.0 μg/mL	Positive (-S9)	Styles and Cross, 1983
Mouse micronucleus test	40.0, 80.0 mg/kg	Negative	Ashby *et al.*,1985
DNA repair; rat hepatocytes	100.0, 200.0 500.0, 1000.0 mg/kg	Negative	Ashby *et al.*, 1985
Chromosome aberration, rat bone marrow cells	30% (dermal application)	Positive	Geshev and Kincheva, 1974

absence of S9. This response was not seen when S9 was added to cell cultures (Styles and Cross, 1983). Ashby et al. (1985) evaluated the ability of TNT to damage DNA in rat hepatocytes (unscheduled DNA synthesis). They found no evidence that TNT induced DNA repair. TNT also gave negative results in the mouse micronucleus test (Ashby et al., 1985).

Carcinogenicity in Animals

The first long-term animal studies designed to detect carcinogenic responses in animals administered TNT in their diet were performed by Furedi et al. (1984a, 1984b). These researchers observed statistically significant increases in the incidence of malignant tumors in rats and mice in two different tissue sites at the highest treatment levels used in the studies. Toxic effects were noted at the dose levels at which the rodents developed malignant tumors; however, no significant differences were observed in survival times of dosed animals and controls.

Carcinogenicity in rats: Furedi et al. (1984a). In this 24-month chronic toxicity study, Fischer-344 rats received TNT in their feed at five dosing levels (i.e., 0.0, 0.4, 2.0, 10.0, and 50.0 mg/kg-d). Each treatment group consisted of 150 rats (75 male and 75 female). The animals were quarantined for 12 days prior to the beginning of the study, and unhealthy rats were eliminated. Healthy rats were assigned to the five treatment groups by a stratified randomization procedure (blocked by body weight). After the treatment group assignments had been completed, all the rats were marked with an identification number. The rats were randomly assigned to cages without regard to dose level or sex. Body weights at the start of the experiment ranged from 53 to 168 g for males and 73 to 128 g for females. Animals were six to seven weeks old at the beginning of treatment.

The TNT used in the bioassay was obtained from an ordnance plant. Military grade TNT contains several impurities (see Table 2-1), and consequently we designate it as TNT(m). The concentration of TNT(m) in the feed given to the animals was calculated to be within 98% of prescribed values for all treatment groups over the duration of the experiment. However, homogeneity of TNT(m) in the rat meal was tested and found by the researchers to be unsatisfactory in some of the test diets at the low dose levels, and

during the period between weeks 36 and 54. Additionally, a slight decrease in TNT(m) concentration was noted in the rat meal a week after it had been placed in the rat cages. Volatility of TNT(m) was cited as a possible explanation; however, this seems unlikely because its vapor pressure at 20°C is about 1.1×10^{-6} torr. The researchers also noted a dose-related decrease in food intake by rats at the 10 and 50 mg/kg-doses. Fluctuation in food consumption was observed in both male and female rats at all dosing levels. No relationship between TNT(m) dose rate and food consumption rates could be found, and therefore the researchers judged this effect to be unrelated to the TNT(m) treatment. No mention was made of how much of the daily diet was actually consumed and how much was wasted.

When any rats died or were sacrificed, the brain, spinal cord, pituitary, spleen, kidneys, heart, liver, and gonads, as well as any gross lesions, were examined histologically. In female rats, the urinary bladder and bone marrow were also examined at all dose levels. During the studies, interim sacrifices of ten animals randomly taken from each treatment group were made at six months and again at 12 months. The few animals that were moribund or died before the completion of the study were also thoroughly examined. No evidence of neoplastic growth was found in any of the animals that were sacrificed or died prematurely. The Fischer-344 rats that were sacrificed at the completion of the study were examined both for the presence of grossly visible lesions and for microscopic abnormalities. The results of these examinations (female animals only) are summarized in Table 3-9. The incidence of each lesion was evaluated statistically. The numbers of treated and control animals with a specific lesion were analyzed by either a chi-square test or a Fisher exact test. The Fisher exact test was used when the expected value of any cell was less than or equal to five.

Male rats dosed at 10.0 and 50.0 mg/kg-d exhibited a dose-related, statistically significant ($p < 0.01$) increase in the incidence of hepatocellular hyperplasia. Significant ($p < 0.01$) increases in the incidence of hyperplastic change were detected in the renal pelvi of females at the highest TNT(m) dosing level. Hyperplasia was also found in the mucosal epithelium of the urinary bladders of female rats. This response was significant ($p < 0.01$) in the high-dose group (50 mg/kg). Papillomas were found in the urinary bladders of female rats, though not at a statistically significant level.

Table 3-9. Incidence of tumors in female rats and mice after two years of consuming feed containing TNT(m). Data are from Furedi *et al*. (1984a, 1984b).

Species	Location of tumor	Dose rate (mg/kg-d)	Incidence
F-344 Rat	Urinary bladder (papilloma)	0.0	0/54
		0.4	0/54
		2.0	0/55
		10.0	1/55
		50.0	5/55[a]
F-344 Rat	Urinary bladder (carcinoma)	0.0	0/54
		0.4	0/54
		2.0	0/55
		10.0	0/55
		50.0	12/55[b]
F-344 Rat	Urinary bladder (papilloma or carcinoma)	0.0	0/54
		0.4	0/54
		2.0	0/55
		10.0	1/55
		50.0	17/55[b]
B6C3F1 Mice	Spleen (leukemia or malignant lymphoma)	0.0	9/54
		1.5	15/54
		10.0	17/54
		70.0	21/54[c]

[a] $p = 0.05$, Fisher exact test.

[b] $p < 0.01$, chi-square test ($p = 0.00014$, Fisher exact test for 0/54 and 12/55 and $p = 0.000002$, Fisher exact test for 0/54 and 17/55).

[c] $p < 0.05$, chi-square test ($p = 0.0024$, Fisher exact test for 9/54 and 21/54).

A significant incidence ($p < 0.01$) of urinary bladder carcinoma was observed in female Fischer-344 rats. It has been recognized that chemicals that induce benign tumors may also induce malignant tumors of a histologically related type. One established protocol of carcinogen risk assessment is to combine the incidence of benign tumors with the incidence of malignant tumors (U.S. EPA, 1986). Both the incidence of urinary bladder carcinomas alone and the combined incidence of urinary bladder papillomas and urinary bladder carcinomas in female rats were significant ($p < 0.01$) only at the highest dose level.

Carcinogenicity in mice: Furedi et al. (1984b). This 24-month study consisted of B6C3F1 mice that received TNT(m) in their feed. Each treatment group had 75 males and 75 females. The daily dose rates for the treatments were 0.0, 1.5, 10.0 and 70.0 mg/kg-d. One week prior to the initiation of treatment, when the mice were six- to seven-weeks old, body weight ranged from 20.8 to 26.9 g for males, and from 15.0 to 17.9 g for females. As in the rat study, all mice were marked with identification numbers. Mice were randomly assigned to cages without regard to dose level or sex.

The TNT(m) used in the study was analyzed three times, with purities of 99.43 wt% (± 2.89 wt%), 99.05 wt% (± 4.11 wt%), and 98.86 wt% (± 2.01 wt%). Concentrations of TNT(m) in the mouse feed were within 98% of the intended concentrations. A slight decrease in TNT(m) concentration was measured after the test diets had been in the animal room for one week. The researchers noted that the volatility of TNT(m) might be the reason for this change. A statistically significent ($p < 0.05$) decrease in food consumption by male mice was seen at the beginning of the study at the highest dose level.

Survival times of the mice were not affected by the TNT(m) doses used in this study. Mean survival times of TNT(m)-treated mice were not significantly different ($p < 0.05$) from those of control groups. Scheduled interim sacrifices were made at six and 12 months, and these mice were examined both for gross effects and for microscopic lesions. The mice that died spontaneously or were sacrificed in a moribund condition were also examined for gross and microscopic lesions. No treatment-related lesions were found to be responsible for these deaths. The researchers found that the administration of TNT(m) for up to 12 months did not induce nonneoplastic or neoplastic lesions in either male or female mice. They concluded that death in these cases was apparently due to naturally occurring disease.

Female mice that received 70 mg/kg-d of TNT(m) had a significant incidence ($p < 0.05$) of leukemia and/or malignant lymphoma of the spleen (see Table 3-9). The leukemia was either a granulocytic or lymphocytic type. The malignant lymphoma was histiocytic, lymphocytic, or a mixed type. These lesions were considered by the researchers to be TNT(m) treatment-related. These neoplasias involved other organs and tissues, including adrenals, bone marrow, brain, gastrointestinal tract, eyes, kidneys, liver, lungs, and the following lymph nodes; axillary cervical, hepatic, inguinal lumbar, mandibular, mesenteric, popliteal, renal, and respiratory. The authors

reported that the incidence of combined leukemia/malignant lymphoma in the spleen of females appeared to be dose-related, but this trend was not statistically significant.

Carcinogenicity in Humans

Reviews of the occupational health literature on TNT completed by Zakhari et al. (1978) and Hathaway (1985) focused on health effects (e.g., anemia, hepatitis, etc.) observed in workers exposed to TNT in workplace air. We were unable to find any epidemiological studies that specifically studied the association between TNT exposures and the incidence of cancer among those exposed.

Estimation of Intake Limits to Protect Public Health

The estimation of a safe intake for TNT depends on several factors, including the evidence for its carcinogenicity, assumptions related to the application of the multistage dose-response model to bioassay data to determine cancer potencies, and the possibility that an impurity in TNT (i.e., 2,6-DNT) contributes to or accounts for its carcinogenic potency. To guide risk assessments of substances that are potential carcinogens, the U.S. EPA has promulgated a set of guidelines (U.S. EPA, 1986). According to the guidelines, quantitative risk assessments should be carried out when the "weight of evidence" from human and animal studies indicates that a substance is a potential carcinogen to humans.

"Sufficient" evidence of carcinogenicity in animals is described by the U.S. EPA as evidence "which indicates that there is an increased incidence of malignant tumors or combined malignant and benign tumors: (a) in multiple species or strains; or (b) in multiple experiments (e.g., with different routes of administration or using different dose levels); or (c) to an unusual degree in a single experiment with regard to high incidence, unusual site or tumor type, or early age at onset" (U.S. EPA, 1986). The chronic bioassays completed by Furedi et al. (1984a, 1984b) demonstrated that the TNT(m) administered in the highest doses to rats and mice caused statistically significant increases in papillomas and carcinomas of the urinary bladder in rats, and leukemia and malignant lymphomas in mice (see Table 3-9). Other

evidence regarding the carcinogenic potential of TNT is from short-term bioassays. As shown in Table 3-8, bioassays completed by several researchers have shown a positive mutagenic response. However, Ashby et al. (1985) suggested that TNT is not a genotoxic carcinogen (as indicated by the Salmonella assays) because it tested negative in both a mouse bone-marrow micronucleus test and in a rat liver assay for unscheduled DNA synthesis. They also suggested that TNT is probably a threshold-dependent carcinogen and that the carcinogenic responses observed in rats and mice by Furedi et al. (1984a, 1984b) were associated with TNT's systemic toxicity. One other plausible mechanism is that a reactive intermediate of TNT metabolism is actually the active carcinogenic agent. According to the U.S. EPA guidelines, TNT(m) can be considered a Group B2 agent, which represents a probable human carcinogen. This "Group B2" categorization is intended for chemicals that have sufficient evidence of carcinogenicity from animal bioassays, but have inadequate or no epidemiological evidence.

Based on a B2 designation for TNT(m), we have quantified its carcinogenic potency using the multistage dose-response model of Crump and Watson (1979) with tumor incidence and dose data from the chronic bioassays conducted by Furedi et al. (1984a, 1984b). The potency values are shown in Table 3-10. The associated acceptable dose rates (ADRs) corresponding to different lifetime cancer risks are presented in Table 3-11. An important issue related to the carcinogenic potency of TNT(m) is whether the tumorigenic responses observed in the chronic bioassays were due to impurities in the TNT(m). Of particular concern is the carcinogenic potential of 2,6-DNT (see Section 5 for a discussion of its carcinogenic potential). To determine whether 2,6-DNT could account for the estimated potency of TNT(m), we compared potencies for 2,6-DNT derived from other bioassay data sets (see Appendix C, Table C-1) with potencies calculated from the estimated dose rate of 2,6-DNT as an impurity in TNT(m) administered to rats and mice in the studies by Furedi et al. (1984a, 1984b) (see Appendix C, Table C-2). The potencies in Table C-1 range from 0.19 to 3.4 kg-d/mg, based on a body-weight extrapolation, and the average potency is 1.8 kg-d/mg. The three potency values based on body weight were 0.8, 2.0, and [illegible] kg-d/mg (Table C-2), with an average value of 1.8 kg-d/mg -- identical to the average value derived from other bioassays. Potencies based on a surface-area extrapolation were also comparable, ranging from 1.3 to 35.8 kg-d/mg (Table C-1) for 2,6-DNT in DNT bioassays and 5 to 33 kg-d/mg for

Table 3-10. TNT(m) cancer potencies based on dose-response data from the chronic biossays conducted by Furedi *et al*. (1984a, 1984b).

Species (strain)	Sex and weight (kg)	Total applied dose (mg/kg-d)	2,4-DNT component of TNT dose[a] (mg/kg-d)	2,6-DNT component of TNT dose[a] (mg/kg-d)	Tumor Type	Tumor Incidence[b]	95% UCL potenc.[c] in kg-d/mg(TNT(m)) BW[d]	95% UCL potenc.[c] in kg-d/mg(TNT(m)) SA[e]
Rats (F-344)	F	0.0	0.0	0.0	Urinary	0/54		
	0.29	0.394	0.002	0.001	bladder:	0/54		
		1.970	0.01	0.005	papilloma	0/55	0.0050	0.031
		9.908	0.05	0.025	or	1/55		
		49.245	0.25	0.123	carcinoma	17/55		
Mice (B6C3F1)	F	0.0	0.0	0.0	Spleen:	9/54		
	0.038	1.509	0.0075	0.00375	leukemia	15/54	0.0068	0.081
		10.10	0.05	0.025	or malignant	17/54		
		70.90	0.35	0.175	lymphoma	21/54		
Rats (F-344)	F	0.0	0.0	0.0	Urinary	0/54		
	0.29	0.394	0.002	0.001	bladder:	0/54		
		1.970	0.01	0.005	carcinoma	0/55	0.0020	0.012
		9.908	0.05	0.025		0/55		
		49.245	0.25	0.123		12/55		

[a] Maximum amounts of 2,4-DNT are approximately 0.5 wt% in TNT(m) and 0.25 wt% for 2,6-DNT (see Table 2-1).

[b] These tumor incidences do not include serial sacrifices.

[c] "Potency" means the low-dose dose-response slope expressed by the value of the linear multistage coefficient, q_1, such that at very low doses, risk = q_1 x dose, according to a multistage prediction model (U.S. EPA, 1980; Anderson *et al*., 1983). 95% UCL = one-tailed 95% upper confidence limit.

[d] BW = body weight interspecies dose extrapolation method; doses in mg/kg are assumed to be equipotent.

[e] SA = surface area interspecies dose extrapolation method; doses in $mg/kg^{2/3}$ are assumed to be equipotent.

Table 3-11. Acceptable dose rates for TNT(m) calculated from the potencies presented in Table 3-10.

Species and sex	Tumor type	Lifetime cancer risk 10^{-5}		10^{-6}		10^{-7}	
		BW[a]	SA[b]	BW[c]	SA[b]	BW[a]	SA[b]
		Dose rate in mg/kg-d					
F-344 Rats (female)	Urinary bladder papilloma or carcinoma	2×10^{-3}	3.2×10^{-4}	2×10^{-4}	3.2×10^{-5}	2×10^{-5}	3.2×10^{-6}
B6C3F1 Mice (female)	Spleen leukemia or malignant lymphoma	1.5×10^{-3}	1.2×10^{-4}	1.5×10^{-4}	1.2×10^{-5}	1.5×10^{-5}	1.2×10^{-6}
F-344 Rats (female)	Urinary bladder carcinoma	5×10^{-3}	8×10^{-4}	5×10^{-4}	8×10^{-5}	5×10^{-5}	8×10^{-6}

[a] Extrapolation based on body weight (see Table 3-10).

[b] Extrapolation based on body surface area (see Table 3-10).

2,6-DNT in TNT(m) (Table C-2). We note, however, that the primary tumor site in the laboratory animals administered the dinitrotoluenes was the liver, whereas in the Furedi et al. bioassays the tumors involved the spleen and urinary tract. It is possible that the elevated amount of TNT, compared with the quantity of 2,6-DNT, alters the pharmacokinetics of the mixture such that the site of action for the 2,6-DNT is different.

If TNT(m) actually exerts its carcinogenic effect by an epigenetic mechanism with an effects threshold or if an impurity such as 2,6-DNT actually is the active carcinogenic agent, then a safe intake for TNT can be estimated using a safety factor with a no-observed-effect-level (NOEL) from a suitable animal study (see Appendix D). Fuerdi et al. (1984a) determined a chronic NOEL for rats as 0.4 mg/kg-d; with a safety factor of 100, the safe intake becomes 0.004 mg/kg-d or 4 µg/kg-d. The NOEL for the chronic bioassay with mice was 1.5 mg/kg-d (Furedi et al., 1984b); the associated safe intake is 0.015 mg/kg-d (15 µg/kg-d), calculated with a safety factor of 100.

TOXIC EFFECTS ON PLANTS

Only one study has been reported on the toxic effects of TNT on terrestrial plants. Palazzo and Leggett (1986) studied the effects of TNT on yellow nutsedge grown in a hydroponic solution. Toxic effects were observed at a concentration of 5 mg/L in the hydroponic solution. Root growth was most affected, with root weight reductions of 95%. TNT and its metabolites, 4-amino-2,6-DNT and 2-amino-4,6-DNT, were detected in all parts of the yellow nutsedge plant.

TOXIC EFFECTS ON AQUATIC SPECIES

Studies involving the toxicity of TNT to aquatic species have examined its acute toxicity under both static and dynamic test conditions. Much of the work on the acute toxicity of TNT was conducted by Liu et al. (1976, 1983a, 1983b), who were primarily interested in the aquatic toxicity of wastewaters from the production of TNT. Table 3-12 summarizes the results of their work, as well as that of other researchers. The most sensitive vertebrate species was rainbow trout (Salmo gairdneri), with a 96-h LC50 of 0.8 mg/L. The other species tested (i.e., fathead minnow (Pimephales promelas); bluegill sunfish

Table 3-12. Acute toxicity of TNT on aquatic organisms.

Species	Concentration (mg/L)	Effect	Reference
Aquatic vertebrates			
Fathead minncw	2.0 (1.9 to 2.2)[a]	96-h LC50[b]	Liu et al., 1976
Fathead minnow	3.0 (2.8 to 3.3)	96-h LC50[b]	Liu et al., 1983b
Rainbow trout	0.8 (0.7 to 1.0)	96-h LC50[c]	Liu et al., 1983a
Bluegill sunfish	2.6 (2.3 to 2.9)	96-h LC50[c]	Liu et al., 1983a
Channel catfish	2.4 (2.0 to 2.7)	96-h LC50[d]	Liu et al., 1983a
Fathead minnow	2.9 (2.6 to 3.2)	96-h LC50[c]	Liu et al., 1983a
Fathead minnow	2.58	96-h LC50[c]	Smock et al., 1976
Fathead minnow	0.46	96-h EC50[f]	Smock et al., 1976
Bluegill	2.3 to 2.8	TLm[e]	Pedersen, 1971, cited in Dacre and Rosenblatt, 1974
Aquatic invertebrates			
Water flea	6.6 (4.5 to 9.7)	48-h LC50[c]	Liu et al., 1976
Water flea	11.9 (11.1 to 12.8)	48-h EC50[b]	Liu et al., 1983b
Midge	27.0 (22.0 to 33.0)	48-h LC50[c]	Liu et al., 1983a
Worm	5.2 (4.5 to 6.0)	48-h LC50[c]	Liu et al., 1983a
Scud	6.5 (5.6 to 7.5)	96-h LC50[c]	Liu et al., 1976
Water flea	11.7 (10.9 to 12.6)	96-h LC50[c]	Liu et al., 1983a

a 95% confidence limit in parentheses.
b Static test.
c Dynamic test (water flow-through).
d Dynamic test with aeration.
e Threshold limit for mortality.
f Moribund response.

(Lepomis machrochirus); and channel catfish (Ictalurus punctatus)) had 96-h LC50 values ranging from 2.0 to 3.0 mg/L. TNT was less toxic to the invertebrate species tested (i.e., water flea (Daphnia magna); scud (Hyalella azteca); midge (Tanytarus dissimilis); and worm (Lumbiculus variegatus)). Liu et al. (1983a) also examined the incipient LC50 of selected species. They defined this LC50 as "the concentration above which 50 percent of the test organisms cannot survive indefinitely". The incipient LC50's for the fathead minnow, bluegill sunfish, channel catfish, and rainbow trout ranged from 1.4 to 1.9 mg/L, based on a 14-d test under flow-through conditions. In contrast, the incipient LC50 for the water flea was 0.19 mg/L and the worm tested had an LC50 of 13.7 mg/L.

Bailey et al. (1985) carried out early life-state (ELS) tests with channel catfish, rainbow trout, and fathead minnows, while chronic toxicity studies were conducted with fathead minnows and water fleas. In the ELS tests there were statistically signficant ($p < 0.05$) decreases in average fry length and weight for rainbow trout at concentrations of 0.49 and 0.93 mg/L. The ELS test with channel catfish was inconclusive because of difficulties associated with the separation of eggs, and the ELS test completed with minnows was only a range-finding study, without statistical analyses of the results. In the chronic test with fathead minnows, statistically significant ($p < 0.05$) effects were observed in reproductive parameters (e.g., spawning pair survival, number of spawns per pair, etc.) at concentrations as low as 0.04 mg/L (the lowest test concentration). The chronic tests with water fleas did not demonstrate any significant effects at concentrations ranging from 0.03 to 1.03 mg/L of TNT.

Liu et al. (1983a) also investigated the effects of water temperature, pH, and hardness on the toxicity of TNT. They found that these parameters affected the acute toxicities by less than a factor of two. In addition, they irradiated TNT solutions with UV light to determine the effect of the resulting photoproducts. For both the vertebrate and invertebrates tested, the toxicity of the water decreased after UV treatment.

Finally, Liu et al. (1983a) examined the effect of TNT on the growth of four species of algae (Selenastrum capricornutum, Microcystis aeruginosa, Anabaena flos-aqua, and Navicula pelliculosa). The basic response was growth inhibition at concentrations as low as 4.1 mg/L. Smock et al. (1976) also studied the effect of TNT on S. capricornutum and M. aeruginosa and found that inhibition occurred at concentrations of 5 and 15 mg/L of TNT, respectively.

4. DATA-BASE ASSESSMENT FOR 2,4-DINITROTOLUENE

The 2,4-DNT isomer of dinitrotoluene is present as an impurity in TNT and as the primary component of military-grade DNT, which is used as an ingredient in some propellant formulations. This isomer has been detected in soils at open-burning and open-detonation grounds (see Appendix B). Our analysis of the likely distribution of this compound in environmental media suggests that most of the 2,4-DNT added to surface soils will eventually reside in the vadose zone and ground water. Toxicological studies conducted on 2,4-DNT and 2,6-DNT in recent years indicate that 2,6-DNT probably accounts for the carcinogenic responses observed in chronic animal bioassays with technical-grade DNT (denoted tDNT), which contains primarily 2,4-DNT. 2,4-DNT is apparently not a cancer initiator, but a weak cancer promotor. Because it was not possible to derive a dose-response relationship for this compound, we estimated a safe intake based on a chronic NOEL for noncarcinogenic effects in laboratory animals.

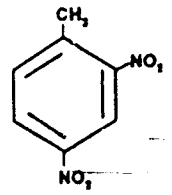
ENVIRONMENTAL PARTITIONING AND TRANSPORT

The chemical identifiers for 2,4-DNT are shown in Table 4-1 and the pertinent physicochemical properties are presented in Table 4-2.

Soil Transport and Adsorption

Spanggord et al. (1980b) studied the adsorption of 2,4-DNT on sediment sterilized with $HgCl_2$ and measured a sediment/water partition coefficient of 12 after 10 d equilibration. They did not state the organic carbon content of the sediment. We estimated a K_{oc} value of 258 from the aqueous solubility and melting point of 2,4-DNT (see Appendix A). If the sediment used by Spanggord et al. had contained between 1 to 3 wt% organic carbon, then the K_d values based on our estimate of K_{oc} would range from 2.6 to 7.7. The higher value of K_d for the sediment could be due to adsorption on clays. Tucker et al. (1985), in an analysis of factors affecting the movement of explosives in soils, noted that diffusion through soil in both vapor and aqueous phases could be an important transport mechanism for DNT's in soils receiving limited water infiltration.

Table 4-1. Chemical identifiers of 2,4-dinitrotoluene.

Empirical formula:	$C_7H_6N_2O_4$	Structural formula:
Molecular weight:	182.14	
CAS reg. no.:	[121-14-2]	
RTECS no.:	XT1575000	

Partition Coefficients: Plant/Soil, Beef-Fat/Diet, and Fish/Water

The only measured values available for the partition coefficients presented in Table 4-3 were for the bioconcentration factor, K_{fw}. Liu *et al.* (1983c) measured the uptake of ^{14}C-2,4-DNT in bluegills, an invertebrate species (*Daphnia magna*), and a green algae (*Selenastrum capricornutum*) over a four-day period. The K_{fw} (calculated as the ratio of residual ^{14}C activity in the organism to the ^{14}C activity in water) for bluegill was 78, 2507 for the algae, and 13 for *Daphnia*. They also conducted a ten-day depuration study and found that essentially all of the radioactivity in the bluegills was gone after approximately four days. Hartley (1981), cited in Etnier (1986), studied uptake by bluegills using ^{14}C-2,4-DNT. The bioconcentration factors ranged from 10.59 for muscle to 102.8 for brain tissue. The whole body factor was 24.8.

Equilibrium Distribution in Reference Landscapes

Table 4-4 presents the fractional distribution of 2,4-DNT among the eight compartments of the reference landscapes for the Western and Southeastern ecoregions. Nearly all the 2,4-DNT added to the surface soils is predicted by the GEOTOX model to distribute to the lower soil zone and to ground water in the two ecoregions. Although the Henry's law constant for 2,4-DNT is higher than TNT's, only a small fraction of the 2,4-DNT added to surface soils is expected to enter the atmosphere. Because 2,4-DNT moves preferentially to the

Table 4-2. Chemical and physical properties of 2,4-DNT. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	300 (decomposes)	Windholz *et al.*, 1983
Freezing/melting point	°C	70	Windholz *et al.*, 1983
Density	g/mL	1.52	Windholz *et al.*, 1983
Vapor pressure	torr	1.3×10^{-4}	Measured value, Pella, 1977
Aqueous solubility	mol/L mg/L	1.5×10^{-3} 273	Windholz *et al.*, 1983
Henry's law constant	(L-torr)/mol	8.7×10^{-2}	Calculated as the ratio of vapor pressure to aqueous solubility.
		0.4[a]	Average of two measured values, Haynes and Smith, 1981.
Diffusion coefficients: Air Water	m^2/s m^2/s	6.4×10^{-6} 6.3×10^{-10}	Estimated[b] Estimated[b]
Log octanol/water partition coefficient (K_{ow})	log K_{ow}	1.98	Hansch and Leo, 1979 Quoted in Nikaitani, D. and Hansel, unpublished

[a] Temperature not given.

[b] See Appendix A.

Table 4-3. Partition coefficients for use in estimating human exposures to 2,4-DNT.

Partition coefficient Value	Parameter Source			Units
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	4	Calculated[b] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	2.7×10^{-3}	Average of values calculated[b] from water solubility and log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	15	Calculated[b] from log K_{ow}
			78	Measured value for bluegill, Liu *et al.*, 1983c
			24.8[c]	Measured value for bluegill, Hartley (1981), cited in Etnier (1986)
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	260	Calculated[b] from solubility in water and melting point
Sediment/water	K_d	(mg/kg sediment)/(mg/kg water)	12	Measured value from Spanggord *et al.*, 1980b

[a] Wet-weights for plant and fish

[b] See Appendix A

[c] Whole body

Table 4-4. Fractional distributions of 2,4-DNT among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of 2,4-DNT to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	2.3×10^{-5}	2.0×10^{-5}
Air particles	6.9×10^{-9}	1.6×10^{-8}
Biota	2.7×10^{-4}	0.026
Upper soil	0.029	0.10
Lower soil	0.43	0.098
Ground water	0.54	0.77
Surface water	0.0019	0.0068
Sediments	1.2×10^{-4}	4.5×10^{-4}

[a] Fractions do not add to one because of rounding.

subsurface, it would be beneficial to make additional measurements on the adsorption of 2,4-DNT to soils and sediments with different clay and organic matter contents.

ENVIRONMENTAL TRANSFORMATION PROCESSES

Photolysis

Spanggord *et al*. (1980b) reported that aqueous solutions of 2,4-DNT absorb light in the near ultraviolet to visible region, with an "absorption coefficient" of 4100 $M^{-1}cm^{-1}$ at 297.5 nm, decreasing to 36 $M^{-1}cm^{-1}$ at 400 nm. They also noted that reaction products enhanced the photolysis of 2,4-DNT. Burlinson and Glover (1977a) found that the photoproducts of 2,4-DNT are analogous to those from TNT.

Spanggord et al. (1980b) measured the initial photolysis rates (prior to rate acceleration) of 2,4-DNT in various waters (see Table 4-5). After the initial photolysis they observed the same rate enhancement due to organic matter as they found for TNT. They also noted that 50% of the 2,4-DNT disappeared in 5 d from a sample of TNT condensate water left in the sun. They also found that anaerobic photolysis was three times faster than aerobic. They calculated a quantum yield of 7.0×10^{-4} at 313 nm for 2,4-DNT in distilled water. This quantum yield was then used to calculate the annual variation of photolysis half-lives in distilled water at several latitudes and seasons. Using the Zepp and Cline (1977) procedure for calculating photolysis rates in aqueous media, and assuming 10 h of sunlight, Spanggord et al. estimated a range of half-lives of 1.8 d for summer at 20°N to 11.5 d for winter at 50°N. Simmons and Zepp (1986) reported rate constants for photolysis of 2,4-DNT in waters containing humic materials (see Table 4-5). Their estimated photolysis rates varied with the quantity, absorbance, and opacity of the humic substances.

Burlinson and Glover (1977a) studied the photolytic transformation of 2,4-DNT in unbuffered solution, using a medium-pressure mercury arc. The pH of the solution dropped from 6.2 to 3.2 during photolysis, and the mixture became a turbid yellow-brown color. They found the photoproducts of 2,4-DNT shown in Table 4-6. These compounds represented 53 wt% of the initial 2,4-DNT.

The mechanism of 2,4-DNT photolysis has not been as rigorously studied as that of TNT; however, Spanggord et al. (1980b) suggest that the mechanism is analogous. Andrews and Osmon (1977) discovered the formation of a highly colored (fluorescent) intermediate in a continuous-flow system and Wettermark and Ricci (1963) and Craig and Atherton (1984) also found the same intermediate in flash-photolysis experiments. The authors of the latter two papers postulated the abstraction of a proton from the methyl group by the ortho-nitro group and subsequent conversion to the anion with resonance stabilization.

Biotransformation

Microorganisms transform 2,4-DNT to products analogous to those of TNT (Parrish, 1977; McCormick et al., 1978); however, 2,4-DNT is biotransformed more slowly and by fewer microorganisms. The aromatic ring in 2,4-DNT has

Table 4-5. Photolysis rates of 2,4-DNT in various waters.

Initial concentration (mg/L)	Water[a]	Rate constant for photolysis (s^{-1})	Ref.
1.0	Holston River	7.4×10^{-5}	Spanggord et al., 1980b
1.0	Searsville Pond	5.2×10^{-5}	Spanggord et al., 1980b
1.0	Waconda Bay	2.0×10^{-5}	Spanggord et al., 1980b
0.2 to 2.0	Aucilla River	2.9×10^{-5}	Simmons and Zepp, 1986
0.2 to 2.0	Wylde Lake	1.5×10^{-5}	Simmons and Zepp, 1986
1.0	Distilled water	4.7×10^{-6}	Spanggord et al., 1980b
0.2 to 2.0	Distilled water	6.3×10^{-6}	Simmons and Zepp, 1986
Unknown	TNT condensate water	1.6×10^{-6}	Spanggord et al., 1980b

[a] Waconda Bay and Holston River represent munition-contaminated waters, and Searsville Pond, Aucilla River, and Wylde Lake are uncontaminated waters.

been mineralized only by cultured organisms from Waconda Bay, Chattanooga, Tennessee (Spanggord et al., 1980b; Chou and Spanggord, 1981).

The biotransformation rate of 2,4-DNT is one-sixth to one-third the rate of TNT (Parrish, 1977; McCormick et al., 1978). Parrish (1977) reported that 2,4-DNT is completely degraded by fungal mycelia in basal salts media after an induction period of 8 h and a reaction time of 24 h. (TNT had an induction time of 2 h, with reaction completion in 4 h.) McCormick et al. (1976) gave relative rate constants for 2,4-DNT and TNT that show that TNT degrades 2.5 to 6 times faster than 2,4-DNT. As a means of estimating the biodegradation rate of 2,4-DNT, we multiplied the microbial degradation half-lives for TNT derived from different experimental studies by 2.5 to 6. The half-lives for microbial transformation of TNT in natural waters range from 7.5 d (calculated from rate data of Burlinson, 1980) to 25 d (calculated from rate data by Spanggord et al., 1980b), and therefore the half-lives for 2,4-DNT could range from 19 (i.e., 7.5 x 2.5) to 150 days (i.e., 25 x 6) in natural waters.

Not only is microbial reduction of 2,4-DNT slower than that of TNT, but there are also fewer organisms that can degrade 2,4-DNT. Parrish (1977) found

Table 4-6. Photoproducts of 2,4-DNT identified by Burlinson and Glover (1977a).

Compound	Composition, wt%
2-Amino-4-nitrobenzoic acid	16
2-Amino-4-nitrobenzaldehyde	10
2,2'-Dicarboxy-5,5'-dinitroazoxybenzene[a]	10
2,4-Dinitrobenzoic acid (DNBA)	7
2,4-Dinitrobenzaldehyde	6
2,2'-Dicarboxaldehyde-5,5'-dinitroazoxybenzene[b]	3
Unknown	2

[a] Reported incorrectly as a carboxy compound.
[b] Reported incorrectly as a carboxaldehyde compound.

that only five of the 190 fungi investigated transformed 2,4-DNT, whereas 183 of them modified TNT. This work was done with a basal salts medium, plus 5% glucose at 29°C, using the washed mycelia of the fungi.

Chou and Spanggord (1981) identified only one reduction product, the yellow 2-amino-4-nitrotoluene, using microorganisms from pond water. Veillonella alkalescens attacks only the 4 position of the DNT molecule according to McCormick et al. (1976). In a later paper, McCormick et al. (1978) identified the following compounds resulting from action by Mucrospoirum sp.:

2-amino-4-NT,
4-amino-2-NT,
2,2'-dinitro-4,4'-azoxytoluene,
4,4'-dinitro-2,2'-azoxytoluene,
4-acetamido-2-nitrotoluene, and an
unidentified compound.

Other studies have used larger cell populations or added nutrients to examine biodegradation processes. The effects of 2,4-DNT concentration as well as oxygen were studied by Spanggord et al. (1980b) with microorganisms from Waconda Bay, Chattanooga, Tennessee. They reported that cells can be gradually acclimated to grow in 120 ppm of 2,4-DNT. In the aerated water, 10 ppm 2,4-DNT was "transformed" with a half-life of 1.8 d and a 2-day lag time. In anaerobic conditions (with a nitrogen head space), the half-life increased to 4.5 d (it was unclear whether or not "transformed" meant mineralized in this experiment). Mineralization experiments were attempted with large cell populations of adapted bacteria. Spanggord et al. (1980b) and Chou and Spanggord (1981) used high-cell-population cultures (10^7 to 10^8 cells/mL) and noted complete mineralization of 2.5 ppm 2,4-DNT with half-lives of 0.41 to 0.27 h, depending on the concentration of cells. When similar experiments were done with uncontaminated eutrophic waters, they found no biotransformation of 2,4-DNT without the addition of nutrients. When a high-cell population (9×10^8 cells/mL) was used, they observed transformation, but not mineralization and the half-life was 2.1 h (Chou and Spanggord, 1981). Chambers et al. (1963) used phenol-adapted bacteria to attempt mineralization of various compounds. With 2,4-DNT they found some oxygen uptake using "respirometric techniques" in a buffered solution of bacteria and 2,4-DNT. Bringmann and Kuhn (1971) also accomplished complete oxidation in a two-step laboratory procedure with Azotobacter agilis and activated sewage sludge.

Experiments by Carpenter et al. (1978) provide evidence that 2,4-DNT reduction products bind to humic materials as do the reduction products of TNT. They made a precipitate from a surrogate lipid residue and a surrogate reduction product of the TNT-type compounds. (The reduction product chosen was 2,4-diaminotoluene, which would be a reduction product of 2,4-DNT if both nitro-groups were microbially reduced (McCormick et al., 1978.)) They found spectral similarities between infrared spectra of the precipitates formed from ^{14}C-TNT in an aerated active sludge reactor and those formed from 2,4-diaminotoluene and glycerol, palmitoyl chloride and sebacyl chloride. The results led them to conclude that the aromatic amine reduction products may undergo condensation reactions with carboxylate groups to form polyamides. Furthermore, they postulated that polyamide polymers formed from biodegradation will be long-lived because amide linkages are resistant to

microbial attack. As the amine by-products are formed, they will probably be scavenged by humic materials.

Hydrolysis

No studies have specifically addressed 2,4-DNT hydrolysis. However, because TNT did not hydrolyze in Hoffsommer and Rosen's (1973) experiments with sea water, we believe that 2,4-DNT will also be resistant to hydrolysis.

TOXIC EFFECTS ON ANIMALS AND HUMANS

The toxicology of 2,4-DNT (the primary isomer in technical-grade DNT) has received considerable attention because of concerns over the potential health risks it poses to exposed individuals (e.g., workers who are involved in its manufacture). Major reviews of its metabolism and toxicology have been prepared by Rickert et al. (1984) and Etnier (1986). Cancer bioassays completed with different technical grades (containing >75 wt% 2,4-DNT with varying amounts of the 2,6-DNT isomer) of tDNT have given both negative results (NCI, 1978) and positive results (i.e., hepatocellular carcinomas in rats) (see Ellis et al., 1979; CIIT, 1982). Subsequent research has focused on the identification of the DNT isomer responsible for the observed carcinomas. It is now thought that 2,6-DNT is the isomer principally responsible for the carcinogenic response observed (see Goldsworthy and Popp, 1986; Leonard et al., 1986; and Dixit et al., 1986).

Toxicokinetics

Absorption. Data on the uptake of 2,4-DNT by humans are available from studies conducted with workers handling DNT. Woolen et al. (1985), for example, measured the amount of the DNT metabolite, 2,4-dinitrobenzoic acid (2,4-DNBA), in the urine of workers exposed to technical-grade DNT (~75 wt% 2,4-DNT). They found that the urinary concentration of 2,4-DNBA rose quickly during the work day, reflecting the rapid uptake of DNT via inhalation and skin absorption. In a separate study, Levine et al. (1985a) identified DNT metabolites in urine from workers exposed to tDNT and DNT isomers by inhalation. They also took skin swipes to determine the extent of skin

contamination. One finding from their investigation was that the quantity of urinary DNT metabolites could not be accounted for by inhalation alone. Levine et al. (1985a) suggested that skin absorption could represent another significant exposure pathway over the course of a work day. Woolen et al (1985) also considered skin absorption an important exposure pathway.

Lee et al. (1978) administered single oral doses of ^{14}C-2,4-DNT to mice, rats, rabbits, dogs, and monkeys. In mice, about 78% of the administered radioactivity was recovered from the feces and the gastrointestinal tract, including its contents, compared with 9.5 to 17.3% recovered from rats, dogs, rabbits, and monkeys. Lee et al. (1978) estimated the net absorption to be about 17% of the oral dose for mice and between 75 to 85% for the other species. Mori et al. (1978) observed a peak concentration of radioactivity in the blood of Wistar rats 6 h after an oral administration of radiolabeled 2,4-DNT.

Rickert et al. (1983) administered ^{14}C-2,4-DNT by gavage to Fischer-344 rats and detected a peak of radioactivity in the liver 1-2 h later. A second peak was observed 8 to 12 h after dosing. Radioactivity peaked in the first half of the small intestine and declined more rapidly than concentrations in the second half of the intestine increased, indicating significant absorption in the first half of the small intestine.

Distribution. Mori et al. (1980) administered ^{3}H-2,4-DNT orally to rats and measured radioactivity in blood, bile, liver, brain, heart, lung, spleen, pancreas, kidney, stomach, small intestine, large intestine, and the testes. The highest levels were found in the liver, adipose tissue, and skin. Control rats received one dose of ^{3}H-2,4-DNT, while treated rats received 0.5 wt% 2,4-DNT in the diet for 4 months prior to receiving a dose of ^{3}H-2,4-DNT. Levels of radioactivity in the control rats were higher than in treated rats, suggesting that repeated ingestion may saturate tissues, and/or increase the rate of elimination.

Lee et al. (1978) investigated the distribution of radioactivity in mice, rats, rabbits, dogs, and monkeys following oral administration of ^{14}C-2,4-DNT. An average of 9.2% of the radiolabel was excreted in the urine of mice, while 75 to 81% was excreted in the urine of rats, dogs, rabbits, and monkeys after 24 h. Radioactivity was detected in the liver, kidney, lungs, brain, and skeletal muscle. Livers contained up to 1.1% of the

radioactivity. Rickert and Long (1980) found that the concentration of radioactivity in the livers and kidneys of rats, which received orally administered ^{14}C-2,4-DNT, was up to ten times higher than in blood plasma or red blood cells. They also measured different quantities of radioactivity in the livers of male and female rats after oral dosing with ^{14}C-2,4-DNT. Male rats had two times the quantity of radioactivity in their livers as female rats 2 to 72 h after dosing. In their review, Rickert et al. (1984) also noted that there are quantitative differences between male and female rats in the excretion of 2,4-DNT metabolites. A greater percentage of the applied dose passes through the bile duct of male rats than of female rats. After entering the intestine from the bile duct, some of these 2,4-DNT metabolites are reabsorbed by the intestinal wall and are transported back to the liver (Medinsky and Dent, 1983). This enterohepatic cycling appears to be an important factor in sex-related differences in 2,4-DNT toxicity and will be discussed in more detail in the metabolism section.

Metabolism. The metabolism of 2,4-DNT has been explored both in vitro and in vivo. Rickert et al. (1984) and Etnier (1986) reviewed several studies (e.g., Dent et al., 1980; Decad et al., 1982; Short et al., 1979) in which 2,4-DNT was incubated with post-mitochondrial supernatant fractions from different species to determine the metabolites produced under aerobic and anaerobic conditions. The various studies demonstrated that aminonitrotoluenes (i.e., 2-ANT) and 4-amino- 2-nitrotoluene (4-ANT)) were the primary products formed under reducing conditions, whereas oxidative conditions resulted in the formation of 2,4-dinitrobenzyl alcohol 2,4-DNBAlc as the principal product. Short et al. (1979) found that there were pronounced sex-related differences in the amounts of the aminonitrotoluenes produced in the post-mitochondrial supernatants of liver. Specifically, the supernatant fractions from females of the species tested (i.e., mouse, rat, rabbit, dog, and monkey) contained less of the aminonitrotoluenes than did the fractions from males.

The oxidative metabolism of 2,4-DNT was examined by different researchers (e.g., Decad et al., 1982; Short et al., 1979; and Bonse and Rickert, 1981) by pretreating test animals with inducers of mixed-function oxidases (MFO), namely, phenobarbital and Aroclor 1254, or an inhibitor, SKF525-A. The amount of dinitrobenzyl alcohol was increased in the supernatants obtained from

animals pretreated with the MFO inducers, and decreased in the fractions from animals treated with the inhibitor.

The role of microorganisms in metabolizing 2,4-DNT has also been studied in vitro. Dent et al. (1980), for example, examined the metabolism of 2,4-DNT under anaerobic and aerobic conditions with rat cecal microflora. No metabolism occurred in the aerobic atmosphere; however, under anaerobic conditions the cecal microflora rapidly reduced 2,4-DNT to 4-ANT and 2-ANT. Reduction of the nitro group at the 4-position occurred more rapidly than at the 2-position. After prolonged incubation, the rat cecal microflora metabolized the aminonitrotoluenes to form 2,4-diaminotoluene (2,4-DAT), a known carcinogen. Guest et al. (1982) examined the metabolism of 2,4-DNT using intestinal microflora from rats, mice, and humans. No metabolism was observed in the presence of oxygen. Under anaerobic conditions, the nitro groups were reduced to 2-nitro-4-nitrosotoluene and 4-nitro-2-nitrosotoluene and then to the corresponding aminonitrotoluenes. Concentration of 2,4-DAT increased as the concentrations of the 2-ANT and 4-ANT decreased. No sex or species differences in metabolic pathways were observed. The rates of disappearance of 2,4-DNT, however, were the fastest with intestinal microflora from mice, followed by rat microflora and then human microflora.

In other work, Mori et al. (1984) identified metabolic products of E. coli, isolated from human intestines. Metabolic products included hydroxylaminonitrotoluenes and monoaminonitrotoluenes. The work of the researchers noted above (Dent et al., 1980; Guest et al., 1982; and Mori et al., 1984) indicates that the microbial metabolism of 2,4-DNT proceeds in the form of sequential reductions. Initially, the nitro groups are reduced to nitrosotoluenes, which are subsequently reduced to aminotoluenes via hydroxylamine intermediates. The aminotoluenes are then reduced to 2,4-DAT.

Metabolites of 2,4-DNT have been measured in the urine of laboratory animals and in the urine of workers exposed to dinitrotoluenes in the workplace. Rickert and Long (1981) administered oral doses of 10, 35, or 100 mg of ^{14}C-2,4-DNT per kg of body weight to male and female Fischer-344 rats and measured four primary metabolites in urine (the primary route of elimination of the radiolabel): 4-(N-acetyl)amino-2-nitrobenzoic acid (4-AANBA), 2,4-DNBA, 2-amino-4-nitrobenzoic acid (2-ANBA), and 2,4-dinitrobenzyl alcohol glucuronide (DNBAG). At doses of 10 and 35 mg/kg, the urine of female rats contained a significantly ($p < 0.05$) greater amount of

DNBAG than did males. Elimination of DNBAG from female rats approached saturation at a dose of 100 mg/kg, but not in male rats. The excretion of 2,4-DNBA became saturated in male and female rats at a dose of 100 mg/kg. Lee et al. (1978) measured the urinary metabolites resulting from a single intragastric administration of ^{14}C-2,4-DNT to mice, rats, rabbits, dogs, and monkeys. The primary metabolites in all the species were 2,4-dinitrobenzyl alcohol and aminonitrobenzyl alcohols (isomers not specified) and associated glucuronide and sulfate conjugates.

Schut et al. (1985) studied the metabolism of 2,4-DNT in A/J mice after intraperitoneal and oral administrations of 100 mg/kg of 2,4-DNT (including small amounts of a tracer radiolabel, ^{3}H-2,4-DNT). The primary neutral metabolite was 2,4-dinitrobenzyl alcohol, regardless of route of administration (maximum of 5.59% of applied dose). Minor amounts of 2,4-DAT, 4-ANT, and 2-ANT were detected.

Rickert et al. (1981) used conventional and axenic Fischer-344 rats to study the role of cecal microflora in the production of metabolites of 2,4-DNT that require both oxidation and reduction (i.e., 4-AANBA and 2-ANBA). The axenic rats, which received oral doses of 35 mg/kg of 2,4-DNT (including ^{14}C-2,4-DNT radiolabel), produced significantly ($p < 0.05$) less 4-AANBA acid and 2-ANBA than did the conventional rats, confirming the results of the in vitro studies that demonstrated the reductive capacity of intestinal microflora. The covalent binding of 2,4-DNT was also investigated by Rickert et al. (1981) in their study with axenic and conventional Fischer-344 rats. They found that the amount of covalently bound ^{14}C was greater in the livers of conventional male rats than in the axenic male rats.

The process of hepatic biotransformation, which produced reactive metabolites capable of covalent binding to critical hepatic molecules, is not well established. Possible activation steps include formation of sulfate esters of benzyl alcohol and/or hydroxylamine metabolites. Kedderis et al. (1984) used male Fischer-344 rats in their experiments designed to examine the possible role of sulfation in the bioactivation of 2,4-DNT. These researchers administered the sulfotransferase inhibitors 2,6-dichloro-4-nitrophenol (DCN) or pentachlorophenol (PCP) to the rats prior to dosing with 2,4-DNT. The sulfotransferase inhibitors decreased the total hepatic macromolecular covalent binding of 2,4-DNT by 33%, compared to that observed in rats that received 2,4-DNT without prior sulfotransferase inhibition. Kedderis et al.

(1984) suggested that most of the covalent binding is caused by sulfation. The other possible pathways that were suggested include N-acetylation followed by N,O-acetyl transfer or direct O-acetylation of the hydroxylamine.

The metabolism of dinitrotoluenes in humans has been examined by Turner et al. (1985), who measured DNT metabolites in the urine of workers exposed to technical-grade DNT. The primary urinary metabolites of 2,4-DNT detected in the urine of four workers were 2,4-DNBA and 2-ANBA. Urine also contained unmetabolized 2,4-DNT and 4-AANBA. Small quantities of 4-amino-2-nitrobenzoic acid were found in the urine of one subject. Although these urinary metabolites are similar to those measured in the urine of rats, Turner et al. (1985) noted that the amounts of metabolites were different. For example, 2-ANBA represented 5% of the urinary 2,4-DNT metabolites in rats (Rickert and Long, 1981), but represented 32% of urinary metabolites in the four human subjects studied.

Excretion. Most of the studies that have examined the metabolism and excretion of 2,4-DNT in laboratory animals (i.e., mice, rats, rabbits, dogs, and monkeys) have shown that the major route of elimination is through the urine (Schut et al., 1985; Rickert and Long, 1981; Medinsky and Dent, 1983; and Bond and Rickert 1981). However, in two strains of mice (CD-1 and B6C3F1) (see Lee et al., 1978) fecal elimination was the predominant route of excretion.

Medinsky and Dent (1983) found biliary excretion to be an important component of the biologic fate of 2,4-DNT. In their examination of biliary excretion, two groups of rats (Fischer-344) were orally dosed with ^{14}C-2,4-DNT; one group had a biliary trap and the other did not. Animals without the biliary trap excreted 60 to 90% of the dose in the urine, while animals with the biliary trap excreted only 20 to 60% of the dose in the urine. Therefore, a significant portion of 2,4-DNT (30 to 40%) actually enters the intestine before entering the urine. Additionally, completion of biliary excretion after a dose of 35 mg/kg took longer for males (24 h) than for females (12 h). Male rats excreted 7% more radiolabel in bile than females. Bond et al. (1981) studied metabolism and biliary excretion of 2,4-DNT in male and female rats using isolated perfused rat livers. After perfusion with 20 μM of 2,4-DNT, livers from male rats excreted 2.5 times more 2,4-DNT in the bile than livers from females. However, after perfusion with

70 μM 2,4-DNT, the increase in biliary excretion was only 1.5 and 3.3 times that observed after perfusion with 20 μM 2,4-DNT. Thus, biliary excretion in males appears to saturate at higher dose levels. The study completed by Turner et al. (1985) with DNT workers showed that urinary metabolites were at the highest concentrations near the end of the workday and rapidly declined to very low or undetectable concentrations by the next work day.

Summary. Metabolic processes in the rat have been comparatively well studied, and appear to be similar to those in dogs, rabbits, monkeys, and mice. 2,4-DNT is initially metabolized by the hepatic mixed-function oxidases, and is subsequently conjugated to glucuronic acid. The predominant metabolite is DNBAG, which is excreted in the urine. In both male and female rats urinary excretion is the dominant method of elimination during the initial 24 hours after dosing. Prior to urinary excretion, biliary transport is greater in males than females. 2,4-DNT metabolites flow into the small intestine through the bile duct, where the glucuronic acid conjugate is hydrolyzed by intestinal microflora. The free alcohol, 2,4-DNBAlc, is lipid-soluble and can either be resorbed by the intestine or further reduced by the microflora and subsequently resorbed. Evidence of resorption comes from Rickert et al. (1981) who found that axenic rats excreted a smaller proportion of a dose in the urine than conventional rats, implying less resorption and more fecal excretion. Absorbed metabolites are then transported back to the liver where they are oxidized, and the cycle continues. The secondary oxidation can include oxidation of amino groups and formation of reactive hydroxylamine intermediates, resulting in liver toxicity. The sex-dependent toxicity seen in males is probably caused by the higher biliary transport and enterohepatic cycling in males, which possibly leads to a greater production of reactive intermediates (Rickert et al., 1984).

Toxic Effects on Organs and Systems

Hematological effects. McGee et al. (1942) observed cyanosis, anemia, and pallor among 154 workers exposed to DNT during the manufacture of military powders. These symptoms are indicative of methemoglobinemia, a common effect of elevated exposures to nitroaromatics. Hematological effects have also been observed in laboratory animals. For example, methemoglobinemia, Heinz bodies,

reduced erythrocyte counts and hemoglobin concentrations, and increased reticulocyctes, were observed in beagles administered 2,4-DNT in their feed (Ellis et al., 1985). Lee et al. (1985) found that subchronic administration of 2,4-DNT to CD rats produced anemia and reticulocytosis.

Circulatory system. Levine et al. (1986) completed a retrospective cohort mortality study of DNT workers. That study was based on 457 male workers from two DNT manufacturing plants who were employed at the plants for more than 30 days between 1951 and 1981. There was a statistically significant ($p = 0.002$) increase in the standardized mortality ratio (SMR) for diseases of the circulatory system. The source of the increased SMR could not readily be explained by other risk factors (e.g., smoking), and the authors recommended further epidemiologic investigations.

Hepatic effects. Administration of 2,4-DNT to laboratory animals has produced liver effects. For example, Kozuka et al. (1979) observed a significant increase in the liver weights ($p < 0.01$) of rats fed approximately 25 mg/kg-d 2,4-DNT for six months. They also observed a significant increase in SGOT activity in the treated rats. Changes in liver weights have also been observed by Lee et al. (1985) in rats and by Hong et al. (1985) in mice.

Neurotoxic effects. Ellis et al. (1985) reported that the main adverse effect in dogs that received 2,4-DNT in subchronic and chronic feeding studies was neuropathy; this was manifested as incoordination and paralysis. Neurotoxic effects were observed in one dog that received 1.5 mg/kg-d for two years. Other dogs developed symptoms after 6 months at a dose rate of 10 mg/kg-d. Ellis et al. noted that neurotoxicity was less pronounced in rats and mice.

Renal effects. McGown et al. (1983) observed hyaline droplet formation in the epithelium of the proximal convoluted tubules of the kidneys in rats of both sexes. Male rats appeared to be more susceptible than females. No dose-dependent treatment response was noted, and it is not clear whether this effect was a treatment-related response.

Dermal effects. Ellis et al. (1979) used a modified Draize method for testing the skin and eye irritation of 2,4-DNT in rabbits. The results showed that this DNT isomer was nonirritating. No cases of skin rash caused by occupational exposure to pure 2,4-DNT have been documented, although cases of nitroaromatic dermatitis have been well documented.

Reproductive effects. Hamill et al. (1982) studied the reproductive hazard for workers exposed to DNT (isomers unspecified) and diaminotoluene at a chemical plant. No reproductive effects (e.g., abnormal sperm count or morphology, etc.) were demonstrated among male DNT workers. In another study, Levine et al. (1985b) studied the fertility of workers exposed to DNT and diaminotoluene at three facilities. They did not find any significant effects on fertility (as measured by standardized fertility ratios) that could be associated with DNT and TDA exposures. In laboratory animals, reproductive effects have included testicular atrophy and decreased spermatogenesis. Dose-dependent degenerative changes in the testes of male rats were observed by McGown et al. (1983) during a 14-d study with 2,4-DNT. Ellis et al. (1979) observed decreased testis weight, atrophy of the testes, and aspermatogenesis in male mice receiving doses of 95 or 900 mg/kg-d 2,4-DNT during chronic feeding studies. Ellis et al. (1979) also carried out a three-generation study using rats, and no treatment-related, adverse reproductive effects were seen. Smith (1983) administered 390 mg/kg of 2,4-DNT to mice by gavage and did not detect any significant differences in the reproductive outcomes (e.g., litter, litter weight, etc.) between controls and treated mice. In another reproductive toxicity study, Lane et al. (1985) administered 2,4-DNT by gavage to male Sprague-Dawley rats at dose rates of 0, 60, 180, or 240 mg/kg-d for five days. The mating period was seven weeks long for all but the highest treatment level, which lasted an additional six weeks in order to study the reversibility of reproductive effects. There were no reproductive effects at the 60 mg/kg-d treatment level. At the next highest level there was a transient effect on one measure of reproductive toxicity, namely, the mating index, which is the ratio of the number of females pregnant to the number of females allowed to mate, multiplied by 100. At the highest treatment level significant reproductive effects were observed (e.g., mating index, and the preimplantation loss index, which is equal to the total number of corpora lutea minus the total number of implantation sites divided by the number of

pregnant females). However, the daily dose of 240 mg/kg also caused weight loss and cyanosis among the rats.

Teratogenicity

In a teratogenic evaluation of technical-grade DNT (76% 2,4-DNT and 19% 2,6-DNT) in Fischer-344 rats, Price et al. (1985) found no statistically significant effects on fetal growth or morphological development. Female rats were dosed by gavage on gestation days (gd) 7 to 20 at levels of 14, 35, 37.5, 75, 100, or 150 mg/kg-d. Only at the high dose was there a significant female mortality rate (46.2%). Sacrifice occurred on gd 20, and signs of hematological toxicity were observed in the 100 mg/kg-d treatment groups (e.g., increased methemoglobin, reticulocyte count, etc.). Dose-related decreases were measured in maternal weight during gestation, and relative liver and spleen weights also decreased. An increase in prenatal mortality was observed at the highest dose rate, but was not statistically different from the controls. No statistically significant increases occurred in the incidence of malformed fetuses in the DNT-treated groups.

Genotoxicity

The genotoxicity of 2,4-DNT has been evaluated in bacteria, yeast, cultured mammalian cells, and in whole animals. These short-term assays have measured the ability of 2,4-DNT to induce forward and reverse mutations, mitotic recombination, chromosome aberrations, chromosome breakage, DNA damage and repair, and abnormal sperm morphology. The results of these studies are discussed in the following paragraphs and are summarized in Table 4-7. Both Etnier (1986) and Rickert et al. (1984) have also reviewed the genotoxicity of 2,4-DNT.

Although 2,4-DNT has not been consistently active in all strains of Salmonella, a number of studies have shown that 2,4-DNT is capable of inducing reverse mutations in these bacteria. Typically, 2,4-DNT has caused a relatively weak mutagenic response (i.e., a two-to-threefold increase in the number of revertants) (Chiu et al., 1978; Ellis et al., 1980; Tokiwa et al., 1981; Couch et al., 1981; Spanggord et al., 1982b; Mori et al., 1982). The standard Ames assay includes a series of samples to which exogenous

Table 4-7. Overview of studies completed on the genotoxicity of 2,4-DNT.

Assay	Genetic endpoint	Sample purity	Dosage	Results +S9	Results -S9	Comments	References
Salmonella plate incorporation TA1535, TA1537, TA1538, TA98, TA100	Reverse mutation	Not specified	0.6, 5.0 15.0, 35.0, 45.0 µg/plate		-		Simmon et al., 1977
Salmonella plate incorporation TA98, TA100	Reverse mutation	Not specified	0.1, 1.0, 10.0 µM		+	Assays not performed with S9	Chiu et al., 1978
Salmonella plate incorporation TA1535, TA1537, TA1538, TA98, TA100	Reverse mutation	≥98.0%	10.0, 30.0, 100.0 300.00, 1000.0 µg/plate	+	+		Ellis et al., 1980
Salmonella plate incorporation TA98, TA100	Reverse mutation	Not specified	500.0 1000.0 µg/plate			Insufficient data to evaluate	Tokiwa et al., 1981
Salmonella plate incorporation TA1535, TA1537 TA1538, TA98, TA100	Reverse mutation	Technical grade	1000.0 µg/plate	+		Single concentration tested. Sample contained 76.5% 2,4-DNT	Couch et al., 1981
		≥99.8%	200.0 µg/plate	+	+	Single concentration tested	
Salmonella plate incorporation TA1535, TA1537 TA1538, TA98, TA100 TA98NR101, TA100NR3	Reverse mutation	Not specified	10.0 to 5000.0 µg/plate	+	+	Positive results only with TA100 and TA100NR3. Specific revertant counts not reported.	Spanggord et al., 1982b

Table 4-7. (continued)

Assay	Genetic endpoint	Sample purity	Dosage	Results +S9	Results -S9	Comments	References
Salmonella plate incorporation TA98, TA100	Reverse mutation	>99.0%	100.0, 200.0, 300.0 µg/plate		+	Concentration-dependent response. Assays not performed with S9.	Mori *et al.*, 1982
Saccharomyces suspension test	Mitotic recombination	Not specified	0.006, 0.008% (v/v)	-	-	Assay may be insensitive	Simmon *et al.*, 1977
Chinese hamster ovary cell	Forward mutation HGPRT locus	Technical grade	2.0 µM	-		Single concentration tested. Sample contained 76.5% 2,4-DNT	Abernethy and Crouch, 1982
		≥99.8%	3.0 µM	-			
Mouse lymphoma cells (P388)	Forward mutation	Technical grade	1.6, 8.0, 40.0, 200.0, 1000.0 µg/mL	-	-	Sample contained 80% 2,4-DNT, 20% 2,6-DNT. Dose-dependent decrease in survival, with and without S9.	Styles and Cross, 1983
		'Pure'		-	+	Dose-dependent decrease in survival, with and without S9.	ibid.
Rat lymphocyte	Chromosome aberration	Not specified	0.2% in diet	+		Abstract. Reported significant increase in frequency of chromatid gaps after 5-19 wk in culture.	Hodgson *et al.*, 1976

Table 4-7. (continued)

Assay	Genetic endpoint	Sample purity	Dosage	Results +S9	Results -S9	Comments	References
Rat dominant-lethal	Forward mutation	Not specified	0.2% (in diet)	+		Abstract. Reported significant increase in number of dead implants/total implants	Hodgson *et al.*, 1976
Rat dominant-lethal	Forward mutation	Not specified	60.0, 180.0 240.0 mg/kg		-	Abstract. Insufficient data to evaluate	Dougherty *et al.*, 1978
Mouse dominant-lethal	Forward mutation	'Purified'	250 mg/kg (2d)	+		Significant decrease in fertility	Soares and Lock, 1980
Mouse dominant-lethal	Forward mutation	≥ 98.0%	0.0015, 0.01, 0.02, 0.07, 0.10, 0.15 0.20, 0.50% in diet for 13 wk.	+		Significant decrease in fertility index and viability index at 0.10 to 0.20%	Ellis *et al.*, 1980
		'Purified'	250 µg/kg (2d)		-		
Mouse bone-marrow	Chromosome aberration	'Pure'	200.0, 400.0 mg/kg		-		Ashby *et al.*, 1985
Mouse sperm morphology	Abnormal sperm morphology	Technical grade	250 mg/kg (2d)		-		Soares and Lock, 1980

Table 4-7. (continued)

Assay	Genetic endpoint	Sample purity	Dosage	Results +S9	Results -S9	Comments	References
Recessive spot test	Forward mutation	Technical grade	100 mg/kg		-		ibid.
Spermatocyte DNA repair	DNA	≥ 99.0%	10.0, 100.0 1000.0 μM		-	Toxic at highest concentration	Working and Butterworth, 1984
Hepatocyte DNA repair	DNA repair	'Purified'	0.001, 0.000 μM		-	Technical-grade 2,4-DNT also gave negative results	Bermudez et al., 1979
Hepatocyte DNA repair	DNA repair	Technical grade	40.0, 80.0, 120.0, 160.0, 160.0, 200.0 mg/kg		+	Sample contained 71% 2,4-DNT. Concentration-related increase, DNA repair. Treatment also stimulated cell proliferation	Mirsalis et al., 1982a
Hepatocyte DNA repair	DNA	>99.9%	50.0, 200.0 mg/kg		+	Treatment also stimulated cell proliferation	Mirsalis et al., 1982b
Hepatocyte DNA repair	DNA	'Pure'	25.0, 100.0, 150.0 mg/kg		+		Ashby et al., 1985

metabolizing enzymes have been added (S9). Although the genotoxicity of many compounds is associated with metabolic activation by the enzymes contained in this fraction, 2,4-DNT has been mutagenic both in the presence and absence of S9 (Table 4-7).

It is not clear whether or not 2,4-DNT induces dominant lethal mutations in rats; both positive and negative results have been reported (Hodgson et al., 1976; Dougherty et al., 1978). In contrast, both Soares and Lock (1980) and Ellis et al. (1980) found that 2,4-DNT was able to induce dominant lethal mutations in mice. 2,4-DNT did not damage DNA in rat spermatocytes (measured as unscheduled DNA synthesis, UDS) (Working and Butterworth, 1984). Mirsalis et al. (1982a) reported that tDNT caused a concentration-dependent increase in UDS in primary hepatocyte cultures. Equivalent results have been obtained with pure 2,4-DNT (Ashby et al., 1985). However, a similar assay by Bermudez et al. (1979) did not find any evidence of UDS. Rat lymphocytes cultured in the presence of 2,4-DNT had a significant increase in the frequency of chromatid gaps (Hodgson et al., 1976).

2,4-DNT has been inactive in assays that measured induction of chromosome aberration (Ashby et al., 1985), mitotic recombination (Simmon et al., 1977), recessive mutation or abnormal sperm morphology (Soares and Lock, 1980) or forward mutation in CHO cells (Abernethy and Couch, 1982). 2,4-DNT was mutagenic to P388 mouse lymphoma cells in the absence of exogenous metabolic activation (Styles and Cross, 1983).

Carcinogenicity

The carcinogenicity of DNT mixtures that include 2,4-DNT has been investigated in several bioassays. The first bioassay was completed by the National Cancer Institute in 1978 (NCI, 1978). Technical-grade DNT (>95 wt% 2,4-DNT) was administered in the feed of Fischer-344 rats and B6C3F1 mice over a period of 78 wk. No statistically significant evidence for carcinogenicity was demonstrated for mice. In rats, the only significant findings were an increase in benign skin tumors ($p < 0.008$) and in mammary gland fibroadenomas ($p = 0.016$). Some rare neoplasms (e.g., hemangiosarcomas of the subcutis and urinary bladder and prostate gland adenocarcinoma) occurred in the high-dose male rats; however, these neoplasms were not considered treatment-related. One unidentified reviewer (NCI, 1978), though, noted that the rats may not

have received a maximum tolerated dose, and consequently the hemangiosarcomas may be "biologically significant." In a study funded by the U.S. Army, Ellis et al. (1979) administered different amounts of 2,4-DNT (98 wt% purity, with approximately 2 wt% 2,6-DNT) in the feed of CD rats, CD-1 mice, and beagle dogs for two years. A positive carcinogenic response was observed in rats, (see Appendix C for dose rates and tumor incidence data), but not in the mice or dogs. The Chemical Industry Institute of Toxicology (CIIT) sponsored a two-year bioassay by Hazleton Laboratories America, Inc. (CIIT, 1982) in which technical-grade DNT (~76 wt%, 2,4-DNT and ~19 wt%, 2,6-DNT) was administered in the feed of male and female Fischer-344 rats. At a dose rate of 14 mg/kg-d (see Appendix C) hepatocellular carcinomas or neoplastic nodules were observed in all of the rats.

The primary difference of interest in the bioassays summarized above was the absence of hepatocellular carcinomas in the Fischer-344 rats of the NCI study and the elevated occurrence of those carcinomas in the Fischer-344 rats used in the Hazleton Laboratories study. One explanation for the discrepancy is the isomeric composition of the DNT mixtures used in the bioassay studies. For example, the daily dose of 2,6-DNT in the Hazleton Laboratories study was a factor of 3.7 times higher than the estimated amount in the NCI study (see Popp and Leonard, 1983). To determine which of the DNT isomers was primarily responsible for the carcinogenic response in rats, CIIT researchers did one-year bioassays with 2,4-DNT, 2,6-DNT, and a tDNT (76 wt% 2,4-DNT, 18 wt% 2,6-DNT) administered in the diets of male Fischer-344 rats. In addition, research was conducted on the hepatic initiation/promotion properties of the two isomers, as well as tDNT.

In the bioassays (Leonard et al., 1987) rats were fed 7 or 14 mg/kg-d of 2,6-DNT, 27 mg/kg-d of 2,4-DNT, or 35 mg/kg-d of tDNT. Rats treated with 2,6-DNT developed hepatocellular carcinomas; however, no hepatocellular carcinomas were observed in the rats treated with 2,4-DNT. Approximately half the rats receiving tDNT developed hepatocellular carcinomas. In the initiation/promotion-related work, Leonard et al. (1983, 1986), used a hepatic initiation-promotion protocol with Fischer-344 rats to show that 2,4-DNT has only promoting activity, whereas 2,6-DNT both initiates and promotes hepatocytes (see also the Carcinogenic Effects subsection in Section 5). Leonard et al. (1986) suggest that the differences between the bioassays using tDNT can be explained by the initiator-promotor characteristics of the two isomers, as well as by the quantities administered to the rats.

One other study dealing with carcinogenicity of 2,4-DNT was conducted by Schut et al. (1982). In their pulmonary tumor bioassay 52A/J mice received intraperitoneal injections of 2,4-DNT totaling 600, 1500, or 3000 mg/kg over a period of 8 weeks, with sacrifices occurring 22 weeks after the last injection. The average number of lung adenomas per mouse was not significantly different ($p > 0.05$) from the averages calculated for the untreated controls or vehicle-treated controls.

Levine et al. (1986) conducted a retrospective epidemiological study of 457 DNT workers and found that the number of deaths caused by cancer was less than expected (standard mortality ratio = 87). Furthermore, there were no specific cancers that had an increased incidence over what was expected. Levine et al. also noted that even if DNT were a human carcinogen, their study would probably not have detected significant increases of rare tumors associated with liver and other organs.

Estimation of Intake Limits to Protect Public Health

The weight of evidence from animal bioassays conducted by Leonard et al. (1987), as well as initiation-promotion studies with hepatocyte foci (Leonard et al., 1986), indicate that 2,4-DNT is probably a cancer promoter, whereas 2,6-DNT is a complete carcinogen with both initiating and promoting activity. Currently there are no methodologies for quantifying the health risks of promoters, and therefore we have reviewed the chronic animal bioassays completed on DNT (tDNT and pure 2,4-DNT) to identify no-effect dose rates for 2,4-DNT that could be used to derive safe intake. The only long-term study that clearly defined a NOEL suitable for estimation of an intake to protect public health was conducted by Ellis et al. (1979) with CD rats. Other studies examined had various deficiencies. For example, the CIIT (1982) study conducted with Fischer-344 rats produced hepatoxicity in the low-dose group (intake of approximately 2.6 mg/kg-d of 2,4-DNT); the NCI (1978) study with Fischer-344 rats and B6C3F1 mice using > 95 wt% pure 2,4-DNT focused on the detection of neoplastic and nonneoplastic lesions in the exposed animals and did not specifically address other forms or measures of toxicity (e.g., relative and absolute organ and body weights). Male rats in that study had a significantly increased incidence of benign skin tumors, and although the treated mice did not have any tumors that could be associated with the DNT

treatments, no other measures of toxicity were reported. Consequently, it is not possible to determine whether the treatments actually represent a no-effect level. A year-long bioassay by Leonard et al. (1987) with pure 2,4-DNT administered at a dose rate of 27 mg/kg-d produced toxic responses (e.g., manifested by significant ($p < 0.05$) increases in liver weights) in male Fischer-344 rats.

In the study by Ellis et al. (1979) male and female CD rats consuming 0.563 and 0.69 mg/kg-d of 2,4-DNT in feed (see Table C-1) for two years did not exhibit any toxic effects. At the next highest treatment level (i.e., 3.8 and 5.0 mg/kg-d) toxic responses (liver toxicity, anemia, etc.) were observed in some animals. To calculate a safe intake, we divided the highest no-effect dose rate (0.69 mg/kg-d) by a safety factor of 100 to obtain an intake of 0.0069 mg/kg-d (6.9 μg/kg-d).

TOXIC EFFECTS ON AQUATIC SPECIES

The literature dealing with the aquatic toxicity of 2,4-DNT has been reviewed by Etnier (1986), and here we present an overview of the major studies that have been completed. The acute toxicity of 2,4-DNT has been evaluated through the use of both static and flow-through tests for vertebrate and invertebrate species. The 96-h LC50 values for fish under static (Table 4-8) and flow-through (Table 4-9) conditions were similar. Incipient LC50 values were lower than 96-h LC50 values determined under static or flow-through tests. The same relationship was found for water fleas and the worm, L. variegatus. Of the vertebrate species, rainbow trout were the most sensitive species in all acute tests, while the midge (I. dissimilis) had the lowest 48-h LC50 and water flea the lowest incipient LC50. Liu et al. (1983c) examined the effects of photolysis and water characteristics on the toxicity of 2,4-DNT. They found that with increased photolysis of 2,4-DNT, the acute toxicity to fathead minnows decreased. In tests with bluegills, they found that temperature had the greatest effect on acute toxicity (i.e., the 96-h LC50 decreased by about a factor of three as water temperature increased from 17.3 to 24.5°C) and that hardness (as $CaCO_3$) and pH had little effect.

The toxic effects of 2,4-DNT to vertebrate and invertebrate species after prolonged exposures have been studied by Bailey (1982), Bailey et al. (1984), and Hartley (1981). Bailey (1982) used a laboratory-scale microcosm to study

Table 4-8. Acute toxicity of 2,4-DNT to aquatic organisms under static conditions.

Species	Concentration (mg/L)	Effect	Reference
Aquatic vertebrates			
Fathead minnow	32.8 (27.3 to 38.0)[a]	96-h LC50	Bailey and Spanggord, 1983
Fathead minnow	28.5 (26.3 to 32.5)	96-h LC50	Liu et al., 1983c
Bluegill	13.5 (12.1 to 15.1)	96-h LC50	Liu et al., 1983c
Channel catfish	24.8 (21.0 to 29.3)	96-h LC50	Liu et al., 1983c
Rainbow trout	13.6 (12.2 to 15.2)	96-h LC50	Liu et al., 1983c
Aquatic invertebrates			
Water flea	47.5 (29.5 to 99.7)	48-h LC50	Liu et al., 1983c
Water flea	38.3 (33.6 to 43.8)	48-h LC50	Liu et al., 1983c
Worm (L. variegatus)	> 83.2	48-h LC50	Liu et al., 1983c
Midge (T. dissimilis)	22.5 (19.8 to 25.5)	48-h LC50	Liu et al., 1983c

[a] 95% confidence interval within parentheses

the effects of a 21-d exposure of 2,4-DNT to water flea and the worm, L. variegatus. Concentrations ranged from 0.6 to 10 mg/L of 2,4-DNT and concentrations at and above 1 mg/L greatly reduced the total number of water fleas. The number of worms in the microcosms were not affected at concentrations below 5.6 mg/L. Bailey et al. (1984), as reported in Etnier (1986), conducted a series of chronic tests with rainbow trout and fathead minnows. Exposure periods ranged from 60 d to six months. In a 90 d exposure with rainbow trout, in which hatching success, fry survival and growth were monitored, a no-effect concentration of 0.56 mg/L was defined in one series with acceptable survival in controls. An early life-stage test with fathead minnows did not show effects on egg hatchability, growth of frys, and fry survival at concentrations up to 3.1 mg/L. In a six-month study, egg

Table 4-9. Acute toxicity of 2,4-DNT to aquatic organisms under flow-through tests (from Liu et al., 1983c).

Species	Concentration (mg/L)	Effect
Fathead minnow	36.1	96-h LC50
	26.0 (23.4 to 28.5)[a]	incipient LC50
Bluegill	16.0	96-h LC50
	9.2 (8.5 to 10.4)	incipient LC50
Channel catfish	32.0	96-h LC50
	16.4 (14.1 to 18.9)	incipient LC50
Rainbow trout	13.9	96-h LC50
	6.3 (5.6 to 7.0)	incipient LC50
Water flea	23.9	96-h LC50
	4.1 (3.0 to 5.3)	incipient LC50
Worm	47.2	96-h LC50
	30.4 (25.8 to 35.8)	incipient LC50

[a] 95% confidence interval, reported only for the incipient LC50 values.

production was reduced ($p < 0.05$) at a concentration of 0.62 mg/L. At the lowest concentration tested (i.e., 0.28 mg/L), there was a significant increase ($p < 0.05$) in fry deformities. Hartley (1981), cited in Etnier (1986), exposed juvenile bluefish to 2,4-DNT under flow-through conditions for eight weeks. The threshold concentration for reduced growth was 0.05 mg/L. Histopathological responses were observed in organs at concentrations above 0.5 mg/L.

In other work, Liu et al. (1983c) studied the effects of 2,4-DNT on the population growth of four algal species. With S. capricornutum a statistically significant ($p < 0.05$) inhibition of growth occurred at all concentrations tested, ranging from 0.9 to 94.4 mg/L for periods of 4 and 14 days. The growth of M. aeruginosa was inhibited at a concentration of 0.5 mg/L, and A. flos-aquae was inhibited at all concentrations tested (0.9 to 94.4 mg/L). N. pelliculosa was inhibited at concentrations above 9.8 mg/L.

5. DATA-BASE ASSESSMENT FOR 2,6-DINITROTOLUENE

The 2,6-DNT isomer of dinitrotoluene is present as a minor constituent in military-grade TNT and tDNT. Nevertheless, it is a soil contaminant at many installations where demilitarization operations have been conducted (see Appendix B). Our analysis of the potential steady-state partitioning of 2,6-DNT among the eight compartments of a reference landscape indicates that it will reside primarily in the vadose zone and ground water. In recent years there has been increased interest in the toxicology of 2,6-DNT because there is strong evidence that it is a hepatocarcinogen in rats. Accordingly, we assess the carcinogenic potency of this DNT isomer in order to calculate safe intake rates for different lifetime cancer risks.

ENVIRONMENTAL PARTITIONING AND TRANSPORT

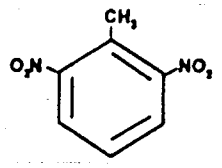
Most of the values for the various chemical and physical properties of the 2,6-DNT isomer (see Table 5-1 for identifiers) have been estimated rather than measured. Vapor pressure measurements have been made (see Table 5-2 and Appendix A), but solubility in water has not been measured and was estimated from K_{OW} and melting point (see Appendix A). The uncertainties associated with the estimate of solubility subsequently affect the Henry's law constant (computed as the ratio of vapor pressure to solubility) and K_{OC}, which is calculated from aqueous solubility and melting point. Partition coefficients for plant/soil, beef-fat/diet, and fish/water are all derived from regression equations (see Table 5-3). The predicted equilibrium distribution of 2,6-DNT among reference landscapes, shown in Table 5-4, is similar to that of 2,4-DNT (Table 4-4). One notable difference, though, is the smaller fraction of 2,6-DNT residing in the lower soil zone. This is due to the lower K_{OC} value for 2,6-DNT, which was derived from its aqueous solubility (estimated) and melting point.

ENVIRONMENTAL TRANSFORMATION PROCESSES

Photolysis

Simmons and Zepp (1986) reported rate constants for the photolysis of nitro compounds in water samples containing humic materials (see Table 5-5).

Table 5-1. Chemical identifiers of 2,6-dinitrotoluene.

Empirical formula:	$C_7H_6N_2O_4$	Structural formula:
Molecular weight:	182.14	
CAS reg. no.:	[606-20-2]	
RTECS no.:	XT1925000	

Their rate constants represent the photolysis rate within an infinitesimally small surface layer of water to eliminate the light attenuation caused by humic substances with depth. All values were relative to a p-nitroanisole actinometer. Using their rate constant for 2,6-DNT, we calculate a half-life of 17 h during sunlight photolysis of distilled water containing 2,6-DNT. From their surface photolysis rates for solutions containing fulvic acids from the Aucilla River in Georgia and Wylde Lake in Canada, we calculate half-lives of 1.0 and 1.5 h, respectively.[a] Similar half-lives were determined for water samples from natural waters in Florida and Georgia containing humic substances. The photolysis of 2,6-DNT is faster than that of 2,4-DNT, and Simmons and Zepp attribute the enhanced rate of 2,6-DNT to steric crowding of the methyl group and the adjacent nitro groups on the 2,6-DNT ring.

In a review, Spanggord et al. (1980a) referred to earlier experimental work in which they observed a 50% decrease in the concentration of 2,6-DNT in condensate water after one day in sunlight. Burlinson and Glover (1977b), exposed an aqueous solution containing 150 ppm 2,6-DNT to sunlight for 5 d, at which time they measured 0.2 ppm 2,6-DNT. From this time-dependent concentration change, we calculate a half-life of approximately 0.5 d. The products were polymers, azo and azoxydimers, and monomers with aldehyde, acid, nitroso, and oxime groups.

Langmuir et al. (1969) reported the fading of short-lived, colored intermediates of 2,6-DNT that are induced by flash photolysis. Their experiments showed that the aci-product, an excited state isomer of 2,6-DNT analogous to structure I shown in Figure 3-1, has an absorption maximum of

[a] Note that these are surface photolysis rates. Rates decrease rapidly with depth in natural water.

Table 5-2. Chemical and physical properties of 2,6-DNT. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	Not available	
Freezing/melting point	°C	64 to 66	Windholz *et al.*, 1983
Density	g/mL	1.28	Windholz *et al.*, 1983
Vapor pressure	torr	3.5×10^{-4}	Measured value, Pella, 1977
Aqueous solubility	mol/L mg/L	5.0×10^{-3} 910	Estimated[a]
Henry's law constant	(L·torr)/mol	0.07	Calculated as the ratio of vapor pressure to aqueous solubility
Diffusion coefficients: Air Water	m^2/s m^2/s	6.4×10^{-6} 6.3×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition coefficient (K_{ow})	log K_{ow}	1.9	Estimated[a]

[a] See Appendix A.

Table 5-3. Partition coefficients for use in estimating human exposures to 2,6-DNT.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	4	Calculated[b] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	3.1×10^{-3}	Calculated[b] from log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	13	Calculated[b] from log K_{ow}
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	34	Calculated[b] from log K_{ow}

[a] Wet-weights for plant and fish

[b] See Appendix A

Table 5-4. Fractional distributions of 2,6-DNT among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of 2,6-DNT to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	2.9×10^{-5}	3.2×10^{-5}
Air particles	1.6×10^{-9}	4.9×10^{-9}
Biota	9.3×10^{-5}	0.012
Upper soil	0.010	0.045
Lower soil	0.26	0.10
Ground water	0.73	0.83
Surface water	0.0033	0.016
Sediments	3.4×10^{-5}	1.7×10^{-4}

[a] Fractions do not add to one because of rounding.

410 nm in the visible region. At environmental pH's, it should lose a proton rapidly because it has a pKa of 1.8. Langmuir et al. proposed a mechanism that is analogous to that of 2,4-DNT and TNT.

Hydrolysis

We could not find any studies dealing with the hydrolysis of 2,6-DNT. However, since TNT does not hydrolyze in sea water (see Hoffsommer and Rosen, 1973), we doubt that 2,6-DNT would hydrolyze.

Biotransformation

Chou and Spanggord (1981) reported that 2,6-DNT was not biotransformed after 6 wk incubation in Searsville Pond water. When 500 ppm yeast extract was added, it disappeared within 6 d. Searsville Pond had no known previous contamination of TNT, DNT, or their degradation products. Organisms from

Table 5-5. Rates of photolytic degradation of 2,6-DNT in water.

Initial concentration (mg/L)	Sample description	Rate constant for photolysis (s^{-1})	Half-life (h)	Reference
0.2 to 2	Distilled water exposed to sunlight	1.1×10^{-4} [a]	18	Simmons and Zepp, 1986
0.2 to 2	Humic-rich water exposed to sunlight	1.3×10^{-4} to 2.0×10^{-4} [b]	1.0 to 1.5	Simmons and Zepp, 1986
Not stated	Condensate water from TNT production, exposed to sunlight	8.0×10^{-6} [c]	~24	Spanggord et al., 1980a
150	Distilled water exposed to sunlight	1.6×10^{-5} [d]	12	Burlinson and Glover, 1977b

[a] Calculated from a rate constant of 0.98 d^{-1}.

[b] Calculated from rate constants of 11 and 17 d^{-1}.

[c] 50% of the 2,6-DNT disappeared in 1 d.

[d] 150 mg/L of 2,6-DNT decreased to 0.2 mg/L in 5 d.

Waconda Bay in Tennessee, which had been previously exposed to these chemicals, were able to mineralize 2,4-DNT, but not 2,6-DNT. The 2,6-DNT was not degraded by these organisms unless yeast extract was added to their growth medium. McCormick et al. (1976) studied biotransformation rates of various nitroaromatics by Veillonella alkalescens in a hydrogen atmosphere. The relative rate constants of 2,6-DNT and TNT showed the latter to be 3.7 times faster. We would therefore expect half-lives ranging from 30 to 93 d for 2,6-DNT, based on the 8 to 25 d half-lives for biotransformation of TNT given by Spanggord et al. (1980b). In other work, Bringmann and Kuhn (1971) accomplished complete oxidation of 2,6-DNT in a two-step laboratory procedure with Azotobacter agilis and activated sewage sludge.

TOXIC EFFECTS ON ANIMALS AND HUMANS

The toxic effects of 2,6-DNT as well as 2,4-DNT have been reviewed by Rickert et al. (1984) and Etnier (1986). Generally, the toxicokinetics and toxicity of 2,6-DNT parallel those of 2,4-DNT; however, as was noted in Section 4, there is considerable evidence that the 2,6-DNT isomer is a hepatocarcinogen. In Appendix C we calculate carcinogenic potencies of the 2,6-DNT isomer using the tumor incidence and dose-rate data from chronic bioassays described later in this section. Based on those potency estimates, we compute dose rates for different lifetime risks of cancer.

Absorption. Rickert et al. (198) administered ^{14}C-2,6-DNT to Fischer-344 rats by gavage in doses of 10 and 35 mg/kg. Rats were sacrificed at 1, 2, 4, 8, 12, 24, 48, 96, 192, and 384 h after the initial dose, and the livers and intestines were removed for subsequent analysis. Radioactivity disappeared quickly from the first quarter of the small intestine, indicating that 2,6-DNT is rapidly absorbed. After 24 h, little radioactivity was measured in the intestines. Radioactivity in the liver peaked within one to two hours, declined, and then attained a second peak eight to 12 h after ingestion. Schut et al. (1983) examined the distribution and elimination of ^{3}H-2,6-DNT in A/J mice after i.p. and oral administrations. Radioactivity was quickly excreted in urine after i.p. administration, and after 4 h, 87.6% of the administered dose (1 mg/kg) appeared in the urine. By comparison, the radioactivity in urine after 4 h amounted to 33.6% when the radiolabel was

administered orally, suggesting a slower rate of absorption. Little radioactivity was detected in the large intestine, which is consistent with the findings of Rickert et al. (1983).

Distribution. After oral and i.p. administrations of ^{3}H-2,6-DNT to A/J mice, Schut et al. (1983) detected the highest levels of radioactivity in the liver, blood, and GI tract. Levels of radioactivity were very low in the brain, heart, and spleen. Radioactivity levels in the liver of orally dosed mice remained constant for up to 8 h after dosing. However, levels of radioactivity in the liver increased during the first hour after i.p. dosing and decreased thereafter. Long and Rickert (1982) administered doses of 10 mg/kg of ^{14}C-2,6-DNT to male and female Fischer-344 rats and measured the urinary and fecal contents. Fifty percent of the dose was accounted for in the urine after 72 h, and 20% was accounted for in the feces. Between two and 72 h after dosing with 2,6-DNT, concentrations in the liver were twice as high in males as in females.

Lee et al. (1975) studied the distribution and excretion of ^{14}C-2,6-DNT administered orally to Charles River rats. After 24 h, 38% of the administered radioactivity was in the feces (27.5%) and GI tract (10.5%), while 60% was recovered in the urine. A small amount of the radioactivity was in the expired air (0.1%). Less than 0.1% of the radioactivity was detected in the spleen, brain, and lungs, while 0.9% was in the liver, 0.1% was in the kidneys, and 0.8% in skeletal muscle.

Metabolism. Dixit et al. (1986) found that 2,6-dinitrobenzyl alcohol (2,6-DNBAlc) (unconjugated or as a glucuronide conjugate) represented the primary metabolite of ^{3}H-2,6-DNT in cultured hepatocytes from A/J mice and Fischer-344 rats. A minor metabolite was 2-amino-6-nitrotoluene. The anaerobic metabolism of ^{3}H-2,6-DNT by extracts of the cecal contents from mice and rats resulted primarily in the formation of 2-amino-6-nitrotoluene and 2-(N-acetylamino)-6-nitrotoluene. Small quantities of 2,6-diaminotoluene were also detected during this in vitro assay No (N-acetylamino) nitrobenzoic acid was found -- as it was in work dealing with the metabolism of 2,4-DNT. Long and Rickert (1982) studied the metabolism of ^{14}C-2,6-DNT in isolated perfused rat livers and also showed that 2,6-DNBAlc and its glucuronide conjugate were the main metabolites. They also examined the in vivo

metabolism following a single oral dose of ^{14}C-2,6-DNT to Fischer-344 rats. Urinary excretion was the principal pathway of elimination, and the major metabolites were 2,6-dinitrobenzoic acid (2,6-DNBA), 2,6-dinitrobenzyl alcohol glucuronide, and 2-amino-6-nitrobenzoic acid.

Urine from four workers exposed to tDNT contained unchanged 2,6-DNT, 2,6-DNBA, and 2,6-DNBAlc (see Turner et al., 1985). These urinary metabolites are similar to those found by Long and Rickert (1982) in rats that had received an oral administration of 2,6-DNT. Turner et al., however, noted that no reduced 2,6-DNT metabolites were detected in the human urine (possibly due to the detection limits). In contrast, 2-amino-4-nitrobenzoic acid (from the 2,4-DNT isomer) constituted 25.6 to 37.7% of the total metabolites in the four workers. The observation of the reduced 2,4-DNT metabolite indicated that nitro group reduction does occur in humans. Nevertheless, because no reduced metabolites of 2,6-DNT were measured, Turner et al. suggest that "the biliary excretion/nitro reduction pathway for bioactivation of 2,6-DNT occurs to a much lesser extent in humans than in rats". The metabolism studies conducted with 2,6-DNT indicate that it undergoes oxidative/reductive transformations analogous to 2,4-DNT. The methyl group of 2,6-DNT is oxidized to an alcohol by the cytochrome P-450 pathway in the liver. This benzyl alcohol is then conjugated with glucuronic acid and excreted in the bile. Twice as much of the glucuronide finds its way into the bile of males as of females (Long and Rickert, 1982). The glucuronide passes through the bile duct and into the small intestine where it undergoes deconjugation and reduction by intestinal microflora. Reduction by the intestinal microflora of the deconjugated metabolite results in an aminonitrobenzyl alcohol. This alcohol can be reabsorbed from the intestine. The amount of pectin in the diet of rats appears to determine the amount of metabolism by the intestinal microflora (Goldsworthy et al. 1986).

The reabsorbed aminonitrobenzyl alcohol reaching the liver must then be transformed to a compound that covalently bonds with hepatic macromolecules (see Rickert et al., 1983 and Rickert et al., 1984). N-hydroxylation with subsequent sulfation of the alcohol moiety has been mentioned as a possible activation process of aminonitrobenzyl alcohol. Kedderis et al. (1984) used sulfotransferase inhibitors to determine whether sulfate ester formation is a necessary step in covalent binding of DNT metabolites to hepatic macromolecules. The effect of the sulfotransferase inhibitors

pentachlorophenol or 2,6-dichloro-4-nitrophenol was to decrease the covalent binding of 2,6-DNT metabolites. The decrease of total hepatic macromolecular binding by 2,6-DNT metabolites was 69%. A 95% decrease was observed in hepatic DNA binding by metabolites of 2,6-DNT.

Excretion. As previously discussed, urine is the major elimination pathway for 2,6-DNT. Long and Rickert (1982) found that both male and female rats excreted approximately half of 10 mg/kg oral doses of ^{14}C-2,6-DNT as urinary metabolites, while almost 20% of the dose was excreted in the feces in 72 h. Urinary excretion was essentially finished after 24 h. Schut et al. (1983) found that mice given an i.p. or oral administration of ^{3}H-2,6-DNT excreted radioactivity more slowly after oral dosing than after i.p. administration. Elimination from the GI tract was negligible after i.p. and oral doses (i.e., <2.4% of the radiolabel). Elimination from the lungs was also negligible (0.03% of the radiolabel). In their study of urinary metabolites of DNT workers, Turner et al. (1985) estimated half-lives for the elimination of 2,6-DNT metabolites. They found that the excretion half-lives for 2,6-DNBA and 2,6-dinitrobenzyl glucuronide ranged between 2.53 and 2.89 h.

Toxic Effects on Organs and Systems

Lee et al. (1976) performed acute and subchronic studies of 2,6-DNT using dogs, rats, and mice. Dogs received the 2,6-DNT in capsule form, whereas the rats and mice received it in their feed. Average dose rates to male and female dogs were 4, 20, and 100 mg/kg-d. Rats and mice received 0.01, 0.05, and 0.25 wt% 2,6-DNT in their feed. Dose rates of 2,6-DNT varied according to the particular period of the 13-week exposures, and in our summaries below we use the dose rates given for the appropriate exposure period. Statistical tests were only performed on selected parameters (i.e., absolute and relative body weights and blood chemistry). The following subsections on organ toxicity are drawn largely from the work of Lee et al. (1976).

Hepatic effects. Dogs orally dosed with 20 to 100 mg/kg-d of 2,6-DNT for 13 weeks had extramedullary hematopoiesis in the liver, bile duct hyperplasia, and degeneration and inflammation of the liver. Bile duct hyperplasia was

observed in male and female rats at average 2,6-DNT doses of 144.7 and 155 mg/kg-d, respectively, over 13 weeks.

Hemotological effects. Dogs that were dosed with 20 mg/kg-d of 2,6-DNT exhibited signs of anemia with decreased hematocrit and hemoglobin concentration, and a compensatory reticulocytosis after two weeks. Small amounts of methemoglobin were detected at eight weeks in the blood of these dogs. Heinz bodies and SGPT increases were noted at the end of 13 weeks.

Average dose rates of 2,6-DNT to male rats (14.7 mg/kg-d) and female rats (15.5 mg/kg-d) produced significantly increased (p level not given) amounts of methemoglobin after 13 weeks. Compensatory reticulocytosis was noted in both male and female rats at these dosages after four weeks. Decreased reticulocyte counts were observed in male and female rats at dose rates of 8.5 and 7.7 mg/kg-d, respectively, after four weeks of dosing. Extramedullary hematopoiesis in the spleen was seen in dogs, rats and mice. This response was observed at the same dosing rate that elicited extramedullary hematopoiesis in the liver. Hemosiderosis was observed in both male and female rats at the low dose rates (i.e., 7.2 and 7.4 mg/kg-d, respectively).

Reproductive effects. A dose rate of 100 mg/kg-d resulted in the depression of spermatogenesis after 13 weeks in a couple of dogs. Male rats had depression of spermatogenesis at 37 and 126 mg/kg-d after four weeks. Testicular atrophy and aspermatogenesis were noted in rats after 13 weeks of 144.7 mg/kg-d dosing. There was testicular atrophy in mice at a dose rate of 326.3 mg/kg-d after four weeks.

Neurological effects. Dogs began experiencing neuromuscular symptoms before four weeks at a dose rate of 100 mg/kg-d. Symptoms began with listlessness, incoordination, and lack of balance. The muscles then became stiff, and the animals were unable to move. No neuromuscular toxicity was noted for either rats or mice.

Dermal effects. Lee *et al*. (1975) found 2,6-DNT to be a mild skin irritant in rabbits.

Genotoxicity

2,6-DNT has been studied for its ability to induce reverse mutations in bacteria, mitotic recombination in yeast, forward mutations in mammalian cells, and unscheduled DNA synthesis in primary hepatocyte cultures. The results of these tests are summarized in Table 5-6, and are discussed in the following section.

A relatively early study reported that 2,6-DNT was inactive in the Ames assay (Simmon et al., 1977). However, more recent studies have found evidence that 2,6-DNT is mutagenic to Salmonella. For example, Couch et al. (1981) reported that 2,6-DNT induced a two-to-fourfold increase in the number of revertants when tested in the presence or absence of S9. The data of Spanggord et al. (1981) confirmed that the addition of S9 does not significantly alter the activity of 2,6-DNT. However, in the latter study, 2,6-DNT was only mutagenic to strain TA100, which is sensitive to base pair mutagens.

Lee et al. (1976) measured a statistically significant increase in the number of chromatid gaps and chromatid breaks in the lymphocytes of rats that were fed 2,6-DNT in the diet (0.05 wt%). Kidney cells from the same animals showed a significant increase in the number of tetraploids.

2,6-DNT has given variable results in assays of UDS, and its activity in primary hepatocytes may depend on the test protocol. When Bermudez et al. (1979) evaluated 2,6-DNT in an in vitro assay, there was no evidence of DNA damage. The more sensitive in vivo-in vitro assay of Mirsalis et al. (1982a, 1982b) demonstrated that 2,6-DNT was a potent inducer of UDS in hepatocytes. 2,6-DNT does not induce UDS in spermatocytes (Working and Butterworth, 1984).

Short-term assays to evaluate the ability of 2,6-DNT to induce mitotic recombination in Saccharomyces or forward mutations in CHO and mouse lymphoma cells have given negative results (Simmon et al. 1977; Abernethy and Couch, 1982; Styles and Cross, 1983; Lee et al., 1976).

Carcinogenicity

In this subsection we review the results of several bioassays carried out on tDNT, as well as the 2,4- and 2,6-DNT isomers. Appendix C contains tumor incidence data for the studies reviewed here. The National Cancer Institute

tudies completed on the genotoxicity of 2,6-DNT.

Sample purity	Dosage	Results +S9	Results -S9	Comments	References
Not specified	0.6, 5.0, 15,0, 35.0, 45.0 µg/plate	-	-		Simmons et al., 1977
Not specified	100.0, 1000.0 µg/plate	?		Insufficient data to evaluate	Tokiwa et al., 1981
Not specified	10.0 to 5000.0 µg/plate	+	+	Positive results only with TA100. Specific revertant counts not reported.	Spanggord et al., 198
≥99.98%	1000.0 µg/plate	+	+	Single concentration tested.	Couch et al., 1981
Not specified	9.6×10^{-5} to 7.0×10^{-3} % (v/v)	-	-	Assay may be insensitive	Simmon et al., 1977
≥99.8%	0.7, 1.4, 2.0 2.5 mM	-	-		Abernethy and Couch, 1
Not specified	0.05 wt% (in diet)	-	-		Lee et al., 1976
'Pure'	1.6, 8.0, 40,0, 200.0, 1000.0 µg/mL	-	-	Dose-dependent decrease in survival, with and without S9.	Styles and Cross, 1983

Table 5-6. (continued)

ay	Genetic endpoint	Sample purity	Dosage	Results +S9	Comments	References
phocyte	Chromosome aberration	Not specified	0.05% (in diet)	+	Significant increase in number of chromatid gaps and breaks.	Lee *et al*., 1978
ney 1	Chromosome aberration	Not specified	0.05% (in diet)	+	Significant increase in number of tetraploid cells.	Lee *et al*., 1976
rmatocyte repair	DNA repair	≥99.0%	10.0, 100.0, 1000.0 μM	-	Toxic at highest concentration	Working and Butterwort 1984
atocyte repair	DNA repair	'Purified'	0.001, 0.0001 M	-		Bermudez *et al*., 1979
atocyte repair	DNA repair	≥99.9%	5.0, 20.0, 100.0 μg/kg	+	Treatment also stimulated cell proliferation	Mirsalis *et al*., 1982a Mirsalis *et al*., 1982b

(NCI, 1978) performed a chronic study on Fischer-344 rats and B6C3F1 mice. The DNT used in the study was "greater than" 95% 2,4-DNT. Impurities were not identified. Groups of 50 male and 50 female rats received tDNT at dietary concentrations of 0.04 and 0.008 wt% (time-weighted concentrations). No statistically significant incidence of malignant tumors was observed. Slight, but not statistically significant, increases in the number of hepatocellular carcinomas were seen in male rats receiving either low or high doses (incidence: 3/49 for low dose vs. 0/45 for low-dose controls; 3/48 for high dose vs. 0/25 for high-dose controls). Neither the high-dose nor the low-dose groups had survival rates significantly different from the corresponding control group. Five males and five females from each dose level were sacrificed at week 78. The dosing regime was discontinued for the remaining rats and they were observed for an additional 26 weeks. Treatment-induced benign tumors found in significant numbers were: fibroma of the skin and subcutaneous tissue in the high-dose ($p = 0.003$) and low-dose ($p = 0.008$) male rats and fibroadenoma of the mammary gland ($p = 0.016$) for high-dose female rats. The maximum tolerated dose was not defined in this study.

Under funding from the U.S. Army, Ellis et al. (1979) performed chronic bioassays using dogs, rats, and mice. The tDNT used in these studies averaged 98 wt% 2,4-DNT and 2 wt% 2,6-DNT. Significant incidences of malignant tumors were found only in the CD rats. Three dosing levels were used in the rat study. Each treated group of rats began with 38 male rats and 38 female rats. The rat feed consumed provided an average 2,4-DNT intake for both sexes (see Table C-1) of 0.63 mg/kg-d (0.01 mg/kg-d 2,6-DNT), 4.4 mg/kg-d (0.09 mg/kg-d 2,6-DNT), and 39.1 mg/kg-d (0.8 mg/kg-d 2,6-DNT). The high dose was very toxic to the rats and caused a shortened lifespan. Incidences of hepatocellular carcinoma were statistically significant only for female rats receiving the high dose. The incidence of carcinomas in the females receiving the high dose was 18/34 compared to 0/23 for the controls ($p = 0.00000071$). Significant numbers of benign tumors were also noted in both rats and mice. Although hepatocellular neoplastic nodules occurred in rats, these data were not combined with the carcinoma data and statistically evaluated. The males receiving the high dose had an increase in hepatocellular carcinomas, but this increase was not statistically significant when compared to controls (6/29 vs 1/25). This result could be due to the high rate of early deaths of the males receiving the high dose.

The Chemical Industry Institute of Toxicology (CIIT, 1982) completed a two-year tDNT feeding study that indicated that tDNT is hepatocarcinogenic in Fischer-344 rats. The tDNT used in this study was composed of 76.4 wt% 2,4-DNT and 18.8 wt% 2,6-DNT. The remaining 4.8 wt% contained four other DNT isomers. Groups consisting of 130 rats of each sex were assigned to treatment groups receiving average intakes of 2,4-DNT of 3.4, 13.5, and 35 mg/kg-d (associated intakes of 2,6-DNT were 0.64, 2.5, and 6.6 mg/kg-d). Statistically significant incidences of hepatocellular carcinomas and hepatic neoplastic nodules were found in both sexes at the high-dose level. Two hepatocellular carcinomas were found in male rats as early as the week-26 serial sacrifice. The combined incidences were 10/10 for males receiving the high dose compared to 0/10 for controls after one year of treatment. Female rats had a 9/10 combined hepatic-neoplastic-nodule/hepatocellular-carcinoma incidence compared to 0/10 for controls. All rats in the high-dose group were sacrificed at week 55 because of the histopathological findings at the earlier serial sacrifices. Male and female rats in the mid-dose groups had high incidences of neoplastic nodules and hepatocellular carcinomas at subsequent serial sacrifices and at the week 104 terminal sacrifice (e.g., male rats: 23/23 vs 0/19 for controls; female rats: 66/68 vs 1/23 for controls at week 104). Cholangiocarcinomas were seen in males receiving mid and high doses and were assumed to be treatment-related, although they were not statistically significant.

Leonard et al. (1987) also carried out one-year bioassays using tDNT (19 wt% 2,6-DNT), pure 2,4-DNT, and pure 2,6-DNT. Significant incidences of hepatic neoplastic nodules and hepatocarcinomas were noted in the Fischer-344 rats receiving the pure 2,6-DNT or tDNT. No malignant tumors were found in the rats administered pure 2,4-DNT in their diets. Multiple carcinomas were frequently seen in rats dosed with either tDNT or 2,6-DNT. Ten percent of these animals had cholangiocarcinomas. Leonard et al. concluded that most, if not all, of the carcinogenicity of tDNT could be attributed to the 2,6-DNT isomer.

In addition to the animal bioassays that have been completed, work has been completed on the initiation/promotion potential of the DNT isomers. Leonard et al. (1983), for example, studied the initiation of the DNT isomers to examine their carcinogenic activity. Four different hepatic initiation-promotion systems using Fischer-344 rat hepatocytes were used to examine the

relationship between DNT dosing and the incidence of gamma-glutamyl transpeptidase positive foci (GGT^+) (> 9 cells/focus). An increased number of GGT^+ foci indicated initiation ability of a DNT isomer. Both 2,6-DNT and tDNT gave positive initiation responses in which a partial hepatectomy (growth initiation) was performed 12 h before administration. When tDNT administration was started at times later than 12 h after a partial hepatectomy, the number of GGT^+ foci decreased, suggesting that DNT-induced promotion may be reversible. Only 2,6-DNT significantly ($p < 0.05$) increased the number of GGT^+ foci. Additional testing showed that GGT^+ foci increased with dose between 37.5 and 100 mg/kg. While tDNT and 2,6-DNT are considered initiators in these tests, compared to other known initiators, they are only weak initiators.

Leonard et al. (1986) also studied the promotional activity of 2,4- and 2,6-DNT. These isomers were given to rats at dietary dose rates of 27 mg/kg-d and 14 mg/kg-d, respectively, for six weeks after receiving the initiator DEN (diethylnitrosamine) in the diet. Both 2,4- and 2,6-DNT significantly increased ($p < 0.05$) the number of GGT^+ foci, when there was prior treatment with DEN. The 2,6 isomer produced twice the number of GGT^+ foci as did 2,4-DNT. However, only 2,6-DNT produced a significant increase ($p < 0.05$) in GGT^+ foci without the use of the DEN initiator. Thus, it appears that 2,6-DNT is an initiator and promoter, whereas 2,4-DNT acts solely as a promoter in this assay. On the basis of the above data, 2,6-DNT appears to be a complete carcinogen, causing both initiation and promotion of carcinogenesis, while 2,4-DNT appears to be only a weak promoter. The data suggest 2,4-DNT exposure must be accompanied with exposure to an initiator to induce carcinogenesis.

Estimation of Intake Limits to Protect Public Health

The hepatocarcinogenicity of 2,6-DNT has been demonstrated with both animal bioassays and studies involving the initiation-promotion of hepatocytes. Under the "weight-of-evidence" protocols adopted by the U.S. EPA (1986b) for use in carrying out risk assessments of suspected carcinogens, 2,6-DNT would be classified as a Group B2 agent (i.e., "sufficient" evidence from animal studies, but with inadequate human data). To assess the carcinogenic potency of 2,6-DNT, we applied the multistage dose-response model (see Appendix C for a discussion of assumptions) to the tumor incidence-dose

rate data in Table C-1. The resulting 95% upper-confidence limits of carcinogenic potency were then used to calculate doses corresponding to different lifetime cancer risks and are shown in Table 5-7. At a lifetime risk of 10^{-5}, the doses range from 1.7×10^{-6} to 5.3×10^{-5} mg/kg-d (based on a body-weight extrapolation) and 2.8×10^{-7} to 7.7×10^{-6} mg/kg-d (based on a surface-area extrapolation).

TOXIC EFFECTS ON AQUATIC SPECIES

Aquatic toxicity data for 2,6-DNT are very limited. Measurements of the acute toxicity in water fleas and fathead minnows were performed by Liu et al. (1983b) and are presented in Table 5-8. 2,6-DNT is about twice as toxic to these two species as is 2,4-DNT. No chronic or bioaccumulation studies have been reported on 2,6-DNT.

Table 5-7. Dose rates for 2,6-DNT corresponding to different lifetime cancer risks. Dose rates are calculated from the potencies presented in Table C-1 of Appendix C.

Species and sex	Tumor type[a]	Lifetime cancer risk 10^{-5} BW[b]	10^{-5} SA[c]	10^{-6} BW[b]	10^{-6} SA[c]	10^{-7} BW[b]	10^{-7} SA[c]	Reference
		Dose rate in mg/kg-d						
Rat male	HCC only	2.9×10^{-6}	5.0×10^{-7}	2.9×10^{-7}	5.0×10^{-8}	2.9×10^{-8}	5.6×10^{-9}	Leonard *et al.*, 1987
Rat male	HCC or NN	1.7×10^{-6}	2.8×10^{-7}	1.7×10^{-7}	2.8×10^{-8}	1.7×10^{-8}	2.8×10^{-9}	Leonard *et al.*, 1987
Rat male	HCC or NN	3.3×10^{-6}	5.6×10^{-7}	3.3×10^{-7}	5.6×10^{-8}	3.3×10^{-8}	5.6×10^{-9}	Leonard *et al.*, 1987
Rat male	HCC only	7.7×10^{-6}	1.3×10^{-6}	7.7×10^{-7}	1.3×10^{-7}	7.7×10^{-8}	1.3×10^{-8}	Leonard *et al.*, 1987
Rat male	HCC or NN	4.5×10^{-5}	7.7×10^{-6}	4.5×10^{-6}	7.7×10^{-7}	4.5×10^{-7}	7.7×10^{-8}	CIIT, 1982
Rat female	HCC or NN	5.3×10^{-5}	7.7×10^{-6}	5.3×10^{-6}	7.7×10^{-7}	5.3×10^{-7}	7.7×10^{-8}	CIIT, 1982
Rat male	HCC or NN	1.4×10^{-5}	2.8×10^{-6}	1.4×10^{-6}	2.8×10^{-7}	1.4×10^{-7}	2.8×10^{-8}	Ellis *et al.*, 1979
Rat female	HCC or NN	6.2×10^{-6}	1.1×10^{-6}	6.2×10^{-7}	1.1×10^{-7}	6.2×10^{-8}	1.1×10^{-8}	Ellis *et al.*, 1979
Rat male	HCC only	1.8×10^{-5}	3.7×10^{-6}	1.8×10^{-6}	3.7×10^{-7}	1.8×10^{-7}	3.7×10^{-8}	Ellis *et al.*, 1979
Rat female	HCC only	1.0×10^{-5}	1.7×10^{-6}	1.0×10^{-6}	1.7×10^{-7}	1.0×10^{-7}	1.7×10^{-8}	Ellis *et al.*, 1979

Table 5-7. Footnotes

[a]. HCC = hepatocellular carcinoma, NN = neoplastic nodule.

[b]. BW = Body weight interspecies dose-extrapolation method; equivalent doses (D) assumed to be in mg/kg, so $D_{human} = D_{animal}$.

[c]. SA = Surface Area interspecies dose-extrapolation method; equivalent doses (D) assumed to be in mg/kg $^{2/3}$, so $D_{human} = D_{animal}$ [(animal weight)/70 kg]$^{1/3}$.

Species	Concentration (mg/L)	Effect	Reference
Water flea	19.3 to 24.6 (21.8)	48 h EC50	Liu *et al*., 1983b
Fathead minnow	17.2 to 20.2 (18.5)	96 h LC50	Liu *et al*., 1983b

6. DATA-BASE ASSESSMENT FOR 1,3-DINITROBENZENE

1,3-Dinitrobenzene (DNB) is an impurity in TNT and as such, it represents a potential co-contaminant of TNT at OB/OD grounds. This particular co-contaminant has been measured in soils at OB/OD areas as well as in the waste waters of munitions factories. An earlier review of the environmental chemistry and toxicology of DNB (Wentsel et al., 1979), addressed most of the relevant literature published prior to 1979. In the following subsections, we update that previous review, focusing primarily on those studies with information pertinent to the quantification of the health and environmental risks of DNB.

ENVIRONMENTAL PARTITIONING AND TRANSPORT

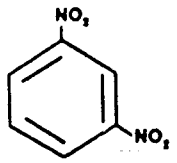
The chemical identifiers for DNB are presented in Table 6-1, its chemical and physical properties are shown in Table 6-2, and its partition coefficients are shown in Table 6-3. Although DNB has the highest Henry's law constant (i.e., 0.14 L-torr/mol) of the explosives and co-contaminants addressed in this report, most of the DNB added to the surface-soil compartments of the reference landscapes under steady-state conditions should distribute to the lower-soil compartment or ground water of those landscapes, as shown in Table 6-4.

ENVIRONMENTAL TRANSFORMATION PROCESSES

Photolysis

Simmons and Zepp (1986) reported rate constants for the photolysis of nitroaromatic compounds, including DNB, in distilled water and aqueous solutions containing humic materials (see Table 6-5). The average annual rate constant for the direct photolysis of DNB in distilled water was 0.03 d^{-1} at 40°N latitude. The near-surface photolysis rates for DNB in a sample of Aucilla River water and in an aqueous solution containing fulvic acid were 0.029 and 0.043 d^{-1}, respectively. The photolysis rates for DNB were considerably lower than those for 2,4-DNT and 2,6-DNT, which Simmons and Zepp also studied. One explanation is that DNB lacks the methyl group that

Table 6-1. Chemical identifiers of 1,3-dinitrobenzene.

Empirical formula:	$C_6H_4(NO_2)_2$	Structural formula:
Molecular weight:	168.11	
CAS reg. no.:	[99-65-0]	
RTECS no.:	CZ7350000	

accelerates the photolytic decomposition of the DNT molecules due to steric interaction with the adjacent nitro groups on the ring. Spanggord et al. (1980a) noted a 50% loss of the DNB component of condensate water after 12 d of exposure to sunlight. However, the same amount was lost after 14 d in the shade. They suggested that volatilization was the primary mode of DNB loss.

Biotransformation

Several researchers have investigated the biotransformation of DNB. Mitchell et al. (1982), for example, examined its aerobic biotransformation in Tennessee River water taken downstream from the Volunteer Army Ammunition Plant, Chatanooga, Tennessee. Controls included sterilized Tennessee River water and local water samples and sediments taken near Frederick, Maryland. When incubated with the Tennessee River water at a concentration of 5 μg/L, DNB was completely transformed in 15 d with a 10-d lag time. These changes were reproducible with samples taken up to a year later. Most local waters and sterilized samples studied did not support the biotransformation of DNB; however one combination of a local sediment and water caused transformation of DNB at approximately the same rate as the water from the Tennessee River. No other sediment caused transformation.

Other researchers have also found that DNB is transformed by microorganisms. McCormick et al. (1976) measured a microbial degradation rate for DNB that was 3.4 times less than that of TNT, using *Viellonella alkalescens*. Cartwright and Cain (1959) demonstrated reduction to arylamines

Table 6-2. Chemical and physical properties of DNB. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	300 to 303 (decomposes)	Windholz *et al.*, 1983
Freezing/melting point	°C	89 to 90	Windholz *et al.*, 1983
Density	g/mL	1.58	Windholz *et al.*, 1983
Vapor pressure	torr	2×10^{-4}	Extrapolated[a] from pressure-temperature data given in Meyer, 1981
Aqueous solubility	mol/L mg/L	1.1×10^{-3} 180	Interpolated from solubility-temperature data given in Freier, 1976
Henry's law constant	(L-torr)/mol	2×10^{-1}	Calculated as the ratio of vapor pressure to aqueous solubility
Diffusion coefficients: Air Water	m^2/s m^2/s	6.8×10^{-6} 6.8×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition coefficient (K_{ow})	log K_{ow}	1.49	Hansch and Leo, 1979

[a] See Appendix A.

Table 6-3. Partition coefficients for use in estimating human exposures to DNB.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	4	Calculated[b] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	2.2×10^{-3}	Calculated[b] from water solubility and log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	6	Calculated[b] from log K_{ow}
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	210	Calculated[b] from solubility in water and melting point

[a] Wet-weights for plant and fish

[b] See Appendix A

Table 6-4. Fractional distributions of DNB among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady- state addition of DNB to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	7.8×10^{-5}	8.9×10^{-5}
Air particles	6.2×10^{-9}	1.4×10^{-8}
Biota	2.5×10^{-4}	0.025
Upper soil	0.027	0.096
Lower soil	0.40	0.098
Ground water	0.57	0.77
Surface water	0.0021	0.0078
Sediments	1.1×10^{-4}	4.2×10^{-4}

[a] Fractions do not add to one because of rounding.

by an extract of Nocardia erythropolis. Bringmann and Kuhn (1971) were able to oxidize DNB in a two-step laboratory procedure with Azotobacter agilis and activated sewage sludge. Chambers et al. (1963) found that some oxygen was utilized in a mineralization experiment with DNB, using phenol-adapted bacteria from sewage sludge. Hallas and Alexander (1983) found that the microbes in a raw sewage effluent were able to transform DNB under both aerobic and anaerobic conditions. The half-lives were less than two weeks for both conditions. Their reported biotransformation product was 3-nitroaniline.

Several other researchers found little or no biodegradation with selected microorganisms. Sanyal and Gupta (1974) found that DNB totally inhibited fungal growth of the five fungi tested when they were looking for a combined corrosion and fungus inhibitor. Alexander and Lustigman (1966) found no ring cleavage after 64 d when using DNB as a sole carbon source with soil microorganisms. They did not examine the solution for transformation products.

Table 6-5. Photolytic degradation of DNB (from Simmons and Zepp, 1986).

Initial concentration (mg/L)	Derivation	Photolysis rate constant (d^{-1})	Half life (d)
0.2	Calculated[a] average annual rate for DNB in distilled water at pH = 5.5	0.03	23
0.1 to 1	Measured in distilled water exposed to sunlight in Athens, GA, corrected with actinometer.	0.01	69
0.1 to 1	Measured in Aucilla River water exposed to sunlight, corrected to surface value with actinometer.	0.029	24
0.1 to 1	Measured in an aqueous solution containing fulvic acid exposed to sunlight, corrected to surface value with actinometer.	0.043	16

[a] Using the method of Zepp and Cline, 1977

In summary, biotransformation will be a significant degradation pathway for DNB only in environmental media with organisms that have adapted to transform it.

Hydrolysis

Hydrolysis is not expected to be a major environmental fate of DNB (Spanggord *et al.*, 1980a).

TOXIC EFFECTS ON ANIMALS AND HUMANS

The toxic effects of DNB have been studied for over one hundred years (see Beritic (1956) for a discussion of the earliest citations). The first analyses of its toxicity were related to occupational exposures in munitions

factories during the World Wars when it was used as an explosive filler. The primary effect to exposed workers was methemoglobinemia. Similar responses have been demonstrated in laboratory animals. Recent studies have shown that DNB also causes toxic effects to the reproductive system of rats.

Toxicokinetics

Absorption. The existing data on the absorption of DNB come mainly from occupational exposures; few animal data exist. A study by Ishihara et al. (1976) has shown that DNB is easily absorbed through skin. Their study was designed to explore the cause of cyanosis in a woman employed in an electronics factory who used rubber gloves to handle parts immersed in a chemical mixture containing 5000 ppm of DNB. They duplicated the exposure condition using a volunteer who handled the immersed parts for 45 min using latex gloves. Blood samples were collected before and after the exposure (i.e., immediately before exposure, at the end of the exposure, and at 3, 5, and 21 h post exposure). Methemoglobin levels reached peak levels immediately after the exposure and declined afterward. Measurements of the breathing-zone air of the volunteer did not detect the presence of DNB; however, a permeation experiment conducted by the researchers showed that DNB passes through the latex gloves -- indicating that dermal absorption was the route of uptake. The rapid increase in methemoglobin levels suggests that this compound is quickly absorbed through skin. The review by Wentsel et al. (1979) also noted an early study conducted by White and Hay (1901) in which DNB was applied in the form of an ointment to the backs of cats -- all of which developed classical symptoms of methemoglobinemia. Okubo and Shigeta (1982) reported several cases of cyanosis that occurred among workers who were exposed to DNB dust particles while removing DNB that had crystallized in a tank. The workers used only gauze masks and rubber gloves. Exposures included both inhalation and skin absorption (particles of DNB adhered to the worker's skin), although the authors considered inhalation the major exposure pathway because the skin contact was with DNB in solid form. However, without measurements of the airborne concentrations of the DNB particles and the deposition on skin, it is not possible to determine which pathway was the most important.

Metabolism and elimination. The primary metabolic reactions of DNB involve reduction of the nitro groups and oxidation of the aromatic ring to a phenol. Parke (1961) examined the metabolism and excretion of radiolabeled DNB in rabbits. After oral (50 to 100 mg/kg) administration, 65 to 93% of the radioactivity was recovered in the urine after 2 d. The feces only contained trace amounts (1 to 5%) of the dose. Of the metabolites detected, 30% were conjugated with glucuronic acid and only 6% with sulfate. The major metabolites found were m-nitroaniline; m-diaminobenzene (together totaling 35% of the dose); 2,4-diaminophenol (31% of the dose); and 2-amino-4-nitrophenol (14% of the dose). Miscellaneous metabolites (20% of the dose) included other oxidation and reduction products, and azoxy dimers. The reduction of nitro groups to amino groups and the oxidation of amino groups to nitro groups involves the same reactive intermediate (hydroxylamine). As noted by Wentsel et al. (1979), there are some limitations to Parke's study. For example, $^{14}CO_2$ was not measured, the analytical methods for identifying and quantifying the metabolites are outdated, and the method of ^{14}C counting (i.e., end-window counter) is not as accurate as the currently accepted technique (i.e., liquid scintillation). The only recent measurement of a metabolite of DNB is contained in the paper by Ishihara et al. (1976), who reported total metabolite production (both amino and nitro compounds) in urine as 2,4-dinitrophenol. Cossum and Rickert (1987) studied the metabolism of DNB isomers in erythrocytes from Fischer-344 rats, rhesus monkeys, and humans. They were unable to detect metabolites of DNB in the erythrocytes from any of the species. The o-DNB and p-DNB isomers, though, were metabolized to S-(nitrophenyl) glutathione conjugates.

Toxic Effects on Organs and Systems

Hematological effects. The primary effect of DNB absorbed into blood is the formation of methemoglobin. The methemoglobin-forming capacity of DNB and other nitrobenzenes has been reviewed by Kiese (1974). That review indicates that DNB is among the more potent formers of methemoglobin. For example, in cats an intraperitoneal (i.p.) administration of 7 mg/kg of DNB produces essentially the same maximum level of methemoglobin as an i.p. administration of 40 mg/kg of TNT. A later study by Watanabe et al. (1976) of the methemoglobin-forming potential of 19 chemicals administered to rats (i.p.

injections of 100 μmol/kg of each chemical) demonstrated that DNB was more effective in producing methemoglobin than all of the other compounds tested, except m-chloronitrobenzene and p-dinitrobenzene. The actual biochemical mechanism of methemoglobin formation by DNB and other nitro compounds is unknown -- in particular, whether the parent compound or a metabolite is the active agent in methemoglobin formation.

Definitive studies on the relationship between intakes of DNB and blood effects in humans or animals are not available. However, the limited studies that have been conducted show that low levels of DNB can cause measurable effects in blood. Kiese (1949a, 1949b), in a series of frequently cited experiments (see Wentsel et al. (1979) for a discussion of that work), detected changes in blood pigment concentrations and the presence of Heinz bodies in the red blood cells of dogs receiving subcutaneous injections of 0.5, 1.0, 1.5, and 2.0 mg/kg of DNB in olive oil for various periods up to 144 d. Blood effects were dose related. More recently, Cody et al. (1981) found that hemoglobin levels were significantly reduced in male and female rats consuming water for 7 weeks containing 50, 100, and 200 mg/L of DNB.

Occupational exposures to DNB have resulted in elevated levels of methemoglobin (Ishihara et al., 1976) and Heinz bodies (Beritic, 1956). Symptoms of acute methemoglobinemia that have been observed after occupational exposures include headache, nausea, and cyanotic appearance. Unfortunately, none of the documented occupational exposures provide data on the amounts of DNB (or other isomers) inhaled or otherwise absorbed systemically. A further complication is that there is usually a large inter-individual variation in the responses to DNB exposures (see Beritic, 1956).

Hepatic effects. Data on the effects of DNB to liver are inconclusive. Watanabe et al. (1976) found that GPT levels in rats measured 5 h after an i.p. injection of 100 μmol/kg were significantly increased over the measured levels in the controls; however, GOT levels were not significantly different. Measurements of GOT and GPT in workers who have had acute exposures to DNB have not shown abnormally high levels of GOT or GPT (Ishihara et al., 1976; Okubo and Shigeta, 1982). Two women who experienced occupational over-exposures to DNB and subsequently exhibited symptoms of cyanosis did exhibit slightly elevated levels of serum bilirubin, an indicator of liver damage. In addition, one of the subjects had an enlarged liver.

Central nervous system effects. Nervous system effects observed in laboratory animals include reduced motor nerve conduction and various behavioral effects observed in subchronic toxicity studies. Pankow et al. (1975) measured the sciatic motor conduction velocity in rats receiving single i.p. injections of DNB (0.15 μmol/kg). Measurements of the nerve conduction velocity were carried out at 1, 2, 7, and 28 d after the initial injection. Only at the one-day measurement was the velocity (in m/s) significantly ($p < 0.001$) reduced from the controls (16% decrease). A subchronic toxicity test with rats consuming water containing 3, 8, and 20 mg/L for 16 wk, showed that at the low concentrations the treated rats had a statistically significant ($p < 0.01$) increase in the use of activity wheels (measured as average revolutions per day) over the controls. In another subchronic study, Linder et al. (1986) observed neurotoxic effects, including impaired movement, vertigo, and rigid muscles in rats gavaged with 6.0 mg/kg of DNB in corn oil for 10 weeks.

Reproductive effects. Effects on the reproductive system were identified by Cody et al. (1981) in their subchronic toxicity study with rats. They observed a statistically significant decrease ($p < 0.001$) in the weights of the testicles of rats consuming 20 mg/L of DNB in their drinking water for 16 weeks. A more recent study completed by Linder et al. (1986) (gavage doses to Sprague-Dawley rats were 0.75, 1.5, 3.0, and 6.0 mg/kg-d)[a] clearly demonstrated that DNB is a testicular toxicant. Weights of testes in the rats gavaged with 3 mg/kg-d of DNB for 12 weeks were 40% of the weights in the controls ($p < 0.05$). The epididymides of the rats at that treatment level were also significantly depressed ($p < 0.05$). The sperm-head counts for rats in the 1.5 mg/kg-d treatment group were significantly reduced ($p < 0.05$) from those of the control group when expressed on either a per testis or per gram testis basis. Sperm morphology parameters (e.g., flagellar defects, atypically shaped heads) of that treatment group were not different from the control rats. At the 0.75 mg/kg-d treatment level no effects to sperm were observed. However, at the 3 mg/kg-d treatment level head counts of testicular sperm and

[a] Treatment at the 6.0 mg/kg-d level was stopped prior to the scheduled sacrifice because of severe toxicity to the rats and consequently, quantitative comparisons with the other treatment groups were not made.

cauda sperm reserves "approached zero." Furthermore, sperm mobility and the number of sperm per mg of epididymal fluid could not be evaluated because of the clumping of degenerated spermatozoa. The seminiferous tubules of the rats in that treatment group were atrophic. The male rats treated with 3 mg/kg-d did not sire litters. The number of pups per litter for the male rats at the 0.75 and 1.5 mg/kg-d treatment levels was slightly lower than the controls ($p < 0.05$), but the changes in litter sizes were not dose-related and were within the normal range. The authors considered the results "equivocal." Our literature review did not reveal any studies dealing with reproductive effects in humans.

Genotoxicity

The genotoxicity of DNB has been assessed in three microbial systems (*Salmonella typhimurium*, *Saccharomyces cerevisiae*, and *Escherichia coli*). Positive results were obtained in three separate investigations using the *S. typhimurium* plate incorporation assay (McGregor *et al.*, 1980; Chiu *et al.*, 1978; Spanggord *et al.*, 1982b). The combined range of doses examined was 1.0 to 1680 µg per plate. The lowest dose to exhibit a positive response was 16.8 µg/plate (Chiu *et al.*, 1978). McGregor *et al.* (1980) found that the addition of rat microsomal enzymes reduced the positive response. DNB did not induce reversions in a strain of *S. typhimurium* (i.e., TA100NR3) lacking nitroreductase activity (Spanggord *et al.*, 1982b). This suggests that nitro group reduction is required for mutagenic activity of this compound.

Results of testing for mitotic recombination with the yeast *S. cerevisiae* were negative in two separate investigations. McGregor *et al.* (1980) used strain D5 in a suspension test with DNB at 4.0 mg/mL both with and without microsomal activation. The compound was not toxic, but did precipitate at this concentration. As the negative data were not presented and there is no report of the investigators examining lower, soluble concentrations of DNB, it is not possible to evaluate these results with *S. cerevisiae*. Simmon *et al.* (1977) studied the mutagenic activity of DNB in *S. typhimurium* with strains TA1535, TA1537, TA1538, TA98, and TA100. Positive responses were not obtained with or without metabolic activation. In the *E. coli* DNA repair assay no differential growth was detected between repair-proficient and

repair-deficient strains following exposure to DNB with or without microsomal activation (McGregor et al., 1980).

Estimation of Intake Limits to Protect Public Health

Table 6-6 summarizes the dose-response data from the subchronic toxicity studies completed by Cody et al. (1981) and Linder et al. (1986). Effects are observed at average dose rates as low as 0.4 mg/kg-d in the Cody et al. study (e.g., significant increases in spleen weights and wheel-running activity of male rats), while Linder et al. noted only a marginal, non-dose-related (though statistically significant) decrease in the sizes of litters sired by rats in the 0.75 mg/kg-d treatment group. Neither study included measurements of methemoglobin formation. The early work by Kiese (1949a, 1949b) with dogs receiving subcutaneous injections of DNB is unsuitable for defining a NOEL because of the small numbers of animals used, lack of statistical tests, and the intermittent nature of the dosing (see Wentsel et al., 1979). Kiese (1949b) did note effects at doses as low as 0.1 and 0.2 mg/kg-d, but the equivalent oral doses are apt to be higher.

Based on the two subchronic toxicity studies, the NOEL for DNB is probably below 0.4 mg/kg-d, which is the lowest-observed-adverse-effect level (LOAEL). With a safety factor of 1000, which accounts for the less-than-lifetime period of the study, the estimated ADI becomes 0.0004 mg/kg-d. By comparison, the oral rat LD50 of 83 mg/kg (Cody et al., 1981) multiplied by a factor of 1×10^{-5} d^{-1} (see Appendix D) yields a lower-bound estimate of an ADI of 0.0008 mg/kg-d.

TOXIC EFFECTS ON AQUATIC SPECIES

Table 6-7 summarizes the results of various studies on the acute toxicity of DNB to aquatic species. Bluegill and rainbow trout were the most sensitive vertebrate species in the acute tests, with 96-h LC50's of 1.44 and 1.70 mg/L, respectively. Van der Schalie (1983) performed a dynamic acute test (using a flow-through system) with rainbow trout that lasted 30 d. The LC50 for that period was 0.37 mg/L, or more than a factor of 4.5 lower than the 96-h LC50 (i.e., 1.70 mg/L). Two early-life-stage (ELS) tests using rainbow trout were also conducted. These tests were used to define concentration-response

Table 6-6. Dose-response relationships for DNB from subchronic toxicity studies by Cody et al. (1981) and Linder et al. (1986).

Species (sex)	Route	No. of animals	Duration (wk)	Dose rate mg/kg-d	Effects	Reference
Rat (male)	Oral	20	16	0.4[a]	Significant increase ($p < 0.01$) in wheel-running activity. Significant increase ($p < 0.01$) in spleen weight.	Cody et al., 1981
				1.1[b]	Significant increase ($p < 0.01$) in wheel-running activity. Significant increase ($p < 0.001$) in spleen weight.	
				2.6[c]	Significant increase ($p < 0.001$) in spleen weight. Significant decreases in hemoglobin at weeks 5 and 10 ($p < 0.05$ and $p < 0.01$, respectively). Significant decrease ($p < 0.001$) in the weight of testicles.	
Rat (female)	Oral	20	16	0.5[a]	Significant increase ($p < 0.01$) in ovary weight.	
				1.3[b]	Significant increase ($p < 0.001$) in spleen weight.	
				3.1[c]	Significant increase ($p < 0.001$) in spleen weight.	

Table 6-6. (Continued)

Species (sex)	Route	No. of animals	Duration (wk)	Dose rate mg/kg-d	Effects	Reference
Rat (male)	Gavage	12	12	0.75	Significant decrease ($p < 0.05$) in litter size[d].	Linder *et al.*, 1986
					Significant increase ($p < 0.05$) in spleen weight. Significant decrease ($p < 0.05$) in testicular sperm-head count. Significant decrease ($p < 0.05$) in litter size[d].	
				3.0	No litters sired. Significant increase ($p < 0.05$) in spleen weight. Significant changes ($p < 0.05$) in sperm morphology. Significant decrease ($p < 0.05$) in testicular sperm-head count. Significant decrease ($p < 0.05$) in cauda sperm reserves.	

[a] Average dose rate derived from consumption of 3 mg/L of DNB in drinking water.

[b] Average dose rate derived from consumption of 8 mg/L of DNB in drinking water.

[c] Average dose rate derived from consumption of 20 mg/L of DNB in drinking water.

[d] The authors note, however, that the number of pups was within the normal litter size.

Table 6-7. Acute toxicity of DNB to aquatic organisms.

Species	Concentration (mg/L)	Effect[a]	Reference
Aquatic vertebrates			
Bluegill	1.44	96-h LC50	van der Schalie, 1983
Rainbow trout	1.70	96-h LC50	van der Schalie, 1983
Fathead minnow	7	96-h LC50	Liu *et al*., 1983b
Fathead minnow	7	96-h LC50	Bailey and Spanggord, 1983
Fathead minnow	16.8	96-h LC50	van der Schalie, 1983
Channel catfish	8.13	96-h LC50	van der Schalie, 1983
Golden orfe	10	LC50 (no time units)	Juhnke and Luedemann, 1978 cited in Wentsel *et al*., 1979
Redbelly dace	8 to 10	6-h TLM	LeClerc, 1960 cited in Wentsel *et al*., 1979
Minnow	8 to 12	6-h TLM	LeClerc, 1960 cited in Wentsel *et al*., 1979
Aquatic invertebrates			
Water flea	27.4	48 h LC50	van der Schalie, 1983
Water flea	49.6	48 h LC50	Liu *et al*., 1983b

[a] LC50: Concentration producing mortality in 50% of test species; TLM: threshold level for mortality.

relationships for endpoints such as hatching success, fry survival, fry deformities, fry length, and fry weight over a 68-d period (60-d post hatch). Lowest-effect concentrations for the two tests were 0.84 and 0.97 mg/L, while the highest-no-effect levels were 0.44 and 0.50 mg/L. However, van der Schalie noted that the 30-d LC50 for rainbow trout was below the no-effect-levels defined in the ELS tests. Based on the concentration-response data for the dynamic acute test and the ELS test, he indicated that the no-effect concentration is 0.16 mg/L.

7. DATA-BASE ASSESSMENT FOR 1,3,5-TRINITROBENZENE (TNB)

TNB is a by-product of both TNT synthesis and TNT photolysis (see Section 2). It is formed during the nitration step of TNT synthesis as a result of the oxidation of methyl groups. Although the complete mechanism of TNB formation during TNT photolysis is unknown, Burlinson (1980) suggested that it is produced from the decarbonylation of TNBAL, a major TNT photoproduct. The primary pathway for TNB appears to be biotransformation to 3,5-dinitroaniline; degradation by photolysis and hydrolysis is minimal. TNB has been measured in natural waters and soils containing TNT, its parent compound.

ENVIRONMENTAL PARTITIONING AND TRANSPORT

Table 7-1 shows the chemical structure of TNB along with alternative names, its Chemical Abstracts Service Identification number (CASID), empirical formula, and molecular weight. Chemical and physical properties that are applicable to environmental assessments are found in Table 7-2. The partition coefficients presented in Table 7-3 are all calculated from regression equations. Most of the TNB continuously added to the upper-soil zones of the two reference landscapes (Table 7-4) should reside in the ground water and in the vadose zone under steady-state conditions.

ENVIRONMENTAL TRANSFORMATION PROCESSES

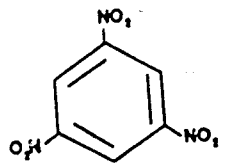
Photolysis

Burlinson *et al*. (1973) reported that TNB remained unchanged in an aqueous solution after 6 h of irradiation by a mercury lamp with a spectrum similar to that of sunlight. Consequently, we do not expect that photolysis will be a significant transformation process for this nitroaromatic compound.

Biotransformation

Several studies have shown that TNB will undergo biotransformation. Mitchell *et al*. (1982), for example, found that only 6% of TNB was transformed

Table 7-1. Chemical identifiers of TNB.

Empirical formula:	$C_6H_3N_3O_6$	Structural formula:
Molecular weight:	213.12	
CAS reg. no.:	[99-35-4]	
RTECS no.:	DC3850000	

after 19 d in a water sample taken from the Tennessee River downstream from the Volunteer Army Ammunition Plant. McCormick et al. (1976) found the rate of reduction of TNB by Veillonella alkalescens to be approximately half that of TNT. The microbial transformation half-lives of TNT shown in Table 3-7 range from 8 to 25 d, and therefore the estimated half-life of TNB should range from approximately 16 to 50 d.

We also calculated a microbial degradation rate for TNB from the experiments of Burlinson (1980), who measured the degradation of TNT in Potomac River water. The TNB formed from the degradation of TNT decreased from 2.4 to 0.8 ppm on days 8 through 14. These concentration changes indicate that the half-life is about 4 d -- considerably lower than the values noted above. Because TNB resists photolysis and hydrolysis, and volatilization is slow, its loss was probably due to microbial action. Also, much less TNB was measured in water samples to which river sediment was added, a further indication of microbial action; although some loss may be due to sediment adsorption. Burlinson suggested that microbial action on TNB caused the formation of the amine, 3,5-DNA (see Figure 2-2).

Kayser et al. (1977) reported that TNB was 99.5% transformed after a 48-h incubation period in a batch study utilizing "activated sludge organisms" supplemented with corn-steep nutrient. The only product isolated was 3,5-DNA. Chambers et al. (1963) found that TNB utilized some oxygen in a mineralization experiment with phenol-adapted bacteria from sewage sludge. They did not state the cell population nor analyze the products. Bringmann

Table 7-2. Chemical and physical properties of TNB. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	Sublimes or explodes	Windholz *et al.*, 1983
Freezing/melting point	°C	122.5	Windholz *et al.*, 1983
Density	g/mL	1.76	Windholz *et al.*, 1983
Vapor pressure	torr	1×10^{-4}	Extrapolated from pressure-temperature data given in Meyer, 1981[a]
Aqueous solubility	mol/L mg/L	1.5×10^{-3} 330	Interpolated[a] from solubility-temperature data in Urbanski, 1986a
Henry's law constant	(L-torr)/mol	7×10^{-2}	Calculated as the ratio of vapor pressure to aqueous solubility
Diffusion coefficients: Air	m^2/s	6.2×10^{-6}	Estimated[a]
Water	m^2/s	6.2×10^{-10}	Estimated[a]
Log octanol/water partition coefficient (K_{ow})	log K_{ow}	1.18	Hansch and Leo, 1979

[a] See Appendix A.

Table 7-3. Partition coefficients for use in estimating human exposures to TNB.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil weight	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	2	Calculated[b] from molecular
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	1.6×10^{-3}	Calculated[b] from water solubility and log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	3	Calculated[b] from log K_{ow}
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	77	Calculated[b] from solubility in water and melting point

[a] Wet-weights for plant and fish

[b] See Appendix A

Table 7-4. Fractional distributions of TNB among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of TNB to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	2.5×10^{-5}	2.5×10^{-5}
Air particles	3.1×10^{-9}	8.8×10^{-9}
Biota	7.3×10^{-5}	0 0085
Upper soil	0.016	0.066
Lower soil	0.31	0.10
Ground water	0.67	0.81
Surface water	0.0029	0.013
Sediments	6.1×10^{-5}	2.7×10^{-4}

[a] Fractions do not add to one because of rounding.

and Kuhn (1971) also accomplished complete oxidation in a two-step laboratory procedure with Azotobacter agilis and activated sewage sludge. We would expect the amine biotransformation products to subsequently react with humic substances.

Hydrolysis

Spanggord et al. (1980a) did not expect hydrolysis to be a significant environmental fate of TNB. Also, Sidgwick (1966) notes that TNB forms salts or complexes with strong alkalis; upon acidification, these salts revert to TNB and not a hydrolysis product.

TOXIC EFFECTS ON ANIMALS AND HUMANS

The toxicology of TNB was reviewed by Wentsel et al. in 1979. In the discussions below, we summarize the salient characteristics of this compound and provide updated information derived from more recent studies.

Toxicokinetics

Data dealing with the toxicokinetics of TNB are sparse. Most of the information on its absorption, distribution, and metabolism is from studies that examined the formation of methemoglobin in animals, or other toxic responses after oral, dermal, and intraperitoneal administrations to laboratory animals. Absorption occurs fairly quickly, as the work of Senczuk et al. (1976), cited in Wentsel et al. (1979), showed. The peak level of methemoglobin (expressed as % of total hemoglobin) in Wistar rats occurred at approximately 3 h after a single dose of 85.2 mg/kg of TNB. Wentsel et al. (1979) also refer to an earlier study by Timofievskaya and Rodionova (1973), who applied TNB in the form of an ointment (33% TNB in fat) to the shaved skins of mice, and observed respiratory effects in the mice and changes in the color of their urine. Watanabe et al. (1976) measured the amounts of diazo-positive metabolites in the urine of male rats that received 100 μM/kg (i.p.) of TNB. They found TNB produced 1.8 times more of the diazo-positive metabolites than did the controls (for comparison, DNB produced 6.0 times more of these metabolites).

Toxic Effects on Organs and Systems

Toxicological data on TNB are quite limited, ostensibly because this compound was never produced in commercial quantities, and thus there were no occupational health issues to prompt toxicity studies or epidemiological investigations. The few studies that have been done indicate that it can cause toxic effects to the liver, kidneys, blood, and the reproductive system.

Hepatic effects. Watanabe et al. (1976) measured the GOT and GPT levels in Wistar rats 5 h after an i.p. administration of 100 μM/kg (i.e., 21.3 mg/kg) of TNB, and did not observe a statistically significant change compared with the controls. Fogleman et al. (1955) administered 37 doses of 25 mg/kg of TNB to two dogs over a 54-d period (equivalent to an average dose of 17 mg/kg-d) and found at autopsy that the livers of the dogs were heavier than controls, yet there were "no grossly observable pathological changes." In addition, sections taken of the liver were "comparable to controls." Neither of the above studies provide conclusive evidence on the potential for

hepatotoxicity -- Watanabe et al. (1976) measured enzyme levels after only one administration, and Fogleman et al. (1955) used just two animals in their study.

Renal effects. Fogleman et al. (1955) found clear signs of renal toxicity in dogs administered doses of TNB ranging from 10 to 100 mg/kg over different time periods. Pathological examinations of kidney sections from two dogs receiving an average dose of 17 mg/kg-d of TNB for 54 days showed that the endothelial and epithelial cells were undergoing proliferation and exhibited cloudy swelling. Other signs of renal toxicity included degeneration of the loops of Henle and changes in the cells of the distal convoluted tubules.

Hematological effects. TNB is a potent methemoglobin former. Intraperitoneal injections to rats of 21.3 mg/kg produced an average of 18% methemoglobin (Watanabe et al., 1976); while for cats, i.p. injections of 5 and 10 mg/kg produced maximum levels of methemoglobin of 35 and 44%, respectively, one hour after administration (Kiese, 1974). Fogleman et al. (1955) also observed varying degrees of methemoglobinemia (0.4 to 33.8% methemoglobin) in two dogs receiving an average dose of 17 mg/kg-d over 54 days. The spleens of those two dogs were heavier than those of the controls (statistical significance not reported), and microscopic examinations of the spleens revealed thickened trabeculae.

Reproductive effects. Korolev et al. (1977) (cited in Wentsel et al., 1979) did not detect any significant changes in spermatogenesis of white rats at doses of up to 2 mg/kg (Wentsel et al. (1979) noted that the doses were presumably administered daily by gavage and that the methods were not clearly explained).

Teratogenicity

There are no data on the teratogenicity of TNB.

Genotoxicity

McGregor et al. (1980) used the E. coli W3110/pol A^+, p3478/pol A^- system to evaluate the ability of TNB to induce DNA damage. In this assay, DNA-repair deficient and DNA-repair proficient strains are cultured with the chemical of interest. Genotoxic effects are scored as the preferential killing of the repair-deficient strain. TNB was toxic to E. coli in the presence and absence of metabolic activation (S9). However, the toxicity of TNB did not differ substantially between repair-deficient and repair-proficient strains. This suggests that TNB does not damage DNA, at least under the conditions of this assay. Attempts by McGregor et al. to study the mutagenicity of TNB in the Saccharomyces cerevisiae assay were unsuccessful. The test compound precipitated in the inoculation medium at 2.0 mg/mL.

Positive responses have been observed using the Ames assay over a broad dose range with several strains of Salmonella typhimurium. The role of metabolic activation in the mutagenic response is not well defined, however. Spanggord et al. (1982b) tested TNB at five doses (10 to 70 µg/plate). These authors considered a test substance to be mutagenic if it elicited a reproducible, dose-related increase in the number of revertants over several dose levels. Based on these criteria, TNB gave a positive response in TA98 and TA100 both with and without S9. TNB was also mutagenic to test strains TA1535 (with S9), and TA1537 and TA1538 (only in the absence of S9).

McGregor et al. (1980) evaluated the mutagenicity of TNB in the Ames assay (0.1 to 100 µg/plate). They concluded that this compound was mutagenic in all strains (TA98, TA100, TA1535, TA1537, and TA1538). However, this response was quite variable and depended on the strain and on the presence or absence of S9. In general, the presence of metabolic activation appeared to increase the number of revertants, suggesting that in Salmonella, TNB is metabolized to a toxic intermediate.

Carcinogenicity

The only study dealing with the carcinogenic potential of TNB, was completed by Gorski (1969). TNB was administered by subcutaneous injections to 20 mice. Unfortunately, the author did not describe important details of

that work regarding the actual doses, number of injections/animal or duration of study. Three mice died prior to the completion of the test, but the remaining 17 did not exhibit tumors.

Estimation of Intake Limits to Protect Public Health

Because of inadequacies in the toxicological data-base for TNB (i.e., no chronic NOELs were defined, inconsistent dosing regimens, etc.), we have derived a provisional, or interim safe intake rate from an oral LD50 value. Fogleman et al. (1955) report the oral LD50 of TNB to rats to be "approximately 505 mg/kg." This value lies in the mid range of other LD50 values reported by Korolev et al. (1977) and Timofievskaya and Rodionova (1973) (cited in Wentsel et al., 1979) for mice, rats, and guinea pigs, which ranged between 280 mg/kg (female rats) and 730 mg/kg (guinea pig). A provisional lower-bound estimate of a safe intake is calculated as the product of 505 mg/kg and a conversion factor of 1×10^{-5} d^{-1} (see Appendix D), or 0.005 mg/kg-d. For comparison, we have calculated a daily intake based on the lowest-observed-effect level from one of the short term exposures of Fogleman et al. in which two dogs received 13 daily doses of 10 mg/kg over a 19-d period, equivalent to an average dose rate of about 7 mg/kg-d (the spleens and livers of these dogs were heavier than those of the controls, but no statistical significance was reported). This dose rate can be adjusted to obtain an estimate of an ADI by dividing by a factor of 1000, giving 0.007 mg/kg-d -- comparable to the value estimated from the LD50.

TOXIC EFFECTS ON AQUATIC SPECIES

The results of acute toxicity studies with TNB are shown in Table 7-5. Dynamic tests conducted by van der Schalie (1983) with fathead minnows resulted in an LC50 of 0.46 mg/L for a 10-d exposure period. The same test with rainbow trout gave an LC50 of 0.43 mg/L for an 18-d exposure period. In addition to the acute tests, van der Schalie (1983) conducted ELS tests with fathead minnows and rainbow trout. The mean no-effect concentration in the ELS test with fathead minnows was 0.08 mg/L. At 0.12 mg/L fry survival was significantly different ($p < 0.01$) from controls and at 0.72 mg/L none of the fry survived. Two ELS tests were carried out with rainbow trout. The

Table 7-5. Acute toxicity of TNB to aquatic organisms.

Species[a]	Concentration (mg/L)	Effect	Reference
Channel catfish	0.38	96 h LC50	van der Schalie, 1983
Fathead minnow	0.49	96 h LC50	van der Schalie, 1983
Rainbow trout	0.52	96 h LC50	van der Schalie, 1983
Bluegill	0.85	96 h LC50	van der Schalie, 1983
Fathead minnow	1.03	96 h LC50	van der Schalie, 1983
Fathead minnow	1.1	96 h LC50	Liu *et al.*, 1983b
Fathead minnow	1.1	96 h LC50	Bailey and Spanggord, 1983
Water flea	2.7	48 h LC50	Liu *et al.*, 1983b
Water flea	2.98	48 h EC50[b]	van der Schalie, 1983

[a] Species: Fathead minnow = *Pimephelas promelas*; Channel catfish = *Ictalurus punctatus*; Rainbow trout = *Salmo gairdneri*; Bluegill = *Lempomis macrochirus*; Water flea = *Daphnia magna*.

[b] The EC50 was for immobilization.

average no-effect concentration for the two tests was approximately 0.08 mg/L. At higher concentrations, fry survival was significantly ($p < 0.01$) reduced from that of the controls. A 21-d chronic toxicity study with daphnia indicated that the no-effect range was between 0.47 and 0.75 mg/L, showing that these invertebrates were less sensitive to TNB than the fathead minnow and rainbow trout. Finally, van der Schalie (1983) performed tests to determine the toxicity of TNB to an algae species, *S. capricornutum*. Growth of the algae was inhibited with respect to the controls at all concentrations tested, ranging from 0.10 to 17.32 mg/L.

8. DATA-BASE ASSESSMENT FOR 2-AMINO-4,6-DINITROTOLUENE AND 4-AMINO-2,6-DINITROTOLUENE

Two important degradation products of TNT found in soils and plants are 2-amino-4,6-DNT (2-A-4,6-DNT) and 4-amino-2,6-DNT (4-A-2,6-DNT) (see Table 8-1 for chemical structure and identifiers). These products are formed by the microbial reduction of nitro groups on the TNT molecule. To date little work has been done on their environmental chemistry and toxicology. In fact, there were no measured values available in the literature on key properties of the two compounds (e.g., vapor pressure and solubility in water) and consequently, these properties had to be estimated (see Tables 8-2 and 8-3 for physical and chemical properties).

Both of the amino compounds will undergo photolysis in surface water, and they are also susceptible to biotransformation. In addition, there is experimental evidence that suggests that the microbial reduction products of 2-A-4,6-DNT and 4-A-2,6-DNT should bind to humic acids and other organic matter. Data for evaluating the potential human-health risks of these compounds are very limited, and no data were available on their toxicity to aquatic species or plants.

ENVIRONMENTAL PARTITIONING AND TRANSPORT

The partition coefficients for 2-A-4,6-DNT and 4-A-2,6-DNT as shown in Tables 8-4 and 8-5, respectively, are similar because the chemical properties from which the coefficients are estimated are not very different. The equilibrium distribution for 2-A-4,6-DNT shown in Table 8-6 indicates that the ground water compartment should retain the largest fraction of the total inventory of that compound in both arid (Western ecoregion) and humid (Southeastern ecoregion) climates.

ENVIRONMENTAL TRANSFORMATION PROCESSES

Photolysis

Burlinson *et al*. (1979a) found that distilled water solutions containing 50 mg/L of 2-A-4,6-DNT required 7-1/2 h for the initial concentration to be

Table 8-1. Chemical identifiers of 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene.

2-amino-4,6-dinitrotoluene:

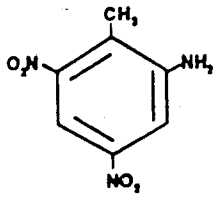
Empirical Formula: $C_7H_7N_3O_4$

Molecular weight: 197.15

CAS reg. no.: [35572-78-2]

RTECS no.: XU6090000

Structural formula:



4-amino-2,6-dinitrotoluene:

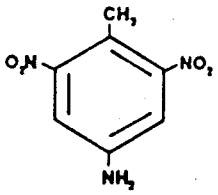
Empirical Formula: $C_7H_7N_3O_4$

Molecular weight: 197.15

CAS reg. no.: [19406-51-0]

RTECS no.: XU6080000

Structural formula:



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diminished photolytically 70%, while 2 h was required for the 4-amino analogue. The estimated half-lives are 4.3 h and 1.2 h, respectively (the TNT photolysis half-life under these conditions was 0.5 h). One by-product of 2-A-4,6-DNT was dinitroindazole (2% of the products). The products for 4-A-2,6-DNT were 4-amino-2,6-dinitrobenzaldehyde and 4-amino-6-nitroanthranil, both in approximately 2% yield. Burlinson (1980) later showed that small amounts of both amino DNT's formed and then disappeared in Potomac River water containing sediment that was exposed to sunlight. This could result from the combined action of photolysis, microbial action, or sorption onto sediment particles.

Biotransformation

Microorganisms reduce 4-A-2,6-DNT to 2,4-diamino-6-nitrotoluene (2,4-diamino-6-NT) under both aerobic and anaerobic conditions. 2-amino-4,6-DNT is reduced to both 2,4- and 2,6-diaminonitrotoluenes. Under

Table 8-2. Chemical and physical properties of 2-amino-4,6-dinitrotoluene. Properties are for an ambient temperature of 20°C.

Property	units	Value	Source
Boiling point	°C	Not available	
Freezing/melting point	°C	176	Heilbron, 1982
Density	g/mL	Not available	
Vapor pressure	torr	$\simeq 4 \times 10^{-5}$	Estimated[a] from melting and decomposition temperature
Aqueous solubility	mol/L mg/L	1.4×10^{-2} 2800	Estimated[a] melting point
Henry's law constant	(L-torr)/mol	$\simeq 3 \times 10^{-3}$	Calculated as the ratio of vapor pressure to aqueous solubility
Diffusion coefficients: Air Water	m^2/s m^2/s	6.1×10^{-6} 6.0×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition coefficient (K_{ow})	log K_{ow}	0.5	Estimated[a] using the fragment-constant method

[a] See Appendix A.

Table 8-3. Chemical and physical properties of 4-amino-2,6-dinitrotoluene. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	Not available	
Freezing/melting point	°C	171	Heilbron, 1982
Density	g/mL	Not available	
Vapor pressure	torr	$\approx 2 \times 10^{-5}$	Estimated[a] from melting and decomposition temperature
Aqueous solubility	mol/L mg/L	1.4×10^{-2} 2800	Estimated[a]
Henry's law constant	(L-torr)/mol	$\approx 1 \times 10^{-3}$	Calculated as the ratio of vapor pressure to aqueous solubility
Diffusion coefficients: Air Water	m^2/s m^2/s	6.1×10^{-6} 6.0×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition coefficient (K_{ow})	$\log_{10} K_{ow}$	0.6	Estimated[a] using the fragment-constant method

[a] See Appendix A.

Table 8-4. Partition coefficients for use in estimating human exposures to 2-amino-4,6-dinitrotoluene.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	3	Calculated[b] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	6.2×10^{-4}	Calculated[b] from log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	1	Calculated[b] from
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	1.4	Calculated[b] from log K_{ow}

[a] Wet-weights for plant and fish

[b] See Appendix A

Table 8-5. Partition coefficients for use in estimating human exposures to 4-amino-2,6-dinitrotoluene.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	3	Calculated[b] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	7.0×10^{-4}	Calculated[b] from log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	1	Calculated[b] from log K_{ow}
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	1.8	Calculated[b] from log K_{ow}

[a] Wet weights for plant and fish

[b] See Appendix A

Table 8-6. Fractional distributions of 2-amino-4,6-dinitrotoluene among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady- state addition of 2-amino-4,6-dinitrotoluene to the upper-soil layer. Not shown are fractional distributions for 4-amino-2,6-dinitrotoluene, which were almost identical. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	1.2×10^{-7}	1.0×10^{-7}
Air particles	7.3×10^{-11}	2.5×10^{-10}
Biota	3.2×10^{-5}	0.0040
Upper soil	0.0046	0.021
Lower soil	0.22	0.10
Ground water	0.78	0.85
Surface water	0.0037	0.021
Sediments	7.5×10^{-6}	4.1×10^{-5}

[a] Fractions do not add to one because of rounding.

aerobic conditions, amine products do not accumulate, but under the anaerobic conditions, they both accumulate and are reduced further to the triamino analogue. Alkaline conditions also favor amine accumulation (see Hoffsommer et al., 1978; McCormick et al., 1976; Kayser et al., 1977).

McCormick et al. (1976) presented experimental evidence showing that the 4-position was favored for the reduction of a TNT nitro group to an amine. They found more 4,4'-azoxy compound than compounds resulting from reduction of the 2- position. Hoffsommer et al. (1978) studied the batch reduction of 4-A-2,6-DNT by activated sludge microorganisms with corn-steep nutrient that resulted in a loss of 4-A-2,6-DNT from 15 to 1.5 ppm in 3 d. The associated half-life is about 0.9 d. In contrast, 2-A-4,6-DNT was reduced from 13 to 6 ppm in 0.33 d (the half-life is 0.3 d). The authors suggested that further reduction of the remaining nitro groups and formation of polar products and polymers are the final fate of microbial action. They also noted

incorporation of ^{14}C-labeled reduction products into the floc components of the sludge after 30 d. The amine products themselves did not, however, accumulate.

Kayser et al. (1977) reported that the amino-DNT's were biodegraded by microorganisms in sewage sludge to 2,4-diamino-6-NT. Transformation of 4-A-2,6-DNT occurred under four different environmental conditions in a study by Burlinson (1980) dealing with degradation of TNT: (1) in a sample of Potomac River water exposed to sunlight; (2) the same as (1) but with sediment added; (3) the same as (1) but in the shade; and (4) the same as (1), but in the dark. The 4-A-2,6-DNT was not observed until after several days had elapsed, although the TNT was disappearing during that time. 4-A-2,6-DNT was found to a far lesser extent (approximately 1% of the initial TNT concentration) in the sunlight and shade samples than in the dark samples (approximately 10% of the initial TNT concentration). It also seemed to increase in concentration from day 7 to day 38 in the dark sample. The data did not demonstrate a significant accumulation of amine products in the 38-d experiment. Rate data for biotransformation in environmental media are not available.

Complex formation

Several authors have shown that 2-A-4,6-DNT, 4-A-2,6-DNT and their microbial reduction products will bind to humic acids, lipids, or proteins. Kaplan and Kaplan (1983) showed that 2,4-diamino-6-NT, which is found in microbial reduction products, becomes increasingly bound with time and with higher humic acid concentration. Carpenter et al. (1978) presented further evidence that TNT is reduced by microbial action and that the reduction products react with the lipid and protein components of an activated-sludge system. Three to five days after addition of ^{14}C-TNT to the aerated sludge, no TNT could be detected. However, the presence of the ^{14}C label in lipids, and protein precipitates, derived from extraction of the sludge, confirmed that TNT-derived products were incorporated in them. To test their hypothesis that the amino-products of TNT microbial reduction were reacting with the lipids and proteins, they prepared a mixture consisting of 2,4-diaminotoluene, as a surrogate for a TNT-reduction product, and glycerol, palmitoyl chloride and sebacyl chloride to represent the lipids and proteins. When they compared

the infrared (IR) spectrum for the surrogate precipitate with the IR spectrum for the TNT-sludge protein and lipid fraction-derived precipitates, they found similar absorbances, especially in the amine region (1630 to 1710 cm^{-1} and 3000 to 3500 cm^{-1}). Their work implied that the amine reduction products remained intact but reacted with lipids, fatty acids, and protein constituents of the microbial flora forming polyamides. They stated that polyamide linkages are known to resist degradation and to be stable.

Hoffsommer et al. (1978) found 65.5% of the ^{14}C activity in a bacterial floc after a 30-d biodegradation study with ^{14}C-TNT and sewage-sludge microorganisms. Only 9% of the total ^{14}C was extracted from the floc with benzene; 57% was extracted from the floc with sulfuric acid; and the remaining approximately 34% was mainly in the aqueous phase. From day 3 to day 30, the amount of ^{14}C-labeled material that required extraction with H_2SO_4 from the floc increased from 40 to 60%. These results demonstrate that approximately 2/3 of the microbial reduction products were bound to the floc. However, Hoffsommer et al. suggest that polymer formation is the ultimate fate of TNT microbial reduction products.

TOXIC EFFECTS ON ANIMALS AND HUMANS

Neither 2-A-4,6-DNT nor 4-A-2,6-DNT are used in the synthesis of organic compounds or as end products, and consequently there has been little interest in their toxic effects on humans or laboratory animals. The limited amount of work that has been completed suggests that they are less toxic than TNT.

Toxicokinetics

The only data available on the toxicokinetics of 2-A-4,6-DNT and 4-A-2,6-DNT are from Ellis et al. (1976). These researchers studied the acute oral toxicity to rats of the compounds and examined the distribution and excretion of radiolabeled 4-A-2,6-DNT. In their toxicity tests to determine the oral LD50 in Charles River rats, they noted toxic responses within 30 min of oral dosing for both isomers, indicating that the rate of absorption is fairly rapid. However, their analyses of the uptake and distribution of radiolabeled 4-A-2,6-DNT showed that only about 50% of that isomer was absorbed. Twenty-four hours after the administration of a single oral dose of

the radiolabel, 30.1% of the initial radioactivity was recovered in urine, 0.2% in air, 44.4% in feces, and 5.5% in the gastrointestinal tract. Minor amounts were found in whole blood (0.2%), spleen (<0.1%), kidneys (0.1%), brain (<0.1%), lungs (<0.1%), and muscle (0.3%). Analysis of the urine sample by thin-layer-chromatography identified two polar components that contained 78 and 22%, respectively, of the total radioactivity in the urine.

Toxic Effects on Organs and Systems

Ellis *et al*. (1978) administered oral doses of 2-A-4,6-DNT and 4-A-2,6-DNT that were acutely toxic to rats and mice. Symptoms of the acute intoxications included unconsciousness, depression, inactivity, and gasping breathing. These responses may have been caused by methemoglobinemia, a toxic response produced by many nitroaromatics (see Kiese, 1974). Table 8-7 summarizes the LD50 values estimated for the rats and mice. Ellis *et al*. (1978) also investigated the dermal and ocular effects of the compounds. The only positive response was for 2-A-4,6-DNT, which proved to be a mild skin irritant.

Teratogenicity

There are no data on the teratogenicity of these compounds.

Genotoxicity

The mutagenicity of 2-A-4,6-DNT and 4-A-2,6-DNT in *S. typhimurium* have been investigated by Won *et al*. (1976) and Spanggord *et al*. (1982b). Won *et al*. reported that the mutagenic activity of TNT metabolites (including both aminodinitrotoluenes) in tester strains of *S. typhimurium* "were essentially similar to controls". Spanggord *et al*. (1982b) used five tester strains (TA1535, TA1537, TA1538, TA98, TA100) with and without metabolic activation. In addition, they used a nitroreductase-deficient strain (TA100NR3) to study the role of this enzyme in the mutagenicity of nitroaromatic compounds. Positive mutagenic responses were defined as those that had a "reproducible dose-related increase in the number of revertant colonies over at least three dose levels." Using this criterion, they found that 2-A-4,6-DNT was positive in strains TA1535, (with S-9) TA1537, TA1538, TA98, and TA100 with and without

Table 8-7. Oral LD50's of 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene for rats and mice (data from Ellis *et al.*, 1978).

Compound	LD50 Dose (mg/kg)			
	Rat		Mouse	
	Male	Female	Male	Female
2-A-4,6-DNT	2240	1394	1722	1522
4-A-2,6-DNT	1360	959	1342	1495

S-9; while 4-A-2,6-DNT was positive in strains TA1535 (with S-9), TA1538 (w/o S-9), TA98 and TA100, with and without S-9. Negative results were obtained using the nitroreductase-deficient strain with both 2-A-4-,6-DNT and 2-A-2,6-DNT.

Carcinogenicity

There are no data on the carcinogenicity of these compounds.

Estimation of Intake Limits to Protect Public Health

No subchronic or chronic toxicity studies have been completed on 2-A-4,6-DNT and 4-A-2,6-DNT, and consequently safe intakes cannot be calculated using standard protocols. Therefore, we have made lower-bound estimates of the ADI's (denoted LB-ADI) of these compounds by multiplying their LD50's (Table 8-7) by a factor of 1×10^{-5} d^{-1} (see Appendix D for a discussion of the methodology). The resulting LB-ADI values for 2-A-4,6-DNT range from 0.014 mg/kg-d (1×10^{-5} d^{-1} x LD50 for female rats) to 0.027 mg/kg-d (1×10^{-5} d^{-1} x LD50 for male rats). For 4-A-2,6-DNT, LB-ADI values range from 0.01 mg/kg-d ($1 \times 10^{-5-1}$d x LD50 for female rats) to 0.015 mg/kg-d ($1 \times 10^{-5-1}$d x LD50 for female mice).

TOXIC EFFECTS ON AQUATIC SPECIES

Data are available on the toxic effects of the aminodinitrotoluenes to *D. magna* and fathead minnows. Liu *et al*. (1983b) reported 48-h EC50 values for 2-A-4,6-DNT and 4-A-2,6-DNT as 4.6 and 5.4 mg/L, respectively, with 95% confidence intervals of 3.5 to 5.8 mg/L and 3.5 to 9.8 mg/L. The 96-h LC50 values of the two isomers for fathead minnows were 15.1 and 6.9 mg/L, with 95% confidence intervals of 14.0 to 16.4 mg/L and 5.6 to 8.1 mg/L.

9. DATA-BASE ASSESSMENT FOR RDX

RDX is the second most important explosive in the demilitarization inventory. Unlike TNT and its associated nitroaromatic co-contaminants, RDX is a heterocyclic compound with three nitro groups bound to three ring nitrogen atoms (see Table 9-1). It can undergo photolytic and microbial degradation, but hydrolysis is unimportant at the pH levels of most natural waters. Biotransformation occurs typically under anaerobic conditions, during which the RDX molecule can be transformed to low molecular-weight products.

ENVIRONMENTAL PARTITIONING AND TRANSPORT

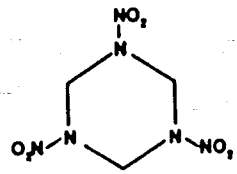
The basic chemical and physical properties of RDX are presented in Table 9-2.

Soil Transport and Adsorption

Hale et al. (1979) measured the movement of ^{14}C-RDX through irrigated soil columns (152-cm long) over a 26-week period. The concentration profile of RDX showed that downward leaching occurred in each of four different soil types. After a two-week period ^{14}C-RDX was detected at depths of 122 to 152 cm in a silty-clay loam and a basic-silt loam. At the conclusion of week 26, RDX was also found at the bottom of the columns.

Measurements of K_d and K_{oc} by Tucker et al. (1985), Spanggord et al. (1980b), Spanggord et al. (1983a), and Sikka et al. (1980) indicate that RDX does not strongly adsorb to soils and sediments. Tucker et al. (1985) conducted soil sorption experiments with different kinds of soils and completed regression analyses of the soil properties and the soil-water partition coefficients. The soils in these tests were of low organic-carbon content; 9 of 12 were less than 1.3 wt% organic carbon. They found that the K_d values for RDX could be estimated by use of regression equations, with independent variables consisting of the organic content of the soil, cation-exchange-capacity, pH, and silt content. They also suggested that an organic content of at least 0.25 wt% would be necessary for adsorption to be a critical process affecting RDX migration through soils.

Table 9-1. Chemical identifiers of RDX.

Empirical Formula:	$C_3H_6N_6O_6$	Structural formula:
Molecular weight:	222.26	
CAS reg. no.:	[121-82-4]	
RTECS no.:	XY9450000	

Tucker et al. (1985) derived a K_{OC} value of 136 from the measurements with different soils. Spanggord et al. (1980b) measured K_d values of 4.2 and 1.4 for two sediments, and the associated K_{OC} values were 127 and 42, respectively. Sikka et al. (1980) measured the K_d of RDX for three sediments with varying clay and organic-carbon content. The K_d values were 4.15 for an organic-clay sediment (52 wt% OC), 3.06 for a clay loam (4.1 wt% OC), and 0.8 for a sandy loam (0.3 wt% OC). The calculated K_{OC} values are 7.7, 74.6, and 266.7, respectively. For comparison, the K_{OC} calculated from the aqueous solubility and melting point of RDX (Appendix A) is 61. Hale et al. (1979) measured the K_d for RDX in four soils with unspecified organic-carbon content. Values of K_d were 7.8, 6.4, 0.2, and 1.8. The geometric mean of the K_d measurements from the different studies above (see Table 9-3) is 2.17 with a geometric standard deviation of 3.18.

Partition Coefficients: Plant/Soil, Beef-Fat/Diet, and Fish/Water

Partition coefficients for plant/soil, beef-fat/diet, and fish/water are presented in Table 9-4. The only measured values for partitioning in environmental media were fish uptake studies conducted by Bentley et al. (1977b). In these studies, three species of fish (bluegill, channel catfish, and fathead minnow) were exposed to concentrations of ^{14}C-RDX for 14 and 28 d. ^{14}C-residues were measured in edible tissue as well as viscera at different periods to determine whether equilibrium conditions had been achieved. The bioconcentration factors, as shown in Table 9-4, ranged from

Table 9-2. Chemical and physical properties of RDX. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	decomposes during melting	Dobratz, 1981
Freezing/melting point	°C	205 to 206	Windholz *et al.*, 1983
Density	g/mL	1.82	Windholz *et al.*, 1983
Vapor pressure	torr	1×10^{-9}	Calculated[a] from a vapor pressure-temperature equation
Aqueous solubility	mol/L mg/L	1.9×10^{-4} 42.2	Average of 9 measured values reported in Sikka *et al.*, 1980
Henry's law constant	(L-torr)/mol	5×10^{-6}	Calculated as the ratio of vapor pressure to aqueous solubility
Diffusion coefficients: Air Water	m^2/s m^2/s	6.0×10^{-6} 5.9×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition	$\log K_{ow}$	0.87	Measured, Banerjee *et al.*, 1980

[a] See Appendix A.

Table 9-3. Summary of measured K_d values for RDX.

K_d	K_{oc}	Ref.
1.4	42	Spanggord et al. (1980b)
4.2	127	Spanggord et al. (1980b)
--	136	Tucker et al. (1985)
4.15	7.7	Sikka et al. (1980)
3.06	74.6	Sikka et al. (1980)
0.8	266.7	Sikka et al. (1980)
7.8	--	Hale et al. (1979)
6.4	--	Hale et al. (1979)
0.2	--	Hale et al. (1979)
1.8	--	Hale et al. (1979)

3.4 for catfish to 5.0 for minnows. These values are slightly higher than the value estimated from K_{ow} (i.e., 1.9); however, the residual ^{14}C activity in the fish tissue includes both RDX and its metabolites.

Equilibrium Distribution in Reference Landscapes

The predicted equilibrium distribution of RDX among the eight compartments of the reference landscapes indicates that it tends to reside in the subsurface (see Table 9-5). This is consistent with the low Henry's law constant for this compound, which limits its volitilization to the atmosphere. One important difference between the fractional distributions of the two ecoregions is that a greater proportion of RDX should reside in the lower-soil zone of the Western ecoregion than in the Southeastern ecoregion. This is due in part to differences in the characteristics of the soil zones (e.g., the lower-soil zone in the Southeastern ecoregion is not as thick as the one for the Western ecoregion and consequently, there is less sorptive capacity for that zone) and the quantity of recharge, which affects the leaching of RDX.

Table 9-4. Partition coefficients for use in estimating human exposures to RDX.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	2	Calculated[b] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	2.2×10^{-3}	Calculated[b] from water solubility and log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	2	Calculated[b] from log K_{ow}
			4.1	Average of two measured values for bluegill, from Bentley *et al.*, 1977a
			3.4	Average of two values for channel catfish, from Bentley *et al.*, 1977a
			5.0	Average of two values for fathead minnows, from Bentley *et al.*, 1977a

[a] Wet-weights for plant and fish
[b] See Appendix A

Table 9-5. Fractional distributions of RDX among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of RDX to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	5.0×10^{-12}	7.5×10^{-12}
Air particles	2.7×10^{-9}	7.7×10^{-9}
Biota	6.5×10^{-5}	0.0078
Upper soil	0.014	0.060
Lower soil	0.30	0.10
Ground water	0.69	0.82
Surface water	0.0031	0.014
Sediments	5.3×10^{-5}	2.4×10^{-4}

[a] Fractions do not add to one because of rounding.

ENVIRONMENTAL TRANSFORMATION PROCESSES

RDX degrades both photolytically and microbially to low-molecular-weight compounds with fragmentation of its six-membered ring. Hydrolysis is significant only in alkaline waters (pH greater than 8).

Photolysis

Transformation of RDX in the environment occurs the fastest by photolysis. Spanggord et al. (1980b) measured the degradation of RDX in distilled and filter-sterilized natural water in sunlight. During a cloudy January period with air temperatures ranging from -2 to 18°C, in Menlo Park, California, they calculated a half-life of 13 d from their measurements of RDX disappearance from distilled water in borosilicate tubes; 14 d for RDX in

Holston River water; and 9 d for RDX in Searsville Pond water (see Table 9-6). In a subsequent experiment during a sunny period in March with water temperatures ranging from 5° to 20°C, the half-life of RDX was 1.8 d in distilled water. Spanggord et al. (1983a) also measured RDX photolysis in a 4-cm-deep dish of lagoon water exposed to sunlight (see Table 9-6), and the decay constant was $6.6 \times 10^{-7} s^{-1}$ (equivalent to a 12-day half-life). Their data did not show an acceleration in the photolysis rate due to photo products or humic substances as was true for the photolysis of TNT. However, a large increase in the photolysis rate was observed with the increase in sunlight and ambient temperatures between the January and March experiments that used distilled water.

Similar experiments by Sikka et al. (1980) using a mercury lamp confirm that RDX photolysis is not enhanced by humic substances in natural waters (see Table 9-6). They observed the disappearance of RDX in water in summer sunlight in Syracuse, New York. From the time-varying concentration data they reported (Table 9-6), we calculate RDX half-lives of 10.5 h in distilled water in August sunlight and 6.9 h in July sunlight in water taken upstream from the Holston Army Ammunition Plant.

Dissolved oxygen and a photosensitizer showed no influence on the photolysis rates of RDX measured by Sikka et al. (1980). Under UV irradiation (254 nm) the amount of photolytic degradation was 34% in a degassed water solution and 31% in an undegassed solution after 15 s. When a photosensitizer (methylene blue) was added, no significant rate enhancement was found in a 25 minute period.

Experimental data on the decrease of RDX photolysis with water depth is provided by Kubose and Hoffsommer (1977). They passed UV light through a succession of 1-cm cells filled with 29.5 ppm RDX in distilled water. They found that RDX disappeared 2200 times faster in the first cm than in the fourth. In addition, Spanggord et al. (1983a) estimated an RDX photolysis rate constant of $4.5 \times 10^{-6}\ s^{-1}$ ($3.9 \times 10^{-1}\ d^{-1}$) at the surface of a waste disposal lagoon in fall and a rate constant of $2.3 \times 10^{-8}\ s^{-1}$ ($2 \times 10^{-3}\ d^{-1}$) at a depth of 15 cm -- a factor of almost 200 lower.

Spanggord et al. (1980b) also used the quantum yield of RDX photolysis (0.16 at 313 nm) and the methods of Zepp and Cline (1977) for calculating aqueous photolysis to predict half-lives of RDX in distilled water for four seasons at 20, 40 and 50°N latitude. Their values ranged from 1.1 d for summer at 20°N to 12.5 d for winter at 50°N. Using values for midday solar

Table 9-6. Summary of data on RDX photolysis rates.

Light source[a]	RDX concentration (mg/L)	Water sample	Reaction vessel	Rate constant for photolysis (s^{-1})	Reference
313 nm	1.3	Distilled water	Tube	3.6×10^{-5}	Spanggord *et al.*, 1980b
Sunlight[b]	1.3	Distilled water	Tube	6.3×10^{-7}	Spanggord *et al.*, 1980b
Sunlight[b]	1.3	Holston R.[c]	Tube	5.8×10^{-7}	Spanggord *et al.*, 1980b
Sunlight[b]	1.3	Searsville Pond[c]	Tube	8.9×10^{-7}	Spanggord *et al.*, 1980b
Sunlight[d]	16	Lagoon water[e]	Dish, 4 cm-deep	6.6×10^{-7}	Spanggord *et al.*, 1980b
Sunlight[d]	16	Distilled water	Dish, 4 cm-deep	6.5×10^{-6}	Spanggord *et al.*, 1980b
300 nm	34.1	Distilled water	Tube	6.7×10^{-4}[f]	Sikka *et al.*, 1980
300 nm	35.1	Holston R.	Tube	8.3×10^{-4}[g]	Sikka *et al.*, 1980
Sunlight[h]	51.0	Holston R.	Dish, 3.8-cm deep	2.8×10^{-5}[i]	Sikka *et al.*, 1980
Sunlight[j]	11.2	distilled water	Dish	1.8×10^{-5}[k]	Sikka *et al.*, 1980

[a] Mercury lamps were used for the single wavelength light sources.

[b] January sunlight, Menlo Park, California.

[c] Natural waters were filter-sterilized.

[d] June sunlight.

[e] Samples from a lagoon at the Louisiana Army Ammunition Plant.

[f] Calculated from a half-life of 13.9 min.

[g] Calculated from a half-life of 17.3 min.

[h] July sunlight.

[i] Calculated from a concentration change of 51.0 to 5.41 mg/L of RDX in 22.4 h.

[j] August sunlight.

[k] Calculated from a concentration change of 11.2 to 1.46 mg/L in 31 h.

radiance at 40°N and extinction coefficients derived from Zepp and Cline (1977), Sikka et al. (1980) estimated photolysis half-lives of 2.0 to 10.4 h, for summer and winter, respectively. They corrected these half-lives for average daily irradiance and calculated half-lives of 6 and 20 h for summer and winter at 43°N (latitude of Syracuse, NY).

The mechanism for RDX photochemical degradation apparently changes between 254 nm UV irradiation and irradiation at 290 nm, the effective lower-bound wavelength of natural sunlight. Kubose and Hoffsommer (1977) propose that the primary photochemical step with wavelengths greater than 280 nm is cleavage of the nitramine bond, producing an azayl radical and NO_2. In water, NO_2^- and NO_3^- will form. Under acidic conditions, the NO_3^- ion becomes NO, which can combine with an azayl radical to form a mononitroso compound. Because NO cannot be formed under neutral conditions, the mononitroso-RDX is not found during photolysis of RDX in tap water with its natural buffering. With shorter wavelengths of light (i.e., 220 nm), mononitroso-RDX is formed directly from cleavage of the N-O bond. This may explain the faster photolysis rate measured by Kubose and Hoffsommer (1977) with 200 nm than with 280 nm light. They also observed different ratios of photolysis products, for example, more formaldehyde was formed at 220 nm than at 280 nm.

Glover and Hoffsommer (1979b) proposed that the photolysis of RDX in water by UV light proceeds by three different initiating steps as shown by Fig. 9-1. One step is the cleavage of the N-O linkage of a nitro group forming mononitroso RDX; the second step is a nitrogen-nitrogen bond cleavage yielding an amine; and finally, the formation of triazine. All these intermediate products are unstable and will react further. Among the "final" products is N-methylamino-N-nitrosamine (NH_2-CH_2-NH-NO), ammonia, formamide, nitrous oxide, nitrate and nitrite ions.

Photolysis of solid RDX produces polymeric products as well as the small molecular-weight products of aqueous photolysis. Stals et al. (1971) exposed polycrystalline RDX to 254 nm UV light and isolated waxes, oils and solids; some of these were branched alkanes or esters. The formation of higher molecular-weight compounds was presumably due to confinement of reaction intermediates. Smetana and Bulusu (1977) reported only gaseous products from the photolysis of polycrystalline RDX at 310 to 330 nm under a vacuum.

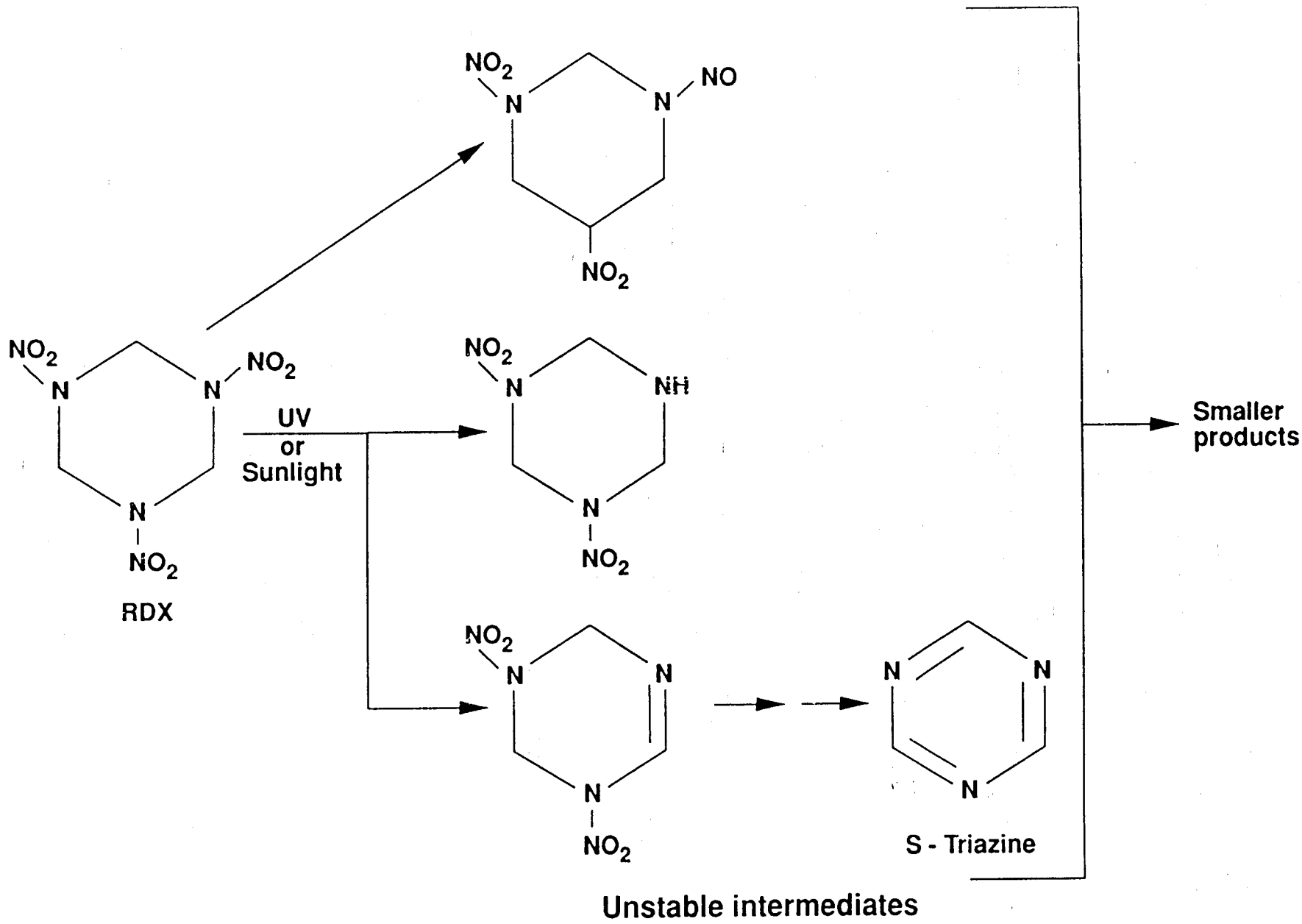


Figure 9-1. Photolytic decomposition of RDX (Glover and Hoffsommer, 1979a, 1979b; Kubose, 1977).

Biotransformation

Microbial reduction of RDX usually takes place under anaerobic conditions. McCormick et al. (1981) found that RDX disappeared from nutrient broth cultures within 4 d of anaerobic incubation, but found no loss in aerobic cultures or controls. The nitro groups were sequentially reduced to nitroso analogues and then to low molecular-weight products. Intermediates include hydrazines and nitrosamines (McCormick et al., 1981).

Spanggord et al. (1980b) found that aqueous solutions of RDX could be aerobically degraded by the adapted microbes in Holston River water containing sediment. After a 20-d lag time, the RDX concentration changed from 10 ppm to 4 ppm over 16 d, and thereafter it remained unchanged. In Holston River water without sediment, 10 ppm RDX was not significantly degraded aerobically. Spanggord et al. (1980b) repeated the above experiment with Searsville Pond water with it's own sediment and with Holston River sediment. No RDX degradation was found except with the Holston River sediment. Again, there was a 20-d lag time, but the half-life was 18 d.

Sikka et al. (1980) observed similar results: a 20-d lag time followed by a half-life of 7 d for RDX dissolved in Holston River water containing 1 wt% sediment. No details were provided on the amount of aeration. They also repeated these experiments and measured the evolution of $^{14}CO_2$ from the biotransformation of ^{14}C-RDX in water. Evolution of $^{14}CO_2$ did not occur until after a lag time of about 10 to 20 d. They also found that the addition of yeast extract to the Holston River water increased the rate of $^{14}CO_2$ evolution.

Spanggord et al. (1980b) reported that RDX does not undergo biotransformation under anaerobic conditions in Holston River or Searsville Pond water without added nutrients. With adapted RDX-transforming bacteria or added nutrients, however, RDX is transformed under anaerobic conditions. In one experiment they found that 25% of the RDX in a sample of Holston River water disappeared in 20 d (half-life of 48 d) with an RDX-transforming bacteria culture. When an aqueous basal-salts medium was used, 50% of the RDX was lost in 4 d. In Searsville Pond water containing 10 ppm RDX and 50 ppm yeast extract, the RDX concentration decreased to 0.1 ppm in 8 d. Spanggord et al. (1983a) also found no loss of RDX in a lagoon water unless yeast extract were added.

In summary, microbial degradation will occur only where sediment microbial populations have adapted to mineralize RDX. Furthermore, it appears that anaerobic conditions and added nutrients of some type are also required, with rare exceptions.

Hydrolysis

Hydrolysis should be a significant environmental fate of RDX only in sufficiently alkaline waters. Sikka et al. (1980) measured hydrolysis rates of RDX in aqueous solutions at pH's from 2 to 9. At pH's of 2 and 5 no hydrolysis of RDX was observed, and at a pH of 7, a 5% concentration drop in all RDX solutions was recorded between days 9 and 12 of the experiment. At a pH of 9 and a temperature of 31°C, 29% of the RDX disappeared due to hydrolysis.

The most useful measurements and data for hydrolysis at environmentally encountered pH's were made by Hoffsommer and Rosen (1973). After 112 d in seawater (pH = approximately 8), 11.6% of the RDX had hydrolyzed. Later, Hoffsommer et al. (1977) measured the alkaline hydrolysis of RDX. Their second-order (in [OH] concentration) rate constant at 25°C was $3.9 \times 10^{-3}\ M^{-1}s^{-1}$ (measured at high pH). We converted pH's of 7, 8, 9, and 12 to the associated concentrations of OH^- and substituted those values into the second-order rate constant. The resulting half-lives were 58 yr, 5.8 yr, 210 d and 4.9 h, respectively.

TOXIC EFFECTS ON ANIMALS AND HUMANS

RDX toxicity has been recently reviewed by Etnier (1986), and therefore this section will focus on studies dealing with the salient toxic properties of RDX and those toxicity studies that could serve as the basis for estimating safe intake rates of RDX.

Toxicokinetics

Absorption. McNamara et al. (1974) reported that RDX did not penetrate the skin of dogs or rabbits when dissolved in DMSO, cyclohexanone, or acetone. This conclusion was based on the lack of physiological response in

dogs and on the absence of RDX in the blood of rabbits after dermal exposure to RDX. Although dermal absorption may be limited, dermatitis in RDX factory workers was noted during World War II (Sunderman, 1944, as cited in Etnier, 1986).

The existing data indicate that absorption of RDX after ingestion is a slow process. Von Oettingen (1949) observed that RDX was still present in the stomachs of dogs four hours after gastric intubation. Schneider et al. (1976) found that 36.8 percent of the initial radioactivity of ^{14}C-RDX (dissolved in DMSO) remained in the guts of rats 24 h after oral dosing. Less than 3 percent was still present after 48 h. Schneider et al. (1976) also noted that the minimum oral lethal dose of RDX in rats depended on the form in which RDX was applied. For example, powdered RDX appeared to be more toxic than granular RDX when administered orally to rats. In a separate study, Schneider et al. (1977) administered ^{14}C-RDX by gavage to swine (100 mg/kg) and rats (50 or 100 mg/kg), and then measured the concentration of RDX in plasma and urine. In rats, the concentration of RDX in plasma reached a maximum within several hours and then declined. RDX plasma concentrations in swine began to increase within an hour of administration, but did not peak until nearly 24 h. Convulsions in the rats occurred sooner than those observed in swine. Berry et al. (1983) noted that epileptiform seizures in a German Shepherd dog began 9.5 h after the ingestion of an unknown quantity of RDX.

Stone et al. (1969) reported that the acute effects (e.g., convulsions) seen in military personnel after ingestion of RDX in a plasticized matrix (C-4) occurred within a few hours of ingestion. Woody et al. (1986) measured RDX in the serum of a three-year-old child who accidentally ingested C-4 (plasticized RDX). The peak concentration of RDX in serum occurred sometime before the first serum measurement, which was made approximately 24 h after ingestion. The peak concentrations in the urine and feces occurred at 48 and 96 h, respectively. According to the case report, generalized tonic-clonic seizures began approximately 20 h after ingestion.

Distribution. Schneider et al. (1977) studied the distribution of ^{14}C-RDX in rats after i.p. or oral administration. Animals received 50 or 500 mg/kg (i.p.) or 100 mg/kg (oral). Although no statistical analysis was performed, these researchers concluded that the distribution of RDX in the rat was unaffected by either the route of administration or the dose. Schneider et al. also observed that RDX accumulated, at least temporarily, in various

tissues. The ratio of tissue-to-plasma concentration ranged from 2.6 for the heart, to 8.8 for the kidney. The brain had a tissue/plasma concentration ratio of 3.4. These researchers also administered RDX by gavage to miniature swine and found even less evidence of preferential uptake between organs. The urine of the miniature swine contained approximately the same concentration of RDX as did the plasma (Schneider et al., 1977).

Woody et al. (1986) measured a cerebral spinal fluid to blood-serum ratio of 0.83 approximately 24 hours after ingestion of C-4 (plasticized RDX) by a three-year-old male child. This ratio was considered to be indicative of extensive distribution or accumulation of RDX in the cerebral spinal fluid.

Metabolism. Bradley (1977) showed that the metabolism of RDX in rats was affected by pretreatment with substances that induce or inhibit the mixed function oxidases, that is, phenobarbital and pyrazole, respectively. When rats received phenobarbital prior to administration of ^{14}C-RDX, there was a significant increase ($p < 0.01$) in the amount of radiolabeled urinary metabolites excreted within a 24 h period. The cumulative percentage of radioactivity excreted in the urine over 4 d was also significantly greater in these animals, compared to controls. Pretreatment with phenobarbital caused a significant increase ($p < 0.01$) in the amount of radiolabel excreted as $^{14}CO_2$ within the first 24 h after dosing. The total excretion of $^{14}CO_2$ over a 4 d period was not affected by phenobarbital, however. Animals given the MFO inhibitor pyrazole excreted significantly smaller ($p < 0.01$) amounts of radioactivity in the urine than controls. Pyrazole treatment also decreased the excretion of $^{14}CO_2$ by approximately 50%. Subchronic administration of RDX appears to inhibit its metabolism. Pretreatment of rats with 20 mg/kg RDX for 4 d or 11 d prior to administration of ^{14}C-RDX caused a significant decrease ($p < 0.01$) in the amount of urinary metabolites. The underlying mechanism of this inhibition is not known.

French et al. (1976) noted hepatocyte alterations in rats 24 h after oral dosing with RDX (100 mg/kg). At 120 hours after dosing, proliferation of the smooth endoplasmic reticulum was observed. This proliferation also suggests that RDX induces the MFO.

Schneider et al. (1978) found that 3 to 5 percent of a dose of ^{14}C-labeled RDX was excreted as the unchanged parent compound in the urine of rats. The majority of the ^{14}C label that was excreted in the urine could not be identified. Various one-carbon metabolites have been identified, including

$^{14}CO_2$ exhaled from the lungs of rats (Schneider et al., 1977, 1978). Bicarbonate ion and formic acid have also been identified (Schneider et al., 1978). No quantification of these metabolic end-products has been reported.

Excretion. Schneider et al. (1977) gave rats a single oral dose of 50 mg/kg ^{14}C-RDX, and followed the excretion of radioactivity for 96 h. Approximately 43 percent of the administered radiolabel was excreted via the lungs as $^{14}CO_2$, while urine accounted for another 34 percent of the ^{14}C label. The feces contained three percent of the radioactivity, and another ten percent was in the rat carcass. Woody et al. (1986) found that unmetabolized RDX was present at higher concentrations in the feces than in the urine of a three-year-old male child following accidental ingestion of an estimated 1.2 g of plasticized RDX (C-4). The apparent half-life for elimination of RDX from the child was 15.06 h.

Toxic Effects on Organs and Systems

Neurotoxicity. Acute RDX toxicity in humans is primarily manifested in the central nervous system. Specific neurotoxic symptoms in humans include: hyperirritability, muscle twitching, generalized epileptiform seizures, and prolonged confusion and amnesia (Stone et al., 1969). Laboratory animals exhibit similar symptoms. For example, Levine et al. (1983b) noted that rats that died during a 104-week chronic study often had convulsions prior to death. This phenomenon was observed only with rats dosed at the 40 mg/kg-d level. Hyperactivity and fighting were also observed more frequently among rats at this dose level than with rats fed at lower doses. Schneider et al. (1977) also observed convulsions in rats given a single i.p. dose of 500 mg/kg RDX.

Hematological effects. Levine et al. (1983b) reported mild anemia in rats that received 40 mg/kg-d of RDX in the diet during a 104-week chronic study. Male rats were affected to a greater extent than females. Extramedullary hematopoiesis, sinusoidal congestion and increased quantities of a hemosiderin-like pigment were also observed in both male and female rats at 52 weeks into the study. The hemosiderin-like pigment was also seen in rats dosed at 1.5 mg/kg-d. This pigment was considered to be a secondary

response to the hemolytic-type anemia. Stone et al. (1969) noted neutrophilic leukocytosis and elevated blood urea nitrogen in humans that had ingested up to 180 g of the plasticized RDX (C-4).

Dermal effects. Sunderman (1944) (as reported in Etnier, 1986) demonstrated that RDX powder acted as a primary irritant when applied to human skin. McConnell et al. (1946b) reported a number of cases of mild dermatitis that were attributed to occupational exposure in explosives plants. It is unclear whether these cases of dermatitis were caused by RDX or TNT.

Renal and urinary bladder effects. Levine et al. (1983b) noted distended bladders in male rats that received dietary doses of 40 mg/kg-d RDX for 52 weeks. The bladders contained dark red fluid, and upon histologic examination, luminal distention and cystitis were found. The kidneys of these rats were dark brown and displayed dilation of the renal pelvis. Renal medullary papillary necrosis was also noted. Ketel and Hughes (1972) documented 40 cases of RDX toxicity within a one year period in Vietnam (cited in Etnier, 1986). Based on 18 complete case reports, adverse effects on the kidney were relatively minor, and occurred in only 16% of the affected individuals.

Reproductive effects. Levine et al. (1983b) found statistically significant ($p < 0.05$) reductions in the weights of testes from rats that received dietary doses of 40 mg/kg-d RDX for 52 weeks. Spermatic granuloma of the prostate was present in some animals. Other histologic lesions included germinal cell degeneration, enlarged seminal vesicles, and enlarged prostates.

Ocular effects. Levine et al. (1983b) found a statistically significant ($p < 0.05$) increase in the number of cataracts in female rats that were given 40 mg/kg-d for of RDX in the diet for 78 weeks. Female rats that received this dose for 104 weeks had a statistically ($p < 0.05$) greater incidence of cataracts than the 78-week group.

Hepatic effects. Liver injury was observed by Levine et al. (1983b) in rats that received a 40 mg/kg-d dietary dose of RDX during a 104-week chronic study. Hepatotoxicity was manifested as hepatomegaly, hypocholesterolemia,

hypotriglyceridemia, and reduced serum-albumin/total-protein levels. French et al. (1976) used conventional transmission electron micrographs to observe hepatocytes with dilation of the rough endoplasmic reticulum, mitochondrial swelling, and concentric membrane arrays 24 h after oral administration of 100 mg/kg-d RDX. At 48 h following RDX administration, there was proliferation of the smooth endoplasmic reticulum in the hepatocytes. Marked proliferation of the smooth endoplasmic reticulum was noted after 120 h.

Immune system effects. Exposure to RDX has been implicated in an excessive incidence of systemic lupus erythematosus (SLE) at a munitions plant. In an attempt to determine whether RDX was the causitive agent, Hathaway and Buck (1977) conducted a cross sectional epidemiologic study of individuals employed at five separate munitions plants. SLE can be characterized by the presence of antinuclear antibodies, and Hathaway and Buck (1977) used the fluorescent antinuclear antibody test (FANA) as a screening tool to detect individuals with SLE. They found no statistically significant differences in the number of individuals who tested positive for the presence of FANA between exposed and unexposed workers.

Teratogenicity

Cholakis et al. (1980) studied the teratogenicity of RDX in rats and rabbits. A known teratogen, hydroxyurea, was used as a positive control. No teratogenic effects were found in either species at dose rates up to 20 mg/kg-d (oral administration). At the higher doses of 40 and 80 mg/kg-d, pregnant rats died before giving birth. A two-generation study with rats did not reveal any statistically significant effects in offspring at dose rates up to 20 mg/kg-d.

Genotoxicity

Simmon et al. (1977), Cholakis et al. (1980), and Whong et al. (1980) conducted in vitro microbial assays with five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, and TA100). No evidence of

mutagenicity was found in any of these studies with or without metabolic activation. Simmon et al. (1977) also studied the genotoxicity of RDX in Saccharomyces cerevisiae. RDX was not mutagenic to S. cerevisiae; however Simmon et al. (1977) noted that this organism is not sensitive to certain classes of chemicals, and thus may not provide a valid indication of the genotoxicity of RDX.

Although RDX has given negative results in all mutagenicity studies conducted to date, mononitroso-RDX, which is structurally similar to RDX, has been consistently active in assays of genotoxicity. Snodgrass (1984) summarized the results of a series of short-term tests on mononitroso-RDX (the actual assays were performed by Litton Bionetics). Mononitroso-RDX was not mutagenic to 5 strains of Salmonella typhimurium, with or without metabolic activation. Incubation of CHO cells with mononitroso-RDX caused a significant increase in the incidence of chromosome aberrations and sister chromatid exchange. Mononitroso-RDX also induced DNA repair in primary rat hepatocytes, and caused a significant increase in the number of mutations in mouse lymphoma cells. There was no evidence that mononitroso-RDX produced dominant lethal effects in mice.

Carcinogenicity

Hart (1976), Levine et al. (1983b), and Lish et al. (1984) conducted lifetime bioassays on RDX. Hart (1976) administered RDX in the diet of Sprague-Dawley rats at 1.0, 3.1, or 10.0 mg/kg-d over a two-year period. No significant evidence of toxicity was observed in the treated animals. Levine et al. (1983b) administered RDX in the diet of Fischer-344 rats at 0.3, 1.5, 8.0, or 40.0 mg/kg-d for 24 months. Although RDX was toxic at doses above 1.5 mg/kg-d (e.g., anemia, splenic lesions, hepatotoxicity, etc.), there was no evidence of carcinogenicity.

In contrast with the previous studies conducted with rats, Lish et al. (1984) found that RDX was carcinogenic to female B6C3F1 mice. In their study, groups of 85 mice consumed RDX in the feed at doses of 1.5, 7.0, 35.0, or 100.0 mg/kg-d. The purity of RDX ranged from 89.2 to 98.7 wt%, with HMX as the primary impurity. Initially, the high-dose group received RDX at 175 mg/kg-d, but increased mortality necessitated a reduction to 100.0 mg/kg-d at test week 11. There was a dose-related increase in hepatocellular carcinomas in female mice, but not male mice, that was not statistically

significant. However, the combined incidence of hepatocellular carcinomas and hepatocellular adenomas was statistically significant ($p < 0.05$) for the female mice in the 7.0 and 35.0 mg/kg-d treatment groups.

Estimation of Intake Limits to Protect Public Health

The preponderance of toxicological data currently available indicate that RDX is probably noncarcinogenic. Short-term tests of genotoxicity have given consistently negative results, and the only statistically significant carcinogenic response in laboratory animals was based on an analysis of the combined incidence of hepatocellular carcinomas and hepatocellular adenomas in female mice (Lish et al., 1984). Moreover, the RDX used in the Lish et al. study included HMX as a major impurity, and no long-term animal toxicity studies have examined its potential carcinogenicity. In consideration of the lack of epidemiological studies on the carcinogenicity of RDX, the negative results from assays of mutagenicity, the equivocal results of chronic toxicity tests with mice, and the absence of malignant tumors in rats after chronic dietary administration (Hart, 1976, Levine et al., 1983b), the most appropriate method for estimating a safe intake is to use a safety factor to adjust an animal NOEL to obtain an equivalent human intake. Etnier (1986) used a NOEL of 0.3 mg/kg-d defined in the chronic rat study conducted by Levine et al. (1983b) to derive an acceptable daily intake of RDX for humans. The resulting intake was 0.003 mg/kg-d (0.21 mg/d for a 70 kg adult) based on an uncertainty factor of 100.

TOXIC EFFECTS ON AQUATIC SPECIES

Bentley et al. (1977a) examined the acute and chronic toxicity of RDX on aquatic organisms. Results from the static acute toxicity experiments are presented in Table 9-7. Chronic studies were conducted with the water flea, midge (larvae), and fathead minnow. Fathead minnows were the most sensitive species to chronic RDX exposure. After 30 d of exposure to 6.3 mg/L RDX, survival of fathead minnow fry was significantly lower than in controls. Bentley et al. (1977a) used an application factor of 0.1 to the bluegill 96-h LC50 value of 3.6 mg/L to calculate a water quality criterion of 0.35 mg/L RDX.

Table 9-7. Acute toxicity of RDX to aquatic organisms from Bentley *et al.*, 1977a).

Species	Concentration (mg/L)	Effect
Vertebrates		
Bluegill	3.6[a] (1.9-6.6)	96-h LC50
Bluegill	7.6[c] (5.-10)	96-h LC50
Bluegill	6.0[d] (5.4-6.5)	96-h LC50
Fathead minnow (7 days post hatch)	3.8[d] (3.0-5.0)	96-h LC50
Channel catfish	13[c] (8.8-20)	96-h LC50
Channel catfish	4.1[d] (3.5-4.9)	96-h LC50
Rainbow trout	6.4[d] (5.4-7.4)	96-h LC50
Invertebrates		
Water flea	>100	48-h EC50[e]
Midge	>100	48-h EC50[e]
Algae		
S. capricornutum	3.2	17% decrease in cell density in 96 h.
M. aeruginosa	32.0	18% decrease in cell density in 96 h.
A. flos-aquae	32.0	14% decrease in cell density in 96 h.
N. pelliculosa	32.0	17% decrease in cell density in 96 h.

[a] For a water temperature of 20°C, a pH of 6.0, and a water hardness of 35 mg/L ($CaCO_2$). This concentration was derived from a static test.

[b] 95% confidence interval

[c] Obtained from a dynamic test

[d] Obtained from a static test

[e] EC50 based on immobilization

10. DATA-BASE ASSESSMENT FOR HMX

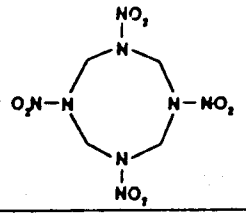
HMX is a by-product of RDX synthesis and is present in military-grade RDX at levels of between 8 and 12 wt%. HMX is also a powerful explosive and is used in different explosive formulations. Because HMX has a chemical structure that is similar to that of RDX (see Table 10-1), its environmental properties parallel those of RDX. For example, the photolytic decay of HMX, although slower than RDX photolysis, is an important environmental transformation process for both explosives. Data on the toxic effects of HMX are limited to the results of two toxicity studies with laboratory animals. Its aquatic toxicity has been evaluated in freshwater algae and fish.

ENVIRONMENTAL PARTITIONING AND TRANSPORT

Pertinent chemical and physical properties of HMX are contained in Table 10-2 and estimated partition coefficients are shown in Table 10-3. The vapor pressure for HMX is extrapolated from approximately 10^{-9} to 10^{-15} torr, and consequently it is subject to some uncertainty. Similarly, the Henry's law constant is only an approximation because of the uncertainty in the vapor pressure estimate. Even with such estimation errors, it is clear from the equilibrium distributions of HMX in the two reference environments shown in Table 10-4 that this compound will preferentially reside in subsurface soils and ground water.

Kayser and Burlinson (1982) analyzed the concentrations of RDX and HMX in leachate from soil-column experiments conducted by Hale et al. (1979). Their concentration data indicated that HMX (present as an impurity in RDX) leached through three different soils at the same rate as RDX over a three-month period. HMX concentrations were consistently about 10% of those of RDX, which is the approximate level of HMX in military-grade RDX. However, when Kayser and Burlinson (1982) extracted the soils, they found the HMX concentration in relation to RDX to be higher than the 10% expected. The proportionately higher concentrations of HMX were more pronounced in some of the soil extracts. The differences could be due to different degradation rates between RDX and HMX or decreased soil adsorption for HMX. Spanggord et al. (1982c) measured the adsorption of HMX onto sediment from the Holston River. They

Table 10-1. Chemical identifiers of HMX.

Empirical formula:	$C_4H_8N_8O_8$	Structural formula:
Molecular weight:	296.17	
CAS reg. no.:	[2691-41-0]	
RTECS no.:	XF7450000	

estimated upper limits for K_d and K_{OC} as 8.7 and 670, respectively. In contrast, the K_{OC} predicted from HMX's aqueous solubility and melting point is 129.

ENVIRONMENTAL TRANSFORMATION PROCESSES

The environmental degradation mechanisms and products of HMX are similar to those of RDX, however; the photolysis and microbial degradation of HMX occur at slower rates.

Photolysis

The photolytic absorption spectrum of HMX is similar to that of RDX and is strongest below 290 nm (Maycock et al., 1969), the point accepted as the effective lower bound of the natural sunlight spectrum (see Lyman et al., 1982). Maycock et al. (1969) also showed that RDX and HMX absorb UV and visible light in a similar manner. Spanggord et al. (1983b) measured the photolytic degradation of HMX and RDX in samples of distilled water, river water, and manufacturing waste water contained in borosilicate tubes or in dishes. Their data showed that the photolytic loss rate of HMX in distilled water exposed to sunlight was faster than the photolysis rate of HMX in lagoon water. They attributed the difference to the increased screening of light caused by suspended partices in the lagoon water (see Table 10-5).

Table 10-2. Chemical and physical properties of HMX. Properties are for an ambient temperature of 20°C unless otherwise noted.

Property	Units	Value	Source
Boiling point	°C	Not available	
Freezing/melting point	°C	285 (decomposes)	Dobratz, 1981
Density	g/mL	1.9	Dobratz, 1981
Vapor pressure	torr	9×10^{-16}	Calculated[a] from a vapor pressure-temperature equation
Aqueous solubility	mol/L mg/L	8.8×10^{-6} 2.6	Spanggord *et al.*, 1982c
	mol/L mg/L	1.7×10^{-5}[b] 5.0	Glover and Hoffsommer, 1973
Henry's law constant	(L-torr)/mol	1×10^{-10}	Calculated as the ratio of vapor pressure to aqueous solubility at 20°C
Diffusion coefficients: Air Water	m^2/s m^2/s	5.1×10^{-6} 5.0×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition coefficient (K_{OW})	log K_{OW}	0.13	Measured, Atlantic Research (1979), cited in Tucker *et al.*, 1985

[a] See Appendix A.

[b] Measured at 22°C.

Table 10-3. Partition coefficients for use in estimating human exposures to HMX.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	1	Calculated[b] from molecular weight
Beef fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	2.9×10^{-3}	Calculated[b] from water solubility and log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	0.5	Calculated[b] from log K_{ow}
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	130	Calculated[b] from solubility in water and melting point
			670	Upper-bound value for a river sediment, based on measured values from Spanggord *et al.*, 1982c

[a] Wet-weights for plant and fish

[b] See Appendix A

Table 10-4. Fractional distributions of HMX among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of HMX to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	6.7×10^{-17}	9.3×10^{-17}
Air particles	4.6×10^{-9}	1.2×10^{-8}
Biota	4.8×10^{-5}	0.0053
Upper soil	0.021	0.082
Lower soil	0.36	0.10
Ground water	0.62	0.80
Surface water	0.0026	0.010
Sediments	8.6×10^{-5}	3.5×10^{-4}

[a] Fractions do not add to one because of rounding.

Spanggord et al. (1983b) calculated the light absorption rate at selected wavelengths for RDX and HMX and found that the RDX absorption rate was twice the HMX absorption rate. In addition, they estimated the quantum yield of HMX to be 0.13. Spanggord et al. (1983b) estimated HMX photolysis rates at depths of 0 to 300 cm in the Holston River, using the procedure of Zepp and Cline (1977) to calculate day-averaged photolysis rate constants. They found that the computer-derived estimates and their measured rate constants differed by a factor of two. Photolytic decay rates for summer and winter (40°N latitude) are summarized in Table 10-5 for different water depths.

Photolytic degradation of HMX and RDX by natural light is caused by absorption of light between 290 and 370 nm (Spanggord et al., 1983b; Maycock et al., 1969; and Smetana and Bulusu, 1977). Maycock et al. (1969) suggested that light absorption in this range causes electronic transitions in the $N-NO_2$ chromophore for both RDX and HMX.

Table 10-5. Rates of photolytic degradation for HMX (derived from data presented in Spanggord et al., 1983b).

Concentration mg/L	Derivation of half-life	Photolysis rate constant (d^{-1})	Calculated half-life (d)
6	Loss from distilled water solution, in borosilicate tube in July sunlight in Menlo Park, CA	0.302	2.2
6	Loss from distilled water solution, in 4-cm-deep dish in June sunlight in Menlo Park, CA	0.19	3.6
6	Loss from lagoon water solution, in borosilicate tube in July sunlight in Menlo Park, CA	0.10	6.9
6	Loss from lagoon water solution, in 4-cm-deep dish in June sunlight in Menlo Park, CA	0.0099	70
not specified	Estimated by method of Zepp and Cline (1977) for Holston River water surface, Summer	0.4	1.7
not specified	Estimated by method of Zepp and Cline (1977) for Holston River water surface, Winter	0.069	10
not specified	Estimated by method of Zepp and Cline (1977) for Holston River water, 300-cm deep, Summer	0.021	33
not specified	Estimated by method of Zepp and Cline (1977) for Holston River water, 300-cm deep, Winter	0.0036	192

The photolysis mechanism probably involves molecular decomposition because no photolytic intermediates accumulate. Torbit (1970) and Maycock et al. (1969) studied the photolytic decomposition of HMX crystals with UV irradiation at a broad spectrum of wavelengths from 235 to 575 nm and at a single wavelength of 254 nm. These authors proposed a mechanism involving rupture of a C-N bond with subsequent loss of the $N-NO_2$ moiety. Torbit's work

also supported cleavage of the C-N bond. He measured the loss of $N-NO_2$ UV absorption with irradiation time. Mass spectral analysis of the products of UV irradiation also indicated cleavage of the C-N bond. Maycock et al. (1969) found three photoproducts present initially. One fragment was too small (mass/charge (m/e) of 14) to use in determining a mechanism. The other two fragments indicated breakage of the C-N linkages of HMX. One had an m/e ratio of 30, which Maycock et al. considered to be formaldehyde; the other had an m/e of 44, which was suspected to be dinitrogen oxide.

These proposed mechanisms are in apparent conflict with those of Kubose and Hoffsommer (1977). They concluded from their study with RDX that irradiation of RDX with light at wavelengths above 280 nm initiated degradation by cleavage of the N-N bond. Irradiation with shorter wavelengths ruptured the N-O bond forming an N-nitroso intermediate. However, Spanggord et al. (1983b) found no evidence for the formation of N-nitroso derivatives in the photolysis of HMX. They proposed a mechanism with breakage of an N-N bond because 50% of the initial NO_2 groups became nitrate or nitrite (Figure 10-1). However, they noted that nitrate and nitrite also undergo further photochemical transformations.

Biotransformation

Biodegradation of HMX occurs aerobically only under very specialized conditions and under anaerobic conditions, the addition of nutrients is necessary.

Spanggord et al. (1983b) found that HMX was not degraded aerobically by naturally-occurring microbes in samples of water from uncontaminated ponds and streams near Menlo Park, California, even with added yeast extract. However, the adapted microbes in the effluent water from the Holston Army Ammunition Plant reduced HMX concentrations by 50% (6.1 to 3 ppm) in 2 d when 50 ppm yeast extract was added to that water. They were unable to culture the organism or find a chemical that was affecting the transformation. They also performed anaerobic biotransformation studies with HMX in a basal salts medium containing yeast extract. They found that the amount of HMX transformed increased as the amount of yeast was increased.

In another set of experiments they studied the effect of different nutrients on biotransformation rates. Glucose, peptone, sodium acetate, and yeast extract did not significantly alter the transformation rates. When

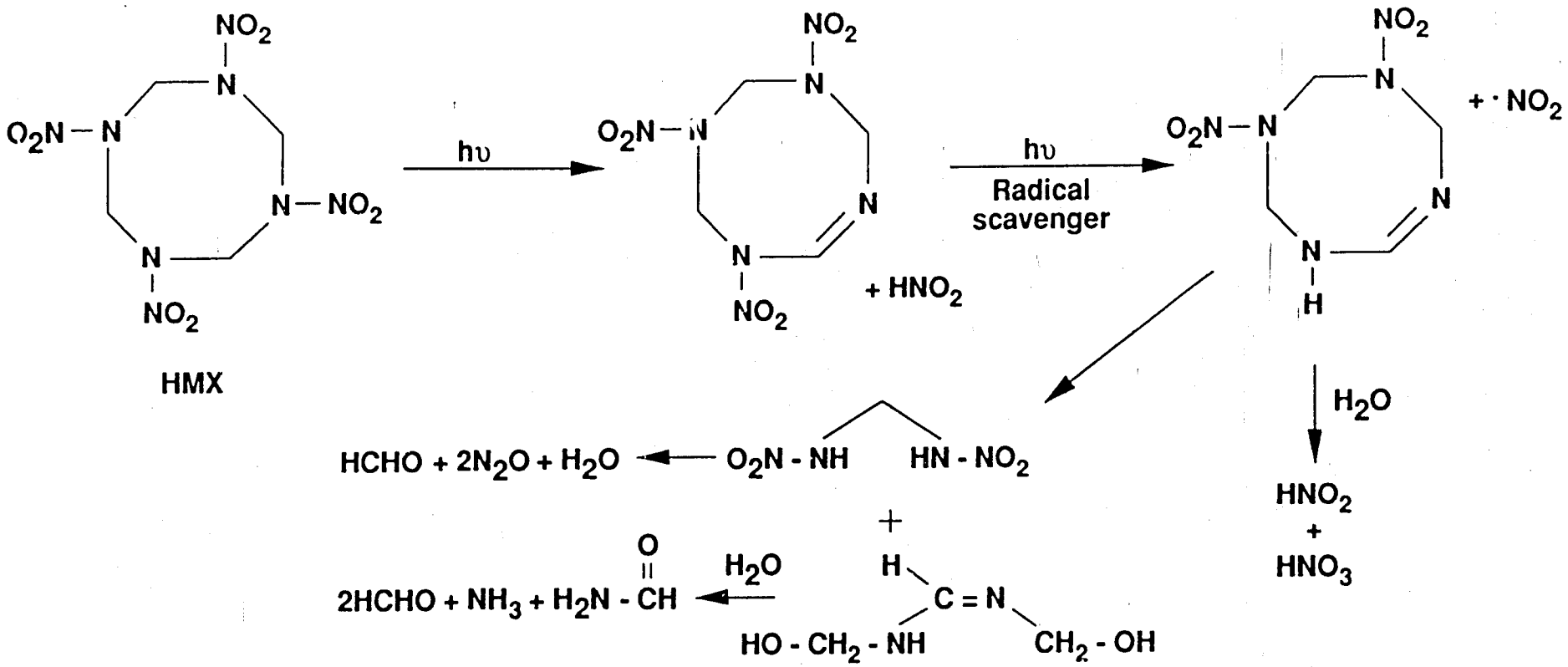


Figure 10-1. Photolytic decomposition mechanism for HMX proposed by Spanggord et al. (1983b).

grown and washed bacterial cells were used, yeast extract was more effective in promoting HMX biodegradation. McCormick et al. (1984) measured the decline of HMX levels in sewage sludge under anaerobic conditions and nutrient broth. They found that HMX degraded more slowly than RDX under the same conditions.

Microbial degradation of HMX begins with non-selective, but sequential reduction of the nitro groups of HMX to their nitroso analogues. Spanggord et al. (1983b) identified mono-, di-, tri-, and tetranitroso-HMX from mass spectral analysis of the metabolites of HMX biodegradation. McCormick et al. (1984) found the same intermediates in anaerobic biodegradation experiments using sewage sludges and nutrient broth. They found no hydrazines among the HMX metabolites, although hydrazine intermediates were detected in similar biodegradation experiments with RDX. They speculated that the absence of hydrazines from HMX degradation was due to a slower initial rate of attack on HMX, which prevented accumulation of hydrazine.

TOXIC EFFECTS ON ANIMALS AND HUMANS

Most of the information on the toxicity of HMX comes from studies conducted by McNamara et al. (1974) and Wilson (1985). The toxicity of HMX has also been discussed by Ryon et al. (1984). In the following data-base assessment, we review the data from these and other studies with respect to the toxicity of HMX to specific organs and systems.

Toxicokinetics

Absorption. Wilson (1985) measured HMX levels in the plasma of mice and rats that had received a single dose of radiolabeled HMX. A peak plasma concentration of 6 to 10 μg/mL was observed in these animals after an oral dose of 500 mg/kg. The time course of plasma levels was similar in both species and the peak was reached 6 h after treatment. In contrast, peak plasma levels of HMX (0.5 to 1.0 μg/mL) occurred within one hour in rats that were given a single intravenous dose of labeled HMX (2.0 mg/kg). These levels remained fairly constant for six hours, but by 24 hours, had decreased to 0.2 μg/mL. Wilson also measured HMX in the plasma of rats that were fed HMX

in their diet for 90 d (treatments ranged from 50 to 4000 mg/kg-d) and concluded that plasma levels of HMX were "extremely low in relation to dose," and that furthermore, the quantity of HMX in the plasma did not increase "significantly"[a] with dose.

McNamara et al. (1974) found substantial differences in the toxicity of HMX to dogs, depending on the route of exposure. In general, dermal application of HMX (17.5 mg/kg in acetone, 21.9 mg/kg in cyclohexanone, or 289.0 mg/kg in DMSO) did not produce consistent or predictable physiological alterations (i.e., changes in the EEG, EKG, blood pressure, heart rate, and respiratory rate). Conversely, intravenous administration of HMX (1.55 or 3.1 mg/kg in cyclohexanone, 2.5 or 6.75 mg/kg in acetone, or 20 or 40 mg/kg in DMSO) caused changes in the EEG, blood pressure, and heart rate. In general, absorption of HMX by rabbits and guinea pigs appears to be similar to that noted in dogs. However, at a high concentration in a very lipophilic solvent (DMSO), HMX can penetrate the skin in amounts great enough to cause death. In particular, dermal application of 1.0 mL HMX (33% in DMSO) was lethal to rabbits after two or five treatments. Some deaths in guinea pigs were also recorded after single dermal applications of 465, 477, 507, or 546 mg/kg (McNamara et al., 1974).

Distribution. Reports of organ toxicity following exposure to HMX indicate that this substance can reach the heart, central nervous system, immune system, liver, and kidneys (McNamara et al., 1974; Wilson, 1985). Wilson (1985) has also noted that following oral (500 mg/kg) or intravenous (2.0 mg/kg) administration of HMX to rats, the highest concentrations were found in the liver and kidneys, and the lowest levels in the brain.

Metabolism and Elimination. Wilson (1985) compared the relative importance of respiratory, fecal, and urinary excretion of HMX by species and route of exposure. Animals were given ^{14}C-HMX orally or intravenously, and excreta were recovered over a four day-period. The results of these experiments are summarized in Table 10-6. Fecal elimination was the dominant route of excretion after oral administration of HMX: 70.0 to 85.0% of the recovered dose was excreted by this pathway. Urinary excretion of the radiolabel represented a minor portion of the recovered dose (3.0 to 4.0%). However, when HMX was given intravenously, urinary excretion became the

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[a] Wilson (1985) did not report the level of statistical significance.

Table 10-6. Excretion of ^{14}C-HMX after oral or intravenous administration (data are from Wilson, 1985).

Species	Route	Concentration or dose (mg/kg)	Percent radiolabel recovered in			
			feces	urine	CO_2	carcass
Rat	Oral	500.0	85.0	4.0	0.7	0.5
Mouse	Oral	500.0	70.0	3.0	0.6	1.0
Rat	i.v.	2.0	3.0	61.0	5.0	6.0

predominant route of elimination (61.0% of the radiolabel was recovered in urine), and fecal excretion decreased to 3.0%. Production of CO_2 represents a relatively minor pathway of HMX elimination (see Table 10-6).

Toxic Effects on Organs and Systems

Hepatic effects. Wilson (1985) reported centrilobular degeneration in the livers of male rats that were fed an average of 8504 mg/kg-d of HMX per day for 14 d. In the same study, female rats that received 1280, 3055, or 3474 mg/kg of HMX daily exhibited hepatocyte hyperplasia. Animals in all dose groups (333 to 9000 mg/kg) had reduced liver weights. Male and female rats that were given HMX in the diet for five weeks (4000 or 1500 mg/kg-d, respectively) had a "significant increase" in levels of alkaline phosphatase relative to controls. After seven additional weeks of treatment, only females still had abnormally high levels of this enzyme. A "significant" number of male rats fed 450, 1350, or 4000 mg/kg-d of HMX (over 90 d) developed enlarged centrilobular cells that had atypically colored nuclei and cytoplasm. In a 90-d experiment, Wilson found that alanine aminotransferase and alkaline phosphatase activity were "slightly depressed" in male mice treated with 5 to 200 mg/kg-d of HMX. Female mice (treatments of 10 to 750 mg/kg-d) also exhibited a slight depression of alkaline phosphatase. Mice that died before the end of a 14-d treatment period with HMX (100 to 5000 mg/kg-d) had a dose-related increase in the incidence of hepatocellular hyperplasia (Wilson, 1985).

Renal effects. Blood urea nitrogen levels were "significantly" elevated in female rats given HMX in their diet (1500 mg/kg-d) for 13 weeks (Wilson, 85). The urine of these same rats had elevated levels of albumin as well as reduced specific gravity and pH, by the twelfth week of treatment. Male rats that were treated with 4000 mg/kg-d of HMX excreted abnormally large amounts of albumin in their urine after five weeks of dosing. Localized atrophy and dilation of the kidney tubules was noted in female rats fed 270, 620, or 1500 mg/kg-d of HMX for 13 weeks. Female rats at all dose levels (50, 115, 270, 620, or 1500 mg/kg-d) exhibited a dose-related increase in kidney weight (Wilson, 1985).

Dermal effects. McNamara et al. (1974) found that a single application of 1.0 mL HMX (33% in DMSO, 75% in cyclohexanone, or 5.7% in acetone) was not overtly irritating to the abraded skin of rabbits. However, mild desquamation of the skin was observed after repeated application (5 d/wk for 4 weeks) of 1.0 mL HMX (33%) in DMSO. Guinea pigs developed localized erythema when 1000 or 2000 mg/kg of HMX in DMSO (33%) was applied to their skin. The erythematous reaction elicited by 1000 mg/kg of HMX was not exacerbated by peated applications (McNamara et al., 1974). A suspension of HMX in physiologic saline (to a concentration of 60%) was "mildly irritating" when applied to the skin of rabbits (Wilson, 1985).

Ocular effects. Cutaneous (2.0 to 33.0%) or intradermal (0.25%) application of HMX in acetone, DMSO, or cyclohexanone (3 d/wk for 3 weeks) did not increase the incidence of cataracts in the eyes of guinea pigs over that of solvent controls (McNamara et al., 1974). Wilson (1985) reported that dry HMX, or HMX in distilled water (60% suspension) was "practically non-irritating" to the eyes of rabbits.

Cardiovascular effects. A single intravenous dose of 40 mg/kg HMX in DMSO caused cardiovascular collapse and death in dogs (McNamara et al., 1974). Prior to death, animals developed bradycardia (slowing of the pulse rate), respiratory alterations, and a change in the EEG. A dog given an i.v.

dose of 2.5 mg/kg of HMX in acetone followed by 6.75 mg/kg of HMX (also in acetone) exhibited a drop in blood pressure, a depressed respiratory rate, and temporary cardiac arrest (McNamara et al., 1974).

Central nervous system effects. Administration of 20 mg/kg intravenously of HMX in DMSO caused hyperreflexia in dogs two hours after dosing (McNamara et al., 1974). Three hours later, one animal was extremely hyperactive, and underwent convulsive seizures. Dietary ingestion of HMX for 13 weeks (1500 or 4000 mg/kg-d) caused an increase in the relative brain weight of male and female rats (Wilson, 1985). Female rats (1500 mg/kg-d) also had elevated absolute brain weight. In the same experiment by Wilson, mice of both sexes given HMX (5 to 750 mg/kg-d) had slightly higher brain weights than untreated controls.

Hematological effects. During weeks five and 12 of a 13-week feeding study with HMX, Wilson (1985) observed a decrease in hemoglobin in high-dose male (4000 mg/kg-d) and female (1500 mg/kg-d) rats. In the twelfth week of this experiment, animals of both sexes also developed a "slight increase" in the levels of methemoglobin and had a reduced red-blood-cell count.

Immune system effects. An unusually high incidence of systemic lupus erythematosus (SLE) at a munitions plant prompted an epidemiologic study to determine if there was a correlation between exposure to explosives, and onset of this disease. The epidemiologic study by Hathaway and Buck (1977) encompassed three plants, where individuals were exposed to RDX, or to RDX in combination with TNT and/or HMX. However, no analysis of HMX concentrations in the air were conducted. Hathaway and Buck found no statistically significant differences in blood chemistry or in the presence of antinuclear antibodies (a predictor of SLE) between exposed and unexposed workers.

Sunderman (1944) used a patch test to evaluate the ability of solid HMX to induce hypersensitivity reactions in occupationally exposed individuals. Although he reported a positive response in some individuals, subsequent reports (Wilson 1985; McNamara et al., 1974) found no evidence that HMX caused allergic response in animals.

Lymphocyte depletion in the thymus and spleen was noted in female rats fed HMX daily, for 14 days (Wilson, 1985). The actual daily dose that these animals received is not clear; although they supposedly belonged in the highest dose group of 9000 mg/kg, the author reported that the average achieved dose was 3055 mg/kg-d. Similar abnormalities were observed in female rats that died before the end of the 14-day treatment period (these animals had received 1280 or 3474 mg/kg-d). A dose-related depletion of thymic and splenic cells was also found in mice given 100 to 5000 mg/kg-d of HMX over 14 d. These effects apparently were found only in animals that died before the end of treatment.

Teratogenicity

There are no data on the teratogencity of HMX.

Genotoxicity

Analytical grade HMX (in DMSO) was evaluated for its ability to induce reverse mutations in five strains of Salmonella (TA98, TA1537, TA1538, TA100, and TA1535) (Whong et al., 1980). In the spot test, concentrations of 0.625 or 1.25 mg/spot reportedly gave negative results. No supporting data were provided, and it is not clear if these results were obtained with or without metabolic activation. HMX was also tested in a plate incorporation assay to which Aroclor-induced S-9 had been added. Apparently, 2.5 mg/plate of HMX was not mutagenic; again, no details of the experiment were included.

Simmon et al. (1977) evaluated the mutagenicity of compounds found in wastewater from munitions plants. Compounds were tested before and after treatment with chlorine disinfection to distinguish the health hazards associated with the treatment process from those inherent in the parent compound. HMX (0.25 mL) was tested in the Ames assay with Salmonella typhimurium, using a standard plate incorporation technique. Mutagenic activity was analyzed in tester strains TA98, TA100, TA1535, TA1537, and TA1538, both with and without metabolic activation. In a separate series of tests, HMX (0.005 to 0.25 mL) was allowed to react with 10 to 1000 ppm of Cl_2 prior to testing in S. typhimurium. HMX did not significantly increase the frequency of reverse mutations in any of the tests. HMX was also examined for

its ability to induce mitotic recombination in Saccharomyces cerevisiae D3. HMX (as well as all other compounds tested) gave a negative response with this organism. Simmon et al. (1977) concluded that S. cerevisiae was not an appropriate organism for an evaluation of the mutagenicity of the compounds tested.

Carcinogenicity

No studies have been conducted to evaluate the carcinogenic potential of HMX.

Estimation of Intake Limits to Protect Public Health

The only toxicological data available to estimate the safe intake rate for HMX are from subchronic (i.e., 13 weeks) toxicity studies that Wilson (1985) conducted with rats and mice. In the study with rats (Fischer-344), five groups consisting of 20 male rats and 20 female rats were administered HMX in their diet. An additional group of male and female rats were used as controls. The daily doses for male rats in the five treatment groups were 50, 150, 450, 1350, and 4000 mg/kg; and for female rats the doses were 50, 115, 270, 620, and 1500 mg/kg. "Significant" (level of statistical significance not given) effects to livers (e.g., "enlarged centrilobular cells with pale nuclei and dark cytoplasm") were observed in males at dose rates at and above 150 mg/kg-d. Kidney effects in the form of focal atrophy and dilation were observed in females at dose rates of 270 mg/kg-d and above. Male and female mice (B6C3F1) were divided into five treatment groups consisting of 20 males and 20 females. Dietary dose rates for the male mice in the different treatment groups were 5, 12, 30, 75, and 200 mg/kg-d, and for the females the dose rates were 10, 30, 90, 250, and 750 mg/kg-d. At the highest dose rates for both sexes mortality was substantial (i.e., 13/20 males, 20/20 females). Laboratory studies of the mice did not reveal any major differences (e.g., in blood chemistry and histopathological examinations) between the exposed mice and the controls. Wilson (1985) did not identify a NOEL for either rats or mice, and no statistical tests were reported for comparisons between the control groups and the treatment groups. Subsequent communication with the author (Wilson, 1985) indicated that a conservative NOEL from these studies is

10 mg/kg-d for mice. An ADI can be derived by dividing 10 mg/kg-d by 1000, which includes adjustments for subchronic to chronic exposure and uncertainties associated with inter- and intra-species differences. The estimated safe intake is therefore 0.01 mg/kg-d. Because of the lack of rigorous statistical tests in the above study, we estimated lower-bound (LB) ADI's by multiplying the oral LD50's determined by Wilson (1985) for mice and rats by an application factor of 1×10^{-5} d^{-1} (see Appendix D). The oral LD50 values ranged from 1960 mg/kg-d for male mice to 7590 mg/kg-d for female rats, and the corresponding LB-ADI's are 0.02 to 0.08 mg/kg-d. The LB-ADIs are higher than the ADI derived from the subchronic study, which suggests that a value of 0.01 mg/kg-d should protect the health of individuals chronically exposed to this explosive.

TOXIC EFFECTS ON AQUATIC SPECIES

Bentley et al. (1977b) performed acute toxicity studies on four species of freshwater vertebrates, four species of invertebrates, and four species of algae. HMX was mixed with acetone, forming a superstock solution for subsequent use in preparing test solutions of HMX with concentrations greater than HMX's solubility in water. Representative data from these studies are listed in Table 10-7. The 7-day-old fry of fathead minnows had the lowest 96 h LC50 at 15 mg/L. All other vertebrate and invertebrate species tested had LC50 or EC50 values greater than 32 mg/L. All four aquatic algae species exposed to concentrations of 3.2 and 10 mg/L HMX showed greater chlorophyll-a content than controls. All four species of algae had an increased cell density at a concentration of 10 mg/L of HMX. Bentley et al. (1977b) calculated a no-effect concentration for HMX of 0.75 mg/L as the product of an application factor (i.e., 0.05) and the 96 h LC50 for 7-day old fathead minnows (i.e., 15 mg/L). Sullivan et al. (1979) examined the results of Bentley et al. (1977b) in order to estimate a 24-h average concentration of HMX that would protect aquatic species. They used an application factor of 0.01 with the LC50 for fathead minnows to derive a safe concentration of 0.15 mg/L.

Table 10-7. Acute toxicity of HMX to aquatic organisms (Bentley *et al*. 1977b).

Species	Concentration (mg/L)	Effect
Vertebrates		
Rainbow trout	>32	96-h LC50
Bluegill	>32	96-h LC50
Fathead minnow	>32	96-h LC50
Fathead minnow (7-days post hatch)	15	96-h LC50
Invertebrates		
Water flea	>32	48-h EC50[a]
Midge	>32	48-h EC50[a]
Aquatic algae		
S. capricornutum	10	9% increase in chlorophyll-a content
A. flos-aquae	10	39% increase in chlorophyll-a content

[a] Effective concentrations causing immobilization.

11. DATA-BASE ASSESSMENT FOR PENTAERYTHRITOL TETRANITRATE (PETN)

PETN is used primarily as a booster explosive or as an energetic ingredient in primer formulations. This explosive is a minor component in the demilitarization inventory of explosives. PETN has been used as a vasodilator in the treatment of angina, and consequently, data are available on its pharmacological properties. Little work, however, has been done on its behavior in the environment.

ENVIRONMENTAL PARTITIONING AND TRANSPORT

Table 11-1 contains the chemical identifiers for PETN. Table 11-2 contains estimated and measured chemical properties, and Table 11-3 presents estimates of the coefficients for equilibrium partitioning between plant/soil, beef-fat/diet, fish/water, and SOC/water. The equilibrium distribution of PETN in the reference landscapes representing the Western and Southeastern ecoregions (Table 11-4) indicates that subsurface transport will be the predominant transport mechanism.

ENVIRONMENTAL TRANSFORMATION PROCESSES

No studies have been completed that examine the transformation of PETN in environmental media. Consequently, our analysis of potential transformation processes is based primarily on inferences drawn from other studies.

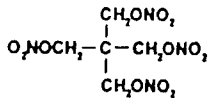
Photolysis

PETN does not absorb light in the natural solar spectrum (Deb, 1957), and therefore no photolytic degradation is expected.

Biotransformation

No information was found on the microbial degradation of PETN in natural waters or soils.

Table 11-1. Chemical identifiers of PETN.

Empirical formula:	$C_5H_8N_4O_{12}$	Structural formula:
Molecular weight:	316.15	
CAS reg. no.:	[78-11-5]	
RTECS no.:	RZ2620000	

Hydrolysis

Although there are no data on the hydrolysis of PETN in natural waters, Fraser (1968) did measure the hydrolysis rates of trinitroglycerine (TNG) and PETN in an ethanol/water/NaOH solution. PETN hydrolysis occurred at a rate that was approximately 1000 times slower than that of TNG. The hydrolysis rates were second-order functions of hydroxide-ion concentration. Capellos et al.(1984) measured the hydrolysis of TNG in a water/$Ca(OH)_2$ solution at a pH of 7 and a temperature of 25°C. The hydrolysis rate for TNG was 0.02 $M^{-1}{}_{OH}s^{-1}$, or a half-life of about 10 years, which suggests (if the above relationship between PETN and TNG holds) that the hydrolysis of PETN should be negligible.

TOXIC EFFECTS ON ANIMALS AND HUMANS

In addition to its use as an explosive, PETN, like its three-carbon analog TNG, has also been used medicinally as a vasodilator in anti-anginal therapy. As a result of the medical use of PETN, relevant pharmacological information is available from low-dose animal tests and over 30 years of administration to angina patients. Studies of PETN have focused primarily on cardiovascular effects.

Toxicokinetics

Absorption. Oral administration of PETN to rats has shown that absorption proceeds fastest from the small intestine, slower from the large intestine, and slowest from the stomach (DiCarlo et al., 1965a). PETN appears to be the only organic nitrate commonly employed as a drug that is not readily

Table 11-2. Chemical and physical properties of PETN. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	Decomposes at 210	Lindner, 1980
Freezing/melting point	°C	140	Windholz *et al.*, 1983
Density	g/mL	1.52	Windholz *et al.*, 1983
Vapor pressure	torr	2×10^{-9}	Calculated[a] from a vapor pressure-temperature equation
Aqueous solubility	mol/L mg/L	6.3×10^{-6} 2	See Small and Rosenblatt, 1974
	mol/L mg/L	1.0×10^{-4} 32	Extrapolated[a] from water solubility at elevated temperatures
Henry's law constant	(L-torr)/mol	$\simeq 3 \times 10^{-4}$	Calculated as the ratio of vapor pressure to an aqueous solubility of 6.3×10^{-6} mol/L.
Diffusion coefficients: Air Water	m^2/s m^2/s	5.1×10^{-6} 4.9×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition coefficient (K_{ow})	log K_{ow}	2.2	Estimated[a]

[a] See Appendix A.

Table 11-3. Partition coefficients for use in estimating human exposures to PETN.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	1	Calculated[b] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	4.4×10^{-3}	Calculated[b] from log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/mg/kg water)	22	Calculated[b] from log K_{ow}
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	68	Calculated[b] from log K_{ow}

a Wet-weight for plants and fish
b See Appendix A.

Table 11-4. Fractional distributions of PETN among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of PETN to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	1.1×10^{-9}	9.0×10^{-10}
Air particles	2.8×10^{-9}	8.2×10^{-9}
Biota	3.4×10^{-5}	0.0041
Upper soil	0.015	0.063
Lower soil	0.30	0.10
Ground water	0.68	0.82
Surface water	0.0030	0.014
Sediments	5.6×10^{-5}	2.6×10^{-4}

[a] Fractions do not add to one because of rounding.

absorbed through the oral mucosa (Gilman et al., 1985). Davidson et al. (1970) reported that radiolabeled PETN was detected in the blood of human volunteers within 15 minutes of administering ^{14}C-PETN. DiCarlo et al. (1967a) administered 10 mg/kg of ^{14}C-PETN by gavage to rats and found that 7 to 8% of the radiolabel was absorbed during the first hour, 14% after 2 h, 24% after 4 h, and 60% after 18 h.

Analyses of urinary metabolites indicate that both mice (see DiCarlo et al., 1964) and rats (see Crew et al. 1966) absorbed over 40% of a 10 mg/kg dose of ^{14}C-PETN in 24 hours. Davidson et al. (1971) reported that 48 h after oral dosing, volunteers had absorbed a minimum of 60% of a 20-mg dose and 50% of a 40-mg dose. PETN remained present in the gastrointestinal tract after the three-day surveillance. Estimates of PETN uptake based on urinary excretion may be low due to enterohepatic circulation of PETN or metabolites followed by fecal excretion (Davidson et al., 1970). Fecal elimination of 33% of an intra-arterial dose of 2 mg/kg of ^{14}C-PETN to rats indicated that enterohepatic recycling of PETN is extensive (King and Fung, 1986).

Since PETN is not found intact in blood or urine, and is slowly absorbed, Davidson et al. (1970) suggested that absorption might follow conversion to

the more readily absorbed compound PE-trinitrate (PE-tri-N) by enzymes or gastrointestinal bacteria. Studies by Carter and Goldman (1976) dealing with the metabolism of PETN in germ-free and conventional rats, however, did not show any differences between the amounts of ^{14}C-PETN and its metabolites in the urine of the two types of rats.

A more exact estimate of the amount of PETN absorbed requires additional data on the extent of metabolism of PETN by gastrointestinal bacteria and the role of enterohepatic recirculation. The presence PETN metabolites in rat feces led King and Fung (1984) to suggest that unabsorbed PETN may be further metabolized by bacteria in the gut. In vitro studies of PETN degradation in rat urine and rat fecal homogenates, sterilized and unsterilized, show rapid and complete degradation of PETN by intestinal microflora (King and Fung, 1984).

Distribution and bioaccumulation. Davidson et al. (1971) proposed that the major portion of an absorbed dose of PETN is rapidly distributed into cells where active nitrate esters are further denitrated with the release of metabolties into systemic circulation. Peak radioactivity levels in blood occurred 2 to 4 h after single oral doses of 40 mg of ^{14}C-PETN were orally administered to patients with coronary heart disease (DiCarlo et al., 1966). These levels remained high for about 6 h and consisted primarily of PE, PE-mononitrate (PEMN), and PE-dinitrate (PEDN). The greatest quantity of ^{14}C in the plasma was 1.44% of the dose, whereas whole blood contained as much as 4.5% of the dose. DiCarlo et al. (1965b) found that erythrocytes can degrade PETN faster than whole blood, and have a strong affinity for PETN and some of its metabolites. PE and PEMN were still found in the blood 24 h after dosing.

Davidson et al. (1970) reported similar results from pharmacokinetic studies in which men received a single dose of 20 mg ^{14}C-PETN. Peak ^{14}C levels in blood occurred 4 to 8 h after dosing. PE and PEMN were the primary blood metabolites. PEDN accounted for 28% of the radioactivity at 4 h, but had completely disappeared at 18 hours. At 24 h the ^{14}C content of blood was 40% of the peak level and at 48 h, it was 10% of the peak. PETN itself was not detected in blood. The distribution of metabolites in the blood of rats dosed with ^{14}C-PETN at 10 mg/kg reported by Crew et al. (1966) is similar to the distribution observed in humans. DiCarlo et al. (1967a) noted that the PETN and/or its metabolites were quickly cleared from blood. Blood clearance

is rapid due to excretion and uptake into organs, where ^{14}C-levels are higher than in blood after 1 h (Litchfield, 1971).

After the oral administration of ^{14}C-PETN (10 mg/kg) to rats (DiCarlo et al., 1967a), the ^{14}C content of the kidneys increased over time from twice as high as the blood level at one hour to three times at four hours, to more than 20 times at 18 h. The ^{14}C content of the liver at 1, 2, and 4 h was about three times greater than that in blood and nine times greater at 18 h. PE accounted for 100% of the radioactivity present in the liver. The spleen concentrated ^{14}C at about twice the liver rate so that at 18 h the ^{14}C level was 19 times higher than in blood. At 18 h, the total radioactivity content of blood and organs examined was less than 3% of the original dose. The level of radioactivity in adipose tissue was always higher than that in blood and increased throughout the experiment. The fat-to-blood proportions increased from two at one hour, to five at two hours, to 33 at 18 h. At 18 h, approximately 56% of the radioactivity still remained in the body: 3% in adipose tissue, about 2% in the blood and organs, 17% in the carcass, and 34% in the gastrointestinal tract.

Metabolism. PETN undergoes a stepwise de-esterification or denitration to PE-tri-N, PEDN, PEMN, and lastly, the complete denitrification to PE (Davidson et al., 1970; DiCarlo et al., 1967a, 1965a, Gilbert et al., 1982). In addition to these metabolites, Crew et al. (1975) found glucuronides of PE-tri-N, PEDN, and PEMN in blood taken from the hearts of rats dosed with ^{14}C-PETN at 10 mg/kg. Crew et al. (1971) discussed the formation of these three glucuronides in rat bile. Litchfield (1971) proposed that the metabolism of PETN and subsequent PE nitrate esters to PE and HNO_2 proceeds by an enzyme-catalyzed reaction with reduced glutathione, which serves as an H donor for the reductive process.

DiCarlo et al. (1965b) reported that PETN is significantly denitrated in human plasma and to a greater extent in whole blood. The enzyme responsible for denitration appears to be concentrated in the erythrocytes. From in vitro studies of rat and human blood, King and Fung (1985) also concluded that enzymes in erythrocytes are important for PETN metabolism. The subcellular fractions of the heart and liver have also been shown to be important in the metabolism of PETN (DiCarlo et al., 1967b).

Due to pharmacologic similarities between PETN and inorganic nitrates such as $NaNO_3$, it was long thought that the vasodilatory mechanism of PETN involved the release of nitrate or nitrite ion. The hypotensive effect, however, is not temporally correlated with the level of inorganic nitrite in the blood (Cass et al., 1962). The vasodilative potency of PETN and other organic nitrate esters is several orders of magnitude greater than that of the inorganic nitrites and nitrates. This increased potency is thought to be related to PETN's ability to more readily cross a cell membrane and to release nitrite within cells.

Ignarro et al. (1981) proposed that PETN exerts its vasodilatory effect after it is denitrated in the presence of cysteine or other compounds with a thiol group to form nitrite that is subsequently reduced to NO. Excess thiol groups combine with NO to form S-nitrosothiols that activate guanylate cyclase, elevate tissue cyclic GMP levels, relax coronary arteries, and decrease systemic arterial pressure.

Excretion. Almost all of a 20-mg dose of ^{14}C-PETN orally administered to humans was recovered from urine and feces in three days (Davidson et al., 1970). The almost complete recovery of radioactivity suggests that the metabolism of PETN to CO_2 is negligible. Crew et al. (1966) reported that 0.28% of ^{14}C-PETN administered to rats was eliminated as $^{14}CO_2$. Urinary excretion rates in humans are similar to those in mice and rats. Davidson et al. (1970) reported that volunteers eliminated 53% of the original radiolabeled dose of PETN in 24 h, and 60% in 48 h. PEMN (51%) and PE (48%) were the primary urinary metabolites. Urinary excretion after a 40-mg oral dose of ^{14}C-PETN to humans amounted to 34% of the initial radioactivity in 24 h (DiCarlo et al., 1966). In a separate study, Davidson et al, (1971) orally administered 20 and 40 mg of ^{14}C-PETN to volunteers and recovered 60 and 50% of the radioactivity in the urine.

Mice orally dosed with ^{14}C-PETN at 10 mg/kg eliminated 36% of the radiolabel in urine after 24 h, 45% in 48 h, and 58% in 72 h (DiCarlo et al., 1965a). In a similar experiment, rats eliminated 28% of ^{14}C in the urine in 18 h after an initial dose of 10 mg/kg of ^{14}C-PETN (DiCarlo et al., 1967a). The average amount of urinary excretion from several pharmacokinetic studies with rats (King and Fung, 1986, 1984; Crew et al., 1966) was about 25% in 24 h. After 72 h rats do not excrete significantly more radioactivity (King and Fung, 1984). In contrast to the metabolic constituents in human urine, PE

is the primary metabolite found in both mouse urine (100% in 24 h) (DiCarlo et al., 1965c) and rat urine (73% at 18 h) (DiCarlo et al., 1967a).

Fecal excretion of ^{14}C in humans following a 20-mg oral dose of radiolabled PETN accounted for 10% of the radioactivity in 18 h and 31.5% in 72 h (Davidson et al., 1970). After a dose of 40 mg of ^{14}C-PETN, 41.2% of the radioactivity was eliminated in the feces in 72 h. Analyses of rat feces showed that rats orally dosed with ^{14}C-PETN at 10 mg/kg eliminated 10% of the radioactivity in 18 h (DiCarlo et al., 1967a). Rats intravenously dosed with 2 mg/kg, eliminated 33% of the radioactivity in 24 h (King and Fung, 1986). The presence of ^{14}C in the feces after intravenous administration indicates that the metabolites of PETN undergo significant enterohepatic recirculation (King and Fung, 1986).

Toxic Effects on Organs and Systems

Dermal effects. An angina patient who had taken 0.6 mg/kg-d continuously for 8 years developed an extensive red rash over most of his body, marked by desquamation of his palms, soles, and perineum, and edema of his arms and legs. Improvement followed after the medication was stopped. The rash recurred when tablets were taken again. Medication with PETN was then stopped altogether and the rash subsided (Ryan, 19[illegible]). Using six New Zealand white rabbits, Ellis and Helton (1975) determined by the Draize procedure that desensitized PETN (includes aluminum and sodium hydroxide) produced no eye irritation and a score of 0.00 on the skin irritation test.

Cardiovascular effects. The desired effect of the medicinal use of PETN is the decreased frequency of angina attacks through vasodilation and the subsequent decrease in systolic and diastolic blood pressure. The decrease in blood pressure is normally accompanied by an increase in heart rate and a decrease in coronary vascular and total peripheral resistance. Daily doses can range from 0.4 to 2.3 mg/kg and are divided into three or four doses (Kastrup et al., 1983). The vasodilatory effect lasts several hours. Occasional adverse effects at therapeutic levels include: rash, headache, nausea, vomiting, weakness, restlessness, and dizziness (Physician's Desk Reference, 1986). Cass et al. (1962) reported that four volunteers developed headaches, but no other symptoms, after a single oral dose of 60 mg

(0.86 mg/kg) of PETN. "No untoward reactions" were observed at the lower levels of 0.29 and 0.57 mg/kg. Two individuals receiving an oral dose of 64 mg (approximately 1 mg/kg) did not complain of any "untoward effects" such as flushed face, headache, or oppression in the chest and no change in blood pressure or heart rate was reported (von Oettingen, 1944).

The effects of PETN to the cardiovascular system have also been investigated in several studies with laboratory animals. Dogs that received PETN at 5 mg/kg by stomach tube experienced a peak decrease in mean arterial blood pressure (MABP) of 29% in 1.5 to 2 hours (von Oettingen, 1944). The MABP gradually returned to normal but was still 16% below normal at 3 hours. Spinal pressure rose to a peak of 35% coincident with the decrease in blood pressure.

Aviado et al. (1969) observed vasodilatory effects, manifested as decreased pulmonary resistance, decreased blood pressure, and increased heart rate in dogs that had received single intravenous doses of PETN at either 100 or 50 µg/kg. Similar effects were not observed at a lower dose of 20 µg/kg. Decreases of MABP by 59, 38, and 17 mm Hg were also observed in dogs intravenously dosed with PETN at 1220, 465, and 100 µg/kg, respectively (Wastila et al., 1976). Wastila et al. (1976) reported decreases in MABP of 30, 27, 18, and 12 mm Hg in rats intravenously dosed with PETN at 500, 250, 100, and 50 µg/kg, respectively. Intravenous doses of PETN caused reduced stress-induced tachycardia at 40 µg/kg (Piris et al., 1978) and a slight decrease in myocardial blood flow at 50 µg/kg (Ashikawa, 1974) in anesthetized dogs.

Respiratory effects. Moderate increases in respiratory rate and volume were observed in dogs dosed with PETN at 5 mg/kg. After an hour, these effects returned to normal (von Oettingen, 1944).

Other effects. Von Oettingen (1944) reported that no pathological changes in lungs, livers, kidneys, spleens, brains, or vascular walls were detected following a one-year chronic feeding study where rats received 2 mg/kg in their food each day. However, most of the kidneys of treated rats appeared to have a greenish fluorescence in contrast to the normal coloration of the control rat kidneys.

Teratogenicity

No information is available.

Genotoxicity

The mutagenicity of PETN was examined by Whong et al. (1980) in an investigation that included other explosives (e.g. tetryl, RDX, HMX). The ability of PETN to induce reversions in five strains (TA1535, TA1537, TA1538, TA100, TA98) of Salmonella typhimurium was examined. Negative results were obtained in the spot test at concentrations of 0.625 and 1.25 mg/spot. In the plate incorporation assay, with Aroclor 1254-induced rat microsomal activation, PETN exhibited no mutagenic effect at concentrations as high as 2.5 mg/plate. The negative data were not provided.

Carcinogenicity

There is no evidence in the literature for the carcinogenicity of PETN. An NTP carcinogenicity bioassay has been completed and is now under review by the Pathology Working Group (Bucher, 1987). Male F344 rats and both sexes of B6C3F1 mice were exposed to a concentration of 2.5 wt% PETN in food or a concentration of 5.0 wt%. Female rats were exposed to a concentration of 0.62 wt% or a concentration of 1.25 wt%. Preliminary results are largely negative. However, there are some questions regarding the significance of Zymbal's gland tumors in the rats (Bucher, 1987).

Carter and Goldman (1976) proposed that highly carcinogenic nitrosamines could potentially be formed in the gastrointestinal tract following the ingestion of PETN. In an attempt to address whether PETN could be a source of nitrite and therefore nitrosamine formation, Boring et al. (1983) found no evidence for the prerequisite nitrite contamination or generation in the gut.

Estimation of Intake Limits to Protect Public Health

Even though PETN has been used therapeutically for many years in the treatment of angina pectoris, little is known about the potential effects of long-term administration of this drug. At therapeutic doses (i.e., 10 to 20 mg three to four times per day) there are infrequent, reversible effects

such as headache, dizziness, nausea, and even allergic skin reactions. Cass et al. (1962), for example, gave 60-mg doses (oral) of Peritrate (PETN) to four subjects, all of whom developed headaches. Doses of 40 mg, however, did not cause any "untoward reactions". Ryan (1972) reported a case of erythroderma in an individual who had taken 40 mg daily (about 0.570 mg/kg-d, assuming a 70-kg body weight) of Peritrate for eight years for treatment of angina. During that time the individual also took occasional tablets of nitroglycerin. It is not clear, though, whether the onset of the skin disorder was directly caused by the cumulative use of PETN over the eight years.

Infants represent a population subgroup that would be particularly sensitive to PETN exposures due to the potential for methemoglobin formation caused by the release of nitrite in blood following PETN uptake. The U.S. EPA (1985a) recommends a level of 1 mg/L as a limit for nitrite-nitrogen in drinking water to protect against methemoglobin formation in all age groups. The equivalent intake of PETN is approximately 7 mg/L (calculated by multiplying 1 mg/L by the ratio of a g-mole of PETN to the g-mole of NO_2 in PETN, or 316.15/46). The equivalent daily intake for an adult consuming 2 L of water per day and weighing 70 kg is 0.2 mg/kg-d. This is nearly a factor of three lower than the dose rate that may have led to the severe skin disorder in the subject receiving PETN to treat angina. A dose rate of 0.2 mg/kg-d could still cause some vasodilation; however, it is unlikely to cause any demonstrable harm and may even be beneficial. Other estimates of safe intakes can be made for comparative purposes. For example, if the daily dose of 2 mg/kg administered to rats over a year, referred to earlier (von Oettingen, 1944), actually defined a chronic NOEL, then a safe intake could be calculated as 0.02 mg/kg-d, based on an uncertainty factor of 100. Ellis and Helton (1975) indicated that the lethal dose of PETN to rats is above 5000 mg/kg, which when multiplied by an application factor of 1 x 10-5 d^{-1} (see Appendix D), gives an intake rate of 0.05 mg/kg-d.

TOXIC EFFECTS ON AQUATIC SPECIES

A literature review completed by Burrows and Dacre (1975) did not reveal any studies dealing with the aquatic toxicity of PETN. Bentley et al. (19 conducted aquatic toxicity tests using desensitized PETN. Unfortunately, they

reported LC50 values that were greater than the solubility of PETN. The elevated values could have been an artifact of both the desensitizing action procedure, which was to boil the PETN in a water solution containing sodium hydroxide and atomized aluminum, and the subsequent neutralization with sulfuric acid that was required to produce a pH of 7 in the test solutions.

12. DATA-BASE ASSESSMENT FOR TETRYL

Our review of the available literature on tetryl, a booster explosive, indicates that there are major gaps in our knowledge of its environmental chemistry and toxicology. Although some aspects of its potential behavior in the environment have been studied recently (i.e., hydrolysis and photolysis), no work has addressed biotransformation processes. Moreover, there is little information on dose-response relationships for different toxic endpoints in humans and laboratory animals -- and thus our ability to derive an acceptable daily intake to protect human health is limited.

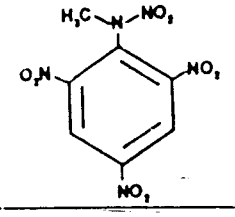
ENVIRONMENTAL PARTITIONING AND TRANSPORT PROPERTIES

Table 12-1 presents the structural formula and other identifiers for tetryl; chemical and physical properties are provided in Table 12-2; and partition coefficients are presented in Table 12-3.

Information on the transport of tetryl in soils is limited primarily to the work of Hale et al. (1979), who studied the migration of tetryl through soil columns. Kayser and Burlinson (1982), in follow-on work, analyzed soil and water samples from the experiments of Hale et al. to obtain additional information on the environmental chemistry of tetryl. After 20 weeks, ^{14}C-tetryl was not detected in the effluent of 5 cm-diameter, 61-cm-long soil columns irrigated at 5 cm per week (Kayser and Burlinson, 1982; Hale et al., 1979). However, examination of the effluent collected periodically showed ^{14}C-labeled compounds as decomposition products. The product found in the largest amount was picric acid (a hydrolysis by-product of tetryl) and the remainder was judged to be composed of polar compounds that were water soluble and nonvolatile (Kayser and Burlinson, 1982).

Hale et al. (1979) also measured the partitioning of tetryl between water and each of the four soils used in the column experiments. The K_d values for the soils (measured by a shake-flask technique) ranged from 7.6 to 35.3 (see Table 12-4). To estimate the K_{OC} values for the soils, we divided the K_d values by the average organic-carbon (OC) content, calculated from data for different column intervals. The average OC values ranged from 0.56 wt% for the Bennington soil to 2.2 wt% for the Genesee soil. The K_{OC} values ranged from 1357 to 2948 (see Table 12-4). Our calculated K_{OC} value, in contrast,

Table 12-1. Chemical identifiers of tetryl.

Empirical formula:	$C_7H_5N_5O_8$	Structural formula:
Molecular weight:	287.15	
CAS reg. no.:	[479-45-8]	
RTECS no.:	BY6300000	

was 271 (Table 12-3). One explanation for the higher measured K_{OC} values is that the methylnitroamino group of tetryl may be binding or complexing with humic materials. Evidence supporting this binding phenomenom comes from Bongiovanni et al. (1984) who found that tetryl was more difficult to extract at low concentrations in soil (i.e., 5 ppm) than other munitions. With one exception, Hale et al. (1979) measured K_ds that were an order of magnitude greater if the soil concentration was derived from the difference between the water concentration before and after partitioning, instead of the soil concentration measured by direct extraction of the soil. In other work, Lakings and Gan (1981) could not extract tetryl from plant tissue matrices, whereas they could extract other munitions with the same methods. They concluded that tetryl was irreversibly adsorbed to protein or other macromolecules. We suspect that the methylnitroamino group reacts with macromolecules in the same way that the amine microbial degradation products of TNT do (see Section 3).

The equilibrium distribution of tetryl, as shown in Table 12-5, ought to be directly affected by its low Henry's law constant (among the lowest of the explosives at 1.5×10^{-6} L-torr/mol) and a moderately high K_{OC} value (i.e., greater than 271). More tetryl (three to ten percent) than the other explosives and co-contaminants examined should reside in the upper soil zone.

ENVIRONMENTAL TRANSFORMATION PROCESSES

The environmental fate of tetryl parallels that of TNT because of the structural similarity of the two compounds. For example, both have comparable photolysis rates (a few days). Tetryl, however, has a functional group, the methylnitroamino group, that is subject to hydrolysis. Also, because of this methylnitroamino group, we suspect that tetryl binds to naturally occurring

Table 12-2. Chemical and physical properties of tetryl. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	Explodes at 180 to 190	Windholz *et al.*, 1983
Freezing/melting point	°C	130 to 132	Windholz *et al.*, 1983
Density	g/mL	1.73	Lindner, 1980
Vapor pressure	torr	4×10^{-10}	Calculated[a] from a vapor pressure-temperature equation
Aqueous solubility	mol/L mg/L	2.6×10^{-4} 75	Taylor and Rinkenback, 1923
Henry's law constant	(L-torr)/mol	2×10^{-6}	Calculated as the ratio of vapor pressure to aqueous solubility.
Diffusion coefficients: Air Water	m^2/s m^2/s	5.3×10^{-6} 5.1×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition coefficient (K_{ow})	log K_{ow}	2	Estimated[a]

[a] See Appendix A.

Table 12-3. Partition coefficients for use in estimating human exposures to tetryl.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	1	Calculated [a]from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	3.7×10^{-3}	Average of values calculated[b] from water solubility and log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	15	Calculated[b] from log K_{ow}
SOC/water	K_{oc}	(mg/kg SOC/(mg/kg water)	270	Estimated[b] from solubility in water and melting point.

[a] Wet-weights for plant and fish

[b] See Appendix A

Table 12-4. Partitioning of tetryl between water and soils (data from Hale et al., 1979)

Soil type	K_d	Organic carbon, wt%	K_{oc}
Brookston	34.3	1.8	1906
Genesee	35.3	2.2	1604
Princeton	11.5	0.39	2948
Bennington	7.6	0.56	1357

macromolecules, lipids, and proteins; whereas only the microbial degradation products of TNT (i.e., 2-A-4,6-DNT and 4-A-2,6-DNT), but not TNT itself, bind or form complexes with these compounds. No information was found on the microbial degradation of tetryl.

Photolysis

Photolysis appears to be a major environmental transformation process for tetryl. Kayser et al. (1984), in studying the hydrolysis of tetryl, found that it disappeared with laboratory room light at a rate that was at least an order of magnitude greater than the hydrolysis loss rate in the dark. The experimental conditions were distilled water and "ambient" temperature. They found that 95% of the tetryl disappeared after 20 d; from these figures we calculate a half-life of 4.5 d. Sunlight photolysis is expected to be faster because of the increased intensity of the light.

Hydrolysis

Hydrolysis is a major environmental fate of tetryl in the absence of light. Kayser et al. (1984) measured the hydrolysis of tetryl in distilled water at a pH of 6.8 and a temperature of 40°C. The calculated half-life was 63 d. They estimated the half-life at 20°C to be about 300 d. Several of their preliminary measurements gave similar half-lives (see Table 12-6).

Table 12-5. Fractional distributions of tetryl among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of tetryl to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	1.1×10^{-12}	1.3×10^{-12}
Air particles	7.1×10^{-9}	1.6×10^{-8}
Biota	6.9×10^{-5}	0.0068
Upper soil	0.030	0.10
Lower soil	0.43	0.099
Ground water	0.54	0.78
Surface water	0.0019	0.0068
Sediments	1.3×10^{-4}	4.7×10^{-4}

[a] Fractions do not add to one because of rounding.

The hydrolysis reaction is second order in hydroxide ion concentration (Kayser et al., 1984), and therefore, a pH change from 7 to 8 should increase the rate by an order of magnitude. The hydrolytic degradation rates determined by Kayser et al. (1984) agree with the measurements of Hoffsommer and Rosen (1973) for tetryl in seawater (pH ≈ 8). Hoffsommer and Rosen found that 88% of the tetryl had hydrolyzed after 101 d. The associated half-life for hydrolysis at pH 8 is approximately 33 d, which is an order of magnitude shorter than that at pH 7 (see Table 12-6).

Biotransformation

No information was found on the microbial degradation of tetryl. However, it is reasonable to assume that microbial action will reduce the nitro groups to amines.

Table 12-6. Hydrolysis half-lives of tetryl.

Concentration (mg/L)	pH	Experimental conditions	Half-life (d)
12	6	Distilled water in the dark at an "ambient temperature" 3.2% of the tetryl was lost in 20 d[a]	430
15	6	Distilled water in the dark at an "ambient temperature." 5.1% of the tetryl was lost in 20 d[a]	260
1 to 20	6.8	Distilled water at 40°C[a]	63
26	8	Seawater at 25°C., 88% of the tetryl disappeared in 101 d[b]	33

[a] Kayser et al., 1984.
[b] Hoffsommer and Rosen, 1973.

TOXIC EFFECTS ON ANIMALS AND HUMANS

Much of the toxicity data available on tetryl is based on occupational exposures associated with its production in ordnance factories during the 1930's and 1940's. Exposures to tetryl dust particles occurred most frequently among workers who were either blending tetryl with graphite in the loading plants, inserting the tetryl into booster bags, or packing it for shipment (Schwartz, 1944). The information derived from the occupational exposures is qualitative in nature. Few animal studies have been completed in the last 20 years, and most of the recent work on this explosive has focused on its genotoxicity.

Toxicokinetics

There are no data available that deal directly with the toxicokinetics of tetryl.

Toxic Effects on Organs and Systems

The primary health effects of tetryl are dermal and respiratory irritation, as demonstrated in various occupational-exposure studies. Generally, though, such studies lacked controlled conditions or did not identify other potential contaminants. Reviews by Bergman (1952) and McConnell et al. (1946b) of the effects of occupational exposures to tetryl indicate that systemic responses were generally not observed. However, Hardy and Maloof (1950) presented limited evidence showing that tetryl workers did experience systemic effects in some instances.

Dermal effects. Tetryl has caused allergic dermatitis among munition plant workers, and Foulger (1953) has noted that contaminated clothing can transmit tetryl dust among workers who subsequently experience dermatitis. A study by McConnell et al. (1946b), which focused specifically on worker health in government-owned ordnance plants during World War II, showed that the number of cases of mild dermatitis attributed to tetryl exposure decreased overall by 81% over a period of approximately two years. The incidence of dermatitis cases decreased due to increased experience in the handling of tetryl, technologic advances, and an increased emphasis on industrial hygiene programs.

Bergman (1952) summarized ten years of experience in dealing with the toxic effects of tetryl among workers at the Picatinny Arsenal. Clinical signs of occupational exposures to tetryl were bright yellow skin and the discoloration of blond hair. According to Bergman, skin yellowing was not due to massive absorption, but rather a skin surface reaction. Initially, the skin became irritated, with erythema, itching, and dermatitis; then in the later stages severe edema occurred with exfoliation of the skin. Most workers become "hardened" one to four weeks after the development of dermatitis and therefore were no longer irritated by tetryl (Schwartz, 1944). The most frequent sites of dermatitis are around the mouth and eyes, and on the cheeks and neck. Dermatitis can also appear on other parts of the body touched by soiled hands (e.g., the arms and genitals).

In a more recent article, Goh (1984) reported a case study of a woman working in an ammunition factory packing detonators with three explosives: TNT, RDX, and tetryl. After four months of work she noticed a yellowish

discoloration of her palms, fingers, and nails, and additionally developed swollen fingers, lips, and hands. A patch test was conducted to determine her sensitivity to these three compounds. After several alterations to the skin test (i.e., a change in vehicles), it was found that she was allergic to tetryl. Subsequently, she was transferred to another section of the factory, where her skin reaction diminished.

Gell (1944) reported on the sensitization of guinea pigs to tetryl. Five different exposure routes were used; intradermal injection, application to scalded skin, subcutaneous implantation of capsules, skin inunction (to assist absorption of fat-soluble substances) in a lanolin base (1% v/v), and smoke inhalation (10% v/v, 30 min, six treatments). Some animals exhibited mild sensitization following the intradermal injection, and others showed a moderate response to the subcutaneous implantation of capsules. A marked skin reaction to inhaled tetryl dust occurred in only one out of eight animals. Six of eight animals in the inhalation group experienced anaphylactic sensitivity. Tetryl applied to a burn or to skin by inunction did not cause sensitization.

Respiratory effects. The inhalation of tetryl dust commonly causes burning, itching, sneezing, and coryza of the nose, which frequently leads to a nosebleed. These symptoms may begin on the first day of exposure or as late as the third month. After cessation of exposure, bronchial complaints continue for 6 to 10 d however, reexposure can cause the recurrence of the symptoms. Hardy and Maloof (1950) described three case reports of workers in a "powder house" where tetryl concentrations in air ranged from 0.83 to 17.7 mg/m^3. One worker who operated a bagging machine in the powder house for five years suffered severe spasmodic coughing and wheezing. Another worker with undefined duties, but only employed for one year, had the same symptoms as the first employee, plus irritation of the nose and throat, vomiting spells, and weight loss. The third case reported involved a man exposed solely to tetryl for four years; he died after suffering from severe asthmatic coughing and common skin irritations. Bergman (1952) also mentioned the potential for lung disease due to tetryl dust exposure.

Gastrointestinal effects. Hardy and Maloof (1950) reported dyspepsia and epigastric pain with nausea and vomiting in tetryl workers. Bergman (1952) found that workers experienced nausea, vomiting, cramps, and diarrhea, but only on an empty stomach.

Central nervous system effects. Irritability, fatigue, general malaise, headache, lassitude, and sleeplessness are symptoms that have been attributed to tetryl exposures (see Bergman, 1952).

Hematological effects. One symptom observed among workers who were overexposed to tetryl is epistaxis (i.e., nosebleed) (Bergman, 1952). To understand this response, Daniele (1964) studied the blood clotting changes (thromboelastograph, prothrombin time, blood prothrombin, labile factor, stable factor, and platelet count) in rabbits administered tetryl. Twelve rabbits and three controls were given tetryl orally at a daily rate of 0.125 g/kg-d for 6 months. Every month the rabbits had their blood drawn and the blood clotting factors examined. Daniele concluded that although some of the factors involved in the blood clotting processes (proaccelerin and platelets) did not change significantly, there was clear evidence of decreased blood clotting capacity.

Cardiovascular effects. Some cases of asymptomatic hypotension in tetryl workers have been observed, but no details of these cases were provided in an article by Bergman (1952).

Hepatic effects. Hardy and Maloof (1950) and Fati and Daniele (1965) briefly cited a study by Wells et al. (1920), in which rabbits and dogs were administered unspecified lethal doses of tetryl. Only the dogs showed prominent changes in the liver, with necrosis of liver lobules and severe fatty degeneration of the bile duct epithelium. In rats a 2 g/kg dose (duration was not mentioned) caused the same effects plus polymorphic nuclei, and frequent mobilization and proliferation of the Kupffer cells (Parmeggiani et al., 1956; cited in Fati and Daniele 1965).

Fati and Daniele (1965) found consistent liver effects in rabbits given tetryl orally. Two groups of male rabbits were administered daily oral doses of 0.125 g/kg body weight for 6 or 9 months, respectively. The first group had 8 rabbits who suffered from swollen livers whose cells had poorly distinguishable boundaries and slightly granulous opaque cytoplasm. The four rabbits in the second group, in addition to the toxicity observed in the first group, had liver necrosis, polymorphic nuclei, and signs of hyperchromatism and pyknosis. The Kupffer cells showed signs of hyperplasia.

Renal effects. A brief review by Fati and Daniele (1965) of the renal effects observed in laboratory animals administered tetryl showed that kidney damage can include tubular swelling and congestion as well as degenerated cells.

Teratogenicity

There are no data currently available on the teratogenicity of tetryl.

Genotoxicity

The mutagenic activity of tetryl has been studied in several microbial test systems. Positive results have been obtained in *S. typhimurium* at tetryl levels ranging from 1 to 33.3 µg/plate in the plate incorporation assay and 2.5 to 30.0 µg/mL in the suspension test (McGregor *et al.*, 1980; Whong *et al.*, 1980). A reduced level of mutagenicity was found in the presence of Aroclor-induced rat microsomal activation. No positive control or activation-requiring positive control was reported by either investigator.

Using the yeast *Saccharomyces cerevisiae* as the indicator organism, both McGregor *et al.* (1980) and Whong *et al.* (1980) found that tetryl induced mitotic recombination. The levels examined were 5.0 to 400 µg/mL in the suspension assay and 5 to 30 µg/plate in the plate incorporation assay. McGregor *et al.* (1980) obtained a negative response in the suspension assay when rat S9 was used.

Whong *et al.* (1980) also found a mutagenic response with tetryl in the fungus *Neurospora crassa*. In both the plate incorporation assay and the suspension test, a concentration-related increase in ad-3+ reversions was obtained. No increases in reversion frequency were found when a frameshift mutant strain of *N. crassa* was employed. Whong *et al.* (1980) suggest that tetryl induces base-pair substitutions. However, McGregor *et al.* (1980) found increases in reversion frequency in both base-pair substitution and frameshift strains of *S. typhimurium*. In the *E. coli* DNA repair assay, McGregor *et al.* (1980) tested tetryl at five doses, ranging from 0.1 to 10 mg/plate. No differential growth was observed in plates containing the strain proficient in DNA repair compared to plates containing the repair-deficient strain.

Carcinogenicity

Griswold et al. (1968) studied the carcinogenicity of tetryl along with 34 other compounds in female Sprague-Dawley rats by gavaging 40-d-old rats with a 40-mg dose every three days over a 30-d period. There were 20 rats in the tetryl treatment group and at the end of the nine-month observation period three rats had died. No details were given on the possible causes of death. The total dose of 400 mg per rat (equivalent to approximately 67 mg/kg-d distributed over the 30-d period, assuming that the rats weighed 0.2 kg) resulted in one rat with a mammary lesion and a stomach adenoma; by comparison, five out of 132 rats in the controls developed mammary lesions: 3-carcinomas, 1-fibroadenoma, and 5-hyperplasias. No statistical comparisons were presented. The primary deficiencies of this study are its short (one month) exposure period and the lack of alternative treatment levels. Consequently, no firm conclusions can be drawn regarding tetryl's carcinogenic potential.

Estimation of Intake Limits to Protect Public Health

None of the toxicity studies reviewed provided dose-response data that clearly defined a no-observed-effect-level. The estimated daily dose of 67 mg/kg-d used in the study by Griswold et al. (1968) was the lowest dose rate reported and the associated effects attributable to that exposure are unclear. At 45 d after the initial 30-d exposure period one rat had died, but whether this was related to tetryl toxicity is unknown. Fati and Daniele (1965) orally administered 125 mg/kg daily to male rabbits for six to nine months and observed liver toxicity. According to EPA protocol (U.S. EPA, 1985b) for using an uncertainty factor (UF) with animal data, a UF of 1000 is used to extrapolate the results of subchronic studies to chronic exposures. For the dose rates noted above, the use of a UF of 1000 gives daily intakes ranging from 0.067 to 0.125 mg/kg-d. However, these dose rates are based on lowest-observed effect levels, and so safe intakes derived on an actual NOEL may be lower. To bound our estimates of a safe intake, we multiplied the maximum tolerated dose (MTD) (the highest single dose that does not cause mortality) from Griswold et al. (1968), which was 2000 mg/kg (400 mg per 0.2-kg rat), by the application factor of 1×10^{-5} d^{-1} (see Appendix D) to obtain a lower-bound estimate of a safe intake rate of 0.02 mg/kg-d. This

value is about a factor of three lower than the 0.067 mg/kg-d value noted above, and so it is reasonable to use the 0.02 mg/kg-d as a provisional intake rate for tetryl.

TOXIC EFFECTS TO AQUATIC SPECIES

Burrows and Dacre (1975) completed a literature review on the aquatic toxicity of tetryl and did not identify any relevant citations. We were unable to locate any recent articles dealing with tetryl's aquatic toxicity.

13. DATA-BASE ASSESSMENT FOR AMMONIUM PICRATE AND PICRIC ACID (PiOH)

Ammonium picrate (Explosive D) was used primarily during the second World War and has subsequently been replaced by other explosives (Kaye, 1978). It now represents less than ten percent of the explosives in the demilitarization inventory. Very little has been published on the environmental chemistry and toxicology of this explosive, apparently because it has not been produced for many years. Ammonium picrate, which is quite soluble in water, hydrolyzes to PiOH; consequently this degradation product is the co-contaminant of greatest importance. Fortunately, there is considerably more information available on PiOH, and for convenience we have covered both compounds in Section 13.

ENVIRONMENTAL PARTITIONING AND TRANSPORT PROPERTIES

Tables 13-1 and 13-2 contain information related to the indentification of ammonium picrate and PiOH. Tables 13-3 and 13-4 present data on the chemical and physical properties of the two compounds. The relatively high solubilities in water (>10,000 mg/L) of ammonium picrate and PiOH distinguish these compounds from the other explosives and co-contaminants. Because of their high solubility, PiOH and ammonium picrate should have little affinity for the organic content of soils. However, the interaction of picrates with soils requires consideration of more than organic-carbon content. The picrate ion has a high affinity for metal cations and will form precipitates; thus it is retained in soils. Picrates also have unusual interactions with plant and animal systems. They bind to proteins, staining them yellow; and they inhibit photosynthetic electron transport (Oettmeier and Masson, 1982).

Soil Transport and Adsorption

Information on the environmental transport of ammonium picrate is limited to inferences drawn from studies that did not specifically examine the factors affecting its behavior in soils and ground waters. Ruchhoft and Norris (1946), for example, found that activated carbon and unspecified soils had limited abilities to remove ammonium picrate dissolved in water. This is consistent with the relatively low value of K_{OC} (i.e., 0.19) predicted from its solubility in water and melting point (see Table 13-5). However, Van Denburgh *et al*. (1980) investigated ground water contamination resulting

Table 13-1. Chemical identifiers of ammonium picrate.

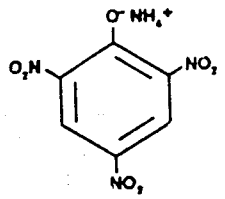
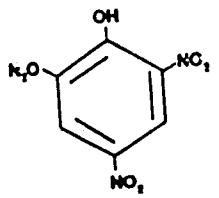
Empirical formula:	$C_6H_6N_4O_7$	Structural formula:
Molecular weight:	246.14	
CAS reg. no.:	[131-74-8]	
RTECS no.:	—	

Table 13-2. Chemical identifiers of PiOH.

Empirical formula:	$C_6H_3N_3O_7$	Structural formula:
Molecular weight:	229.11	
CAS reg. no.:	[88-89-1]	
RTECS no.:	TJ7875000	

from the leaching of ammonium picrate, TNT, and other substances from disposal beds at an ammunition plant, and discovered that TNT had migrated further in ground water than ammonium picrate (measured as PiOH). One explanation for the greater mobility of TNT -- despite its considerably higher K_{OC} value -- is that the adsorption of the ammonium picrate/PiHO is controlled primarily by the clay content of the aquifer material rather than the organic content, and consequently the movement of ammonium picrate/PiOH was retarded relative to TNT. Evidence supporting this comes from Chang and Anderson (1968), who showed that PiOH was an effective flocculater of clays.

Nose (1983) calculated a Freundlich adsorption coefficient of 3.84 for PiOH using a "volcanic ash upland soil" that had a pH of 5.6, a carbon content of 4.8 wt%, and a CEC of 22 meq/100 g. The estimated K_{OC} value for this soil type is 80 (i.e., 3.84/0.048), which is almost a factor of 20 higher than the K_{OC} estimate of 4.1 calculated from PiOH's melting point and water solubility (see Table 13-6).

Table 13-3. Chemical and physical properties of ammonium picrate. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	Not applicable	
Freezing/melting point	°C	Decomposes w/o melting at >265	Kaye, 1978
Density	g/mL	1.72	Kaye, 1978
Vapor pressure	torr	No data	
Aqueous solubility	mol/L mg/L	4.1×10^{-2} 10,000	Kaye, 1978
Henry's law constant[a]	(L-torr)/mol		
Diffusion coefficients: Air Water	m^2/s m^2/s	5.7×10^{-6} 5.7×10^{-10}	Estimated[b] Estimated[b]
Log octanol/water partition coefficient (K_{ow})	log K_{ow}	--[b]	

[a] Cannot be calculated without an estimated vapor pressure.

[b] See Appendix A.

Table 13-4. Chemical and physical properties of PiOH. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	>300 (explodes)	Verschueren, 1983
Freezing/melting point	°C	121.8	Verschueren, 1983
Density	g/mL	1.76	Kaye, 1978
Vapor pressure	torr	2×10^{-8}	Extrapolated from temperature-pressure data given in Meyer, 1981.
Aqueous solubility	mol/L mg/L	4.8×10^{-2} 11,000	Freier, 1976
Henry's law constant	(L-torr)/mol	4×10^{-7}	Calculated as the ratio of vapor pressure to aqueous solubility.
Diffusion coefficients: Air Water	m^2/s m^2/s	6.1×10^{-6} 6.0×10^{-10}	Estimated[b] Estimated[b]
Log octanol/water partition coefficient (K_{OW})	log K_{OW}	2.03	Anderson (no date), as cited by Hansch and Leo, 1979

[a] See Appendix A.

Table 13-5. Partition coefficients for use in estimating human exposures to ammonium picrate.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	2	Calculated[b] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	--	
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	--	
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	0.2	Calculated[b] from solubility in water and melting point.

[a] Wet-weights for plant and fish

[b] See Appendix A

Table 13-6. Partition coefficients for use in estimating human exposures to PiOH.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	2	Calculated[b] from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	1.1×10^{-3}	Average of values calculated[b] from water solubility and log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	16	Calculated[b] from log K_{ow}
			1	Measured value for skin of rainbow trout, Cooper et al., 1984
SOC/water	K_{oc}	(mg/kg SOC)/(mg/kg water)	4.4	Calculated[b] from melting point and water solubility.
Soil/water	K_d	(mg/kg soil)/(mg/kg water)	3.84	Freudlich adsorption coefficent determined by Nose (1983)

[a] Wet-weights for plant and fish

[b] See Appendix A

Partition Coefficients: Plant/Soil, Beef-Fat/Diet, and Fish/Water

All of the partition coefficients for ammonium picrate shown in Table 13-5 were estimated from regression equations. For PiOH, data were available on its bioconcentration in rainbow trout (Table 13-6). Cooper et al. (1984) exposed rainbow trout to water containing 0.45 mg/L of ^{14}C-PiOH for 42 d. The bioconcentration factor varied from approximately one for the skin of the fish to under one for muscle, liver, gills, and intestines. They attributed the preferential uptake in skin to the ability of PiOH to bind with proteins (PiOH stains proteins yellow). Burton et al. (1984) exposed American oysters to water containing 0.45 or 0.05 mg/L of ^{14}C-PiOH for 42 d. The uptake of the PiOH followed a biphasic pattern, but equilibrium conditions were not attained at either exposure. The accumulation factors (underestimates of the true uptake or bioconcentration under equilibrium conditions) ranged from 65.5 (0.45 mg/L exposure) to 16.6 (0.05 mg/L exposure).

Equilibrium Distribution in Reference Landscapes

Without an estimate of the Henry's law constant for ammonium picrate, we were unable to estimate its equilibrium distribution among the reference landscapes. However, PiOH, which has similar chemical properties, distributes primarily to ground water, as shown in Table 13-7.

ENVIRONMENTAL TRANSFORMATION PROCESSES

In this subsection we examine environmental transformation data on ammonium picrate and PiOH. The main transformation processes for PiOH are photolysis and dissociation. Dissociation to picrate ion is rapid for PiOH. At low PiOH concentrations, the degree of dissociation depends on the pH and buffering capacity of the aqueous system; because PiOH is a relatively strong acid (pKa = 0.29, Oettemeier and Masson, 1982) dissociation in dilute solutions is essentially complete.

Table 13-7. Fractional distributions of PiOH among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of PiOH to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	4.7×10^{-13}	8.6×10^{-13}
Air particles	2.3×10^{-10}	7.8×10^{-10}
Biota	2.4×10^{-5}	0.0030
Upper soil	0.0051	0.024
Lower soil	0.22	0.10
Ground water	0.77	0.85
Surface water	0.0037	0.020
Sediments	1.0×10^{-5}	5.6×10^{-5}

[a] Fractions do not add to one because of rounding.

Photolysis

Ammonium picrate is stable in sunlight according to work done by Ruchhoft and Norris (1946). Unfortunately, additional details on their experiments were not reported.

Biotransformation

Neither ammonium picrate nor PiOH is transformed readily by microbial action. Ruchhoft and Norris (1946) found that in 1% sewage sludge, there was no degradation of ammonium picrate at any concentration from 0 to 1000 ppm during a 5-day incubation period. Concentrations of ammonium picrate above 100 ppm reduced the BOD of the medium. Using fresh sewage, activated sludge, and 300 ppm ammonium picrate, they observed (1) an immediate loss of 100 ppm ammonium picrate, but no further loss during aeration; (2) loss of BOD after

2 h aeration; and (3) no change in suspended solids. When they added ammonium picrate to strong sewage, they observed a "reduction." After 17 d they recovered only 33.6% of the ammonium picrate. No mention of products was given, but hydrolysis to PiOH and reduction of a nitro group to form picramic acid are the likely by-products. Osmon and Klausmeier (1973) found that microorganisms growing in a complex medium saturated with ammonium picrate were unable to degrade that explosive. It is conceivable, though, that the ammonium picrate served as a biocide at its solubility limit.

Pitter et al. (1974) found that PiOH resisted biodegradation by "adapted activated sludge" in a comparison study with other nitrophenols. The lack of biodegradation can be attributed to the toxicity of PiOH to microbes. Mondal and Mukherjee (1975) studied antibacterial activity of several chemicals including PiOH on two plant pathogens, Xanthomonas oryzae and X. citri. PiOH was one of the best growth inhibitors. Vaicum and Eminovici (1974) treated "laboratory scale activated sludge units" with PiOH as a representative industrial toxicant. Ten days after injection of 50 ppm PiOH, four of the five sludge characteristics had returned to normal. COD, protein content, glucide content and dehydrogenase activity recovered but catalase activity was an order of magnitude below the initial value. When 200 ppm PiOH was added, no stable recovery was noted for any criteria.

On the other hand, several authors report PiOH being readily biodegraded. Mogilevich et al. (1972) reported that two bacteria, Pseudomonas aurantiaca and P. fluorescens, reduced the para and then the ortho NO_2-groups of PiOH to amines in water. Also, McCormick et al. (1976) determined the rate of reduction of PiOH in a hydrogen atmosphere to be 1/2 the rate of TNT with Veillonella alkalescens in a phosphate buffer solution.

Wyman et al. (1979) studied the biotransformation of PiOH using a specially adapted bacterium, Pseudomonas aeruginosa, isolated from TNT waste ponds. The PiOH was added to a basal salts medium containing 0.1% yeast extract as a nutrient. Anaerobic conditions were maintained by continuously bubbling argon gas through the fermentation solution. After 30 d, 22% of the PiOH had disappeared (the associated half-life is 84 d). They reported that two percent of the product was picramic acid, but did not isolate other products. However, they observed no biotransformation of PiOH with organisms from activated sludge, compost, garden soil, or estuarine sediments. Their conclusion was that ammonium picrate and PiOH will persist in the environment.

Hydrolysis

Ammonium picrate dissolved in water at a neutral pH will quickly dissociate to the picrate ion because of its pKa (~0.29; Oettmeier and Masson, 1982). Also, a rapid metabolic "hydrolysis" occurs in biological systems. Weeks et al. (1983) measured the picrate ion concentration in the blood and urine of rodents following dermal and intraperitoneal administration of PiOH and ammonium picrate. After dermal administration of PiOH to rabbits, picrate ion concentration was greatest 6 h later in the blood and 24 h later in the urine.

TOXIC EFFECTS ON ANIMALS AND HUMANS

There is very little information on the biological effects of ammonium picrate. At physiologic pH, ammonium picrate dissociates to PiOH and the PiOH subsequently dissociates to form the picrate ion, which exists in equilibrium with its protonated form, PiOH. Previous reviews of the toxicity of these substances include those of Berkowitz et al. (1978) and the National Research Council (NRC) (1982).

Toxicokinetics

Absorption. No experimental studies have evaluated the rate or extent of absorption of ammonium picrate in humans. However, reports of toxicity experienced by munitions workers suggest that ammonium picrate can be absorbed through the skin and/or lungs (Schwartz, 1944). The data of Sunderman et al. (1945) imply that ammonium picrate can be absorbed through the lungs of rabbits or guinea pigs (see section on Toxic Effects to Organs and Systems). PiOH is absorbed through the skin of humans when applied topically (Dennie et al., 1929; Schwartz, 1944; Baer, 1976). Absorption of PiOH can also occur across the gastrointestinal tract following ingestion in drinking water (Harris et al., 1946).

Von Oettingen (1949) cited the work of Ilzhofer (1918) as evidence that PiOH is not absorbed through the skin. However, Dennie et al. (1929) applied a 5% solution of PiOH to the intact skin of a dog, and within 24 h the blood tested positive for PiOH. PiOH was also measured in the blood and urine of

dogs after subcutaneous injection of either 0.05 g/kg or 0.075 g/kg (Dennie et al., 1929).

Distribution and bioaccumulation. Sunderman et al. (1945) noted staining of the skin and nasal cavity, and discoloration of the hair in individuals occupationally exposed to ammonium picrate dust. Experimental exposure of rabbits and guinea pigs to ammonium picrate dust under similar occupational conditions (i.e., 8 h/d exposure) revealed granular deposits (presumably of picrate) in the blood, liver, kidneys, lungs, heart, thyroid, and adrenal gland.

Yellow staining of the nails and skin has been observed after PiOH was applied in ointment (staining was noted at sites separate from the area of application) (Dennie et al., 1929). Malmejac (1917) was cited by Dennie et al. (1929) as having measured PiOH in the blood and spinal fluid of humans exposed to PiOH. Occasional reports of central nervous system (CNS), hepatic, and renal toxicity after PiOH exposure imply that it is widely distributed (Dennie et al., 1929; Harris et al., 1946; von Oettingen, 1949).

Autopsies of dogs that were exposed to PiOH by a single subcutaneous injection (0.05, 0.075, or 0.10 g/kg) found PiOH in the blood, urine, subcutaneous fat, intestine, liver and kidney (Dennie et al., 1929). PiOH's effects on the CNS, liver, and kidney suggest that its distribution is extensive (von Oettingen, 1949; Huidobro, 1971).

Metabolism and elimination. At physiologic pH, ammonium picrate exists as picrate ion in equilibrium with a trace of PiOH. Aromatic nitro compounds such as PiOH typically undergo metabolic reduction to amines (Parris, 1980). There is some evidence to suggest that in vivo one of the ortho nitro groups of PiOH is reduced to the corresponding amine, forming picramic acid (see Barral, 1915; Guerbet and Mayer, 1932, cited in von Oettingen, 1949).

Weeks et al. (1983) gave rabbits a single intraperitoneal (i.p.) dose (60 mg/L) or a single dermal application (600 mg/kg) of ammonium picrate. Blood and urine were then analyzed for the presence of picrate. After dermal exposure, picrate ion was still detectable in the blood and urine seven days after exposure. Following i.p. administration, picrate was measured in blood samples taken between six and 24 h, but was not present after this time. Picrate was not detectable in urine after 48 h.

Von Oettingen (1949) cited early reports by Karplus (1883) and Walko (1901), that provided qualitative information on the metabolic fate of PiOH in humans. Exposure to PiOH was followed by excretion of unchanged PiOH, picrate, and picramic acid in urine. Walko (1901) reported that dogs and rabbits given subcutaneous or intravenous injections of PiOH eliminated an unspecified portion of the dose as picramic acid. Elimination of picramic acid in the urine of humans exposed to PiOH was also measured by Barral (1915). Harris et al. (1946) identified unchanged PiOH in the urine of individuals who had ingested PiOH in drinking water for four weeks. This suggests that PiOH is slowly eliminated from the body.

Twenty four hours after a single dermal application of PiOH (600 mg/kg), rabbits had measurable quantities of picrate ion in the urine. Picrate was still present in the urine after 7 d. Following a single i.p. dose of PiOH, picrate was detectable in blood and urine for 24 and 72 h, respectively (Weeks et al., 1983). Dennie et al. (1929) injected a dog subcutaneously with 0.05 g/kg of PiOH and recovered unmetabolized PiOH in the urine.

Toxic Effects on Organs and Systems

Hepatic effects. A six-week exposure to ammonium picrate dust (approximately 0.0088 to 0.1942 mg/m^3, 8 h per day) produced glycogen infiltration and periductal fibrosis in the liver of rabbits. Rabbits exposed to ammonium picrate dust for a year developed granular deposits in the liver and one animal had "moderate" cirrhosis. Guinea pigs exposed under identical conditions for nine or 12 months had chronic congestion of the liver (Sunderman et al., 1945).

Harris et al. (1946) observed a variety of toxic symptoms in navy personnel who had ingested unknown amounts of PiOH in drinking water (exposure occurred over a period of several weeks). Of 20 individuals whose blood was analyzed clinically, one had a "slight but definite elevation of bilirubin". Although Harris et al. (1946) did not discuss the implications of this finding, elevated blood bilirubin is characteristic of jaundice. Von Oettingen (1949) also noted that ingestion of relatively large amounts of PiOH (1 teaspoon and 5 g) caused jaundice. Furthermore, inhalation exposure to PiOH has been associated with urobilinogen and urobilin in the urine (specific data were not given) (von Oettingen, 1949). Increased levels of

these substances in urine can be due to impaired liver function or to increased urobilinogen production following hemolytic disease (Krupp et al., 1982). It has been suggested that PiOH may cause destruction of RBC; therefore, it is not clear whether liver function is impaired, or if increased lysis of RBC leads to an increase in heme breakdown products (von Oettingen, 1949). Dogs given a single subcutaneous injection of 0.05, 0.075, or 0.10 g/kg of PiOH developed cloudy swelling of the liver (Dennie et al., 1929).

Renal effects. Rabbits and guinea pigs exposed to ammonium picrate dust 8 h per day for nine to 12 months (approximately 0.0088 to 0.1942 mg/m^3) developed various pathological abnormalities of the kidneys (Sunderman et al., 1945). The kidneys of rabbits displayed cloudy swelling and brown granular deposits; guinea pigs had globular deposits in the kidney tubules, and one animal had several "minute" hemorrhages. Microscopic hematuria (red blood cells in the urine) was observed in 138 out of 383 individuals who had consumed water contaminated with PiOH (Harris et al., 1946). Although three individuals developed hematuria within 24 h of exposure, the duration of exposure for the remaining 135 was not discussed. Harris et al. (1946) noted that the widespread hematuria was only circumstantially related to urinary tract involvement. Of all of the instances of hematuria, only three men showed clinical evidence of kidney impairment. One had gross hematuria, while the others developed hematuria, albuminuria, and generalized edema. Von Oettingen (1949) cited Rutledge (1936) who reported two cases of nephritis (accompanied by albuminuria, hematuria, and edema) after a solution of PiOH was used in nasal spray. Dennie et al. (1929) observed glomerulitis, cloudy swelling, and gelatinous degeneration of the kidney in dogs injected subcutaneously with single doses of PiOH (0.05, 0.075, or 0.10 g/kg). Non-specific kidney damage was also reported in dogs given repeated doses of 1.8 mg/kg of PiOH (route not specified) (Koizumi, 1915, in von Oettingen, 1949).

Ocular effects. Direct contact of PiOH with the eye has reportedly produced corneal ulcers in humans, however, neither the concentration of PiOH or the exposure period were given (Kittsteiner (1902), cited in von Oettingen, 1949). Inhalation of PiOH dust caused severe irritation of the

mucous membranes of the eyes and respiratory tract of cats (Ilzhofer 1918, cited in von Oettingen, 1949).

Dermal effects. Exposure to ammonium picrate and PiOH has caused dermatitis in both humans and animals. There is evidence that this is due to involvement of the immune system (allergic sensitization) rather than to any inherent primary irritant capacity of these compounds. However, because it is difficult to distinguish between the phenomenon of immunologic sensitization and the physical manifestation of this response (dermatitis), we have not attempted to separate the two.

Occupational exposure to ammonium picrate dust at 0.0088 to 0.1947 mg/m^3 produced dermatitis among workers; the length of exposure ranged from two to 24 months (Sunderman et al., 1945). Although dermatitis was correlated with exposed areas of skin (hands, face, forearm), there was not a clearly defined relationship between exposure concentration and development of dermatitis (i.e., there were 7 reports of dermatitis among 71 individuals exposed to ammonium picrate; however, no cases were reported among those individuals exposed to the greatest concentrations). In an attempt to determine whether ammonium picrate was a primary irritant or if it induced a delayed-type hypersensitivity reaction, Sunderman et al. (1945) conducted a patch test of immunologic hypersensitivity on 23 volunteers (12 of these individuals had not been previously exposed to ammonium picrate). No primary reactions were observed in any of these individuals, which suggests that ammonium picrate is not a primary irritant. However, reapplication of the ammonium picrate patch to the same area of skin produced a positive reaction in one person. Additionally, a factory guard who submitted to patch-testing with ammonium picrate developed an allergic reaction that was not restricted to the localized area of application.

Schwartz (1944) also described sensitization dermatitis among munitions workers that handled ammonium picrate (specific exposure conditions were not given). This dermatitis was characterized by edema, papules, vesicles, and desquamation. Ammonium picrate was not irritating to rabbits when applied topically as a dry powder. However, ten intradermal injections of 0.1, 1.0, or 10.0 % ammonium picrate (0.1 mL each) produced "mild" sensitization in rabbits (Weeks et al., 1983).

PiOH exposure produces dermatitis that is similar to that caused by ammonium picrate. PiOH-induced dermatitis is characterized by papules,

vesicles, excoriations, and edema (Dennie et al., 1929; von Oettingen, 1949; Schwartz, 1944; Baer, 1976). There are many anecdotal case reports that have described severe dermatitis following local application of ointments containing PiOH. Dermatitis developed on the face, hands, feet, anal, and scrotal regions. In most instances, the dermatitis remained localized, but in some individuals the reaction became systemic (Dennie et al., 1929; von Oettingen, 1949; Schwartz, 1944; Baer, 1976). In several cases, a generalized exfoliating dermatitis developed (with universal edema) that required hospitalization. Although ultimately reversible, recovery often took six weeks or longer (Dennie et al., 1929; Sulzberger and Wise, 1933).

Sulzberger and Wise (1933) described sensitization dermatitis (delayed-type hypersensitivity) that developed after application of butesin picrate. However, Baer (1976) noted that both butesin and PiOH are allergens, and it is difficult to distinguish individual effects of each drug.

PiOH is a potent allergen that causes delayed-type hypersensitivity reactions in animals. Maguire and Chase (1972) and Chase and Maguire (1973) have characterized the sensitization response to PiOH by guinea pigs. The reaction is distinguished by vesiculation of the epidermis, edema, and exocytosis. The initial response to PiOH took two to three days to develop; after this period of time, animals became increasingly sensitive. After three or more days of repeated applications, 0.06 to 0.006% solutions induced dermatitis. Weeks et al. (1983) reported that dry PiOH was a 'slight' irritant when 0.5 g was applied to intact or abraded skin of rabbits.

Central nervous system effects. An early report (Dennie et al., 1929) discusses what appears to be CNS involvement after dermal application of PiOH to an individual. The individual developed slowed speech, difficulty in walking, and unequal dilation of the pupils approximately six weeks after a severe allergic response to PiOH.

Pretreatment of mice with PiOH enhanced the analgesic effect of morphine, and prolonged the sleeping time induced by phenobarbital (Huidobro, 1971). A single oral dose of 0.5 g of PiOH caused increasing paralysis in cats that ultimately resulted in respiratory failure (Ilzhofer, 1918; von Oettingen, 1949).

Teratogenicity

There is no information available on the teratogenicity of ammonium picrate or PiOH.

Genotoxicity

Microbial assays. Ammonium picrate was tested by Litton Bionetics (1979a) for its ability to induce reverse mutations in *Salmonella typhimurium* (Ames test). In a standard plate incorporation assay, ammonium picrate was added at 0.50, 1.0, 10.0, 100.0, 500.0, or 1000.0 mg per plate. Ammonium picrate was not mutagenic to test strains TA1535, TA1537, TA1538, TA98, or TA100, either with or without metabolic activation. Although solvent and positive controls were used, it appears that replicate tests were not conducted.

Litton Bionetics (1979a) also measured the frequency of forward mutations in *Saccharomyces cerevisiae* induced by ammonium picrate. The concentrations and test protocol were essentially as noted above for *S. typhimurium*; however, different incubation times and temperatures were used for the two organisms. Ammonium picrate was not mutagenic at any of the concentrations used, either with or without exogenous metabolic activation.

Chiu *et al*. (1978) tested PiOH along with 52 aromatic, heterocyclic, and aliphatic nitro compounds for mutagenicity in *S. typhimurium* strains TA98 and TA100. A standard plate incorporation assay was conducted without the addition of S9. At concentrations ranging from 0.1 to 10.0 μM per plate, PiOH did not induce a significant increase in the reversion frequency of either strain. In contrast to the results of Chiu *et al*. (1978), Gocke *et al*. (1981) found that PiOH induced a positive, concentration-dependent response in *S. typhimurium* strains TA1538 and TA98. These results were obtained at concentrations ranging from 1.0 to 10.0 mg per plate and were independent of metabolic activation.

The ability of PiOH and picramic acid to induce mutations in four strains of *S. typhimurium* was examined by Wyman *et al*. (1979). PiOH was not mutagenic to strains TA98 or TA100 in the absence of S9. However, addition of S9 produced a positive response in both strains. The data for these experiments were not presented. Picramic acid at 1.0 to 200.0 mg per plate caused a

dose-related increase in the number of revertants in strains TA98, TA1538, and TA1535. Strain TA100 also gave a dose-dependent increase in revertants, but only at doses up to 100 mg per plate. These responses were obtained without the addition of metabolic activation. The effect of S9 on the picramic acid-induced mutation rate was not reported.

Yoshikawa et al. (1976) reported that PiOH and picramic acid were mutagenic to S. typhimurium, strain TA98. PiOH and picramic acid were tested at 15, 50, or 150 mg per plate, either with or without S9. Both compounds gave an apparent dose response when S9 was present (the number of revertants increased with concentration). However, the authors' interpretation of a positive mutagenic response was not supported by statistical analysis, nor by any control data.

In an abstract, Won (1977) stated that PiOH was not mutagenic to S. typhimurium (strains TA98 and TA100) in the absence of metabolic activation. When S9 was added, PiOH induced revertants in both strains. Picramic acid was mutagenic in strains TA98, TA100, TA1537, and TA1538, but was negative in TA1535. It is not clear if the results with picramic acid were obtained with or without S9. Furthermore, no information was provided on the concentrations, specific protocol used, or actual revertant counts.

PiOH induced reverse mutations in a streptomycin-dependent strain of Escherichia coli, B/Sd-4 (Demerec et al., 1951). Bacteria were incubated in 0.010 to 0.018% PiOH for three hours, prior to plating on streptomycin-free agar. Treatment of E. coli with 0.010% PiOH did not affect the number of mutants. At levels of 0.017 to 0.018% of PiOH there was a six to ten-fold increase in the number of reverse mutations. However, these concentrations greatly reduced the survival of colonies: depending on the experiment, survival varied from 1.9 to 34%.

Chromosomal aberrations. Ammonium picrate without metabolic activation induced a statistically significant ($p < 0.01$) increase in the frequency of sister chromatid exchanges (SCE) in mouse lymphoma cells, compared to solvent controls (see Litton Bionetics, 1979a). These increases were observed at all concentrations (15.6, 31.3, 62.5, 125.0, and 250 µg/mL). The highest concentration of ammonium picrate (250 µg/mL) induced an increase in SCE that was statistically significant ($p < 0.01$) compared to both solvent and negative controls. When exogenous activation was added to lymphoma cells, the number

of SCE produced in solvent controls was greater than that seen without metabolic activation. However, the frequency of SCE in exposed cells was not significantly different from controls. Metabolic activation apparently increased the toxicity of ammonium picrate; this necessitated the use of much lower concentrations of ammonium picrate in the assays that included S9. The greatest concentration used was 31.3 µg/mL (range of 1.0, 2.0, 3.9, 7.8, 15.6, or 31.3 µg/mL). Amounts greater than 31.3 µg/mL apparently inhibited replication, as cells at these concentrations did not enter second metaphase (Litton Bionetics, 1979a). In contrast to ammonium picrate, picramic acid did not induce a statistically significant increase in SCE, either with or without metabolic activation. Picramic acid (0.625, 1.25, 2.50, 5.0, 10.0, or 20.0 µg/mL) was added to mouse lymphoma cells, and the number of SCE per chromosome, as well as the number of SCE per cell were tabulated. When these results were compared against those obtained with a solvent control, no significant difference in the frequency of SCE was observed (Litton Bionetics, 1979b).

Oral exposure of HA/ICR mice to ammonium picrate did not cause overt chromosomal breakage in bone marrow cells. Animals received either a single dose, or five separate doses of ammonium picrate (separated by 24 h). Cells were harvested at six, 24, or 48 h after treatment. At the three dose levels studied (2.23, 7.43 or 22.3 mg/kg), ammonium picrate did not induce a statistically significant increase in chromosomal aberrations (Litton Bionetics, 1979a). The clastogenic activity of picramic acid was also analyzed by Litton Bionetics (1979c). The dosing protocol was the same as that used to evaluate ammonium picrate, except that mice received doses of 7.6, 25.2, or 75.6 mg/kg. The number of chromosomal aberrations in cells from treated animals was not significantly different from controls (Litton Bionetics, 1979c).

A study by Litton Bionetics (1979a) examined the activity of ammonium picrate in the mouse dominant lethal assay, which evaluates the ability of a compound to induce genetic damage in spermatozoa of treated male mice. The endpoints used to assess damage included a fertility index, the average number of implants per female, and the number of resorptions per female. Ammonium picrate given by gavage to male mice (2.23, 7.43 or 22.3 mg/kg) did not cause statistically significant increases in any of the indices. In a similar assay

with picramic acid administered at doses of 7.56, 25.2, or 75.6 mg/kg to mice, no dominant lethal mutations were observed (Litton Bionetics, 1979d).

PiOH did not induce micronuclei in mouse-bone marrow erythrocytes (Gocke et al., 1981). Negative results were obtained in mice treated orally with 1.0, 1.5, or 2.0 mmol/kg, or with 0.1, 0.3, or 0.4 mmol/kg intraperitoneally.

Gene Mutations in Drosophila. PiOH was tested with 52 additional coded compounds for mutagenicity in Drosophila melanogaster under contract to the National Toxicology Program. The chemicals were tested in three laboratories (University of Wisconsin, Brown University, and Bowling Green University) by the sex-linked recessive lethal mutation and the reciprocal translocation tests. The results on the mutagenic activity of the compounds assayed were presented, along with the data, by Woodruff et al. (1985). PiOH did not induce sex-linked recessive lethal mutations when administered by feeding. Compounds with negative results in feeding studies were re-examined by injection.

Negative results were obtained at the University of Wisconsin following injection of PiOH at a concentration of 400 ppm. Tests conducted at Brown University had equivocal results at 1000 and 1500 ppm PiOH. Clear, positive results were obtained following an injection of 1500 ppm PiOH at Bowling Green State University. Examination of the injection study data from all three laboratories showed that the percentage of recessive-lethal mutations increased with increasing concentration of PiOH. Comparison of the total treated population to the total control population revealed the percentage of lethals in the treated group was significantly higher ($p = 0.02$) than controls. PiOH did not induce reciprocal translocations in Drosophila following 1500 ppm injection (Woodruff et al., 1985).

Gocke et al. (1981) examined the ability of PiOH to induce sex-linked recessive mutations in D. melanogaster. Males received a single dose of 1.25 mM PiOH dissolved in 5% saccharose. The percentage of sex-linked recessive lethals was significantly higher ($p \leq 0.05$) in the treated population (0.53%) compared to controls (0.27%).

Gene Mutation Assays in Mammalian Cells. The mouse lymphoma forward mutation assay uses L5178Y/Tk cells to detect gene mutations that affect the thymidine kinase locus. Cells incubated with 500.0, 1000.0, 2000.0, 2500.0, or 3000.0 µg/mL of ammonium picrate (without S9) did not induce a

statistically significant increase in mutations. Although different concentrations of ammonium picrate were used to evaluate mutagenicity with S9 (31.0, 125.0, 300.0, 400.0, 500.0, or 1000.0 µg/mL), metabolic activation did not increase the mutagenicity of ammonium picrate (Litton Bionetics, 1979a).

Summary. None of the investigations reviewed here found ammonium picrate to be mutagenic. However, the clearly positive response obtained in SCE experiments indicate that ammonium picrate can cause chromosomal damage. PiOH induced mutations in four separate studies with *S. typhimurium*. However, it is not clear whether or not metabolic activation is required. When injected into *D. melanogaster*, PiOH gave positive results in the recessive lethal mutation assay, but was negative for reciprocal translocations. In contrast, conflicting results were reported when PiOH was fed to *D. melanogaster*. PiOH did not cause chromosomal damage in the mouse micronucleus assay. The ability of picramic acid to induce mutation or chromosomal damage has not been adequately characterized. Available information indicates that it causes reverse mutations in *Salmonella*. The role of metabolic activation in this response has not been defined.

Carcinogenicity

Van Esch *et al*. (1957) conducted a very limited study of the carcinogenicity of PiOH. In this experiment, Wistar rats received 500 ppm PiOH in their diet daily for 2 1/2 years. At the end of this time, autopsies were conducted on all animals. Although specific data were not given, treated males reportedly did not develop a greater incidence of tumors than controls. The only tumor data reported for female rats was the incidence and time to appearance of mammary gland tumors. The incidence of these tumors was greater in controls (67%) than in treated animals (57%). The average time to appearance of mammary gland tumors was 22 months in treated animals compared to 25 months in untreated controls. The data reported in this study were extremely sketchy, and interpretation is limited by a number of factors. They used a very small sample size (10 male and 10 female rats), and administered PiOH at only one dose level. Very little specific pathology data were given, and it is not possible to determine the sensitivity of techniques used to detect tumors. Finally, virtually nothing is known about the gastrointestinal

absorption or subsequent metabolism of PiOH, and it is impossible to know what proportion of the administered dose actually entered the bloodstream. In summary, this study is inadequate to evaluate the carcinogenicity of PiOH.

Estimation of Intake Limits to Protect Public Health

The limited toxicity data on ammonium picrate and PiOH provide few studies from which a safe and acceptable daily intake can be calculated. None of the studies that are available adequately define a no-observed-adverse-effect-level (NOAEL) for either compound. Consequently, we have derived alternative intake estimates based on the existing studies and have compared them with a lower-bound estimate of an acceptable daily intake computed from a lethal dose, using the methology described in Appendix D. The two studies used to define minimum-effect dose rates were those of Sunderman et al. (1945) and Van Esch et al. (1957).

Sunderman et al. (1945) evaluated the effects of ammonium picrate on rabbits and guinea pigs as part of a larger study that examined the health effects on workers occupationally exposed to ammonium picrate. (As noted in the discussion of Toxic Effects on Organs and Systems, ammonium picrate elicited allergic sensitization reactions in some individuals.) Sunderman et al. collected a series of air samples at the site of "milling" and "preforming" operations to estimate the concentration of ammonium picrate present in the atmosphere of the plant. Measured values of ammonium picrate ranged from 0.0088 to 0.1942 mg/m^3 (geometric mean = 0.04 mg/m^3). To replicate human exposure conditions, rabbits and guinea pigs were placed at the sampling locations (i.e., milling and preforming operations) for six or 52 wks. Although the number of animals involved in this study was small (for rabbits, n=4; for guinea pigs, n=8), and no control animals were used, the results of this investigation represent the only data that have documented specific exposure conditions and specific adverse effects of ammmonium picrate on animals. Analagous data for humans do not exist.

Rabbits exposed to ammonium picrate 8 h/d, 5 d/wk for 6 wk exhibited glycogen infiltration and periductal fibrosis of the liver, tracheal congestion, and "acute catarrhal tracheitis". When exposure lasted for 12 months (8 h/d, 5 d/wk), Sunderman et al. documented bronchial degeneration, granular deposits in cardiac muscle and hepatic tissue, "moderate" hepatic

cirrhosis (one animal), and cloudy swelling of the kidneys. A similar spectrum of effects was observed in guinea pigs exposed under identical circumstances. Three of the guinea pigs died before the end of the experiment, but at widely different times. It is not known whether or not exposure to ammonium picrate was a factor in their deaths.

To calculate the daily dose that animals received, we assumed that both species absorbed 100 % of inhaled ammonium picrate. Because ventilation rates for rabbits and guinea pigs were not available to us, these rates were calculated by multiplying the alveolar ventilation rate of a standard, 0.25 kg rat (7.02 L/h) times the ratio of rabbit or guinea pig body weight to rat body weight, raised to the 0.74 power (U.S. EPA, 1988). This approach was taken because physiological parameters such as this are assumed to vary in proportion to body weight to the 0.74 power (Paustenbach et al., 1989). Because Sunderman et al. did not provide the body weights for experimental animals, a representative body weight for each species was obtained from Lehman (1954), as cited in Lu (1988). The specific values were 2.0 kg for a rabbit, and 0.75 kg for a guinea pig.

The daily inhaled dose (DID) of rabbits calculated from the geometric mean concentration of ammonium picrate is:

$$\text{DID} = 0.04\ \frac{\text{mg}}{\text{m}^3} \times \frac{1\text{m}^3}{1000\ \text{L}} \times \frac{32.7\ \text{L}}{1\ \text{h}} \times \frac{8\ \text{h}}{\text{d}} \times \frac{5\ \text{d}}{7\ \text{d}} \times \frac{1}{2.0\ \text{kg}} \qquad (13\text{-}1)$$

$$= 0.0038\ \text{mg/kg-d}\ (3.8\ \mu\text{g/kg-d})$$

The DID of guinea pigs calculated from the geometric mean concentration of ammonium picrate is:

$$\text{DID} = 0.04\ \frac{\text{mg}}{\text{m}^3} \times \frac{1\text{m}^3}{1000\ \text{L}} \times \frac{15.8\ \text{L}}{1\ \text{h}} \times \frac{8\ \text{h}}{\text{d}} \times \frac{5\ \text{d}}{7\ \text{d}} \times \frac{1}{0.75\ \text{kg}} \qquad (13\text{-}2)$$

$$= 0.0048\ \text{mg/kg-d}\ (4.8\ \mu\text{g/kg-d})$$

If it is assumed that the smaller of these two values, the DID of rabbits, represents a chronic LOAEL, a safe intake can be calculated by dividing the LOAEL by an uncertainty factor (UF). The EPA guidelines (U.S.EPA, 1985) for calculation of an acceptable daily intake propose that an UF of 100 be applied if extrapolating from valid, long-term animal studies when human data are not available. Alternatively, the guidelines propose that

an UF of 1000 be applied when no human data are available, and extrapolation is from animal studies of less than chronic exposure. Although Sunderman et al. utilized a long exposure period (52 wk) the small number of experimental animals and complete absence of control animals make it difficult to interpret the relative validity of reported results. Consequently, an UF of 1000 is probably appropriate. If this UF is applied to the calculated inhaled dose, an intake of 0.0038 μg/kg-d is obtained.

There are no lifetime studies of the effects of ammonium picrate in animals or humans. However, Van Esch et al. (1957) administered PiOH to rats over a 2-1/2 year period. Animals received 500 ppm daily in their diet. As discussed earlier, Van Esch et al. (1957) reported that treated males did not have a greater incidence of tumors than control animals. Female rats had a somewhat lower incidence of mammary gland tumors than untreated controls. The authors concluded that PiOH was not carcinogenic and that it did not cause adverse effects in animals at the dose used in this study. However, this study was extremely limited in its scope, in that the only biological endpoint evaluated was the incidence of tumors. It is not at all apparent if other adverse effects occurred, nor is it evident that they were looked for. Furthermore, it appears that tumors were documented only on the basis of gross examination. Although on the surface this study appears to document a NOAEL of 500 ppm (500 mg/kg), it merely suggests that under the limited conditions of this study, PiOH did not elicit an increase in the incidence of tumors. This study is also flawed by the use of a very small number of animals (10 of each sex). Also, although PiOH was given to animals in the diet, no analysis of food was conducted and there is no assurance that rats actually received the full dose.

Even with all of these limitations, the Van Esch et al. (1957) report remains the only lifetime study of PiOH toxicity, and we used the apparent NOAEL to calculate a safe intake. To derive the safe intake rate, we made assumptions regarding the average weight of rats and the average amount of food consumed. Specifically, we assumed that rats weighed 200 g and that they consumed 15 g of food per day (Mehlman et al., 1976). Therefore, the estimated daily intake (DI) of PiOH is

$$DI = 500 \frac{mg}{kg} \times \frac{0.015\ kg}{d} \times \frac{1}{0.2\ kg} = 37.5\ mg/kg\text{-}d \qquad (13\text{-}3)$$

As noted in the discussion of Sunderman et al. (1945), selection of a specific UF depends on the source and quality of the data (U.S. EPA, 1985b). Although Van Esch et al. (1957) studied rats treated with PiOH over a 2-1/2 year period, there are enough deficiencies in the study to warrant selection of an UF of 1000. This UF is used when "acute or chronic data in all species are limited or incomplete" (U.S. EPA, 1985b). Calculation of an ADI using a UF of 1000 gives a value of 0.0375 mg/kg-d (37.5 µg/kg-d).

A final alternative for calculation of an ADI involves the use of LD50 or LDlow values, where LDlow is defined as the lowest lethal dose reported in the literature. As discussed in Appendix D, LD50 or LDlow values expressed in mg/kg can be used to calculate a lower-bound ADI if adequate toxicological data are not available. Although there are no documented LD50 values for ammonium picrate or PiOH, Lewis and Sweet (1984) reported that the LDlow values for orally administered PiOH were 100 mg/kg (guinea pig) and 120 mg/kg (rabbit). Application of a factor of 1×10^{-5} d^{-1} to the LDlow values gives a lower-bound intake rate of approximately 0.001 mg/kg-d (1.0 µg/kg-d).

TOXIC EFFECTS TO AQUATIC SPECIES

Dawson et al. (1977), in a study of the aquatic toxicity of 47 industrial chemicals, determined the 96-h LC50 of ammonium picrate to bluegill sunfish to be 220 mg/L. In earlier work, Grindley (1946) found that it took an average of 26 h before minnows (Phoxinus phoxinus L.) overturned at a concentration of 200 mg/L of sodium picrate. Goodfellow et al. (1983) studied the acute toxicity of PiOH and picramic acid to rainbow trout (Salmo gairdneri) and American oysters (Crassostrea virginica). The 96-h LC50 for LC50 was 46.2 mg/L. For comparison, Heitmuller et al. (1981) reported a 96-h LC50 of 130 mg/L for sheephead minnows (Cyprinodon variegatus), exposed to PiOH. For bluegills (Lepomis macochirus), Buccafusco et al. (1981) reported a PiOH - 96-h LC50 of 170 mg/L. The work of Goodfellow et al. (1983) also showed that picramic acid was more toxic than PiOH to American oysters, with the associated 144-h LC50 values of 69.8 and 245.9 mg/L, respectively.

14. HEALTH AND ENVIRONMENTAL DATA AVAILABLE ON EXPLOSIVES AND ASSOCIATED CO-CONTAMINANTS

The health and environmental data available on the various explosives and associated co-contaminants of concern were reviewed in the previous sections. In this concluding section, we will identify deficiencies in the data bases for the different substances and make recommendations for research to address the deficiencies. Our individual assessments were organized around four primary topics: Environmental Partitioning and Transport Properties, Environmental Transformation Properties, Toxic Effects on Animals and Humans, Toxic Effects on Aquatic Species, and Toxic Effects on Plants. These topical areas cover subjects that are relevant to assessments of the potential risks of operations involving the disposal or destruction of conventional ordnance. In our reviews of the available data, we observed deficiencies in information common to most of the explosives, as well as deficiencies specific to each substance. We begin the following discussions, therefore, with a review of the general deficiencies in the data bases, with subsequent discussions focusing on individual compounds.

DATA-BASE DEFICIENCIES APPLICABLE TO MOST COMPOUNDS

Of the four topics considered, data on the effects of the explosives on plants were generally lacking. We did not find any information in the literature indicating that plant effects had been observed at sites with residual levels of explosives in soils. Only one study demonstrated phytotoxicity, and that study dealt with the effects of TNT on nutsedge grown in a hydroponic solution. However, unless there is demonstrable evidence that residual explosives in soils have caused phytotoxic effects, we do not recommend studies on plant effects. Consequently, it would be prudent to conduct a survey of OB/OD grounds to ascertain whether such damage has occurred.

A more important limitation of the existing data bases is the absence of data on the uptake of the explosives by plants. Uptake by food-chain crops is important because it represents one possible exposure pathway to soil contaminants. We note, however, that plant uptake data are generally unavailable on other organic compounds as well. Unfortunately, there are no

reliable structure-activity relationships available that can be used to predict uptake for specific crops, and thus there is no alternative method of estimating this factor. For the purposes of the data-base assessment, we derived upper-bound estimates of uptake using an empirical relationship reported by Topp et al. (1986) for barley. Clearly, more research is needed to address the various factors influencing the uptake of organic contaminants such as those addressed in this report (see the discussion in Appendix A for additional details). We recommend that studies be initiated that examine the uptake of radiolabeled explosives in selected crops.

The lack of data on bioconcentration factors for the various compounds in beef and dairy products was another important limitation of the data bases. In lieu of actual data on these factors, we used regression equations developed by Kanaga (1980) that predicted bioconcentration in beef fat as a function of a compound's log K_{ow} or aqueous solubility. These equations, though, only accounted for about 60% of the variance in the measured bioconcentration factors of selected organic compounds in cattle. It would be beneficial to have specific studies conducted on the major explosives (i.e., TNT and RDX) to determine the extent to which they are bioconcentrated in beef fat.

One of the more important parameters affecting the transport of explosives and associated co-contaminants is the soil/water partition coefficient, K_d. Often this parameter is estimated from the soil organic carbon (SOC)/water partition coefficient, K_{oc}, and the organic carbon content of soil. Unfortunately, other factors such as clay content, cation exchange capacity, and pH can also influence the partitioning of an organic substance between water and soil, and these factors need to be explored more fully. Ideally, K_d measurements should be conducted with a broad range of native soils or aquifer materials from OB/OD grounds to determine the variability in K_d values across different sites. Similarly, additional studies are needed on the extent of aerobic and anaerobic biodegradation of explosives in native soils, as well as ground and surface waters associated with OB/OD sites. Many of the studies we examined used nutrients to enhance biodegradation--this practice may be helpful in determining whether biotransformation is possible, but it does not provide transformation-rate data useful for modeling of the transport and transformation of organic substances in soils and waters.

Another general data deficiency for the various explosives and co-contaminants was the lack of studies involving teratogenic effects. For the sake of completeness, teratogenicity studies should be performed on the major explosives. Consideration should also be given to the development of more systematic approaches to the development of pharmacokinetic and toxicity data on the compounds of concern.

DATA-BASE REQUIREMENTS FOR SPECIFIC EXPLOSIVES AND CO-CONTAMINANTS

The individual data bases on the explosives and associated co-contaminants we assessed also varied a great deal in their coverage of the four various health and environmental topics considered. Explosives like TNT and RDX had obviously been the subject of considerable research, reflecting their importance in conventional weapons, while other explosives such as tetryl only had limited amounts of data--probably due to the fact that it has largely been replaced by other explosives. In the subsections below, we review the principal limitations of the data bases on each of the compounds considered, and make appropriate recommendations for dealing with the deficiencies identified.

TNT

The data base for TNT was the most complete, except for the general deficiencies common to all the compounds considered. One concern that was raised in our analysis of the cancer bioassays completed using military-grade TNT was the possibility that an impurity (such as 2,6-DNT) was the source of the carcinogenic responses observed in the bioassays conducted with rats and mice. This topic needs to be investigated further because of the potential implications for defining a safe daily intake of TNT.

2,4-DNT

This nitro aromatic compound has been detected in the soils at many OB/OD sites. However, only one investigator has reported a K_d value for 2,4-DNT (Spanggord *et al.*, 1980b). Therefore, we recommend that additional partitioning studies be carried out using native soils from OB/OD sites.

Also, it would be useful to carry out further studies on the biotransformation of 2,4-DNT in different soils.

2-6-DNT

The weakest components of the data base on 2,6-DNT were related to its partitioning and transport in environmental media and its toxicity to aquatic species. For example, its solubility in water has not been measured, and all of the partition coefficients (i.e., K_{sp}, K_{fd}, K_{fw}, and K_{oc}) were estimated, not measured. Thus, we recommend that the aqueous solubility be experimentally determined along with its log octanol/water partition coefficient, which is useful for estimating other properties (see Appendix A). Similarly, more studies are needed to ascertain whether soil microbes are capable of degrading this compound. Furthermore, additional studies should be considered to define its toxicity to a variety of aquatic species. However, before such aquatic toxicity studies are commissioned, their usefulness for assessing the impacts of 2,6-DNT at OB/OD sites needs to be determined.

DNB

Measurements are needed of the soil/water partition coefficient for DNB in selected soils as well as its bioconcentration factor in aquatic species. Although the toxicity of DNB has been examined in two subchronic studies, a clearly defined NOEL was not defined. Consequently, it would be prudent to institute studies to clearly define a subchronic NOEL.

TNB

The various partition coefficients for TNB have yet to be measured, but transformation studies have been completed that indicate that it can undergo biotransformation. The weakest aspect of the data base is the absence of a suitable toxicity study that can be used to establish an acceptable intake to protect public health. Supporting studies are also needed to describe its pharmacokinetic properties.

2-A-4,6-DNT and 4-A-2,6-DNT

As discussed earlier, the amino dinitrotoluenes are degradation products of TNT in the environment. Little work has been done on either compound, and consequently a number of studies need to be performed, starting with experimental measurements of all key properties (e.g., vapor pressure, solubility in water, and octanol/water partition coefficient). Other studies should address factors affecting their transport and transformation in soil and water. Finally, both pharmacokinetic and toxicity studies are necessary to derive estimates of acceptable intakes.

RDX

RDX, like the other major explosive TNT, has been the subject of considerable research over the years. We did not identify any major gaps in the data base on this compound that need to be addressed at this time.

HMX

HMX is the primary impurity in RDX and is also a high explosive with applications of its own. The data available on the transport- and transformation-related parameters are generally adequate, but there needs to be a lot more research focused on its pharmacokinetic and toxic properties. In particular, subchronic and chronic toxicity studies need to be completed in parallel with pharmacokinetic studies.

PETN

The primary deficiency of the data base on PETN is the absence of any studies dealing with its transformation in environmental media. Of particular interest are studies on biotransformation in soils and water because metabolic studies with animals indicate that it will probably be degraded through a step-wise denitration process. Additionally, experiments are needed to determine the various partitioning factors discussed above. Finally, there have been no acute studies on the aquatic toxicity of PETN, and at a minimum one or two species should be tested to determine a 96-h LC50 value.

Tetryl

The aqueous solubility and vapor pressure of tetryl need to be determined, as does its log octanol/water partition coefficient and its bioconcentration factor for one or more aquatic species. The biotransformation of tetryl has not yet been studied, and we recommend that it be given a high priority. Also, there have been no toxicity studies with this explosive within the last 20 years, and little is known about its pharmacokinetic properties. Similarly, no studies have been completed on its aquatic toxicity. Thus, toxicity and pharmacokinetic studies should also receive a high priority.

Ammonium Picrate and Picric Acid

Despite the fact ammonium picrate accounts for almost 10% of the explosives in the demilitarization inventory, hardly any research has been conducted on this explosive in years. Fortunately, ammonium picrate does dissociate to picric acid, and more is known about the environmental chemistry and toxicology of that compound. Nevertheless, we believe that studies are indeed needed on the pharmacokinetic and toxicologic properties of ammonium picrate and on its behavior in the environment. It is especially important to conduct toxicity studies that can be used to define NOELs for ammonium picrate and picric acid that can be used to define acceptable intakes to protect public health.

APPENDIX A

CHEMICAL/PHYSICAL PROPERTIES AND ENVIRONMENTAL PARTITION COEFFICIENTS: CALCULATIONS AND SUPPORTING DATA

In this Appendix we present estimates of the chemical properties and environmental partition coefficients that are needed to predict the transport of explosives and their co-contaminants in the environment and to predict food-chain transfers to man. The chemical and physical properties considered include vapor pressure, octanol/water partition coefficient, aqueous solubility, Henry's law constant, and molecular-diffusion coefficients in air and in water. Vapor pressure and aqueous solubility are estimated at a reference temperature of 20°C; estimates are based on extrapolations or interpolations of these parameters at various temperatures, as well as on predictions derived from other chemical properties. Partition coefficients for the individual compounds include soil/water (K_d), soil or sediment organic carbon (SOC)/water (K_{oc}), soil/plant (K_{sp}), fish/water (K_{fw}), and beef-fat/diet (K_{fd}). Estimates of these parameters are based on regression equations relating various properties (e.g., water solubility, octanol/water partition coefficient, or molecular weight) to the partition coefficient of interest.

VAPOR PRESSURE

The vapor pressure of a solid or liquid chemical is the pressure of the gas phase that is in equilibrium with the solid or liquid phase at any given temperature. The evaporative escape, or volatilization, of a chemical depends on the vapor pressure of the compound, as well as the environmental conditions that influence diffusion from the solid or liquid surface. Combined with aqueous solubilities at the same temperature, vapor-pressure data permit the calculation of Henry's law constants that can be used along with the diffusion coefficient of a compound in water to determine the the volitization rate of organic chemicals dissolved in water. Organic chemicals with relatively low vapor pressure, high aqueous solubility, or high adsorptivity to solids are less likely to vaporize and become airborne than compounds with high vapor pressure, low solubility in water, or low adsorption to solids and sediments.

Most of the equations and correlations in the literature for estimating vapor pressure are designed for accuracy between the normal boiling point (T_b) and the critical temperature (T_c), a range of primary interest in process engineering. However, environmental studies usually involve temperatures below the boiling point, where the above-mentioned estimation methods are less accurate. Hence, there is a need for reliable vapor-pressure estimates for a wide variety of explosive compounds, preferably at 20°C. There is a particular need for a method of estimating very low vapor pressures (i.e., «1 torr), where it is very difficult to determine accurate pressure values experimentally.

Vapor-Pressure Equations

Many equations have been proposed that relate the pressure and temperature of the gas phase in equilibrium with the condensed phase. Good correlation with the experimental values is obtained with the Antoine equation:

$$\log P_v = A - \frac{B}{t + C} . \qquad \text{(A-1)}$$

A simpler equation is frequently suitable over limited temperature ranges:

$$\log P_v = A - \frac{B}{T} . \qquad \text{(A-2)}$$

In these equations, the P_v term is the vapor pressure of the compound in torr (mm Hg), t is the temperature in °C, T is the absolute temperature in K (Kelvins), and A, B, and C are empirical constants that are unique for each chemical compound (see, for example, Lange's Handbook of Chemistry (Dean, 1985), 13th ed., pp. 1028-1053, for a tabulation of these constants, based on least-square fits of experimental data, and applicable temperature ranges of several hundred organic compounds). Dobratz (1981) has provided a comprehensive table of modified Antoine equations, as well as melting points and boiling points, for chemical explosives. In Table A-1 we provide a summary of P_v-T equations for the explosives and co-contaminants from various literature sources.

Table A-1. Vapor pressure/temperature equations for selected military explosives and co-contaminants.

Explosive	Equation[a]	Temperature range (°C)	Reference
TNT	$\log P_v = 12.31 - 5175/T$	15 to 75	Pella, 1977
2,4-DNT	$\log P_v = 13.08 - 4992/T$	4 to 71	Pella, 1977
2,6-DNT	$\log P_v = 13.99 - 5139/T$	4 to 50	Pella, 1977
1,3,5-TNB	$\log P_v = 5.5345 - 993.6/(t + 11.2)$	202 to 312	Dean, 1985
1,3-DNB	$\log P_v = 4.337 - 229.2/(t - 137)$	252 to 292	Dean, 1985
RDX	$\log P_v = 14.18 - 6798/T$	55.7 to 97.7	Rosen and Dickinson, 1969
HMX	$\log P_v = 16.18 - 9154/T$	97.6 to 129.3	Rosen and Dickinson, 1969
PETN	$\log P_v = 16.73 - 7750/T$	---	Edwards, 1953,
Tetryl	$\log P_v = 13.71 - 6776/T$	85 to 106	Dobratz, 1981

[a] In these equations, P_v represents vapor pressure in torr; T, absolute temperature in K; and t, temperature in °C.

Graphical Methods

In the absence of applicable vapor pressure/temperature equations for some explosives, it is possible to plot measured data from the literature on semilogarithmic paper, with vapor pressure on the logarithmic ordinate, and inverse absolute temperature (T^{-1}) on the abscissa. A best-fit straight line may be drawn through the data points, and the vapor pressure determined at 20°C on the resultant plot, either by interpolation or extrapolation. It should be stressed that extensive extrapolation of the data will result in less reliable pressure values than those obtained by interpolation. Table A-2 summarizes measured vapor-pressure data for the compounds of interest.

A second technique, which is equivalent to graphical extrapolation of the data, involves the insertion of a selected temperature (i.e, 20°C) in an Antoine equation even though the equation is valid for a different temperature range, and calculation of the corresponding vapor pressure. Again, it should

Table A-2. Measured vapor pressures for selected military explosives and co-contaminants.

Explosive	Measured values: Vapor pressure (torr)	Measured values: Temperature (°C)	Reference
TNT	0.27×10^{-6}	12.0	Leggett, 1977
	1.10×10^{-6}	20.0	
	1.72×10^{-6}	21.5	
	3.98×10^{-6}	25.5	
	8.82×10^{-6}	30.0	
	4.24×10^{-5}	40.0	
2,4-DNT	1.23×10^{-5}	4.0	Pella, 1977
	2.85×10^{-5}	10.0	
	1.32×10^{-4}	20.0	
	3.40×10^{-4}	30.0	
	1.28×10^{-3}	40.0	
	5.21×10^{-3}	50.0	
	1.25×10^{-2}	60.0	
	3.97×10^{-2}	71.0	
2,6-DNT	2.56×10^{-5}	4.0	Pella, 1977
	8.02×10^{-5}	10.0	
	3.53×10^{-4}	20.0	
	1.10×10^{-3}	30.0	
	3.62×10^{-3}	40.0	
	1.29×10^{-2}	50.0	
1,3,5-TNB	3.75×10^{-1}	122	Meyer, 1981
	1.50×10^{0}	150	
	1.05×10^{0}	200	
	1.00×10^{2}	270	
1,3-DNB	7.50×10^{-2}	90	Meyer, 1981
	2.63×10^{0}	130	Jordan, 1954
	3.75×10^{0}	150	Meyer, 1981
	4.31×10^{0}	140	Jordan, 1954
	7.03×10^{0}	150	
	1.11×10^{1}	160	
	1.70×10^{1}	170	
	3.75×10^{1}	200	Meyer, 1981
	19.95×10^{1}	250	
	68.55×10^{1}	290	
RDX	1.00×10^{-7}	48	Rosen and Dickinson, 1969
	4.05×10^{-4}	110	Meyer, 1981
	1.05×10^{-3}	121	
	2.55×10^{-3}	131	
	3.98×10^{-3}	138.5	

Table A-2. (Continued).

Explosive	Measured values: Vapor pressure (torr)	Measured values: Temperature (°C)	Reference
HMX	3.3×10^{-9}	99	Rosen and Dickinson, 1969
	1.8×10^{-8}	109	
	4.0×10^{-8}	115	
	3.0×10^{-7}	129	
PETN	8.25×10^{-4}	97	Meyer, 1981
	3.15×10^{-3}	110.6	
	1.13×10^{-2}	121	
	3.75×10^{-2}	131.6	
	7.05×10^{-2}	138.8	
Picric acid	7.5×10^{-3}	122	Meyer, 1981
	2.0×10^{0}	195	
	5.0×10^{1}	255	

be noted that the resultant estimated value of vapor pressure will be less reliable the greater the discrepancy between 20°C and the applicable temperature range.

Vapor Pressure Estimation with Limited Data

Because there are apparently no measured vapor pressure/temperature data or Antoine-type equations in the literature for 2-A-4,6-DNT and 4-A-2,6-DNT, it was necessary to use the following equation developed by Mackay et al. (1982), based on boiling point, melting point, and ambient temperature (i.e., 20°C):

$$\ln P_v = -(4.4 + \ln T_b)[1.803(T_b/T - 1) - 0.803 \ln(T_b/T)] - 6.8(T_m/T - 1) \quad \text{(A-3)}$$

where P_v = vapor pressure (atm), T_b = normal boiling point (K), T = temperature corresponding to estimated pressure (K), and T_m = melting point (K). Furthermore, since no measured boiling-points were available for the two amino compounds, decomposition temperatures measured by Nielsen et al.

(1979) were used to estimate an upper limit for the vapor pressure at 20°C. (The liquid will decompose at or below its boiling point.)

2-A-4,6-DNT. With a measured melting point of 176°C (449 K) (Heilbron, 1982) and a decomposition temperature of 307°C (580 K), the vapor pressure can be calculated from Eq. A-3 as,

$$\ln P_V = -(4.4 + \ln 580)\left[1.803\left(\frac{580}{293} - 1\right) - 0.803 \ln\left(\frac{580}{293}\right)\right] - 6.8\left(\frac{449}{293} - 1\right)$$

$$= -16.72$$

$$P_V = 5.5 \times 10^{-8} \text{ atm} \simeq 4 \times 10^{-5} \text{ torr.}$$

4-A-2,6-DNT. This compound, with a measured melting point of 171°C (444 K) (Heilbron, 1982) and a decomposition temperature of 325°C (598 K) has an estimated P_V of:

$$\ln P_V = -(4.4 + \ln 598)\left[1.803\left(\frac{598}{293} - 1\right) - 0.803 \ln\left(\frac{598}{293}\right)\right] - 6.8\left(\frac{444}{293} - 1\right)$$

$$= -17.57$$

$$P_V = 2.3 \times 10^{-8} \text{ atm} \simeq 2 \times 10^{-5} \text{ torr.}$$

Vapor Pressures for Explosives and Co-contaminants

Although Tables A-1 and A-2 provide vapor pressure-temperature equations and measured vapor pressure data for a number of military explosives over a wide range of temperature, the primary interest in this report is in pressures at 20°C. In Table A-3, P_V values at 20°C are summarized for all explosives (except ammonium picrate) or co-contaminants under consideration, and the source or estimation method used in each case is identified.

ESTIMATION METHODS FOR K_{OW}

Three basic methods of estimating the octanol/water partition coefficient (K_{OW}) are from retention times (RT) on reverse phase HPLC, fragment constants, and from other solvent/water partition coefficients. Logarithms of reverse-

Table A-3. Summary of vapor-pressure values at 20°C for selected military explosives and their co-contaminants.

Explosive or co-contaminant	Vapor pressure[a] (torr)	Source/estimation method
TNT	1.1×10^{-6}	See Table A-2 measured (Leggett, 1977)
2,4-DNT	1.3×10^{-4}	See Table A-2 measured (Pella, 1977)
2,6-DNT	3.5×10^{-4}	See Table A-2 measured (Pella, 1977)
1,3-DNB	2×10^{-4}	See Table A-2 (extrap.[b]) (Meyer, 1981)
1,3,5-TNB	1×10^{-4}	See Table A-2 (extrap.[b]) (Meyer, 1981)
2-A-4,6-DNT	$\simeq 4 \times 10^{-5}$	Calculated (Mackay et al., 1982)
4-A-2,6-DNT	$\simeq 2 \times 10^{-5}$	Calculated (Mackay et al., 1982)
RDX	1×10^{-9}	See Table A-1 (equation) (Rosen and Dickinson, 1969)
HMX	9×10^{-16}	See Table A-1 (equation) (Rosen and Dickinson, 1969)
PETN	2×10^{-9}	See Table A-1 (equation) (Edwards, 1953)
Tetryl	4×10^{-10}	See Table A-1 (equation) (Dobratz, 1981)
Ammonium picrate	--	--
Picric acid	2×10^{-8}	See Table A-2 (extrap.[b]) (Meyer, 1981)

[a] Vapor pressures that are extrapolated or calculated have one significant figure.
[b] Extrapolated graphically.

phase HPLC capacity factors are proportional to log K_{ow}. For the fragment-constant method, only a knowledge of chemical structure is needed. For molecules that are structurally complex, it is desirable to have a measured value of K_{ow} for a compound with a similar structure. Sets of fragment constants have been reported by Hansch and Leo (1979). It also is possible to estimate K_{ow} from linear regression equations that relate the solubility of a compound in different solvents to log K_{ow}.

Fragment-Constant Method

In the fragment-constant method (Hansch and Leo, 1979) for estimation octanol/water partition coefficient, empirically derived atomic or group

fragment constants (f) and structural factors (F) are used. The calculations are made in terms of log K_{OW} = sum of fragments (f) and factors (F). Hansch and Leo (1979) have provided fragment values (f) for more than 100 atoms and atom groups. Each fragment has two f values that depend on whether it is bonded to an aliphatic or aromatic structure. In addition, 14 different factors (F) must be considered, related to molecular flexibility, unsaturation, multiple halogenation, branching, and interactions with H-polar fragments.

For complex molecules (e.g., 2-amino-4,6-dinitrotoluene) it is highly desirable to have a measured log K_{OW} value for a structurally similar compound (e.g., 2,4-dinitrotoluene). This measured value can then be modified by the addition or subtraction of appropriate f or F values:

$$\log K_{OW} \text{ (new chemical)} = \log K_{OW} \text{ (similar chemical)} \pm \text{fragments (f)} \pm \text{factors (F)} . \quad \text{(A-4)}$$

HPLC Method

The log K_{OW} of an organic compound is proportional to the log of its capacity factor on reverse-phase HPLC (Garst and Wilson, 1984). The capacity factor is proportional to the retention time (RT) of the compound minus the RT of the solvent alone. Thus, an increase in retention time on reverse phase HPLC is expected with an increase in log K_{OW}. In Table A-4, we list the retention times of energetic materials along with measured log K_{OW} values. It is evident that RTs generally increase with increasing log K_{OW}, except where values are close. The RT values can therefore be used to estimate the log K_{OW} of the compounds without measured log K_{OW}. For example, tetryl will have a log K_{OW} of approximately 1.5; 2,6-DNT will have a log K_{OW} of slightly less than 1.98; and PETN will have a log K_{OW} of 2 to 2.5. Likewise, we expect the amino-DNT isomers to have log K_{OW} values greater than that of TNT.

Regression Equation Based on Water Solubility

Regression equations are available for estimating K_{OW} from a compound's solubility in water in cases where it is difficult to apply the fragment-constant method because of the lack of a suitable "backbone" chemical,

Table A-4. Measured log K_{ow} and retention time (RT) on reverse phase HPLC for energetic materials. Compounds of unknown log K_{ow} placed by RT order.

Compound	Measured log K_{ow}	Kayser RT[a]	Voyksner RT[b]	Maskarinec RT[c]	Lloyd RT[d]	Burlinson RT[e]	Kaplan RT[f]
Compounds that do not significantly ionize in water							
HMX	0.13[g]	2.0	8.3	10	2		
RDX	0.87[h]	3.4	12.75	13	2.7		
EGDN	1.16[i]				3		
1,3,5-TNG	1.18[j]	5.3				6.8	
1,3-DNB	1.49[k]				3.2		
Tetryl		5.8	17	28	4.3		
TNT	1.60[l]	7.8	17.25	29	5.1	10.6	5.85
TNG	1.62[i]				4.8		
2,6-DNT				35	5.7		
2,4-DNT	1.98[j]	11.6		36	6.0		
4-nitrotoluene	2.37[m]				7.3		
PETN		12.0	19.8	48	8.1		
4-amino-2,6-DNT						14	6.4
1-amino-4,6-DNT						16	6.3
Compounds that ionize in water							
Ammonium picrate			13.25				
PiOH	2.03[n]	0.8			2.3		

[a] Kayser (1983) reported RT.
[b] Voyksner and Yinon (1980), estimated RT from chromatograms.
[c] Maskarinec *et al*. (1984) estimated RT from chromatograms.
[d] Lloyd (1983) estimated RT from chromatograms.
[e] Burlinson (1980) reported RT.
[f] Kaplan and Kaplan (1982b) reported RT.
[g] Atlantic Research (1979), cited by Tucker *et al*. (1985).

Footnotes continued, Table A-4

[h] Banerjee *et al*. (1980).
[i] Cossum and Roberts (1981), cited by Hansch and Leo (1986).
[j] Nikaitani and Hansch (no date), cited by Hansch and Leo (1979).
[k] Verschueren (1983).
[l] Gould and Hansch (1986), cited by Hansch and Leo (1986).
[m] Fujita *et al*. (1964), cited by Hansch and Leo (1979).
[n] Anderson (1979), cited by Hansch and Leo (1979).

inadequate fragment constants, or the need to add or subtract fragments, which can lead to unacceptable errors. One such equation is from Chiou et al. (1977):

$$\log K_{ow} = 5.00 - 0.670 \log S \qquad \text{(A-5)}$$

where S is the aqueous solubility of the organic compound in μmol/L. Equation A-5 is based on 34 organic compounds and has an r^2 value of 0.97.

Estimation of Log K_{ow} Values

We have estimated log K_{ow} values for those military explosives or co-contaminants for which measured values have not been found in the literature. To minimize uncertainty in the estimates using the fragment-constant method, similar "backbones" were selected for each compound. In most cases, measured log K_{ow} values were available (Hansch and Leo, 1979), and only limited modification of the backbones was necessary to predict log K_{ow}'s for the corresponding explosive compounds.

2,6-Dinitrotoluene

Using 2,4-DNT as a building block (log K_{ow} = 1.98 (Nitaitani and Hansch (no date), cited by Hansch and Leo, 1979)),

$$\begin{aligned}\log K_{ow}(2,6\text{-DNT}) &= \log K_{ow}(2,4\text{-DNT}) + F^{\varphi}_{P-2}\,(NO_2,\ CH_3)\\ &= 1.98 + (-(0.08)(-0.03 + .87))\\ &= 1.98 - 0.067\\ &= 1.91 \simeq 1.9\end{aligned}$$

The log K_{ow} of 2,6-DNT is slightly less than the log K_{ow} of 2,4-DNT as we predicted from its HPLC RT.

2-Amino-4,6-dinitrotoluene and 4-Amino-2,6-dinitrotoluene

Values of log K_{OW} calculated from several structurally similar compounds yielded log K_{OW} values of less than two. For example, starting with TNT, and then substracting a nitro group factor and adding an amino factor:

$$\log K_{OW}\ (\text{4-amino-DNT}) = \log K_{OW}\ (\text{TNT}) - f\varphi(-NO_2) + f\varphi(-NH_2)$$
$$= 1.60 - (-0.03) + (-1.0)$$
$$= 0.63 \simeq 0.6$$

The value of 0.63 is reasonable when compared with the log K_{OW} values obtained by substituting either an ortho or a para amino group for a nitro group in nitrotoluene:

log K_{OW} (P-nitrotoluene)	= 2.42	(Tichy and Bocek (no date), as cited by Hansch and Leo, 1979)
log K_{OW} (p-aminotoluene)	= 1.41	(Tichy and Bocek (no date), as cited by Hansch and Leo, 1979)
difference	= 1.01	

and

log K_{OW} (o-nitrotoluene)	= 2.40	(Tichy and Bocek (no date) as cited by Hansch and Leo, 1979)
log K_{OW} (o-aminotoluene)	= 1.32	(Tichy and Bocek (no date) as cited by Hansch and Leo, 1979)
difference	= 1.08	

Furthermore, because the log K_{OW} of o-aminotoluene is 0.09 less than its para isomer, we suggest that an estimate for the log K_{OW} of 2-amino-4,6-DNT should be approximately 0.09 less than the 4-amino-2,6-DNT. If we are correct in calculating a log K_{OW} for 4-amino-4,6-DNT from TNT, then 2-amino-4,6-DNT should have a log K_{OW} of approximately 0.54 ($\simeq$ 0.5). The lack of agreement with the predicted log K_{OW} from HPLC could be attributed to hydrogen extraction from the water, making the amino-DNTs partially ionic.

Tetryl

Calculation of the log K_{OW} for tetryl from substituent factors is complicated by the lack of a factor for the methyl nitro amino group. We therefore used solubility measurements to calculate log K_{OW}. Because tetryl has a relatively high melting point, we used a formula that incorporates a melting point correction.

$$\log K_{OW} = 6.5 - 0.89 \log S - 0.015\, T_M \quad \text{(Banerjee } \underline{\text{et}}\ \underline{\text{al}}\text{., 1980)} \qquad \text{(A-6)}$$

S = micromoles/L = 260

T_M = melting point in °C = 131

$$\log K_{OW} = 2.39\ (\simeq 2.4)$$

This value is almost an order of magnitude greater than that predicted from HPLC measurements (log $K_{OW} \cong 1.5$). It is conceivable that the reported solubility is incorrect or that Eq. A-6 is inappropriate for this type of compound. In lieu of experimental data, we recommend a log K_{OW} of 2.

PETN

In order to calculate the log K_{OW} of PETN it is necessary to derive values for the substituent factors needed. We approached the calculations by two routes. First, we derived the factor for $-ONO_2$ from the log K_{OW}s of TNG and propane with proximity factor corrections, and obtained a value of -0.03. Using this factor and proximity corrections, we calculated a value of log K_{OW} for PETN of 2:

$$\log K_{OW}\ (\text{TNG})^a = \log K_{OW}\ (\text{propane})^b + 3f(-ONO_2) - 3f(H) + 2F_{P-2}$$

$$1.62 = 2.36 + 3f(-ONO_2) - 0.69 - 1.04f(-ONO_2)$$

$$-0.05 = 1.96f(-ONO_2)$$

$$f(-ONO_2) = -0.03$$

---

[a]Roberts et al. (1983).

[b]Jow and Hansch (1979), as cited by Hansch and Leo, (1979).

and

$$\log K_{ow}\ (PETN) = \log K_{ow}\ (neopentane)^{a} + 4f(-ONO_2) - 4f(H) + 6F_{p-3}$$
$$\log K_{ow}\ (PETN) = 3.11 - 0.12 - 0.92 - 0.04$$
$$\log K_{ow}\ (PETN) = 2.03 \simeq 2$$

Next, we calculated the log K_{ow} of PETN from ethyleneglycoldinitrate by deriving a factor for the entire $-CH_2-ONO_2$ group. (It was unclear if additional proximity factors were needed because models showed somewhat similar interactions between the nitro groups.)

$$f(-CH_2-ONO_2) = \tfrac{1}{2} \log K_{ow}\ (EGDN)^{b}$$
$$= 0.58$$

$$\log K_{ow}\ (PETN) = f(C) + 4f(-CH_2-ONO_2)$$
$$= 0.20 + 4(0.58)$$
$$= 2.52 \simeq 2.5$$

These values for the log K_{ow} of PETN are in reasonable agreement with the estimates from HPLC retention times. Therefore, we expect the log K_{ow} for PETN to be between 2 and 2.5. As a best estimate, we have selected the mean of these two values, which is 2.2.

Ammonium Picrate

Methods for estimating the log K_{ow} of ammonium picrate are not available because ammonium picrate exists as an ion pair in water solution.

Summary

Table A-5 summarizes the derivation of the log K_{ow} values for the explosives and related co-contaminants.

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[a]Jow and Hansch (1979), as cited by Hansch and Leo, (1979).
[b]Roberts et al. (1983).

Table A-5. Summary of log K_{ow} values for selected military explosives and associated co-contaminants.

Compound	log K_{ow}	Source/method	Reference
TNT	1.84	Estimation	Rosenblatt (1981)
	1.60	Measurement	Gould and Hansch (1986), cited by Hansch and Leo (1986)
2,4-DNT	1.98	Measurement	Nikaitani and Hansch (no date), cited by Hansch and Leo (1979)
2,6-DNT	1.9	Estimation (fragment-constant)	See footnote (a)
1,3-DNB	1.49	Measurement	Vershueren (1983)
1,3,5-TNB	1.18	Measurement	Nikaitani and Hansch (no date), cited by Hansch and Leo (1979)
2-A-4,6-DNT	0.5	Estimation (fragment-constant)	See footnote (a)
4-A-2,6-DNT	0.6	Estimation (fragment-constant)	See footnote (a)
RDX	0.87	Measurement	Banerjee et al. (1980)
HMX	0.13	Measurement	Atlantic Research (1979), cited in Tucker et al. (1985)
PETN	2.2 (2 to 2.5)	Estimation (fragment-constant)	See footnote (a)
Tetryl	2 (1.5 to 2.4)	Estimation (solubility regression equation)	See footnote (a)
Picric acid	2.03	Measurement	Anderson (1979), cited by Hansch and Leo (1979)

[a] See calculations in this appendix.

WATER SOLUBILITY

The aqueous solubility of a chemical compound is an important parameter in predicting its environmental transport and distribution. The aqueous solubility of a chemical can be defined as the maximum amount of that chemical that will dissolve in pure water at a specific temperature. For consistency in the calculation or estimation of vapor pressures, solubilities, and Henry's law constants, we have expressed solubilities in moles/L and mg/L at 20°C. In general, chemicals that are highly soluble in water are distributed more easily and rapidly in surface and ground waters than are chemicals of low solubility. Very soluble compounds tend to have relatively low adsorption coefficients for soils and sediments, as well as comparatively low bioconcentration factors in aquatic life. They are also more susceptible to biodegradation by microorganisms in soils and surface waters. Water solubility can also affect transformation by hydrolysis, photolysis, oxidation, and reduction in water. In this discussion, primary emphasis will be placed on the correlation of aqueous solubility with temperature, the identification of sources of solubility data for chemical explosives, and on methods of estimating solubility in the absence of experimental data. The solubilities of the explosives and co-contaminants are summarized in Table A-6.

Experimental Data

Frequently, solubility data are presented in the literature for 20 to 25°C, but occasionally at other temperatures. In the latter case, it is possible to draw a straight line through the data points on a semilogarithmic plot (solubility vs T^{-1}) and then either extrapolate or interpolate to the S value corresponding to $(293K)^{-1}$ on the abscissa.

Regression Equations ($S - K_{OW}$)

Regression equations (Lyman et al., 1982) have been derived for many classes of organic compounds (e.g., alcohol, esters, aromatics, etc.), which correlate water solubility at 25°C with the octanol/water partition coefficient (K_{OW}). Yalkowsky et al. (1983) present the following regression

Table A-6. Aqueous solubilities of explosives and co-contaminants. In cases where there are two values listed, the preferred value is listed first.

Explosive or Co-contaminant	Solubility mg/L	Solubility Mol/L	Temp. °C	Source/Method	Reference
TNT	124	5.4×10^{-4}	20	Average of two measured values[a]	Spanggord et al., 1980a and Urbanski, 1986a
2,4-DNT	270	1.5×10^{-3}	22	Measurement	Department of the Army, 1967a
2,6-DNT	910	5.0×10^{-3}	20 to 25	Average of two estimated values[b]	
1,3,5-TNB	330	1.5×10^{-3}	20	Interpolated[c] from two measured values	Urbanski, 1986a
1,3-DNB	180	1.1×10^{-3}	20	Interpolated[d] from two measured values	Freier, 1976
2-A-4,6-DNT	2800	1.4×10^{-2}	20 to 25	Average of two estimated values[e]	
4-A-2,6-DNT	2800	1.4×10^{-2}	20 to 25	Average of two estimated values[f]	
RDX	42.2	1.9×10^{-4}	20	Average of nine measured values[g]	Sikka et al., 1980
HMX	2.6	8.8×10^{-6}	20	Measurement	Spanggord et al. 1982a
	5	1.7×10^{-5}	22	Measurement	Glover and Hoffsommer, 1973
PETN	2	6.3×10^{-6}	20	Measurements	See Small and Rosenblatt, 1974
	32	1.0×10^{-4}	20	Extrapolated using solubilities at elevated temperatures[h]	Department of the Army, 1967b
Tetryl	75	2.6×10^{-4}	20	Measurement	Urbanski, 1986b
Ammonium picrate	10,000	4.1×10^{-2}	20	Measurement	Kaye, 1978
Picric acid	11,000	4.8×10^{-2}	20	Measurement	Urbanski, 1986a

[a] Values are 130 mg/L (Urbanski, 1986a) and 117 mg/L (Spanggord, 1977 as cited in Spanggord et al., 1980a).
[b] Estimated values are 7.6×10^{-3} mol/L from Eq. A-7 and 2.5×10^{-3} mol/L using the UNIFAC method (see text).
[c] Measured values given in Urbanski (1986a) are 300 mg/L at 17°C and 1100 mg/L at 50°C.
[d] Measured values given in Freier (1976) are 70 mg/L at 13°C and 650 mg/L at 30°C.
[e] Estimated values are 9.07×10^{-4} mol/L from Eq. A-7 and 2.7×10^{-2} mol/L using the UNIFAC method (see text).
[f] Estimated values are 1.06×10^{-3} mol/L from Eq. A-7 and 2.7×10^{-2} mol/L using the UNIFAC method (see text).
[g] The measured values at 20°C reported in Sikka et al. (1980) are 43.4, 42.7, 41.9, 41.6, 41.8, 42.4, 41.9, 42.5, and 42.8 mg/L.
[h] The solubilities reported in Dept. of the Army (1967b) are 43 mg/L at a temperature of 25°C and 180 mg/L at 96°C.

equation, relating aqueous solubility S (mol/L) to K_{OW} and melting point (MP) (°C), which has been found satisfactory for estimating the aqueous solubilities of 162 aromatic compounds:

$$\log S = -0.944 \log K_{OW} - 0.01MP + 0.323 \ . \qquad \text{(A-7)}$$

UNIFAC Method

The UNIFAC method for determining the solubility of an organic compound in water or other solvent is based on the estimation of the compound's activity coefficient in the aqueous phase. The solubility of an organic liquid in water is calculated (see Arbuckle, 1983) as

$$S = \frac{55.6\ a}{\gamma^{\infty}} \qquad \text{(A-8)}$$

where S is in mol/L; a is the activity of the organic liquid in the aqueous phase (unitless); and γ^{∞} is the infinite dilution activity coefficient of the organic substance in water. The activity (a) of hydrophobic compounds in water is approximately one (Banerjee, 1985). For solids in solution, γ^{∞} should be adjusted by multiplying it by $\exp[-0.023\ (T_m-293)]$ where T_m is the melting point (K) of the organic compound (Arbuckle, 1983). Banerjee (1985) improved the accuracy of Eq. A-8 by developing a regression equation relating γ^{∞} to aqueous solubilities of 50 organic compounds. The resulting equation ($r^2 = 0.96$) is

$$\log S = 1.20 + 0.782 \log (1/\gamma^{\infty}) \ . \qquad \text{(A-9)}$$

An activity coefficient in the UNIFAC estimation system is equal to the sum of two terms, one term representing the combinatorial effect of different functional groups of the molecule (represented by group volume and surface area parameters) and the other a residual term accounting for the interaction of the functional groups within the solvent system (see Fredenslund et al., 1975). To calculate the combinatorial and residual terms, the compound's structure must be broken down into functional groups or fragments to estimate

the appropriate coefficients (see Grain (1982) for a discussion of the UNIFAC method and tables covering group volume and surface area parameters as well as group interaction parameters).

Estimation of Water Solubilities

We were unable to find solubility data for 2,6-DNT, 2-amino-4,6-DNT, and 4-A-2,6-DNT, and so we estimated their solubilities using Eq. A-7 and the UNIFAC method. To avoid reliance on either method, we took the average of the two solubilities for each compound to represent our best estimate.

2,6-DNT. For 2,6-DNT the solubility estimated from Eq. A-7 (with K_{OW} = 1.9 and MP = 65°C) was 7.6 x 10^{-3} mol/L. The value of γ^{∞} estimated from the UNIFAC method was 73,330 and from Eq. A-9, S = 2.5 x 10^{-3} mol/L. The average solubility is therefore 5.0 x 10^{-3} mol/L.

2-Amino-4,6-DNT and 4-Amino-2,6-DNT. The solubilities of 2-amino-4,6-DNT and 4-amino-2,6-DNT were estimated from Eq. A-7 as 9.07 x 10^{-4} and 1.06 x 10^{-3} mol/L, respectively, calculated from K_{OW} values of 1.74 and 1.68, and melting points of 176 and 171°C. The value of γ^{∞} from the UNIFAC method for both compounds was 3482, resulting in a solubility of 2.69 x 10^{-2} mol/L. The average solubility of the compounds from the two methods is 1.4 x 10^{-2} mol/L.

DIFFUSION COEFFICIENTS OF CHEMICALS IN AIR AND WATER

The diffusion coefficient of a compound in air is used in GEOTOX to estimate the flux of a compound from soil to the atmosphere, and the diffusion coefficient of a compound in water is used to estimate the flux from sediments to water. Methods for estimating the diffusion coefficients of chemical compounds in air have been reviewed by Tucker and Nelken (1982). One method they suggest is both simple and accurate for nonpolar species at low to moderate temperatures. The method is based on the following correlation:

$$D_G = \frac{10^{-7}\, T^{1.75}\, M_r^{0.5}}{P\left[V_A^{1/3} + V_B^{1/3}\right]^2} \qquad \text{(A-10)}$$

where

D_G = diffusion coefficient of species B in air, m^2/s;

T = temperature, K;

M_r = $(M_A + M_B)/M_A M_B$ in which M_A and M_B are respectively, the molecular weights of air and species B;

P = pressure, atm;

V_A = molar volume of air, $cm^3/mole$; and

V_B = molar volume of species B, $cm^3/mole$.

The method recommended by Tucker and Nelken (1982) for estimating the diffusion coefficient of a compound in water is

$$D_L = \frac{13.26 \times 10^{-9}}{\eta_w^{1.14} \, V_B^{0.589}} , \qquad \text{(A-11)}$$

where

D_L = diffusion coefficient of species B in water, m^2/s;

η_w = viscosity of the water, cP; and

V_B = molar volume of species B, $cm^3/mole$.

Table A-7 contains the estimates of D_G and D_L for the explosives and associated co-contaminants. Calculations were based on the following parameter values: $T = 293$ K; $V_A = 20.1$ cm^3/mol; $M_A = 29$ g/mol; $P = 1$ atm; and $\eta_w = 1.0023$ cP. V_B was calculated using the LeBas method as described in Tucker and Nelken (1982).

COEFFICIENTS FOR EQUILIBRIUM DISTRIBUTIONS OF CHEMICALS BETWEEN SOIL/WATER (K_d) AND SOIL-ORGANIC-CARBON/WATER (K_{oc})

The key parameter in the analysis of the transport and fate of the explosives and their co-contaminants in surface soils (or sediments) is the equilibrium partitioning of those substances between soil and water. The distribution of a chemical between the solid and liquid phases in soil is described by the partition coefficient K_d that relates the amount of chemical sorbed to soil particles to the concentration in water, or

Table A-7. Diffusion coefficients in air and water for explosives and co-contaminants.

Compound	Empirical formula	Molecular weight (g-mole)	LeBas molar volume (cm^3/mol)	Coefficient for diffusion in air (m^2/sec)	Coefficient for for diffusion in water (m^2/sec)
TNT	$C_7H_5N_3O_6$	227.06	203.7	5.9E-06	5.8E-10
2,4-DNT	$C_7H_6N_2O_4$	182.06	175.2	6.4E-06	6.3E-10
2,6-DNT	$C_7H_6N_2O_4$	182.06	175.2	6.4E-06	6.3E-10
1,3-DNB	$C_6H_4(NO_2)_2$	168.05	153.0	6.8E-06	6.8E-10
1,3,5-TNB	$C_6H_3N_3O_6$	213.05	181.5	6.2E-06	6.2E-10
2-A-4,6-DNT	$C_7H_7N_3O_4$	197.08	189.4	6.1E-06	6.0E-10
4-A-2,6-DNT	$C_7H_7N_3O_4$	197.08	189.4	6.1E-06	6.0E-10
RDX	$C_3H_6N_6O_6$	222.09	195.0	6.0E-06	5.9E-10
HMX	$C_4H_8N_8O_8$	296.12	265.0	5.1E-06	5.0E-10
PETN	$C_5H_8N_4O_{12}$	316.09	265.6	5.1E-06	4.9E-10
Tetryl	$C_7H_5N_5O_8$	287.08	251.5	5.3E-06	5.1E-10
Ammonium picrate	$C_6H_6N_4O_7$	246.08	211.4	5.7E-06	5.7E-10
Picric acid	$C_6H_3N_3O_7$	229.05	189.8	6.1E-06	6.0E-10

$$K_d = \frac{C_s}{C_w} = \frac{mg/kg(soil)}{mg/kg(water)} \quad . \qquad (A-12)$$

The actual value of K_d for a chemical in a soil matrix is a function of the properties of the chemical (e.g., hydrophobicity) and the host soil (e.g., organic-carbon content of the soil, pH of the soil, cation-exchange capacity, particle-size distribution, etc.). For discussions of these and other factors, see Lyman et al. (1982). Because of the inherent variability in the soil properties at different sites, the preferred approach for developing values of K_d for assessment of the transport of explosives and related compounds at OB/OD sites is to actually measure K_d for the compounds and soils of interest.

Different studies have shown that the organic carbon content of soils governs the equilibrium distribution of neutral organic compounds between soil water and soil solids. More precisely, the K_d of these types of organic compounds increases as the soil organic carbon (SOC) content increases (Karickhoff et al., 1979; Corwin and Farmer, 1984). From this relationship, K_d can be estimated as the product of the wt% SOC for a given soil (denoted by f_{oc}) and a partition coefficient that describes the partitioning of a chemical between organic carbon and soil water (i.e., K_{oc}). Expressed mathematically,

$$K_d = K_{oc} \times f_{oc}/100 \quad , \qquad (A-13)$$

where

$$K_{oc} = \frac{\text{mg chemical/kg of SOC}}{\text{mg chemical/kg of soil water}}$$

and

$$f_{oc} = \text{wt\% SOC.}$$

There are a number of empirical relationships for estimating the K_{oc} of a chemical from its water solubility or log octanol/water partition coefficient, K_{ow} (see Lyman et al., 1982). We selected the following equation reported by Karickhoff (1985):

$$\log K_{OC} = -0.83 \log SOL - 0.01(MP - 25) - 0.93 , \quad (A-14)$$

where SOL is the mole fraction solubility, and MP is the melting point in °C. This regression equation was based on data for 47 organic compounds representing five chemical families. The r^2 value is 0.93. For some of the explosives there were no measured solubility values (e.g., 2,6-DNT) or the measured values were orders of magnitude apart (e.g., PETN). For these two chemicals we calculated log K_{OC} from the log K_{OW} using the formula derived by Karickhoff (1981):

$$\log K_{OC} = 0.989 \log K_{OW} - 0.346 , \quad (A-15)$$

This equation was derived from linear regression of data for 47 organic compounds. Table A-8 presents the estimated values of K_{OC} for the explosives and co-contaminants.

PLANT/SOIL PARTITION COEFFICIENT (K_{SP})

The uptake of a soil contaminant by a food-chain crop can be estimated by a partition coefficient, K_{SP} (or plant concentration factor), that is defined as

$$K_{SP} = \frac{\text{Concentration of contaminant in crop (mg/g fresh wt)}}{\text{Concentration of contaminant in soil (mg/g dry wt)}} \quad (A-16)$$

Values of K_{SP} are available for a relatively small number of organic compounds, primarily pesticides and herbicides that are used in agricultural applications. The plant concentration factor is a complex function of several dynamic processes. The availability of a soil contaminant for subsequent uptake, for example, depends largely on the binding or adsorption of the contaminant to the soil matrix and the rate of depletion from processes (e.g., leaching, biotransformation, volatilization, etc.) that lower the concentration of the organic contaminant in the root zone. Consequently, the bioavailability of a contaminant is controlled by its chemical/physical properties and the properties of the soil environment (e.g., organic-carbon content, particle size, etc.), and the climate at a specific site (e.g., OB/OD

Table A-8. Calculated values of K_{OC} from Eq. A-14 or A-15.

Explosive or co-contaminant	Solubility in water (moles/L)	Melting point (°C)	Solubility[a] (mole fraction)	log K_{OW}	log K_{OC}	K_{OC}
TNT	5.4E-04	80.7	9.73E-06		2.67	470
2,4-DNT	1.5E-03	70.0	2.70E-05		2.41	260
2,6-DNT	--	--	--	1.9	1.53	34
1,3-DNB	1.1E-03	89.5	1.98E-05		2.33	210
1,3,5-TNB	1.5E-03	122.5	2.79E-05		1.88	77
2-A-4,6-DNT	--	--	--	0.5	0.15	1.4
4-A-2,6-DNT	--	--	--	0.6	0.25	1.8
RDX	1.9E-04	205.5	3.42E-06		1.80	63
HMX	8.8E-06	285.0	1.6E-07		2.11	130
PETN	--	--	--	2.2	1.83	68
Tetryl	2.6E-04	131.0	4.69E-06		2.43	270
Picric acid	4.8E-02	121.8	8.65E-04		0.64	4.4

[a] At low concentrations, the solubility in mole fractions is equal to the ratio of moles of solute to the moles of water. A liter of water contains 55.5 moles.

[b] Values are given to two significant figures.

grounds). Plant uptake of contaminants occurs not only through the rootsystem, but also through the deposition of vapors and particles on foliage. The concentration of a contaminant in a plant will change with time in response to differences in the rates of uptake and loss. Generally, the uptake rate is greatest during the early growth of a plant and subsequently slows as the plant matures (Casterline et al., 1985; Schaunert et al., 1985). Losses of a contaminant can occur through transpiration and metabolism. Because the concentrations of a contaminant in soil and plant vary with time, the values of K_{sp} also change. In fact, it can take most of the growing season before equilibrium is reached between plant and soil concentrations. Kloskowski et al. (1986) also have shown that the uptake of a soil contaminant is influenced by the "aging" of the contaminant; that is, plant uptake of a

freshly added organic compound is different from uptake of a substance that has resided for a long period in soil.

Given the variability in the different chemical, soil, climate, and plant properties that affect the uptake of organic chemicals by plants, it is not surprising that little work has focused on the development of techniques for estimating plant uptake of organic chemicals. Topp et al. (1986), however, have examined some of the factors affecting the uptake of organic chemicals by plants and have derived a regression equation in which the K_{sp} for barley is calculated as the molecular weight of an organic chemical, that is,

$$\log K_{sp} = 5.943 - 2.385 \log MW, \qquad \text{(A-17)}$$

where MW is the gram-molecular weight of an organic compound. This equation was based on measured uptake values for 14 different compounds, and the r^2 value is 0.89. These researchers examined other regression equations, using K_{oc} (r^2 = 0.70) and K_{ow} (r^2 = 0.80), but MW had the highest r^2 value. The uptake measurements, though, are for short-term (i.e., 7-d) uptake experiments, and therefore Eq. A-17 may not be generally applicable to the estimation of the uptake that occurs over the entire period of plant growth prior to harvest. Furthermore, the estimate of K_{sp} is for barley, and its applicability to other crops is unknown. Plants with greater lipid content or with oil channels are expected to have much greater uptake of lipophilic compounds. Despite these limitations, the K_{sp} predicted from Eq. A-17 represents the best estimate of plant uptake, and for the purposes of the data-base assessments, we have adopted these values as default K_{sp} values in lieu of measured data. In Table A-9 we have listed the estimated uptake factors for all of the explosives and their co-contaminants. These values are within the range of plant bioconcentration factors recommended by Small (1984) for different classes of organic chemicals.

BIOCONCENTRATION FACTORS FOR FISH (K_{fw})

The ratio between the concentration of an organic compound in fish (units of mg contaminant/kg fish weight) and the concentration of the contaminant in water (mg/kg) under steady-state conditions is commonly known as the bioconcentration factor, which is denoted as K_{fw} in our notation (the more

Table A-9. Estimated barley uptake factors (K_{sp}) for explosives and their co-contaminants. K_{sp} values predicted from Eq. A-17.

Explosive or co-contaminant	Gram-molecular weight	Predicted K_{sp}[a]
TNT	227.1	2
1,3,5-TNB	213.1	2
1,3-DNB	168.1	4
2,4-DNT (2,6-DNT)	182.1	4
2-A-4,6-DNT (4-A-2,6-DNT)	197.1	3
RDX	222.1	2
HMX	296.1	1
PETN	316.1	1
Tetryl	287.1	1
Picric acid	229.1	2

[a] Values have been rounded off to the nearest integer.

common term is BCF, and we shall use it interchangeably with K_{fw}). The BCF for an organic chemical is a function of dynamic uptake and elimination processes in fish that are in contact with the chemical dissolved in water; see Bysshe (1982) and Spacie and Hamelink (1982) for details. As might be expected, this partition coefficient is positively correlated with the lipophilicity of a compound (as measured by its octanol/water partition coefficient, K_{ow}) and inversely correlated with its aqueous solubility.

Many regression equations have been published that predict bioconcentration as a function of K_{ow}. These equations are of the general form $\log K_{fw} = a \log K_{ow} + b$. A review of the literature on the predictive equations for K_{fw} by Van Gestel et al. (1985) indicates that the most reliable equation is one developed by Veith and Kosian (1983), or

$$\log K_{fw} = 0.79 \log K_{ow} - 0.40 \quad . \qquad \text{(A-18)}$$

Equation A-17 was based on bioconcentration data for 122 chemicals and has an r^2 of 0.86 ($r = 0.928$). This is an improvement over an earlier equation by Veith et al. (1980) for 84 compounds (i.e., $\log K_{fw} = 0.76 \log K_{ow} - 0.23$, with $r^2 = 0.82$).

Although regression equations like Eq. A-18 are valid for many organic compounds, some exceptions should be noted. First, organic compounds incorporated in sediments are also available for fish uptake--a pathway that is not directly accounted for in this equation. Knezovich et al. (1987), in a review of the literature on the bioavailability of organic compounds sorbed to sediments, have shown that the bioaccumulation of xenobiotic compounds from contaminated sediments can be important. Nevertheless, there are presently no methods for predicting bioaccumulation that directly account for the various uptake pathways (i.e., the water column, sediment, or even diet).

In Table A-10 we have tabulated the values of K_{fw} for the compounds considered in this report.

BEEF-FAT/DIET PARTITION COEFFICIENT (K_{fd})

Kenaga (1980) has developed regression equations relating the octanol/water partition coefficient and aqueous solubility of organic chemicals to their partitioning between the fat of cattle and their diet, defined by the beef-fat/diet partition coefficient, K_{fd}. Based on 28-d feeding studies for 23 organic chemicals (including eight chlorinated hydrocarbon insecticides and 15 miscellaneous chemicals), the resulting regression equations using K_{ow} and C_{sol} (in mg/L) were:

$$\log K_{fd} = 0.5 \log K_{ow} - 3.457 , \qquad (n = 23,\ r^2 = 0.62) \qquad \text{(A-19)}$$

and

$$\log K_{fd} = -0.495 \log C_{sol} - 1.476. \qquad (n = 23,\ r^2 = 0.67) \qquad \text{(A-20)}$$

The values of K_{fd} for the explosives and co-contaminants are presented in Table A-11. For the purposes of the data-base assessments, we have taken the average of two K_{fd} values for each compound. For PETN and 2,6-DNT we have included only the values calculated from $\log K_{ow}$ because the solubility measurements varied greatly for PETN and were unavailable for 2,6-DNT.

Table A-10. Estimates of K_{fw} for military explosives and related co-contaminants. K_{fw} values predicted from $\log K_{fw} = 0.79 \log K_{ow} - 0.40$.

Explosive or co-contaminant	$\log K_{ow}$	$\log K_{fw}$	K_{fw}
TNT	1.6	8.64E-01	7
2,4-DNT	1.98	1.16E+00	15
2,6-DNT	1.9	1.10E+00	13
1,3-DNB	1.49	7.77E-01	6
1,3,5-TNB	1.18	5.32E-01	3
2-A-4,6-DNT	0.5	-9.75E-01	1
4-A-2,6-DNT	0.6	-9.27E-01	1
RDX	0.87	2.87E-01	2
HMX	0.13	-2.97E-01	0.5
PETN	2.2	1.34E+00	22
Tetryl	2	6.03E-01	15
Picric acid	2.03	1.20E+00	16

Table A-11. Estimates of the beef fat/diet partition coefficient, K_{fd}, for explosives and their co-contaminants using the two regression equations from Kenaga (1980).

Explosive or co-contaminant	K_{ow}-based estimates			C_{sol}-based estimates[a]			Average[b]
	log K_{ow}	log K_{fd}	K_{fd}	log C_{sol}	log K_{fd}	K_{fd}	K_{fd}
TNT	1.6	-2.66	2.2E-03	2.09	-2.51	3.1E-03	2.6E-03
2,4-DNT	1.98	-2.47	3.4E-03	2.43	-2.68	2.1E-03	2.7E-03
2,6-DNT	1.9	-2.51	3.1E-03	--	--	--	--
1,3-DNB	1.49	-2.71	1.9E-03	2.26	-2.59	2.6E-03	2.2E-03
1,3,5-TNB	1.18	-2.87	1.4E-03	2.52	-2.72	1.9E-03	1.6E-03
2-A-4,6-DNT	0.5	-3.21	6.2E-04	--	--	--	--
4-A-2,6-DNT	0.6	-3.16	7.0E-04	--	--	--	--
RDX	0.87	-3.02	9.5E-04	1.63	-2.28	5.2E-03	2.2E-03
HMX	0.13	-3.39	4.1E-04	0.41	-1.68	2.1E-02	2.9E-03
PETN	2.2	-2.36	4.4E-03	--	--	--	--
Tetryl	2	-2.46	3.5E-03	1.88	-2.41	3.9E-03	3.7E-03
Picric acid	2.03	-2.44	3.6E-03	4.04	-3.48	3.3E-04	1.1E-03

[a] C_{sol} expressed in mg/L.

[b] Average calculated from the log K_{fd} values (i.e., $10^{(\log K_{fd} + \log K_{fd}/2)}$).

APPENDIX B

CONCENTRATIONS OF EXPLOSIVES IN ENVIRONMENTAL MEDIA: MODELING, DETECTION METHODS, AND MEASURED LEVELS

The concentrations of explosives and associated co-contaminants in environmental media resulting from demilitarization operations can be measured directly or predicted using environmental transport models. This appendix provides background information on the GEOTOX model referred to in earlier sections that we used to estimate the equilibrium distribution of contaminants in reference ecoregions. Later in the appendix we identify the analytical methods available for detecting explosives, and present data on concentrations of explosives and co-contaminants that have been measured in environmental media at different locations in the U.S.

GEOTOX MODEL

The GEOTOX model simulates the transport and transformation of a contaminant in a multi-media system comprised of several environmental compartments (e.g., soil, ground water, atmosphere, etc.) Figure B-1 depicts the compartmental structure of GEOTOX. This model was used in our Phase I effort (Layton et al., 1986; McKone and Layton, 1986) to determine the distribution of demilitarization by-products in environmental media and to estimate human exposures from different pathways (e.g., inhalation, water ingestion, etc.). We used GEOTOX in our data-base assessments in order to indicate which environmental media are potentially the most important in terms of transport from the surface soil layer, which is the primary source-term region for residuals associated with open-burning and open-detonation operations.

Table B-1 shows the values used to define the landscapes or compartment properties of the Western and Southeastern ecoregions. These two ecoregions represent humid and arid areas where demilitarization facilities are located. Our Phase I report provides data on the derivation of the various landscape properties. Important differences between the two landscapes are the amount of annual precipitation, thickness of the soil zones, and the ground water inventory. Our estimates of the fractional distributions of explosives and

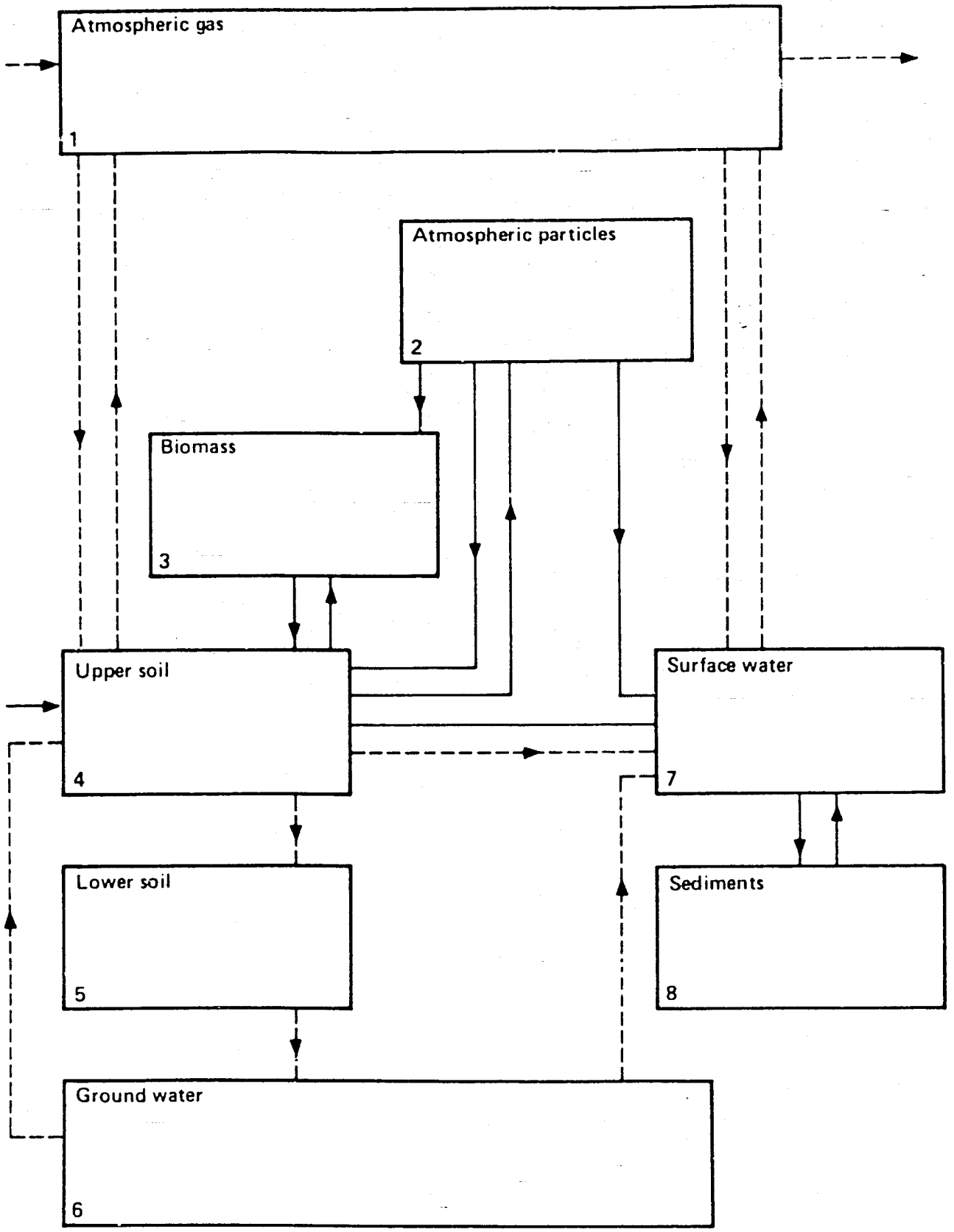

Fig. B-1. Diagram of the eight-compartment structure of GEOTOX. Dashed lines represent flows of liquids and gases, while solid lines represent flows of solids.

Table 3-1. Parameter values used in GEOTOX for the Western and Southeastern ecoregions.

Landscape property	Units	Parameter Value: Western ecoregion		Parameter Value: Southeastern ecoregion	
1 area	km^2	1.00	e00	1.00	e00
2 height of the air compartment	(m)	1.00	e03	1.00	e03
3 humidity	(kg/L)	5.00	e-6	1.14	e-5
4 precipitation onto land	(cm/yr)	3.20	e01	9.90	e01
5 precipitation onto surface water	(cm/yr)	0.30	e00	2.00	e00
6 total surface water runoff	(cm/yr)	1.26	e01	4.00	e01
7 land surface runoff	(cm/yr)	1.10	e01	3.50	e01
8 soil flux to lower atmosphere	($kg/km^2/yr$)	7.50	e03	7.50	e03
9 residence time of particles in the lower atmosphere	(yr)	8.20	e-3	8.20	e-3
10 biota dry mass inventory	(kg/km^2)	7.00	e05	3.10	e07
11 biota dry mass production	($kg/km^2/yr$)	9.00	e04	1.60	e06
12 biota dry mass fraction		3.30	e-1	3.30	e-1
13 evapotranspiration from soil	(cm/yr)	1.88	e01	5.98	e01
14 evaporation from surface water	(cm/yr)	0.15	e00	1.20	e00
15 thickness of the a soil horizon	(m)	1.60	e-1	2.50	e-1
16 bulk density of the soil in the a horizon	(kg/L)	1.50	e00	1.50	e00
17 water content of the soil in the a horizon	(kg/L)	3.80	e-1	4.20	e-1
18 volumetric air content of the soil	(L/L)	7.00	e-2	3.00	e-2
19 mechanical erosion rate	($kg/km^2/yr$)	1.19	e06	4.11	e05
20 irrigation from ground water	(cm/yr)	0.68	e00	2.00	e00
21 thickness of the b soil horizon	(m)	1.00	e01	2.00	e00
22 water content of the soil in the b horizon	(kg/L)	3.00	e-1	2.80	e-1
23 bulk density of the soil in the b horizon	(kg/L)	1.70	e00	2.00	e00
24 volumetric air content in the b horizon	(l/L)	5.00	e-2	2.00	e-2
25 groundwater inventory	(kg/km^2)	1.10	e10	4.60	e09
26 porosity of rock in the ground water zone	(L/L)	3.50	e-1	1.00	e-1
27 density of rock in the ground water zone	(kg/L)	1.70	e00	2.33	e00
28 fraction of the total surface area in surface water		8.42	e-3	1.90	e-2
29 average depth of surface waters	(m)	6.00	e00	6.00	e00

Table B-1. (Continued)

Landscape property	Units	Parameter Value: Western ecoregion	Parameter Value: Southeastern ecoregion
30 suspended sediment load in surface water	(kg/L)	8.90 e-3	8.90 e-4
31 deposition rate of suspended sediment	(kg/m^2/yr)	3.83 e03	3.90 e02
32 thickness of the sediment layer	(m)	5.00 e-2	5.00 e-2
33 bulk density of the sediment layer	(kg/L)	1.50 e00	1.50 e00
34 porosity of the sediment zone		0.20 e00	0.20 e00
35 resuspension rate from the sediment layer	(kg/m^2/yr)	3.83 e03	3.90 e02
36 ambient environmental temperature	(k)	2.83 e02	2.90 e02
37 boundary-layer thickness at air/soil interface	(m)	1.00 e-2	1.00 e-2
38 boundary-layer thickness at water/air interface	(m)	1.00 e-2	1.00 e-2
39 boundary-layer thickness at sediment/water interface	(m)	2.00 e-2	2.00 e-2
40 fraction organic carbon in the upper soil zone		1.20 e-2	1.60 e-2
41 fraction organic carbon in the lower soil zone		2.00 e-3	1.00 e-3
42 fraction organic carbon in the groundwater zone		2.87 e-4	2.87 e-4
43 fraction organic carbon in the sediment zone		2.00 e-2	2.00 e-2
44 wet deposition scavenging efficiency		1.00 e00	1.00 e00

co-contaminants among the compartments are based on the continuous addition to the surface soil layer without subsequent chemical transformation of a contaminant. Chemical-specific input parameters consisted of the gram-molecular weight of a compound, Henry's law constant, diffusion coefficients in air and water, and these partition coefficients: K_{sp}, K_{oc}, K_{fw}, and K_{fd} (for both meat- and milk-fat).

To gain more insight into the relative importance of the various landscape and physicochemical parameters on lifetime exposures, we performed an analysis of the sensitivity of lifetime exposures to TNT to 10% decreases in the input parameters to GEOTOX. Table B-2 shows the results of that analysis. Precipitation had the strongest influence on lifetime exposures, followed by the organic carbon content of the upper soil layer and K_{oc}. Many of the parameters that affect exposure are related to the subsurface environment, and this is reasonable because under steady-state conditions, TNT resides primarily in soil and ground water.

ANALYTICAL METHODS

Various demands for identification and detection of trace quantities of explosives have prompted the development of methods for the analysis and detection of explosive residues (Cumming and Park, 1983). Military requirements have led to sensitive field-detection methods. Chromatography is used in most methods, and specialized detectors have been developed. Older schemes are being replaced by resin concentration in the field, followed by a minimum of cleanup and separation by either gas chromatography (GC) or high-pressure liquid chromatography (HPLC). UV detection is being replaced by methods specific to NO_2 groups.

Field Methods for Analysis

Malotky and Downes (1983) developed a "kit" with the capability to analyze 28 explosives and energetic materials. The kit weighs 5.9 kg and occupies only 14 L. It contains three simultaneous thin-layer chromatography (TLC) experiments using different hexane-ethyl acetate solvent mixtures. Spots of reference samples of TNT, RDX, picric acid, and tetryl are chromatographed beside the sample. The silica gel on the TLC plate contains a

Table B-2. Sensitivity of lifetime average exposure (mg/kg-d) to environmental transport model inputs for TNT.

Parameter (x)	Local sensitivity dE/dx
Precipitation (cm/y)	-3.10
Organic-carbon content in the upper soil layer (unitless)	-0.22
K_{OC}, soil organic-carbon/water partition coefficient (unitless)	-0.18
Bulk density of the upper soil layer (kg/L)	-0.10
Water content of the upper soil layer (kg/L)	0.095
Ambient environmental temperature (K)	0.037
Henry's law constant (torr-L/mol)	-0.031
Porosity of rock in the groundwater zone (unitless)	-0.024
Rock density in the groundwater zone (kg/L)	0.022
Organic-carbon fraction in the ground water zone	0.022
Boundary-layer thickness at the soil/air interface (cm)	0.019
Diffusion coefficient in air (m^2/s)	0.017
Wet deposition scavenging efficiency (unitless)	0.015
Yearly average wind speed (m/s)	-0.015
Height of the air compartment	-0.015

dye that fluoresces orange when excited by a lantern-type, 250-nm UV source. The compound then remains as a gray spot.

Wyant (1977) developed a kit to detect explosives on the surface of letters and other materials. His goal was a non-toxic, non-defacing reagent that would yield a color when sprayed. The chemicals should have a long shelf life and high specificity (few false positives or negatives). The target explosives were dynamite (TNG and ethyleneglycol dinitrate, EGDN), TNT, RDX, and PETN. The reagent chosen for TNT detection was 1,3-diphenylacetone-

tetraethylammonium hydroxide. The test produced a red or red-orange color when TNT was sprayed with the reagent. For nitrate esters, the reagent chosen was procaine and N,N-dimethyl-1-naphthylamine in equal parts of acetic acid and distilled water. The surface to be tested was first sprayed with 120 g zinc dust in 100 mL benzene and then with the above reagent, yielding a red or red-violet color. This reagent system was also chosen for RDX detection at the same level. The only surface that interfered with these tests was leather, probably due to its dark color.

Spangler et al. (1985) described an ion-mobility spectrometer for detecting explosive vapors using a membrane inlet system. It was a portable system without gas bottles that analyzed vapors by either reactant ion (background) subtraction or chloride ion/molecular reaction chemistry. The explosives studied were TNT, 2,4-DNT, RDX, Composition B, and dynamite. Their method involved "sniffing" the headspace over the compound of interest. Their detection limit of TNT vapors was 1 to 10 pg.

Preservation Methods

Improved preservation methods for explosives include chemical preservation and storage on resins. Miller et al. (1983) developed a sampling and preservation protocol for munitions in water samples. The munitions were dinitrophenol (DNP), RDX, TNB, DNB, 2,4-DNT, 2,4,6-TNT, tetryl, diphenylamine (DPA), nitrobenzene (NB), 2,6-DNT, TNG, and PiOH. Preservation was achieved by

(1) adding acetonitrile to make a 10% solution;
(2) acidifying with glacial acetic acid to pH 3.5;
(3) removing sediment by centrifugation;
(4) sealing in amber glass bottles with Teflon-lined caps; and
(5) storing at 4°C in the dark.

Differences in the chemicals necessitated their being prepared for HPLC analysis by two methods: 1) DNP, RDX, 1,3-DNB, 1,3,5-TNB, 2,4-DNT, TNT, tetryl and DPA were extracted by adding 10 g NaCl to 100 mL of explosive-laden water; adjusting the pH to 3.0 with glacial acetic acid; extracting with three 20-mL portions of methylene chloride. It was then concentrated and exchanged into acetonitrile and 2) NB, 2,6-DNT, TNG, and PiOH were recovered by adding

10 g NaCl to 100 mL of water; extracting with one 20-mL portion of methylene chloride; adding 1 mL 0.005 M t-butyl ammonium hydroxide to form an ion pair with the PiOH; extracting with three additional 20-mL portions of methylene chloride. Finally, it was evaporated and exchanged the solvent for acetonitrile. A field test using well water from the Sunflower Army Ammunition Plant showed an equal recovery of munitions on day 1 and, after storage, day 21. However, the amount recovered on day 1 ranged from 23 to 88% of that initially added (Miller et al., 1983). This loss was not addressed: instead, the authors claimed the method was validated for storage.

The same authors extracted sediment samples in a 10-to-1 ratio of solvent-volume to sediment-weight with 1 h of "wrist-action shaking". DNP, RDX, TNB, DNB, 2,4-DNT, 1NT, and DPA were extracted into a 95-to-5 ratio of methylene-chloride to methanol-solution. Hexane was the solvent of choice for TNG, NB, and 2,6-DNT. PiOH and tetryl adhered to the sediments.

Pereira et al. (1979) extracted TNT and its metabolites from 1 L of ground water from the Hawthorne Naval Ammunition Depot, Nevada, using three 50 mL portions of benzene. The combined extracts were dried over anhydrous sodium sulfate, filtered through glass wool, and concentrated. The extract was separated by column chromatography on deactivated alumina, eluted with benzene, and concentrated. The authors did not mention recovery percentages.

Maskarinec et al. (1984) studied several resin systems for the concentration of munitions in water in the field. Resin not only eliminated the transport of refrigerated water bottles, but helped preserve the samples. HMX and RDX were stable in water samples for 3 weeks, but were stable for more than 9 weeks on the resins. TNT degraded equally in both situations. They concluded that the Porapak-R and Porapak-S resins were superior to the Amberlite XAD-4 resin. The Poropaks are hydrophilic and thus attract the polar munition compounds. Less breakthrough occurred with munition-waste-water effluent passing through the Poropaks as well. The explosives studied included HMX, RDX, TNT, 2,6-DNT, 2,4-DNT, PETN, and TNG. Detection limits approached 1 mg/L when followed by high-pressure liquid chromatography (HPLC).

Richard and Junk (1986) used XAD-4 resin and claimed measurement of munition levels of 0.1 mg/L. They showed above 97% recovery for TNT, 2,4-DNT, 2,6-DNT, 1,3,5-TNB, 1,3-DNB, TNB, and RDX using 60-80 mesh (150-180 μm diameter) XAD-4 columns. They reported RDX concentrations in ground water extracted by three different methods: (1) methylene chloride extraction;

(2) 20-50 mesh (300 to 800 μm in diameter) XAD-2, XAD-4, and XAD-7 resins; and (3) the 60-80 mesh XAD-4 mentioned above. Methods (1) and (3) gave nearly equivalent results, while (2) had about 65 to 90% recovery of the compounds. The recovery with coarser mesh was thus much poorer.

Douse (1985) used a more polar resin, Amberlite XAD-7, for cleanup of gunfiring handswabs. He prepared a column with a 2.5 mm (interior diameter) soda-glass tube and 18 mg of XAD-7 beads (100 to 200 μm in diameter). Ethyl acetate was used to remove the explosives (again, the smaller-diameter particles appear to have given better recovery).

Strobel and Tontarski (1983) proposed a cleanup scheme for post-blast debris employing several steps: (1) an aqueous solution of the explosive was put on a nonpolar column (they recommended a column packed with a silica gel coated with a cyclohexyl-bonded phase); the column is washed with acetonitrile/water (20:80) to remove polar waste; (2) the nonpolar column is eluted onto a polar column (they recommended a column coated with a cyanopropyl bonded phase) with methylene chloride/cyclohexane (1:1); it is washed with hexane, eliminating the nonpolar contaminants; and (3) it is then eluted with acetonitrile/water (70:30) for HPLC analysis. They found more than 94% recovery for this procedure with RDX, TNT, tetryl and PETN. They only recovered 24% of the HMX. The columns contained 500 mg of sorbent and had a volume of 2.8 mL. The explosives were eluted by two 500-mL washes placed on top of the columns; the columns were then placed in a centrifuge and the eluant collected.

Laboratory Methods

Separation of munitions can be achieved by several chromatographic methods. HPLC is preferred over gas chromatography (GC) because munitions are thermally labile, and the heating and possible degradation of compounds is avoided. In the last few years this equipment has become more sophisticated, and nitro-specific detectors are lowering the detection limits.

Maskarinec *et al*. (1984) listed the special detectors that can be used with HPLC and GC to optimize the detection of explosives. The GC detectors included nitrogen-phosphorus detectors, electron-capture detectors, thermionic-ionization detectors, and thermal-energy analyzers. The HPLC detectors work by UV absorption; chemical reduction to the corresponding amine

followed by fluorescence detection; photolytic cleavage of the C-NO_2 bond, forming nitrite ions, which are electrochemically oxidized to nitrate; and electrochemical reduction of the nitro group to the corresponding amine. Ultraviolet detection is not as effective for explosives as the nitro-specific detectors because many other contaminants also absorb in the UV, masking the munition peaks. In addition, HMX and PETN do not absorb at 254 nm, a common UV detector wavelength. At shorter wavelengths they do absorb, but so do many other organic compounds, increasing background noise.

Goff et al. (1983) described the use of the TEA detector for both GC and HPLC. TEA involves a pyrolysis of the exit stream from the chromatograph, cleaving the R-NO_2 bond and then catalytically reducing the NO_2 to NO. This is followed by a cold trap, which traps solvent and pyrolysis products, but not the NO. The NO reacts with ozone, producing the monitored, characteristic, chemiluminescent reaction. TEA is thus highly selective for NO_2 and NO functional groups. Using a laboratory mixture of TNT, 2,4-DNT, RDX, tetryl, EGDN, and TNG, they found sensitivity in the 4- to 125-pg level on GC and with the inclusion of PETN, in the nanogram level for HPLC. They suggested that HPLC is the better method for analyzing thermally labile HMX and PETN. The detector response was linear over three orders of magnitude. They also provided a list of compounds that do not interfere with the TEA analyzer.

Douse (1985) compared the TEA detector with the electron capture detector (ECD). Examples of GC separations of explosive mixtures showed excellent separations on chromatograms employing both detection methods. Explosive mixtures (150 to 500 pg) containing TNT, 2,4-DNT, RDX, tetryl, TNG, EGDN and other nitroaromatics showed slightly better separation on chromatograms using ECD detection. 2,4-DNT and TNB eluted closely but were separated with TEA. When handswabs were used, far greater interference from extraneous substances obscured sample peaks using ECD detection. Douse concluded that TEA approaches the sensitivity of ECD and exceeds it in selectivity for nitro compounds in gunfiring handswabs. Low nanogram levels were detected.

Pereira et al. (1979) also used GC coupled with electron capture to analyze ground water samples from the Hawthorne Naval Ammunition Depot (the extraction and separation are described above). They obtained the spectrum presented in Fig. B-2 showing 2,4-DNT, TNT, 4-amino-2,6-DNT, and 2-amino-4,6-DNT in the ground water. They identified the compounds by

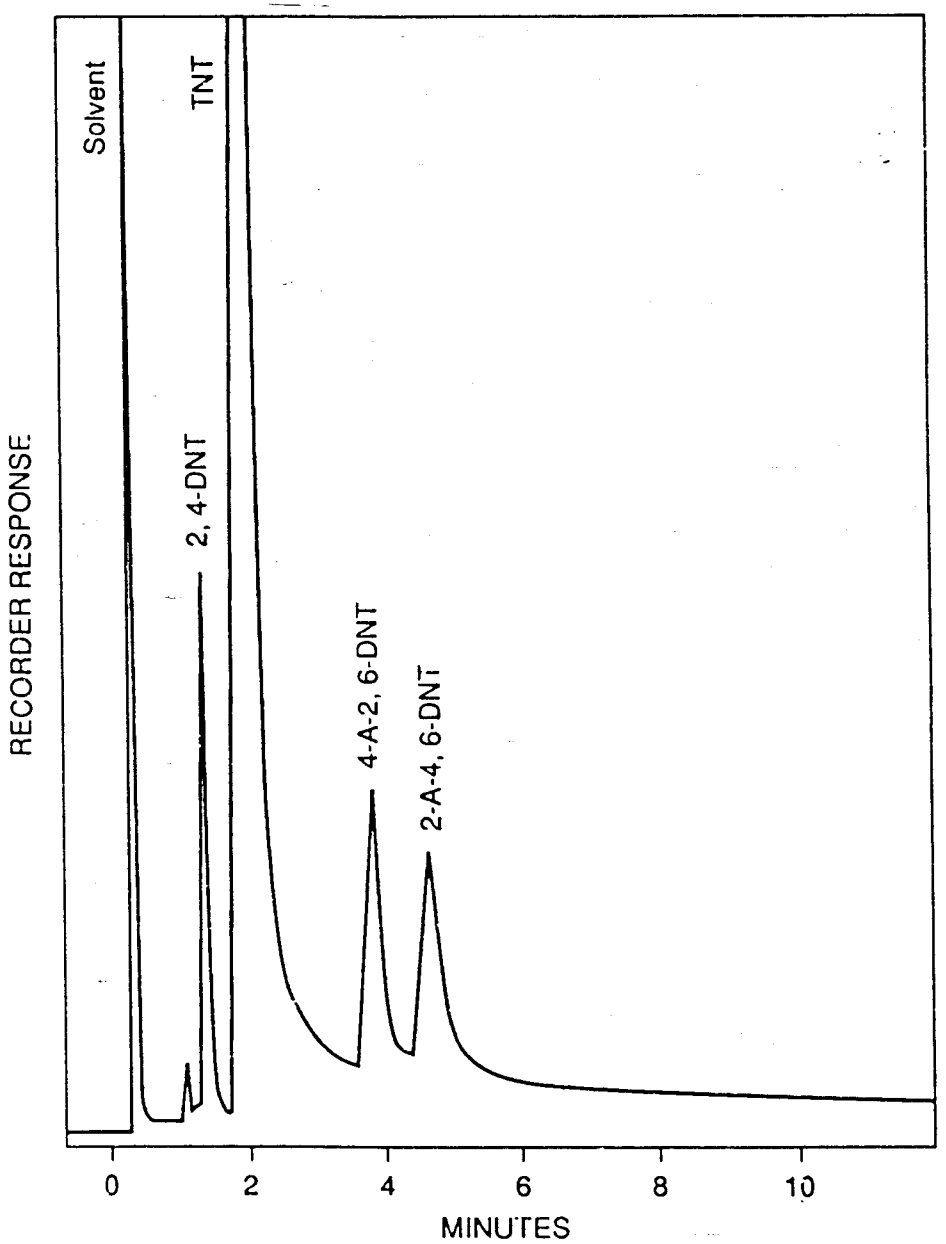

Figure B-2. Gas Chromatography with electron capture detection (GC-ECD) of ground water from Hawthorne Naval Ammunition Depot, Nevada (adapted from Pereira et al., 1979).

coupling the GC to a mass spectrometer (MS) (the mass spectra for these compounds are shown in their paper). Their focus was identification, not quantification.

To use the electrochemical reduction detection scheme (ED), Maskarinec et al. (1984) modified an amperometric detector. They carefully prepared and maintained the gold-mercury thin-film electrode and replaced it weekly. Identification and quantification of HMX, RDX, TNT, 2,4-DNT, 2,6-DNT, and PETN were achieved with ED, while HMX and RDX were lost in the absorption of other organic compounds that are detected by UV at 210 nm (See Fig. B-3). The best separation was achieved with DuPont Zorbax-ODS columns. The combination of Poropak resins and HPLC-ED yielded a detection limit of 1 mg/L for environmental water samples.

Bratin et al. (1981) used a similar procedure to analyze the explosive content in ethanol-acetone handswabs after gunfiring. Table B-3 provides their results on detection limits of UV and ED detectors for explosives. They noted that UV-detection limits improved at 200 nm; however, detector sensitivity still did not approach ED and selectivity was significantly decreased.

Bowermaster and McNair (1983) described improvement in the detection of explosives by using microbore columns for HPLC. They analyzed blast samples containing TNT, RDX, and PETN. They also found improvement in signal, but more noise by using a longer pathlength in the UV detector. PETN was enhanced by detection at 210 nm. They concluded that for a dirty RDX sample, TEA, ED, or other nitro-specific detector would have been better. They claimed detection limits in the 100- to 500-pg range.

Miller et al. (1983) also used HPLC with UV detection of an explosive mixture to test their preservation protocol discussed above. Using a reverse phase with gradient elution from 100% to 10% acetonitrile, they achieved separations with detection limits between 50 and 90 ppb for DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA. For NB, 2,6-DNT, TNG, and PiOH, they used an isocratic separation of 35/65 v/v with acetonitrile-0.005 M t-butyl ammonium hydroxide/water as the solvent. Detection limits were similar to the above except for TNG, which had a detection limit of 1.6 ppm.

Meier et al. (1978) also used HPLC coupled with UV (230 nm) detection for analysis of explosives. They separated TNT, tetryl, and their degradation products with a 40% acetonitrile/water mobile phase and separated HMX and RDX

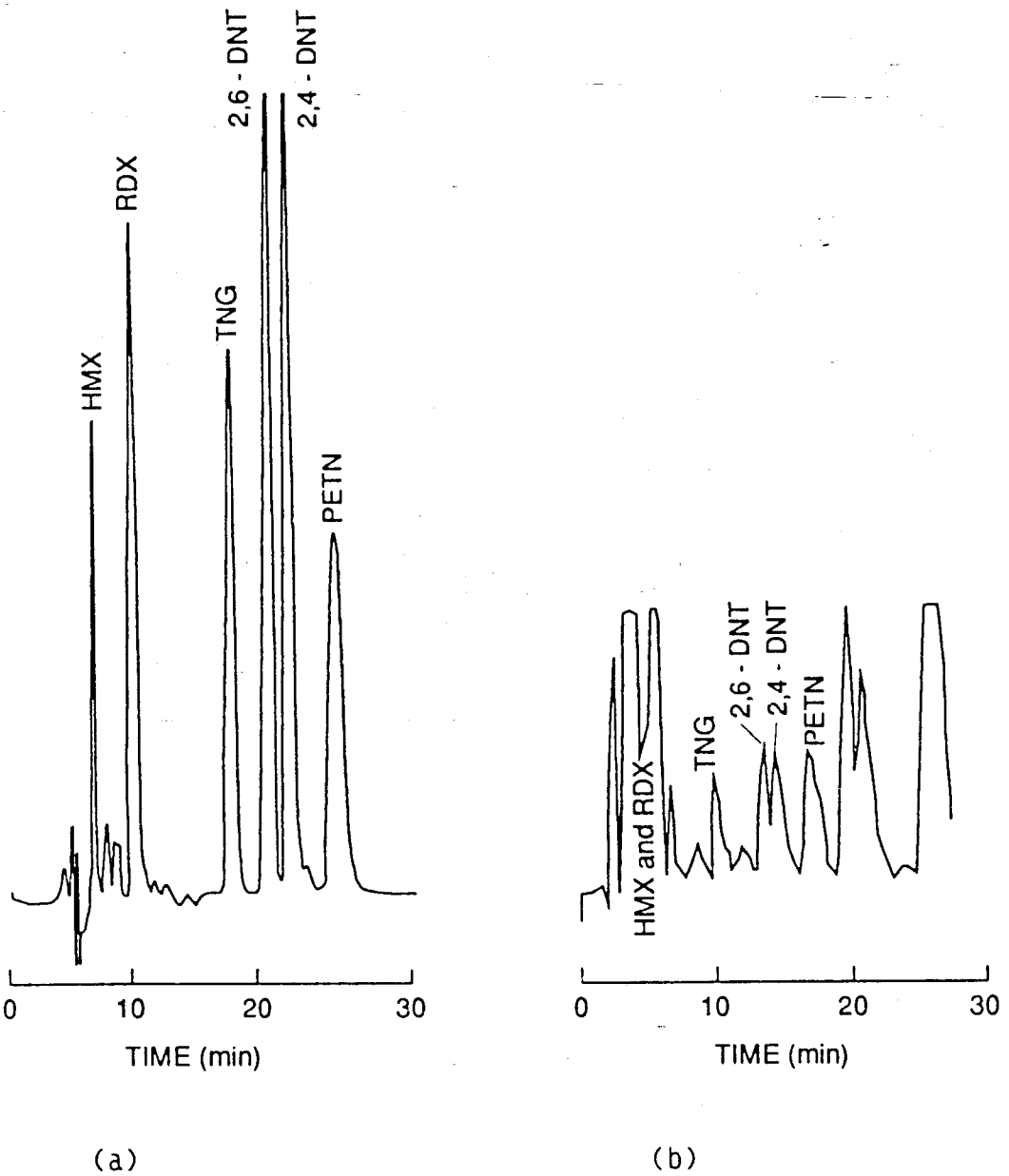

Figure B-3. Electrochemical detection (ED) contrasted with ultraviolet (UV) detection of HPLC of explosive mixture after sorption and desorption on Porapak-R resin. (a) ED (20 L injected). (b) UV absorption at 210 nm (50 L injected) (from Maskarinec et al., 1984).

Table B-3. Detection limits of unltraviolet (UV) and electrochemical reduction (ED) detectors (Bratin et al., 1981).

Explosive	Detection limits (ng) UV at 254 nm	ED at -0.1 V
TNT	0.65	0.14
2,4-DNT	0.57	0.16
2,6-DNT	1.2	0.17
Tetryl	0.77	0.21
Picric acid	0.47	0.065
RDX	0.88	0.17
HMX	1.5	0.29
PETN	---	0.40
TNG	160.0	0.38

with a 30% acetonitrile/water mobile phase. With direct injections of 1 mL of contaminated water, detection limits were 50 ppb.

Another method of detection following GC separation is mass spectrometry. Cumming and Park (1983) agreed that conventional MS is limited because of extensive fragmentation. They suggested new techniques such as "Single Ion Monitoring" (SIM) of the most abundant ion and the negative-ion mode (NI) as detectors of nanogram levels of explosives. SIM and NI spectra are given for explosives TNT, RDX, and PETN, and the propellents EGDN and TNG.

MEASURED CONCENTRATIONS OF MUNITIONS IN ENVIRONMENTAL MEDIA

Obsolete munitions have been detonated and burned as means of disposal since the turn of the century. Results of selected ground- and water-contamination studies on various sites are listed in Tables B-4 and B-5. These tables provide an overview of the concentrations detected in different

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Table B-4. Concentrations of TNT and associated co-contaminants found in soils and waters by various investigators.

Compound	Media	Depth	Description	Concentration (ppm)	Reference
TNT	Soil	Surface	Savanna AD, a soil analysis	88,100-406,000	Noland et al., 1984
		Surface	Blue Grass Facility, washout lagoon area	3.74-7.78	Mizell et al., 1982
		Surface	Blue Grass Facility, burning/demolition area	0.22-1760	Mizell et al., 1982
		Surface	Blue Grass Facility, detonation area	0.978	Mizell et al., 1982
		1 ft	Blue Grass Facility, detonation area	0.223	Mizell et al., 1982
		4 ft	Blue Grass Facility, detonation area	0.239	Mizell et al., 1982
		Not stated	Cornhusker AAP, core samples, leaching pits	70-30,000	Rosenblatt, 1986
		Not stated	Cornhusker AAP, core samples, cesspools	3-300	Rosenblatt, 1986
		Not stated	Alabama Army Ammunition Plant Surveys		
			old burning ground	<0.037-0.694	Rosenblatt & Small, 1981
			old flashing ground	<0.037-2.35	Rosenblatt & Small, 1981
		Surface	Savanna AD dry lagoon, flood plains	290,000 max	Rosenblatt, 1981
			dry lagoons upland	12,000-181,000	Rosenblatt, 1981
		0.05 m	Louisiana AAP, lagoons (discontinued 1981)	9340	Spanggord et al., 1983a
		0.15-0.20	Louisiana AAP, lagoons (discontinued 1981)	1250	Spanggord et al., 1983a
		0.46-0.51	Louisiana AAP, lagoons (discontinued 1981)	400	Spanggord et al., 1983a
		Not stated	Iowa AAP, lagoon sediment	0.012-0.210	Clear and Collins, 1982
		Surface	Hawthorne AAP, open burning area	1230	Newell, 1984
		0-6 in	Iowa AAP, open burning area	2700-12,900	Newell, 1984
		6-18 in	Iowa AAP, open burning area	1370-6600	Newell, 1984
		Residue	Iowa AAP, open burning area	2400-91,500	Newell, 1984
		Surface	Iowa AAP, open burning area	3360	Newell, 1984
		0-6 in	Kansas AAP, open burning area	2350-21,800	Newell, 1984
		0-6 in	Louisiana AAP, open burning area	1250-13,400	Newell, 1984
		6-18 in	Louisiana AAP, open burning area	2080-15,800	Newell, 1984
		Residue	Louisiana AAP, open burning area	1470-56,100	Newell, 1984
		Surface	Louisiana AAP, open burning area	20,000	Newell, 1984
		Residue	Radford AAP, open burning area	23,307	Newell, 1984
		Surface	Radford AAP, open burning area	12,945	Newell, 1984
		0-6 in	Ravenna AAP, open burning area	2263	Newell, 1984

Table b-4. (continued).

Compound	Media	Depth	Description	Concentration (ppm)	Reference
TNT (cont'd.)	Soil	6-18 in	Fort Wingate AAP, open burning area	2810	Newell, 1984
		Surface	Fort Wingate AAP, open burning area	3180	Newell, 1984
		0-6 in	Seneca AAP, open burning area	9270	Newell, 1984
		0-6 in	Redstone ARS, open burning area	2467	Newell, 1984
		Surface	Lone Star AAP, open detonation area	3510	Newell, 1984
		Surface	Volunteer AAP, open detonation area	2470-14,400	Newell, 1984
		Not stated	Alabama AAP, unspecified	7900 max	Tucker et al., 1985
		Not stated	Bluegrass, unspecified	1758 max	Tucker et al., 1985
		Not stated	Cornhusker AAP, unspecified	38,000 max	Tucker et al., 1985
		Not stated	Ft. Wingate DA, unspecified	4936 max	Tucker et al., 1985
		Not stated	Iowa AAP, unspecified	2000 max	Tucker et al., 1985
		Not stated	Joliet AAP, unspecified	482,000 max	Tucker et al., 1985
		Not stated	Longhorn AAP, unspecified	4.6 max	Tucker et al., 1985
		Not stated	Milan AAP, unspecified	13 max	Tucker et al., 1985
		Not stated	Navajo DA, unspecified	188,800 max	Tucker et al., 1985
		Not stated	Savanna AD, unspecified	140 max	Tucker et al., 1985
		Not stated	Tooele AD, unspecified	100 max	Tucker et al., 1985
		Not stated	Umatilla AD, unspecified	2800 max	Tucker et al., 1985
		Not stated	Volunteer AAP, unspecified	3400 max	Tucker et al., 1985
	River	Surface	Blue Grass Facility, seep of ground water	0.0194	Mizell et al., 1982
		Sediment	Savanna AD - TNT reclamation washout	0.289	Rosenblatt, 1981
	Ground water	Shallow	Hawthorne Naval Ammunition Depot, Nevada beneath and downgradient from disposal beds	0.62	Pereira et al., 1979
		27 ft	Blue Grass Facility, downgradient from lagoon, bedrock well	0.139	Mizell et al., 1982
		20-23 ft	Hawthorne Naval Ammunition Depot, Nevada beneath and downgradient from disposal beds used from 1952 to 1968	0.002-0.11	Van Denburgh et al., 1980

Table B-4. (continued).

Compound	Media	Depth	Description	Concentration (ppm)	Reference
TNT (cont'd.)	Ground water	Not stated	Alabama AAP	21.9 max	Tucker et al., 1985
		Not stated	Milan AAP	15.8 max	Tucker et al., 1985
		Not stated	Savanna Depot, limestone bedrock under lagoons	0.42	Rosenblatt, 1981
		20 ft	Iowa AAP, Lagoon (discontinued, 1955)	0.172-0.850	Clear and Collins, 1982
2,4-DNT	Soil	Surface	Blue Grass Facility, washout lagoon area	up to 0.56	Mizell et al., 1982
		Surface	Blue Grass Facility, burning/demolition area	0.98-27.6	Mizell et al., 1982
		Surface	Blue Grass Facility, detonation area	2.06	Mizell et al., 1982
		1 ft	Blue Grass Facility, detonation area	9.31	Mizell et al., 1982
		4 ft	Blue Grass Facility, detonation area	1.84	Mizell et al., 1982
		Not stated	Cornhusker AAP, core samples, leaching pits	0-2 max	Rosenblatt, 1986
		Not stated	Cornhusker AAP, core samples, cesspools	0-3 max	Rosenblatt, 1986
		Not stated	Alabama Army Ammunition Plant Surveys (Both DNT's)		
			old burning ground	<0.102-0.875	Rosenblatt & Small, 1981
			old flashing ground	<0.102-1.845	Rosenblatt & Small, 1981
			smokeless powder manufacturing area	<0.112-1.44	Rosenblatt & Small, 1981
			magazine area	<0.208	Rosenblatt & Small, 1981
			propellant shipping area	<0.208	Rosenblatt & Small, 1981
			rifle-powder finishing area	<0.112-6.095	Rosenblatt & Small, 1981
		Surface	Savanna Army Depot, dry lagoon flood plains, some 2,6-DNT	94,000 max	Rosenblatt, 1981
		6-18 in	Badger AAP, open burning area	4690-20,100	Newell, 1984
		Residue	Radford AAP, open buring area	62,572	Newell, 1984
		Surface	Volunteer AAP, open detonation area	2790-19,400	Newell, 1984
		Not stated	Alabama AAP, unspecified	16 max	Tucker et al., 1985
		Not stated	Bluegrass, unspecified	27 max	Tucker et al., 1985
		Not stated	Cornhusker AAP, unspecified	26 max	Tucker et al., 1985
		Not stated	Ft. Wingate DA, unspecified	0.3 max	Tucker et al., 1985

Table B-4. (continued).

Compound	Media	Depth	Description	Concentration (ppm)	Reference
2,4-DNT (cont'd.)	Soil	Not stated	Iowa AAP, unspecified	30 max	Tucker et al., 1985
		Not stated	Joliet AAP, unspecified	9210 max	Tucker et al., 1985
		Not stated	Milan AAP, unspecified	1 max	Tucker et al., 1985
		Not stated	Navajo DA, unspecified	67 max	Tucker et al., 1985
		Not stated	Savanna AD, unspecified	38 max	Tucker et al., 1985
		Not stated	Tooele AD, unspecified	80 max	Tucker et al., 1985
		Not stated	Umatilla AD, unspecified	4.3 max	Tucker et al., 1985
		Not stated	Volunteer AAP, unspecified	660 max	Tucker et al., 1985
	River	Surface	Iowa AAP Long Creek watershed	0.002	Clear and Collins, 1982
	Ground water	27 ft	Blue Grass Facility, downgradient from lagoon, bedrock well	0.0033	Mizell et al., 1982
		20 ft	Iowa AAP, Lagoon (Discontinued 1955)	0.029-0.035	Clear and Collins, 1982
		Not stated	Alabama AAP	4.342 max	Tucker et al., 1985
		Not stated	Volunteer AAP	8.7 max	Tucker et al., 1985
2,6-DNT	Soil	Maximum	Cornhusker AAP, core samples, cesspools	0-4	Rosenblatt, 1986
		Surface	Blue Grass Facility, burning/demolition area	5.07 max	Mizell et al., 1982
		Surface	Blue Grass Facility, detonation area	0.985	Mizell et al., 198"
		1 ft	Blue Grass Facility, detonation area	2.1	Mizell et al., 1982
		Not stated	Alabama Army Ammunition, Plant surveys (both DNT's)		
		Not stated	old burning ground	<0.102-0.875	Rosenblatt & Small, 1981
		Not stated	old flashing ground	<0.102-1.845	Rosenblatt & Small, 1981
		Not stated	smokeless powder manufacturing area	<0.112-1.44	Rosenblatt & Small, 1981
		Not stated	magazine area	<0.208	Rosenblatt & Small, 1981
		Not stated	propellant shipping area	<0.208	Rosenblatt & Small, 1981
		Not stated	rifle-powder finishing area	<0.112-6.095	Rosenblatt & Small, 1981

Table B-4. (continued).

Compound	Media	Depth	Description	Concentration (ppm)	Reference
2,6-DNT	Soil	Not stated	Alabama AAP	4.8 max	Tucker et al., 1985
		Not stated	Bluegrass Facility	5.1 max	Tucker et al., 1985
		Not stated	Cornhusker AAP	3.8 max	Tucker et al., 1985
(cont'd.)		Not stated	Iowa AAP	6 max	Tucker et al., 1985
		Not stated	Joliet AAP	2540 max	Tucker et al., 1985
		Not stated	Milan AAP	0.88 max	Tucker et al., 1985
		Not stated	Navajo DA	5.4 max	Tucker et al., 1985
		Not stated	Tooele AD	300 max	Tucker et al., 1985
		Not stated	Umatilla AD	5.4 max	Tucker et al., 1985
		Not stated	Volunteer AAP	300 max	Tucker et al., 1985
	Ground water	20 ft	Iowa AAP, Lagoon (discontinued, 1955)	0.008-0.060	Clear and Collins, 1982
		Not stated	Alabama AAP	1.085 max	Tucker et al., 1985
		Not stated	Volunteer AAP	6.9 max	Tucker et al., 1985
	Soil	Surface	Savanna AD, soil analysis	nd-35.1	Noland et al., 1984
		Surface	Blue Grass Facility, burning/demolition area	0.48-2.35	Mizell et al., 1982
		Surface	Blue Grass Facility, detonation area	1.24	Mizell et al., 1982
		1 ft	Blue Grass Facility, detonation area	2.35	Mizell et al., 1982
		4 ft	Blue Grass Facility, detonation area	0.482	Mizell et al., 1982
	Ground water	20 ft	Iowa AAP, Lagoon (discontinued, 1955)	0.036-0.050	Clear and Collins, 1982
1,3,5-TNB	Soil	Surface	Blue Grass Facility, washout lagoon area	1.16	Mizell et al., 1982
		10 ft	Blue Grass Facility, washout lagoon area	7.14	Mizell et al., 1982
		Surface	Blue Grass Facility, burning/demolition area	up to 1.66	Mizell et al., 1982
		1 ft	Blue Grass Facility, detonation area	9.31	Mizell et al., 1982
		Surface	Savanna AD, soil analysis	57.0-2.56	Noland et al., 1984

Table B-4. (continued).

Compound	Media	Depth	Description	Concentration (ppm)	Reference
1,3,5-TNB (cont'd.)	Soil	Maximum	Cornhusker AAP, core samples, leaching pits	4-100	Rosenblatt, 1986
		Maximum	Cornhusker AAP, core samples, cesspools	0-30	Rosenblatt, 1986
			Alabama Army Ammunition Plant Surveys		
		Not stated	magazine area	<0.368-2.54	Rosenblatt & Small, 1981
		Not stated	old flashing ground	<0.368-3.92	Rosenblatt & Small, 1981
		Not stated	smokeless powder manufacturing area	<0.614	Rosenblatt & Small, 1981
		Not stated	Alabama AAP	3.9 max	Tucker et al., 1985
		Not stated	Bluegrass	7.1 max	Tucker et al., 1985
		Not stated	Cornhusker AAP	1109 max	Tucker et al., 1985
		Not stated	Ft. Wingate DA	7.8 max	Tucker et al., 1985
		Not stated	Iowa AAP	200 max	Tucker et al., 1985
		Not stated	Joliet AAP	2610 max	Tucker et al., 1985
		Not stated	Milan AAP	54 max	Tucker et al., 1985
		Not stated	Navajo DA	3.7 max	Tucker et al., 1985
		Not stated	Savanna AD	96 max	Tucker et al., 1985
		Not stated	Umatilla AD	7 max	Tucker et al., 1985
		Not stated	Volunteer AAP	390 max	Tucker et al., 1985
	Lagoon water	Not stated	Louisiana AAP, disposal lagoon (discontinued 1981)	0.2-4.17	Spanggord et al., 1983a
	River water	Not stated	Savanna AD, limestone bedrock under lagoons	0.314	Rosenblatt, 1981
	Ground water	27 ft	Blue Grass, downgradient from lagoon, bed rock well	0.072	Mizell et al., 1982
		20 ft	Iowa AAP, lagoon (discontinued, 1955)	0.010-1,900	Clear and Collins, 1982
		Not stated	Alabama AAP	4.484 max	Tucker et al., 1985
		Not stated	Joliet AAP	1.610 max	Tucker et al., 1985
		Not stated	Iowa AAP	2.3 max	Tucker et al., 1985

Table B-4. (continued).

Compound	Media	Depth	Description	Concentration (ppm)	Reference
4-A-2,6-DNT	Soil	Not stated	Holston River sediment	0.097 max	Spanggord et al., 1981
	Lagoon water	Not stated	Louisiana AAP, disposal lagoon (discontinued 1981)	0.42-2.35	Spanggord et al., 1983a
	Ground water	20-23ft	Hawthorne Naval Ammunition Depot, Nevada beneath and downgradient from disposal beds used from 1952 - 1968	detected	Van Denburgh et al., 1980
		Shallow	Hawthorne Naval Ammunition Depot, Nevada beneath and downgradient from disposal beds	detected	Pereira et al., 1979
2-A-4,6-DNT	Soil	Not stated	Savanna AD, soil analysis	27.9 max	Noland et al., 1984
		Not stated	Holston River sediment	0.0675 max	Spanggord et al., 1981
	Lagoon water	Not stated	Louisiana AAP, disposal lagoon (discontinued 1981)	0.77-1.48	Spanggord et al., 1983a
	Ground water	Shallow	Hawthorne Naval Ammunition Depot, Nevada beneath and downgradient from disposal beds	found	Pereira et al., 1979

[a] AAP = Army Ammunition Plant, AD = Army Depot, ARS = Arsenal and DA = Depot Activity.

Table B-5. Amounts of RDX, HMX, tetryl, PETN, ammonium picrate, and co-contaminants found in soils and waters.

Compound	Media	Depth	Description	Concentration (ppm)	Reference
RDX	Soil	Surface	Savanna AD, a soil analysis	28.6-145	Noland et al., 1984
		Maximum	Cornhusker AAP, core samples, leaching pits	2-40	Rosenblatt, 1986
		Maximum	Cornhusker AAP, core samples, cesspools	0.7-4	Rosenblatt, 1986
		Surface	Savanna AD, dry lagoons, upland	3000-4000	Rosenblatt, 1981
		Surface	Holston AAP, open burning	74,000	Newell, 1984
		0-6 in	Holston AAP, open burning	1834-10,200	Newell, 1984
		Residue	Holston AAP, open burning	3513-5339	Newell, 1984
		Residue	Iowa AAP, open burning	4600-5200	Newell, 1984
		Residue	Kansas AAP, open burning	1340	Newell, 1984
		0-6 in	Louisiana AAP, open burning	2120-2230	Newell, 1984
		6-18 in	Louisiana AAP, open burning	1400-41,300	Newell, 1984
		Residue	Louisiana AAP, open burning	2230-50,400	Newell, 1984
		Surface	Louisiana AAP, open burning	1400-39,100	Newell, 1984
		0-6 in	Ravenna AAP, open burning	2976	Newell, 1984
		6-18 in	Fort Wingate AAP, open burning	3110	Newell, 1984
		0-6 in	Fort Wingate AAP, open burning	3060	Newell, 1984
		0-6 in	Picatinny ARS, open burning	1219	Newell, 1984
		0-6 in	Milan AAP, open detonation	6780	Newell, 1984
		5 ft	Blue Grass, washout lagoons	4.7	Mizell et al., 1982
		10 ft	Blue Grass, washout lagoons	7.9	Mizell et al., 1982
		Sludge	Lone Star Ammunition Plant, settling ponds	58,000	Phung and Bulot, 1981
		1.2-1.8 m	Lone Star Ammunition Plant, settling ponds	11.3	Phung and Bulot, 1981
		3.6-4.5 m	Lone Star Ammunition Plant, settling ponds	3.7	Phung and Bulot, 1981
		Surface	Savanna AD, dry lagoon sediment	3,000-4,000	Rosenblatt, 1981
		.05 m	Lousiana AAP, lagoon sediment (inactive, 1981)	120,000	Spanggord et al., 1983a
		.15-.20 m	Lousiana AAP, lagoon sediment (inactive, 1981)	3,000	Spanggord et al., 1983a
		.46-.51 m	Lousiana AAP, lagoon sediment (inactive, 1981)	400	Spanggord et al., 1983a
	Lagoon water	Not stated	Iowa AAP, pink water lagoon (inactive, 1955)	0.166-.219	Clear and Collins, 1982
		Not stated	Lousiana AAP, lagoon water (inactive, 1981)	5.6-28.9	Spanggord et al., 1983a
		Not stated	LLNL Site 300 lagoon	0.03-2.1	Raber, 1933

Table B-5. (continued).

Compound	Media	Depth	Description	Concentration (ppm)	Reference
RDX (cont'd.)	River water	Surface	Blue Grass, ground water seep	0.036	Mizell et al., 1982
		Surface	Iowa AAP, Spring Creek, facility boundry	0.062	Clear and Collins, 1982
		Surface	Iowa AAP, Brush Creek watershed	0.086-0.185	Clear and Collins, 1982
		Not stated	Holston River, North bank, 0.1 km	0.055	Spanggord et al., 1981
		Not stated	Holston River, 33 km	0.0065	Spanggord et al., 1981
	Ground water	27 ft	Blue Grass, downgradient from lagoon, bedrock well	0.201	Mizell et al., 1982
			Iowa AAP, bedrock well, demolition area	0.018	Clear and Collins, 1982
			Iowa AAP, Brush Creek wells	1.5 max	Clear and Collins, 1982
		20 ft	Iowa AAP, pink lagoon area (discontinued 1955)	14.0	Clear and Collins, 1982
		Not stated	Iowa AAP	36.0 max	Tucker et al., 1985
		Not stated	Milan AAP	30.0 max	Tucker et al., 1985
		Not stated	Louisiana AAP	17.8 max	Tucker et al., 1985
HMX	Soil	Surface	Holston AAP, open burning	33,339	Newell, 1984
		0-6 in	Holston AAP, open burning	1693-5996	Newell, 1984
		Residue	Holston AAP, open burning	1730-7268	Newell, 1984
		Residue	Iowa AAP, open burning	5900	Newell, 1984
		0-6 in	Louisiana AAP, open burning	1360-1590	Newell, 1984
		6-18 in	Louisiana AAP, open burning	2670-9850	Newell, 1984
		Residue	Louisiana AAP, open burning	1690-5900	Newell, 1984
		Surface	Louisiana AAP, open burning	5950	Newell, 1984
		Residue	Ravenna AAP, open detonation	19,598	Newell, 1984
		0-6 in	Milan AAP, open detonation	1380	Newell, 1984
	River water	Not stated	Holston River, North bank, 0.1 km	0.0075 max	Spanggord et al., 1981
		Not stated	Holston River, 8 km	0.0003 max	Spanggord et al., 1981

Compound	Media	Depth	Description	Concentration (ppm)	Reference
	Lagoon water	Not ctated	Lousiana AAP, lagoon water (inactive, 1981)	2.87-6.36	Spanggord et al., 1983a
			LLNL Site 300 lagoon	0.3-4.4	Raber, 1983
	Ground water	Not stated	Milan AAP	2.6	Tucker et al., 1985
		Not stated	Newport AAP	0.59	Bauer, 1985
Tetryl	Soil	Surface	Alabama Army Amunition Plant, demolition landfill	0.554	Rosenblatt & Small, 1981
		Surface	Alabama Army Amunition Plant, rifle powder finish	>0.500	Rosenblatt & Small, 1981
		Surface	AMC-wide all analysis, open burning	Detected 4.8%	Newell, 1984
		Surface	AMC-wide all analysis, open detonation	Detected 3.7%	Newell, 1984
		0-8 in	Picatinny Arsenal, open burning	176.0	Bauer, 1985
		8-14 in	Picatinny Arsenal, open burning	35.1	Bauer, 1985
			Picatinny Arsenal, open detonation	2.5	Bauer, 1985
	Ground water	20 ft	Iowa AAP, pink lagoon area (discontinued, 1955)	0.046	Clear and Collins, 1982
Ammonium Picrate	Waste water	Not stated	Wash waters and evaporation ponds	100-300	Ruchhoft and Norris, 1946

[a] AAP = Army Ammunition Plant; ARS = Arsenal; AD = Army Depot.

environmental samples. More comprehensive data on the extent of soil and water contamination at open-burning and open-detonation sites are contained in a series of reports completed by the U.S. Army Environmental Hygiene Agency (see, for example, Newell, 1984; Bauer, 1985; and Resch, 1985).

APPENDIX C

CARCINOGENIC POTENCY ASSESSMENT FOR 2,6-DNT

The term carcinogenic "potency" as used here refers to the quantitative expression of increased tumorigenic response per unit dose rate at the characteristically low dose levels of environmental concern. Responses are predicted from tumor response-dose data using the "linearized" multistage dose-response extrapolation model that has been adopted for environmental regulatory purposes by the U.S. EPA and by similar agencies in many states (U.S. EPA, 1986; Anderson et al., 1983; CDHS, 1985). The following carcinogenic potency assessment proceeds in three steps: (1) selection of bioassay data sets indicative of DNT carcinogenicity suitable for dose-response assessment, (2) selection of appropriate response and dose information to use in the process of dose-response assessment for the animal bioassay data, and (3) potency quantification using the latter data.

SELECTION OF BIOASSAY DATA INDICATIVE OF DNT CARCINOGENICITY

The rodent bioassay data sets used for this potency assessment include all those that reflect a significantly increased incidence of a given type of malignant tumor, or of a histogenically related set of benign and malignant tumors, among animals exposed to 2,6-DNT compared to those not exposed to this compound. These data sets include studies of the carcinogenicity to rodents of pure 2,6-DNT as well as mixtures containing 2,6-DNT, either as a major component (along with 2,4-DNT in DNT mixtures such as tDNT) or as a trace contaminant (in TNT). The potencies for 2,6-DNT derived here from the latter data sets involving mixtures are not strictly comparable to potencies based on tumor incidence in animals exposed to pure 2,6-DNT. Nevertheless, 2,6-DNT-based potencies describing increased tumor incidence associated with exposure to DNT mixtures are presented here to allow a qualitative test of the hypothesis, currently advanced by several researchers (Long and Rickert, 1982; Kedderis et al., 1984; Rickert et al., 1984; Goldsworthy and Popp, 1986; 1986; Leonard et al., 1987) that 2,6-DNT is the DNT isomer responsible for the carcinogenic activity of DNT observed in rodents. We also include a potency assessment for the 2,6-DNT component of the TNT used in the rodent bioassays

completed by Furedi et al. (1984a, 1984b) to investigate whether the positive response observed in this study is consistent with the hypothesis that 2,6-DNT is again the causative agent, rather than TNT itself.

All of the studies referred to above are listed in Tables C-1, and C-2. These bioassay data sets are used here because they represent the only chronic exposure studies with well-defined exposure-response data that indicate a positive carcinogenic response for DNT in animals, and because comparable human epidemiological data are not available.

ADJUSTMENT OF ANIMAL BIOASSAY DOSE-RESPONSE DATA

For those bioassay data sets considered here, lifetime, time-weighted average (TWA) dose rates of DNT in mg/kg-d are presented for each study, and these values are listed in Table C-1. In each of these dietary bioassays, treated feed was continuously available to the exposed animals. Therefore, no adjustment was necessary to convert the TWA experimental administered doses to lifetime equivalent doses.

For all bioassays involving DNT mixtures or TNT (i.e., all studies except the bioassay of Leonard et al. (1987) in which pure 2,6-DNT was administered in the feed of rats), the corresponding lifetime TWA dose of 2,6-DNT used here for potency analysis is shown in Tables C-1 and C-2. These values are derived directly from the respective studies cited in these Tables, except for the Furedi et al. (1984a, 1984b) and CIIT (1982) studies where 2,6-DNT was assumed to comprise 0.25 and 18.8 wt%, respectively, of the total DNT dose administered.

Although 2,6-DNT does show some potential for direct mutagenic activity in in vitro mutagenicity assays, in vivo hepatocellular assays in rodents suggest that the proximate cause of DNT toxicity is most likely a reactive metabolite of a gut-microfloral metabolite of a liver-generated primary DNT-metabolite, which has been secreted into bile and recycled, after microbial activation, to the liver for further metaboiic activitation (Goldsworthy and Popp, 1986). Therefore, it is likely that the most appropriate input dose use for carcinogenic potency analysis would be the "effective" TWA dose (rate) of proximate (or at least primary) DNT-metabolite formed (e.g., per day). In both rodents and humans, a given DNT dose appears to be almost completely metabolized to primarily urinary metabolites within a

Table C-1. Carcinogenic potencies calculated from 2,6-DNT component of DNT dose.

Study, species, (strain)	Sex and weight (kg)	Dose (mg/kg-d) Total DNT applied dose	2,4-DNT component of DNT dose	2,6-DNT component of DNT dose	Tumor Type[a]	Incidence	95% UCL potency values in kg-d/mg of 2,6-DNT BW[b]	SA[c]
Leonard et al. 1987[d] Rats (F-344)	Male 0.325	0	0	0	HCC	0/20	3.4	20
		7.0	0	7.0		17/20		
		14.0	0	14.0		20/20		
		0	0	0	HCC or NN	0/20	6.0	35.8
		7.0	0	7.0		19/20		
		14.0	0	14.0		20/20		
		0	0	0	HCC or NN	0/20	3.0	18
		35.0	26.6	6.6		15/19		
		0	0	0	HCC	0/20	1.30	7.8
		35.0	26.6	6.6		9/19		
CIIT, 1982[e] Rats (F-344) (albino CD-F)	M 0.32	0	0	0	HCC or NN	10/61	0.224	1.3
		3.388	2.588[f]	0.637[f]		19/70[g]		
		13.451	10.276	2.529		23/23[g]		
	F 0.24	0	0	0		5/57	0.19	1.3
		3.379	2.581[e]	0.637[f]		12/61		
		13.633	10.416	2.563		66/68		

Table C-1. (Continued).

Study, species, (strain)	Sex and weight (kg)	Dose (mg/kg-d) Total DNT applied dose	2,4-DNT component of DNT dose	2,6-DNT component of DNT dose	Tumor Type[a]	Incidence	95% UCL potency values in kg-d/mg of 2,6-DNT BW[b]	SA[c]
Ellis *et al.*, 1979[h] Rat (CD Charles River)	M 0.6	0	0	0	NN or HCC	1/25		
		0.575	0.563[i]	0.0112[i]		2/28		
		3.92	3.84	0.08		2/19	0.73	3.5
		34.5	33.8	0.7		8/29		
	F 0.41	0	0	0	0/23			
		0.706	0.69[i]	0.014[i]		0/35		
		5.14	5.0	0.1		1/27	1.6	9.5
		45.3	44.4	0.9		19/35		
	M 0.6	0	0	0	HCC	1/25[j]		
		0.575	0.563[i]	0.0112[i]		2/28		
		3.92	3.84	0.08		2/19[j]	0.55	2.7
		34.5	33.8	0.7		6/29		
	F 0.41	0	0	0		0/23		
		0.706	0.69[i]	0.014[i]		0/35		
		5.14	5.0	0.1		1/27	1.0	5.8
		45.3	44.4	0.9		19/35[j]		

Footnotes for Table C-1.

a HCC = Hepatocellular carcinoma, NN = Neoplastic nodule.

b BW = Body weight interspecies dose extrapolation method; equipotent doses assumed to be in mg/kg.

c SA = surface area interspecies dose extrapolation method; equipotent doses assumed to be in mg/kg $^{2/3}$.

d Twelve month chronic study using male F-344 rats.

e A 24 month chronic study with serial sacrifices (hepatocellular carcinomas observed in males at the week 26 sacrafice and in females at the week 55 sacrifice).

f Percents of 2,4-DNT (76%) and 2,6-DNT (19%) in the tDNT used in this study also listed in Rickert et al. (1984) and not in the original report CIIT (1982).

g Tumor incidence at the week 104 scheduled sacrifice was used. The high-dose group, in which all animals were sacrificed at 55 weeks, was not used.

h Two-year study using CD rats. Incidence data are from the 24-month scheduled sacrifice and also include unscheduled deaths.

i Percents of 2,4-DNT (98%) and 2,6-DNT (2%) in the DNT formulation used in this study are given in the original report. The dose values given here have been calculated from those percentages.

j Incidence derived from raw data tables in Ellis et al. (1979). These figures are slightly different from those in the summary table presented in original report. The summary table figures are as follows: 0/28 (low-dose males), 1/19 (mid-dose males), 18/34 (high-dose females), respectively.

Table C-2. Carcinogenic potencies for 2,6-DNT in TNT.

Study, species, (strain)	Sex and weight (kg)	Dose (mg/kg-d) Total DNT applied dose	2,4-DNT component of DNT dose[a]	2,6-DNT component of DNT dose[a]	Tumor Type	Incidence	95% UCL potency values in kg-d/mg of 2,6-DNT BW[b]	SA[c]
Furedi *et al.* (1984a) Rat (F-344)	F	0.0	0.0	0.0	Urinary	0/54		
	0.29	0.394	0.002	0.001	bladder:	0/54		
		1.970	0.01	0.005	papilloma	0/55	2.0	13
		9.908	0.05	0.025	or	1/55		
		49.245	0.25	0.123	carcinoma	17/55		
Furedi *et al.* (1984b) Mice (B6C3F1)	F	0.0	0.0	0.0	Spleen	9/54		
	0.038	1.509	0.0075	0.00375	leukemia	15/54	2.7	33
		10.10	0.05	0.025	or malignant	17/54		
		70.90	0.35	0.175	lymphoma	21/54		
Furedi *et al.* (1984a) Rat (F-344)	F	0.0	0.0	0.0	Urinary	0/54		
	0.29	0.394	0.002	0.001	bladder:	0/54		
		1.970	0.01	0.005	carcinoma	0/55	0.80	5.0
		9.908	0.05	0.025		0/55		
		49.245	0.25	0.123		12/55		

[a] Maximum amounts of 2,4-DNT are approximatley 0.5 wt% in TNT(m) and 0.25 wt% for 2,6-DNT (see Table 2-1).

[b] BW = body weight interspecies dose extrapolation method; doses in mg/kg are assumed to be equipotent.

[c] SA = Surface area interspecies dose extrapolation method; doses in $mg/kg^{2/3}$ are assumed to be equipotent.

period of one to several days (Long and Rickert, 1982; Rickert et al., 1981; Levine et al., 1985a); measurable amounts of unmetabolized DNT have been observed to be excreted in occupationally exposed humans, but not in experimentally exposed rats (Turner et al., 1985). In the absence of adequate pharmacokinetic data on DNT metabolism in both rodents (used in the bioassays considered here) and in humans (the target population for whom risk is to be extrapolated from rodent bioassay data), the TWA administered dose of DNT is used as input to the potency analysis. This is equivalent to the assumption that a constant fraction of the applied TWA dose is consistently converted to the effective metabolized or metabolite dose in both rodents and humans, and that this fraction is the same for both rodents and humans. We note, however, that Turner et al. (1985) did find that there are differences in DNT metabolism (see section on metabolism in Sections 4 and 5) between humans and rodents.

For the purpose of potency assessment by the "linearized" multistage model described below, it is appropriate to adjust tumor incidence data for any significantly increased tumor type in order to provide an approximate control for competing causes of death that might act to eliminate animals before they have a chance to get tumors of that type. This is done by removing from all incidence denominators those animals dying prior to the occurrence of the first tumor of the type being counted. This type of adjustment was not performed on any of the bioassay data sets considered here, primarily because the relevant time-to-tumors information was not available to us. Time-to-tumors information was available for the Ellis et al. (1979) and CIIT (1982) studies, but the information indicated that adjustment was not necessary for the data on tumor incidence from these two studies.

CARCINOGENIC POTENCY EXTRAPOLATION BASED ON ANIMAL BIOASSAY DATA

Recall that carcinogenic "potency" refers to a quantitative expression of increased tumorigenic response per unit of incurred dose at very low dose levels, and that the following carcinogenic potency assessment is based on a quantitative analysis of animal bioassay data sets under the assumption that 2,6-DNT is carcinogenic to both animals and humans at the low environmental dose levels of potential concern. The rationale for using this assumption for a suspect human carcinogen, such as 2,6-DNT, is the potential for this

compound, or one of its reactive metabolites, to covalently bind to critical cellular macromolecules in an "independent hit" fashion, which is presumed to be necessary by the "linearized" multistage dose-response extrapolation model used in this potency assessment. This rationale is discussed in detail elsewhere (e.g., U.S. EPA, 1986; Anderson et al., 1983; CDHS, 1985). Arguments against using this assumption in dose-response extrapolation focus on the possibility that observed carcinogenicity of compounds like TNT in bioassays conducted at high doses may be caused primarily by increased cellular and/or subcellular (e.g., peroxisome) proliferation (i.e., by tumor promotion or some epigenetic mechanism, rather than by initiation or some genotoxic mechanism), and that this induced cell behavior has a threshold-type dose-response relationship.

Low-dose potency extrapolation from dichotomous tumor-response information in animal bioassay data sets was performed with a version of the computer program GLOBAL79 (Crump and Watson, 1979) to numerically fit parameters to the multistage dose-response extrapolation model where

$$\text{Increased risk} = R = 1 - e^{-\sum_{i=1}^{g} q_i D^i} \qquad \text{(C-1)}$$

in which g is the number of exposed groups in the bioassay, D is the dose level in mg/kg-d at which the risk function is evaluated, and the q_i values are the multistage parameters (with q_1 being the low-dose "potency" parameter) in $(\text{mg/kg-d})^{-1}$ to be fitted, in accordance with a widely used regulatory procedure (Anderson et al., 1983; CDHS, 1985). The input to this program for each data set consisted of the values for total lifetime TWA applied dose, D (in mg/kg-d) and corresponding tumor incidence data given in Tables C-1 and C-2. For each data set, we used GLOBAL79 to calculate a one-tailed 95% upper-confidence limit (UCL) for the linear parameter, q_1, of the multistage model, denoted $q_1^*(D)$ in units of $(\text{mg/kg-d})^{-1}$. At any given, very low dose, the multistage model predicts an increased risk that is approximately equal to the product of q_1 and dose. Calculated values of $q_1^*(D)$, corresponding to each of the bioassay data sets considered here are given in the Tables C-1 and C-2. Note that, according to the multistage model implemented in GLOBAL79, the value of q_1 must be greater than or equal to 0, whereas the value of q_1^* is restricted to being greater than 0.

Interspecies Dose Equivalence

Following the suggestion of Mantel and Schneiderman (1975), the U.S. Environmental Protection Agency and similar agencies in many states assume, for regulatory purposes, that mg/(surface area) is an equivalent measure of lifetime TWA dose between species for carcinogens (Anderson et al., 1983). Specifically, for purposes of carcinogen risk assessment, the EPA assumes that a TWA lifetime dose expressed as a dose rate in units of $mg/kg^{2/3}$-d is equivalent between species, because to a close approximation, surface area is proportional to the 2/3rd power of body weight, as would be the case for perfect spheres of equal uniform density. Given this assumption and a lifetime dose rate for an animal of D_a mg/kg-d, then the equivalent human TWA dose rate would be M_a/f, where the dose equivalence factor f is here given by

$$f = \left[\frac{W_a}{W_h} \right]^{1/3} , \qquad \text{(C-2)}$$

in which W_h and W_a are the weights of humans (generally assumed to be 70 kg) and the test animal, respectively.

Analyses of available bioassay data suggest that extrapolation of lifetime animal cancer risk estimates based on either a mg/(surface area) or a mg/kg dosage scale may lead to reasonable projections of corresponding human cancer risk (Crouch and Wilson, 1979; Hogan and Hoel, 1982; Crouch, 1983). However, the basis upon which interspecies potency correlations were made on either dosage scale in these particular studies has been called into question (Bernstein et al., 1985). The Mantel and Schneiderman (1975) suggestion relied on by the U.S. Environmental Protection Agency explicitly offered only a "first approximation...[where the] basic assumption is that the locus of action of any drug is on some surface area" -- an approximation that was suggested for use because "[n]o suitable data exist on the quantitative extrapolation of carcinogenic effects from animal to man." But following the logic of the original Mantel and Schneiderman suggestion, it would be reasonable to use a mg/kg dose-equivalence assumption whenever a chemical's carcinogenic effect could reasonably be attributed to steady-state action at potential sites distributed throughout a target tissue mass. Even if the suspected susceptible cellular targets are restricted to lie within a thin layer in susceptible

organs (as may be the case for certain stem-cell populations), the mg/kg dose-equivalence assumption would still be appropriate if the dose is fairly equally distributed throughout the body mass, rather than concentrated within the susceptible layer, for its duration in the body.

In the absence of definitive empirical data, however, it is simply not known which interspecies dose-extrapolation assumption better reflects reality in the context of extrapolating tumor response data in animals to anticipated response in humans. Existing data do not rule out either approach. Thus, extrapolation of potency values from animals to humans is here carried out using both a dose per body weight (BW) and a dose per body surface area (SA) extrapolation method.

Potency Assessment for 2,6-DNT

The results of the potency assessments for all the chronic bioassays with technical grade DNT and pure 2,6-DNT are shown in Table C-1. Potencies of 2,6-DNT ranged from 0.19 to 6.0 kg-d/mg, a factor of over 30 different (i.e., 6/0.19). If 2,6-DNT were indeed the isomer that accounts for the tumorgenic responses observed in the animal bioassays, then we would expect to have less variability in the 2,6-DNT potencies than in the 2,4-DNT potencies. To examine this, we calculated the potencies for 2,4-DNT by multiplying the potencies (based on body weight) for 2,6-DNT in Table C-1 by the ratios of the 2,6-DNT dose rates to the 2,4-DNT dose rates, which is justified because only a linear transformation in applied dose is involved. The resulting 2,4-DNT potencies ranged from 0.011 to 0.74 kg-d/mg, a range of 67. That this range is more than two times greater than the range of calculated 2,6-DNT potencies is qualitatively consistent with the hypothesis that 2,6-DNT alone can explain the tumor-response data considered here. To further test this hypothesis, we calculated the ratio of the geometric variance of the 2,4-DNT potencies to that of the 2,6-DNT potencies. This ratio is 2.28, which is the critical value of an F-distribution with seven degrees of freedom and α equal to 0.15. Although not conclusive, this analysis is also consistent with the hypothesis that 2,6-DNT is the active isomer.

We also calculated the potencies of 2,6-DNT in TNT(m) administered to rats and mice (see Table C-2). These potencies are well within the range of the values presented in Table C-1, again suggesting that 2,6-DNT could be the

agent responsible for tumorigenesis. The tumor sites involved in TNT(m)-induced carcinogenesis, however, were different. One possible explanation for the latter difference is that the pharmacokinetics of the 2,6-DNT were affected by the comparatively large amount of TNT administered so that the site of action was altered. Alternatively, another impurity in the TNT(m) could be responsible for the observed tumors.

APPENDIX D

ESTIMATION OF DAILY INTAKES FOR TOXIC SUBSTANCES LACKING CHRONIC TOXICITY DATA

Several of the explosives and associated co-contaminants addressed in the data-base assessments have limited toxicological data. This presents a problem in situations where decisions need to be made regarding "safe" levels of those substances in soils, water, etc. Normally, a safe intake level or acceptable daily intake (ADI) is calculated from a no-observed-effect-level (NOEL) defined in a chronic toxicity study conducted with laboratory animals. A safety or uncertainty factor is used to adjust the NOEL intake rate (mg-kg/d) to a safe intake for humans, that is,

$$ADI = \frac{NOEL}{SF} \quad . \qquad \text{(D-1)}$$

Often the only type of toxiological data on a substance is an oral LD50 (i.e., the median lethal dose to a population of laboratory animals). To determine whether this parameter could be used to derive lower-bound (LB) estimates of the chronic NOEL's of organic chemicals, we statistically analyzed the ratios of chronic NOEL's to oral LD50's for sets of organic chemicals administered to different test species (i.e., rat, mouse, guinea pig, cat, dog, rabbit, hamster, and monkey) (see Layton et al., 1987). Based on our statistical analyses, the NOEL (mg/kg-d)/LD50 (mg/kg) ratios representing the 5th cumulative percentile of lognormal distributions ranged from approximately 5×10^{-4} to 1×10^{-3} d^{-1}. The ratios were then used to estimate a LB-ADI from

$$LB\text{-}ADI = \frac{R(p) \times LD50\ (oral)}{SF} \quad . \qquad \text{(D-2)}$$

where R (p) is a NOEL/LD50 ratio that corresponds to a specific cumulative percentile value p on a lognormal distribution. With a safety factor of 100, the resulting application factors for estimating a LB-ADI (see Eq. D-2)

from an oral LD50 range from 5×10^{-6} to 1×10^{-5} d^{-1}. For the purposes of our data-base assessments, we have used a value of 1×10^{-5} d^{-1}, which will yield LB-ADI values that have a high probability (~95%) of being below an ADI based on a chronic NOEL--if one were available in the literature.

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