TOXICOLOGICAL PROFILE FOR DINITROCRESOLS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

August 1995

DISCLAIMER

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UPDATE STATEMENT

Toxicological profiles are revised and republished as necessary, but no less than once every three years. For information regarding the update status of previously released profiles contact ATSDR at:

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Atlanta, Georgia 30333

FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by ATSDR and EPA. The original guidelines were published in the Federal Register on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance being described. Each profile identifies and reviews the key literature (that has been peer-reviewed) that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

Each toxicological profile begins with a public health statement, that describes in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protect public health will be identified by ATSDR and EPA. The focus of the profiles is on health and toxicologic information; therefore, we have included this information in the beginning of the document.

Each profile must include the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects.

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects.

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the federal, state, and local levels, interested private sector organizations and groups, and members of the public.

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the Environmental Protection Agency (EPA). The availability of the revised priority list of 275 hazardous substances was announced in the Federal Register on February 28, 1994 (59 FR 9486). For prior versions of the list of substances, see Federal Register notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); and October 17, 1991 (56 FR 52166); and October 28, 1992 (57 FR 48801).

Foreword

Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

This profile reflects our assessment of all relevant toxicologic testing and information that has been peer reviewed. It has been reviewed by scientists from ATSDR, the Centers for Disease Control and Prevention (CDC), and other federal agencies. It has also been reviewed by a panel of nongovernment peer reviewers and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Green Border Review. Green Border review assures consistency with ATSDR policy.

2. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

3. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific minimal risk levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

4. Quality Assurance Review. The Quality Assurance Branch assures that consistency across profiles is maintained, identifies any significant problems in format or content, and establishes that Guidance has been followed.

PEER REVIEW

A peer review panel was assembled for dinitrocresols. The panel consisted of the following members:

1. Dr. Martin Alexander, Professor of Soil Microbiology, Cornell University, Ithaca, New York;

2. Dr. Arthur Gregory, Private Consultant, Techto Enterprises, Sterling, Virginia; and

3. Dr. Lyman Skory, Private Consultant, Skory Consulting, Midland, Michigan.

These experts collectively have knowledge of the dinitrocresols' physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(i)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. PUBLIC HEALTH STATEMENT

This statement was prepared to give you information about dinitrocresols and to emphasize the human health effects that may result from exposure to them. The Environmental Protection Agency (EPA) has identified 1,350 hazardous waste sites as the most serious in the nation. These sites make up the National Priorities List (NPL) and are the sites targeted for long-term federal cleanup activities. Dinitrocresols have been found in at least 50 of the sites on the NPL. However, the number of NPL sites evaluated for dinitrocresols is not known. As EPA evaluates more sites, the number of sites at which dinitrocresols is found may increase. This information is important because exposure to dinitrocresols may cause harmful health effects and because these sites are potential or actual sources of human exposure to dinitrocresols.

When a substance is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. This release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking substances containing the substance or by skin contact with it.

If you are exposed to substances such as dinitrocresols, many factors will determine whether harmful health effects will occur and what the type and severity of those health effects will be. These factors include the dose (how much), the duration (how long), the route or pathway by which you are exposed (breathing, eating, drinking, or skin contact), the other chemicals to which you are exposed, and your individual characteristics such as age, sex, nutritional status, family traits, lifestyle, and state of health.

1.1 WHAT ARE DINITROCRESOLS?

Dinitrocresols are a group of organic chemicals that can contain up to 18 individual compounds. This document contains information on mainly one dinitrocresol that is commercially most important. This dinitrocresol is called 4,6-dinitro-*o*-cresol and is

abbreviated as DNOC. Industries manufacture dinitrocresols, and this is the major source of exposure. DNOC is sold under many trade names, some of which are Antinonnin, Detal, and Dinitrol. EPA has canceled the registration of these pesticides. DNOC is a yellow solid with no smell. The taste of DNOC is not known. It dissolves slightly in water. DNOC in water and soil does not easily evaporate to air. DNOC was primarily used to protect fruit trees and other food crops from insect damage. Another less expensive chemical that is more effective in controlling pests is replacing DNOC. In the 1930s, DNOC was used in pills for reducing weight. It is no longer used for this purpose because of bad effects on health. You will find further information on the physical properties and uses of DNOC in Chapters 3 and 4 of this profile.

1.2 WHAT HAPPENS TO DINITROCRESOLS WHEN THEY ENTER THE ENVIRONMENT?

DNOC enters the air, water, and soil during its manufacture and transport. It also enters the environment when formulated products are prepared and used. Very small amounts of DNOC may form in the atmosphere in the presence of other compounds. Wastes containing DNOC are produced during its manufacture and use. These DNOC-containing wastes are often disposed in landfills. DNOC enters the environment from these landfills. DNOC also enters the environment from accidental spills during manufacture and transport and from leaks during storage.

DNOC destruction in air from chemical reactions with other pollutants or from interaction with sunlight may be insignificant. It eventually returns from air to land and water by settling and washout by snow and rainwater. We do not know how long DNOC stays in the air before it is fully removed. No known chemical reaction removes significant amounts of DNOC from water. DNOC in water may be broken down slowly by microorganisms. DNOC does not appreciably evaporate from water. Some of the DNOC sticks to particles present in water. This process partially transfers DNOC from water to the bottom sediment. When DNOC was accidentally spilled into the Rhine River in Germany, the level of DNOC in water decreased to half its initial value in an estimated 30 days. No known chemical reaction

removes significant amounts of DNOC from soil. Microorganisms break down DNOC in soil. The loss of DNOC from soil by evaporation is not significant. DNOC has been found in groundwater from fields where it was applied. The level of DNOC in soil may decrease to half its original level in an estimated 14 days to 1 month or longer. You will find further information about the fate and movement of DNOC in the environment in Chapter 5.

1.3 HOW MIGHT I BE EXPOSED TO DINITROCRESOLS?

People can be exposed to DNOC by breathing contaminated air, drinking contaminated water, or eating contaminated food. Other than in certain workplaces, levels of DNOC in the air we commonly breathe in the United States have not been measured. However, the ambient level is expected to be very low. The levels of DNOC in drinking water and food also have not been detected. Certain people may be exposed to slightly higher levels of DNOC. People who live near sites containing DNOC wastes may be exposed primarily by breathing contaminated air. Children playing at or near these sites will be exposed by touching and eating soil if that soil contains DNOC. You may be exposed to DNOC if your work involves manufacturing, preparing, or using formulated DNOC products. You may be exposed if you work as a sprayer of DNOC. You also may be exposed to DNOC if your work involves incinerating waste containing DNOC or cleaning up sites contaminated with DNOC. According to one study, the estimated skin contact of workers spraying apple orchards was 22.5 milligrams of DNOC per hour (mg/hour) (1 milligram is one thousandth of a gram or a 30,000th fraction of an ounce). The workers also breathed less than 0.05-0.4 mg DNOC per hour while spraying. The blood and urine of some of the spray operators contained DNOC. You will find more information about DNOC exposure in Chapter 5 of this profile.

1.4 HOW CAN DINITROCRESOLS ENTER AND LEAVE MY BODY?

DNOC can readily enter your body through the lungs if breathed in, through the stomach and intestines if swallowed, or through the skin if touched. The amount of DNOC that enters your body depends on the amount in air, food, and water, and the length of time you are exposed. After DNOC enters your body, your blood can carry it to your lungs, brain, liver,

kidneys, spleen, muscles, and heart. DNOC can build up in these organs and tissues if you are repeatedly exposed. Animal studies show that DNOC is broken down to harmless products that do not cause health effects, but leave the body in urine, feces, and exhaled air. We do not know whether DNOC is broken down in people the same way it is in animals. However, we do know that DNOC leaves the bodies of people more slowly than it leaves the bodies of animals. This might mean there are other differences in the way people and animals break it down. DNOC can be found in human urine for as long as 20 days after the last exposure. Chapter 2 provides more information on how DNOC enters and leaves your body.

1.5 HOW CAN DINITROCRESOLS AFFECT MY HEALTH?

Adverse health effects can result from breathing too much DNOC, from excessive skin contact, and from swallowing too much of it. Some of what we know about how DNOC can affect your health comes from reports of workers who became ill after making DNOC in factories or spraying it on crops. These workers breathed in the DNOC dusts or had skin contact with it, but we do not know to how much they were exposed. Most of what we know about how DNOC can affect your health comes from old reports from doctors who prescribed DNOC for their patients who wanted to lose weight. DNOC has not been used as a diet pill for almost 60 years because of the harmful effects to those patients. The amount of DNOC that the patients took in pill form was as low as 0.35 milligram of DNOC per kilogram of body weight per day (mg/kg/day). DNOC increases your basal metabolic rate, which can increase your pulse and heart rates, and cause profuse sweating and fever. These effects can occur after breathing in, swallowing, or having skin contact with DNOC for a short period. DNOC also may make it difficult for you to breathe and causes headaches, drowsiness, dizziness, and weight loss. DNOC stains the whites of the eyes and the skin yellow, and can cause mild damage to the stomach, the kidneys, and the liver. If swallowed for long periods, DNOC may cause cataracts in your eyes and skin rashes. If you breathe in, swallow, or have skin contact with large amounts of DNOC for short periods, you could have convulsions, become unconscious, and even die. High environmental temperatures, such as in tropical climates, can worsen these effects.

1. PUBLIC HEALTH STATEMENT

DNOC causes similar health effects in animals. In addition, injection of other dinitrocresols into animals caused similar effects. High environmental temperatures can worsen the harmful effects in some animals that swallow DNOC. Some animals exposed to DNOC for a long period show blood cell changes. Ducklings given high levels of DNOC in the diet for a short period developed cataracts.

We do not know whether DNOC causes reproductive effects, birth defects, or cancer in people. One animal study suggests that swallowing DNOC may decrease the number of sperm in the testes of males or cause damage to the ovaries of females. Swallowing DNOC does not appear to cause developmental effects in animals. We do not know whether DNOC causes cancer in animals. More information on the harmful effects of DNOC can be found in Chapter 2.

1.6 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO DINITROCRESOLS?

DNOC can be measured in the blood, urine, and feces of exposed people. DNOC has been detected in human blood as many as 40 days after the last dose was swallowed. Measuring the amount of DNOC in blood may not be a reliable test to determine how much DNOC you were exposed to or for how long, but it can be used to predict whether you would experience harmful effects, such as headache and depression. DNOC has been found in urine for more than 13-20 days after the last exposure, but much of the DNOC also remains in the body. This means that measuring DNOC in the urine may not be a reliable test to determine how much you were exposed to or for how long. Testing urine can determine only whether or not you have been exposed to DNOC, not whether you will experience any harmful health effects. Breakdown products of DNOC are reported one study of human exposure and have been found in the urine of exposed animals. Yellow-stained skin and eyes can alert a doctor that you may have been exposed to DNOC, but other similar chemicals also cause yellow staining. Chapters 2 and 6 provide more information on medical tests.

1. PUBLIC HEALTH STATEMENT

1.7 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

EPA lists DNOC as a hazardous air pollutant. The Occupational Safety and Health Administration (OSHA) regulates DNOC levels in the workplace. The occupational exposure limit for an S-hour workday, 40-hour workweek, is 0.2 milligrams of DNOC per cubic meter of air (mg/m^3). The National Institute for Occupational Safety and Health (NIOSH) recommends that exposure in air not exceed 0.2 mg $DNOC/m^3$ for a l0-hour workday, 40-hour workweek.

Federal regulations limit the amount of DNOC that factories can release into waste water. The EPA requires industries to report releases or spills of 10 pounds or more. For more information on recommendations of the federal government, please see Chapter 7.

1.8 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department or:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE, E-29
Atlanta, Georgia 30333
(404) 639-6000

This agency can also provide you with information on the location of occupational and environmental health clinics. These clinics specialize in the recognition, evaluation, and treatment of illness resulting from exposure to hazardous substances.

2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of dinitrocresols. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure -inhalation, oral, and dermal; and then by health effect--death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt

at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for 4,6-dinitro-*o*-cresol. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1989), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2. HEALTH EFFECTS

Studies regarding toxic effects of dinitrocresols conducted by the inhalation, oral, or dermal routes of exposure were located only for 4,6-dinitro-*o*-cresol. Therefore, the focus of this profile is on 4,6-dinitro-*o*-cresol. As discussed in Section 2.4, 4,6-dinitro-*m*-cresol and 2,6-dinitro-*p*-cresol have been tested for toxicity in animals by parenteral routes and for genotoxicity in bacteria in only a few studies. These studies indicate that 2,6-dinitro-*p*-cresol is similar to 4,6-DNOC in potency and action, but 4,6-dinitro-*m*-cresol is the least toxic. 4,6-Dinitro-*o*-cresol is the most industrially and toxicologically important isomer since it is used as a pesticide and was used in the past as a weight reducing drug. It is commonly called DNOC, the name used in this profile. It should be noted that in the United Kingdom, 4,6-dinitro-*o*-cresol (or more correctly 2-methyl-4,6-dinitrophenol) is often called 3,5-dinitro-*o*-cresol, which is not to be confused with genuine 3,5-dinitro-*o*-cresol (or more correctly 2-methyl-3,5-dinitrophenol) (King and Harvey 1953a). In addition, ChemID (1993) lists 2,4-dinitro-*o*-cresol as a synonym for 4,6-dinitro-*o*-cresol.

2.2.1 Inhalation Exposure

2.2.1.l Death

Information regarding death of humans after inhalation exposure to DNOC is limited. A case report of a spray operator who inhaled a dense DNOC mist for an unspecified, but apparently acute, duration noted that he died after lapsing into a coma while being treated in a hospital (van Noort et al. 1960). In a survey of 133 spray operators who applied DNOC to cereal crops 5 days per week for 6 weeks, 4 developed signs of acute poisoning (not otherwise specified), one of whom died (Bidstrup et al. 1952). The amount or concentration of inhaled DNOC was not reported in the survey.

Only one study was located regarding death in animals after inhalation exposure to DNOC aerosols (Burkatskaya 1965a). In this study, 1 of 3 and 2 of 6 cats died after being exposed to 40 and 100 mg/m^3 of an aerosol of DNOC solution for 4 hours, respectively. Two of six cats died after being exposed to 100 mg/m^3 DNOC solid aerosols (dusts). The data suggest that the DNOC solution aerosol was no more toxic than the dust. In addition, 2 of 3 cats died after being exposed to an aerosol of 2.0 mg/m^3 DNOC in solution 4 hours/day for =l month.

2. HEALTH EFFECTS

2.2.1.2 Systemic Effects

Studies regarding systemic effects in humans and animals after inhalation exposure to DNOC are described below. In the studies reporting effects in humans, exposure concentrations were not known. Only two inhalation studies were located regarding systemic effects in animals. The highest NOAEL values and all LOAEL values from each reliable study for systemic end points in each species and duration category are recorded in Table 2-l and plotted in Figure 2- 1.

Respiratory Effects. Respiratory effects have been observed in both humans and animals following inhalation exposure to DNOC. The limited data suggest that respiratory rates were increased due to DNOC exposure. It is possible that DNOC may act as a respiratory stimulant. Shortly after an acute exposure to a dense DNOC mist, a spray operator became dyspneic and had an elevated respiration rate (van Noort et al. 1960). A male factory worker who had been pouring DNOC powder for 17 days became dyspneic and weak (Hunter 1950). His hands and feet were stained yellow, suggesting dermal exposure. In addition, the employee reported that he had periodically inhaled DNOC aerosols. Respiratory rates were slightly elevated in an employee involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal.

Respiratory rates were also increased in rats exposed to 100 mg/m^3 DNOC for 4 hours (King and Harvey 1953a). Respiratory rates were still elevated 20 hours after the rats were removed from the DNOC aerosols. Dyspnea, sneezing, and/or nasal secretions were observed in cats that were exposed to aerosols of DNOC in solution at 36 mg/m3 or as a dust at 40 mg/m^3 for 4 hours (Burkatskaya 1965a).

Cardiovascular Effects. Elevated pulse rates have been observed in humans exposed to DNOC by inhalation. A male factory worker who had been employed for 17 days pouring DNOC powder had a pulse rate of 130 beats per minute (Hunter 1950). Although his yellow-stained hands and feet indicated dermal exposure, he reported that he had periodically inhaled DNOC aerosols. A pulse rate of 100 beats per minute, a blood pressure of 155/70 mm Hg, and a normal electrocardiogram were found for an employee who was involved with mixing DNOC, refilling sprayer tanks with DNOC, and

TABLE 2-1. Levels of Significant Exposure to Dinitrocresol - Inhalation

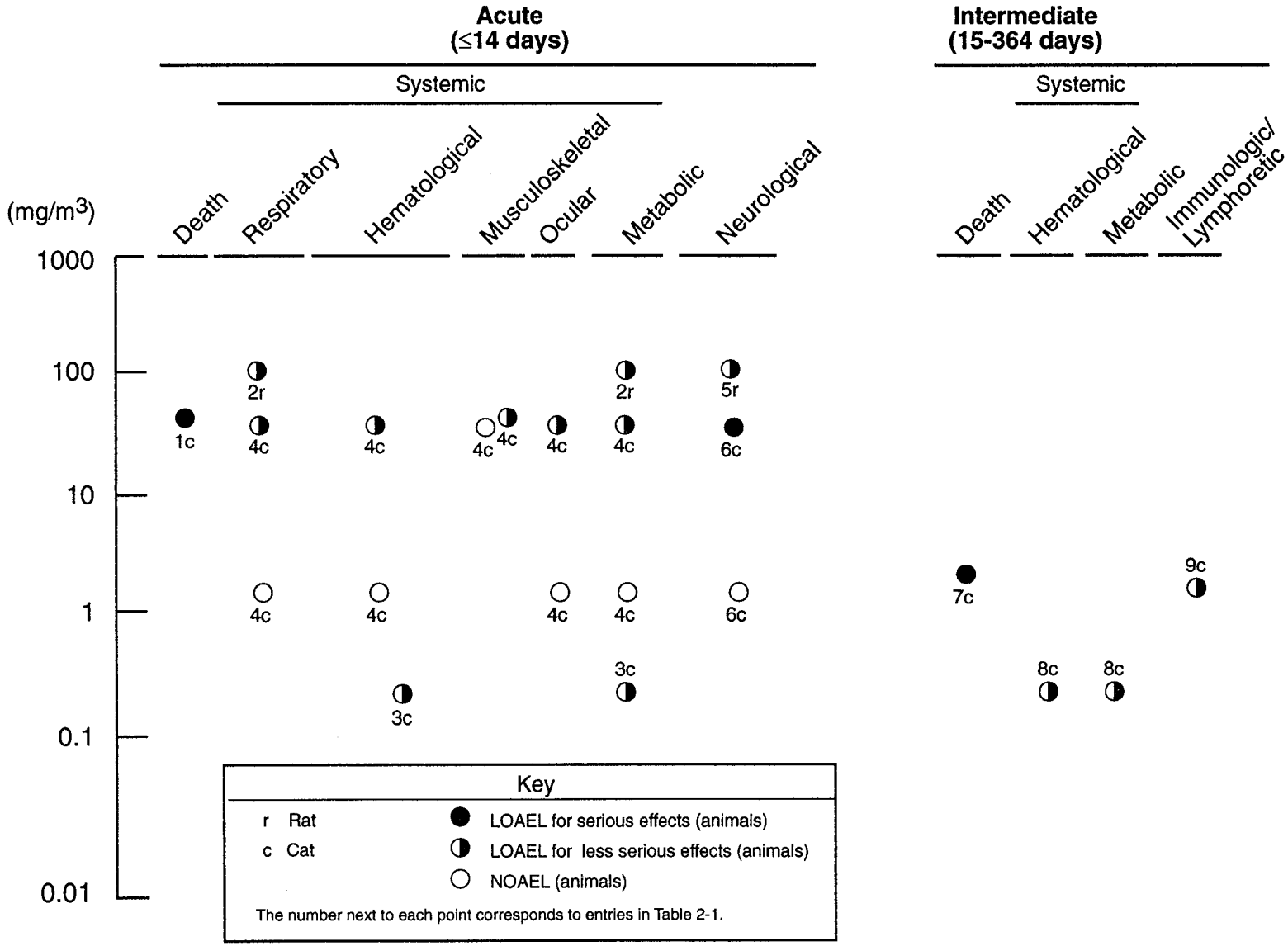
Key[a] to figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
	ACUTE EXPOSURE						
	Death						
1	Cat (NS)	4hr				40 (1/3 died)	Burkatskaya 1965a (4,6-DNOC)
	Systemic						
2	Rat (albino and hooded)	4-5 hr	Resp		100 (10% increase in respiration rates 16 hours after exposure)		King and Harvey 1953a (4,6-DNOC)
			Metabolic		100 (0.7 °C increase in body temperatures 16 hours after exposure)		
3	Cat (NS)	1-2 wk 4hr/d	Hemato		0.2 (acceleration of erythrocyte sedimentation rate; increased leukocyte count)		Burkatskaya 1965a (4,6-DNOC)
			Metabolic		0.2 (increased blood sugar)		
4	Cat (NS)	4hr	Resp	1.4	36 (dyspnea, sneezing, nasal secretions)		Burkatskaya 1965a (4,6-DNOC)
			Hemato	1.4	36 (accelerated erythrocyte sedimentation rate, increased leukocyte count)		
			Musc/skel	36	40 (loss of muscle tone)		
			Ocular	1.4	36 (lacrimation and blepharospasm)		
			Metabolic	1.4	36 (increased body temperature, anorexia, 20-25% increase in blood sugar)		

TABLE 2-1. Levels of Significant Exposure to Dinitrocresol - Inhalation (continued)

Key[a] to figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)		LOAEL Serious (mg/m3)		Reference
	Neurological								
5	Rat (albino)	4-5 hr			100	(lethargy)			King and Harvey 1953a (4,6-DNOC)
6	Cat (NS)	4hr		1.4			36	(twitching and tremors, ataxia, sluggishness)	Burkatskaya 1965a (4,6-DNOC)
	INTERMEDIATE EXPOSURE								
	Death								
7	Cat (NS)	1-3 mo 4hr/d					2.0	(2/3 died)	Burkatskaya 1965a (4,6-DNOC)
	Systemic								
8	Cat (NS)	1 month 4hr/d	Hemato		0.2	(acceleration of erythrocyte sedimentation rate; decrease in erythrocyte and hemoglobin levels; increased leukocyte count)			Burkatskaya 1965a (4,6-DNOC)
			Metabolic		0.2	(increased blood sugar)			
	Immuno./Lymphor								
9	Cat (NS)	1 month 4hr/d			2	(increased leukocyte count; change in differential white count; increase in % neutrophils; decrease in % lymphocytes)			Burkatskaya 1965a (4,6-DNOC)

[a]The number corresponds to entries in Figure 2-1.
d = day(s); h = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory

Figure 2-1. Levels of Significant Exposure to 4,6-Dinitro-o-cresol – Inhalation



occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal.

No studies were located regarding cardiovascular effects in animals after inhalation exposure to DNOC.

Gastrointestinal Effects. A spray operator who subsequently died after exposure to a dense DNOC mist for an acute duration complained of nausea (van Noort et al. 1960). However, vomiting or other gastrointestinal effects were not reported in this employee.

No studies were located regarding gastrointestinal effects in animals after inhalation exposure to DNOC.

Hematological Effects. No abnormal hematological parameters were observed in an employee who was involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951).

In the only study located regarding hematological effects in animals after inhalation exposure to DNOC, significantly decreased hemoglobin content and erythrocyte counts were observed in cats exposed to an aerosol of DNOC dust at 36 mg/m^3 or DNOC solution (mist) at 40 mg/m^3 for 4 hours (Burkatskaya 1965a). In addition, accelerated erythrocyte sedimentation rates and increased leucocyte counts were found in the cats exposed to the dust. In the same study, similar hematological effects were observed when the cats were exposed to DNOC dust at 0.2 mg/m^3 for 2 or 3 months. In the latter experiment, the hematological changes occurred within 1-2 weeks of exposure to the aerosol and were not aggravated with subsequent exposure to DNOC.

Musculoskeletal Effects. Information regarding musculoskeletal effects in humans or animals after inhalation exposure to DNOC is limited. Continuous involuntary contraction of leg muscles and pain in calf muscles were observed in a spray operator who inhaled a dense DNOC mist for an acute period (van Noort et al. 1960). Exposure to an aerosol of DNOC in solution at 40 mg/m^3 for 4 hours resulted in loss of muscle tone in cats (Burkatskaya 1965a). Similar effects in cats exposed to DNOC dust were not reported.

2. HEALTH EFFECTS

Hepatic Effects. DNOC is a yellow compound that stains human (Hunter 1950) and animal (Ambrose 1942) skin on contact. Absorption of DNOC by any route and subsequent distribution to tissues results in a characteristic yellow staining of visceral organs and tissues including the conjunctiva and sclera of the eye (Ibrahim et al. 1934; Pollard and Filbee 1957), blood serum, skeletal tissues, and urine (Ambrose 1942). The yellow staining of the skin and sclera of patients exposed to DNOC prompted physicians to test for liver effects. Results for the icteric index and the Van den Bergh tests have been consistently negative (Dodds and Robertson 1933; Gordon and Wallfield 1935; Plotz 1936), indicating that the yellow color was not due to liver damage.

No studies were located regarding hepatic effects in animals after inhalation exposure to DNOC.

Renal Effects. An elevated blood urea nitrogen (BUN) level was observed in an employee involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951). The patient's clinical history suggested that exposure was probably a combination of inhalation and dermal.

No studies were located regarding renal effects in animals after inhalation exposure to DNOC.

Dermal Effects. As noted above for Hepatic Effects, DNOC is a yellow compound that stains human (Hunter 1950; Pollard and Filbee 1951; van Noort et al. 1960) and animal (Ambrose 1942) skin on contact. While the yellow staining of the skin may be unsightly, such cosmetic effects are not regarded as adverse.

Ocular Effects. Contact with the eyes or absorption of DNOC also results in a characteristic yellow staining of the conjunctiva and sclera of the eye (Dodds and Robertson 1933; Gordon and Wallfield 1935; Ibrahim et al. 1934; Plotz 1936; Pollard and Filbee 1951). While the yellow staining of the sclera may be unsightly, such cosmetic effects are not regarded as adverse.

Blepharospasm and excessive lacrimation were observed in cats exposed to 36 or 60 mg/m^3 DNOC dust for 4 hours (Burkatskaya 1965a). Since these effects were not reported in the cats similarly exposed to a mist of DNOC in solution, they were probably due to an irritating effect of the dust particles on the eyes, rather than to DNOC per se. Furthermore, they were probably due to direct ocular contact (see Section 2.2.3.2).

2. HEALTH EFFECTS

Metabolic Effects. A primary effect of DNOC is oxidative phosphorylative uncoupling. This mitochondrial change is reflected in increased basal metabolism, increased body temperatures and the resulting increased perspiration. A spray operator who died after inhaling a dense DNOC mist for an acute period perspired profusely and had a body temperature of 38.7 ºC upon admission to the hospital and 44 ºC one-half hour after death (van Noort et al. 1960). Elevated body temperature, an 80% increase in basal metabolic rate, and profuse sweating were observed in a male factory worker who had been employed for 17 days pouring DNOC powder (Hunter 1950). Although the yellow hand and feet stains suggest dermal exposure, the employee reported inhaling DNOC aerosols periodically. Elevated body temperature (38.9 ºC), basal metabolic rate, and profuse sweating were observed in an employee who was involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal. Elevated basal metabolic rates were also observed in workers who applied DNOC to cereal crops for ≈6 weeks (Bidstrup et al. 1952).

Other Systemic Effects. Other systemic effects observed in humans and animals include effects on body temperature and blood sugar. These effects are most probably related to uncoupling of oxidative phosphorylation (see Section 2.3.5).

An elevated body temperature was observed in rats exposed to 100 mg/m^3 DNOC for 4 hours (King and Harvey 1953a). The body temperature was still elevated 20 hours after the animals were removed from DNOC aerosols. Food and water consumption was reduced during the exposure period, but was probably due to the lethargic condition of the rats (see Section 2.2.1.4).

Increased blood glucose (20-48%) was observed in cats exposed to DNOC dust at 36 mg/m^3 or to an aerosol of DNOC in solution (mist) at 40 mg/m^3 DNOC for 4 hours (Burkatskaya 1965a). Body temperatures were increased by 0.6-1.4 ºC. Increased blood glucose was also found in cats exposed to 2 mg/m^3 of the DNOC mist for 2-3 months. These increases were first noted during the first l-2 weeks of exposure.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans or animals after inhalation exposure to DNOC.

2. HEALTH EFFECTS

2.2.1.4 Neurological Effects

Although data are limited, depression and lethargy appear to be common neurological signs observed in both humans and animals exposed to DNOC aerosols. These effects are most probably related to uncoupling of oxidative phosphorylation (see Section 2.3.5).

A spray operator who had inhaled a dense DNOC mist for an acute duration developed seizures and went into a coma prior to death (van Noort et al. 1960). No tremors or exophthalmos were observed in a male factory worker who had been employed for 17 days pouring DNOC powder (Hunter 1950). Although the yellow staining of his hands and feet suggested limited dermal exposure, the employee reported having periodically inhaled DNOC aerosols. An employee who was involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks complained of headache and lassitude prior to hospital admission (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal.

Lethargy was observed in rats 30 minutes after exposure to 0.1 or 100 mg/m^3 DNOC (King and Harvey 1953a). They remained lethargic for the 4-hour duration of exposure, and drinking and eating activities were reduced. Twitching, tremors, ataxia, or sluggishness were observed in cats that were exposed to aerosols of DNOC, either as a mist of the solution or as DNOC dust, for 4 hours at concentrations ≥36 mg/m^3 (Burkatskaya 1965a). The LOAEL values for neurological effects in rats and cats is recorded in Table 2-l and plotted in Figure 2-l.

No studies were located regarding the following health effects in humans or animals after inhalation exposure to DNOC:

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2. HEALTH EFFECTS

2.2.1.8 Cancer

No studies were located regarding cancer in humans or animals after inhalation exposure to DNOC.

2.2.2 Oral Exposure

2.2.2.1 Death

Only one study was located regarding death in humans after oral exposure to DNOC (Bidstrup and Payne 1951). In this case report, a spray operator was found dead after consuming an unknown amount of DNOC from a contaminated fresh water tank. The worker had also been exposed to DNOC aerosols for 3 weeks prior to death, but the ingestion of DNOC was believed to be the cause of death.

Mortality following ingestion of DNOC was reported in studies involving rats, mice, cats, ducks, and chickens. In attempts to produce cataracts in ducks and chickens, a diet of 2,500 ppm DNOC resulted in 56% mortality among a group of ducklings (Spencer et al. 1948), and a dose of 4.95 mg/kg resulted in death of an unspecified number of chickens (Buschke 1947). LD_{50} values ranged from 25 to 40 mg/kg in rats of an unspecified sex (Ben-Dyke et al. 1970; Jones et al. 1968). Acute oral exposure of rats to DNOC doses ranging from 20 to 60 mg/kg/day has also resulted in high rates of mortality in studies not designed to determine LD_{50} values statistically (King and Harvey 1953a; Parker et al. 1951; Spencer et al. 1948). Similar results were reported when rats received oral doses of the sodium dinitro-*o*-cresol (salt) (Ambrose 1942). Single oral doses in the range of 10-35 mg/kg DNOC were lethal for 3 of 30 to 20 of 20 mice (Arustamyan 1972). Doses in the range of the derived oral LD_{50} for mice (16.4 mg/kg) caused death within 3-7 hours. A single oral dose of 50 mg/kg DNOC caused death in 50% of an unspecified number of cats, while a dose of 100 mg/kg DNOC was lethal for all cats (Burkatskaya 1965b). However, this study was reported almost in abstract form with limited experimental details and data.

Environmental temperatures influenced the mortality rate among rats orally dosed with DNOC (King and Harvey 1953a). Six of 12 rats died after receiving 20 mg/kg at 37-40 ºC, while only 2 of 12 rats died after receiving twice the dose (40 mg/kg) at almost half the temperature (20-22 ºC). Therefore, increased environmental temperatures increased the toxicity of DNOC in rats. The investigators further demonstrated that increased environmental temperatures did not alter DNOC blood levels.

2. HEALTH EFFECTS

Because DNOC uncouples oxidative phosphorylation, an increase in heat production and body temperature occurs (see Section 2.35). Elevated environmental temperatures lower the rate of heat dissipation and further exacerbate the signs of DNOC toxicity, which may become fatal.

Treatment of mice with 3 mg/kg/day DNOC resulted in 100% mortality within 8-32 days when the vehicle was water and within 9-38 days when the vehicle was oil (Arustamyan 1972). In an intermediate-duration study, 5 of 20 male and female rats died when given a diet providing 20 mg/kg/day DNOC for 90 days (Den Tonkelaar et al. 1983). The intermittent nature of exposure during feeding was less likely to cause mortality, compared to administration of a single bolus dose and probably explains why only 25% of the rats died after receiving a daily dose close to the acute LD_{50} value.

The LD_{50} values and the doses resulting in death of rats, mice, and cats in each duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

The systemic effects in humans and animals after oral exposure to DNOC are described below. The highest NOAEL values and all LOAEL values from each reliable study for systemic end points in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. Respiratory rates were not affected in volunteers who ingested 0.92-1.27 mg/kg/day for 5-7 days (Harvey et al. 1951). However, congestion, edema, and hemorrhage were observed in an employee who had accidentally ingested an unknown amount of DNOC and subsequently died (Bidstrup and Payne 1951).

Signs of respiratory distress (dyspnea and asphyxial convulsions) were observed prior to death in rats given single doses of 36-90 mg/kg DNOC (Ambrose 1942) as the sodium salt. These signs were not seen at 27 mg/kg. Mice that received single oral doses in the range of 10-35 mg/kg DNOC became dyspneic within 60-80 minutes (Arustamyan 1972). Necropsy examination revealed bloody fluid in the thoracic cavity of some mice. A single oral dose of 25 mg/kg DNOC caused accelerated heavy breathing and dyspnea in cats within the first hour (Burkatskaya 1965b). These signs persisted for

TABLE 2-2. Levels of Significant Exposure to Dinitrocresol - Oral

Key to figure [a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Rat (NS)	once (G)				27 M (10% deaths)	Ambrose 1942 (sodium 4,6-DNOC)
2	Rat (NS)	once (G)				25 (LD50)	Ben-Dyke et al. 1970; Jones et al. 1968 (4,6-DNOC)
3	Rat (NS)	once (GW)				20 (6/12 deaths at 37-40 °C)	King and Harvey 1953a (4,6-DNOC)
						40 (2/12 deaths at 20-22 °C)	
4	Rat (NS)	10 d 1x/d (GW)				25 (3/6 deaths)	King and Harvey 1953a (4,6-DNOC)
5	Rat (albino)	4-10 d ad lib (F)				60 (4/12 died)	Parker et al. 1951 (4,6-DNOC)
6	Rat (NS)	once (GO)				20 (3/20 died)	Spencer et al. 1948 (4,6-DNOC)
7	Mouse (white)	once (GW)				10 (3/30 died)	Arustamyan 1972 (4,6-DNOC)
8	Mouse (white)	once (GW)				16.4 (LD50)	Arustamyan 1972 (4,6-DNOC)

TABLE 2-2. Levels of Significant Exposure to Dinitrocresol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Systemic						
9	Human	1-4 d 1x/d (C)	Cardio	3			Dodds and Robertson 1933 (4,6-DNOC)
			Ocular		3 (unspecified ocular effects)		
			Metabolic			3 (>50% increase basal metabolic rate)	
10	Human	11 d 1x/d (C)	Cardio		2.27 F (pulse rate 90/min; swelling of fingers and hands)		Gordon and Wallfield 1935 (4,6-DNOC)
			Gastro		2.27 F (nausea, vomiting)		
			Hemato	2.27 F			
			Hepatic	2.27 F			
			Dermal		2.27 F (maculopapular eruption on skin)		
11	Human	5-7 d 1x/d (C)	Resp	1.27 M			Harvey et al. 1951 (4,6-DNOC)
			Cardio	1.27 M			
			Hemato	1.27 M			
			Dermal	1.27 M			
			Bd Wt	1.27 M			
12	Human	3-5 d 1x/d (C)	Metabolic		0.35 M (increased perspiration and fatigue, elevated temperature 38.2 °C)		Plotz 1936 (4,6-DNOC)
13	Rat (NS)	once (G)	Resp	27 M		36 M (dyspnea, asphyxial convulsions)	Ambrose 1942 (sodium 4,6-DNOC)
			Hemato	27 M		36 M (cyanosis)	

TABLE 2-2. Levels of Significant Exposure to Dinitrocresol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
14	Rat (NS)	10 d 1x/d (GW)	Bd Wt	25			King and Harvey 1953a (4,6-DNOC)
15	Mouse (white)	once (GW)	Resp			10 (dyspnea, hemothorax)	Arustamyan 1972 (4,6-DNOC)
			Gastro			10 (coagulative necrosis, in stomach mucosa; catarrhal inflammation of small intestine)	
			Hepatic			10 (enlarged liver with foci of hemorrhage and necrosis)	
16	Chicken (NS)	once (GO)	Ocular			2.5 (cataract formation)	Buschke 1947 (4,6-DNOC)
			Metabolic		2.5 (decrease in body temperature by 2 °F) 4.0 (increased oxygen uptake)		
	Neurological						
17	Human	1-4 d 1x/d (C)			3 (lethargy, headache, loss of appetite)		Dodds and Robertson 1933 (4,6-DNOC)
18	Human	11 d 1x/d (C)			2.3 F (drowsiness, headache, ringing in ears)		Gordon and Wallfield 1935 (4,6-DNOC)
19	Human	5-7d 1x/d (C)			0.92 M (malaise, lassitude, and headache)		Harvey et al. 1951 (4,6-DNOC)

TABLE 2-2. Levels of Significant Exposure to Dinitrocresol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
20	Human	3-5 d 1x/d (C)			0.35 [b] M (fatigue, dizziness)		Plotz 1936 (4,6-DNOC)
21	Rat (NS)	once (G)		18M	27M (depression)		Ambrose 1942 (sodium 4,6-DNOC)
22	Rat (Wistar)	once (GO)			20M (90-120% increased brain blood flow)		Verschoyle et al. 1987 (4,6-DNOC)
23	Mouse (white)	once (GW)				10 (severe agitation, muscle twitches, prostration)	Arustamyan 1972 (4,6-DNOC)
	Reproductive						
24	Mouse (C3H, C57BL/6)	5 d 1x/d (GW)		12M			Quinto et al. 1989 (4,6-DNOC)
	Developmental						
25	Mouse (DBA and CFLP)	4 d Gd 11-14 1x/d (GW)		8			Nehez et al. 1981 (DNOC)
	INTERMEDIATE EXPOSURE						
	Death						
26	Rat (Wistar)	90 d ad lib (F)				20 (5/20 deaths)	Den Tonkelaar et al. 1983 (4,6-DNOC)

TABLE 2-2. Levels of Significant Exposure to Dinitrocresol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
27	Mouse (white)	32 d 1x/d (GW)				3 (100% mortality)	Arustamyan 1972 (4,6-DNOC)
	Systemic						
28	Human	14-63 d 1x/d (C)	Cardio	1.05			Ibrahim et al. 1934 (4,6-DNOC)
			Ocular	1.05			
			Bd Wt			1.05 (weight loss of 0.45 kg/wk)	
			Metabolic			1.05 (34-77% increase in basal metabolic rate, excessive thirst and perspiration, 40 °C body temperature)	
29	Human	4-11 wk 7d/wk 1x/d (C)	Cardio		0.75 (palpitations)		Plotz 1936 (4,6-DNOC)
			Dermal		0.75 (urticarial eruptions)		
			Bd Wt		0.75 (decrease in body weight of 0.6 kg/wk)		
			Metabolic		0.58 (2 °F avg increase in body temperature)		
30	Rat (Wistar, albino)	105 d ad lib (F)	Bd Wt	7.6M	18M (15% growth inhibition)		Ambrose 1942 (sodium 4,6-DNOC)

TABLE 2-2. Levels of Significant Exposure to Dinitrocresol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference
31	Rat (Wistar)	90 d ad lib (F)	Cardio	20					Den Tonkelaar et al. 1983 (4,6-DNOC)
			Gastro	10	20	(fewer HCl-cells in fundus, small acini and no granules in salivary glands)			
			Hemato	2.5	5	(increase in hemoglobin, hematocrit, and MCH/MCV)			
			Hepatic	10	20	(increased SGPT)			
			Renal	2.5	5	(increased blood urea nitrogen, decreased urinary creatinine)			
			Metabolic		2.5	(decreased carbohydrate and increased fat metabolism)			
			Endocr		2.5	(decreased thyroid hormones	20	(fewer acidophilic cells in pituitary, vacuolization of acini, no clear zona fasiculata in adrenals, atrophy of Islet of Langerhans)	
			Other	2.5	5	(decreased food efficiency)			

TABLE 2-2. Levels of Significant Exposure to Dinitrocresol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
32	Rat (white)	77-182 d ad lib (F)	Resp	25M			Spencer et al. 1948 (4,6-DNOC)
			Cardio	25M			
			Gastro	25M			
			Hepatic	25M			
			Renal	10M	25M (increased blood urea nitrogen)		
			Ocular	25M			
			Bd Wt	10M	25M (18% decreased body weight, depletion of body fat)		
33	Rat (white)	6 mo 1x/d (G)	Hepatic	5F	10F (fatty degeneration)		Vashakidze 1967 (4,6-DNOC)
			Bd Wt	5F	10F (10-18% reduced body weight gain)		
34	Rat (Wistar)	3 wk ad lib (F)	Hemato	20M			Vos et al. 1983 (DNOC >99%)
			Hepatic	5M	20M (increased relative liver weight)		
			Renal	20M			
			Endocr	20M			
	Immunological/Lymphoreticular						
35	Rat (Wistar)	90 d ad lib (F)		10		20 (atrophy or underdevelopment of thymus, spleen, lymph nodes; decreased circulating lymphocytes)	Den Tonkelaar et al. 1983 (4,6-DNOC)

TABLE 2-2. Levels of Significant Exposure to Dinitrocresol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
36	Rat (white)	77-182 d ad lib (F)		10M	25M (hemosiderosis and congestion of the spleen)		Spencer et al. 1948 (4,6-DNOC)
37	Rat (Wistar)	3 wk ad lib (F)		20M			Vos et al. 1983 (DNOC >99%)
	Neurological						
38	Human	14-63 d 1x/d (C)			1.05 (lethargy, depression)		Ibrahim et al. 1934 (4,6-DNOC)
39	Human	4-11 wk 7d/wk 1x/d (C)			0.75 (headache and lassitude)		Plotz 1936 (4,6-DNOC)
40	Rat (Wistar)	90 d ad lib (F)		5	10 (increased relative brain weight)		Den Tonkelaar et al. 1983 (4,6-DNOC)
	Reproductive						
41	Rat (Wistar)	90 d ad lib (F)		10		20 (no corpora lutea in ovaries; juvenile uteri; aspermatogenesis)	Den Tonkelaar et al. 1983 (4,6-DNOC)
42	Rat (white)	77-182 d ad lib (F)		25M			Spencer et al. 1948 (4,6-DNOC)

TABLE 2-2. Levels of Significant Exposure to Dinitrocresol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
43	Rat (Wistar)	3 wk ad lib (F)		20 M			Vos et al. 1983 (DNOC >99%)

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive both an acute and an intermediate minimal risk level (MRL) of 0.004 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability).

ad lib = ad libitum; (C) = capsule; Cardio = cardiovascular; d = day(s); Derm = dermal; (F) = feed; F = female; (G) = gavage; Gastro= gastrointestinal; GD = gestation day; (GO) = gavage in oil vehicle; (GW) = gavage in water vehicle; HCl = hydrochloric acid; Hemato = hematological; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; SGPT = serum glutamic-pyruvate transaminase; wk = week(s); x = times; > = increased

Figure 2-2. Levels of Significant Exposure to 4,6-Dinitro-o-cresol – Oral

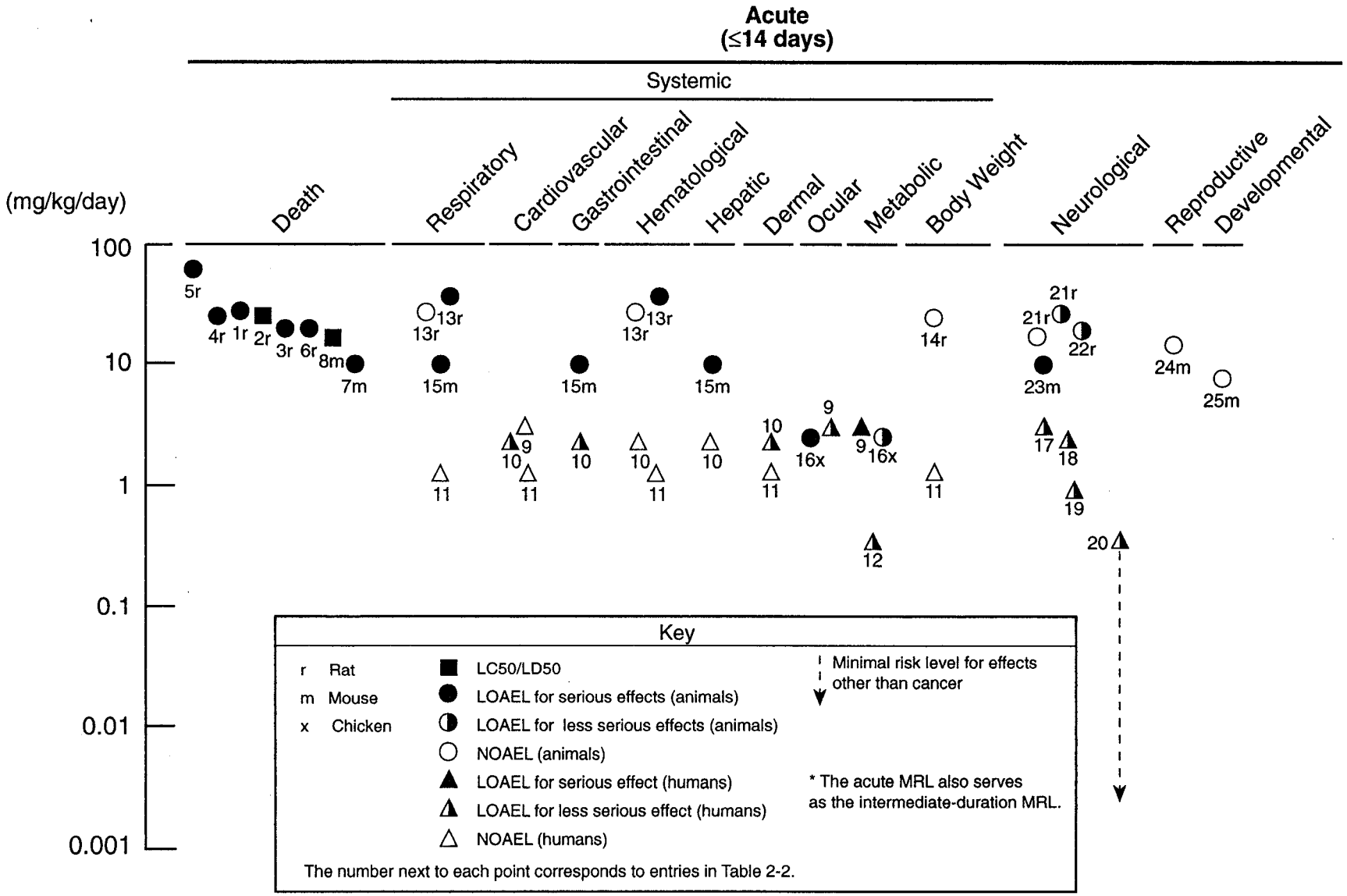
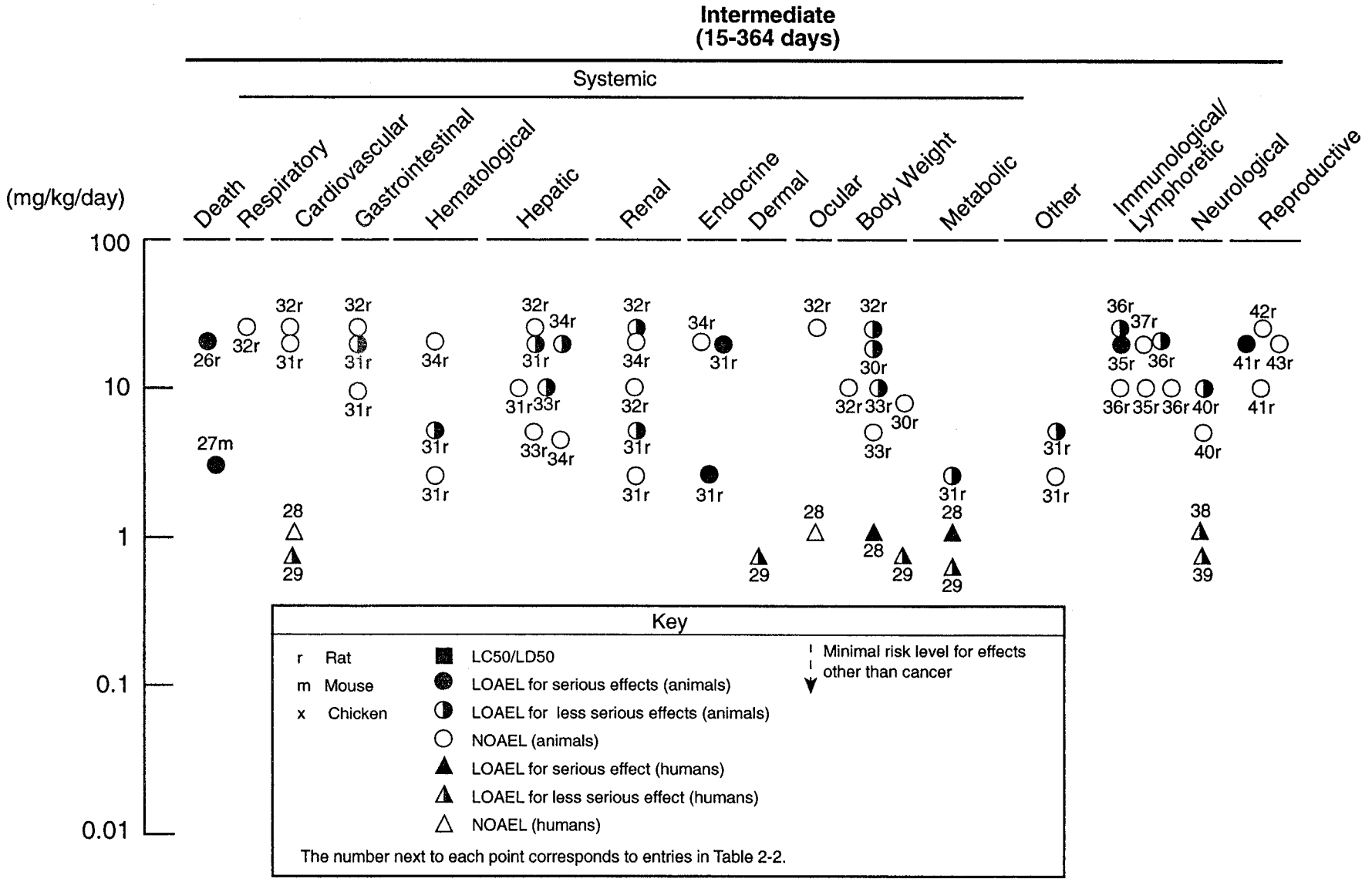


Figure 2-2. Levels of Significant Exposure to 4,6-Dinitro-o-cresol – Oral (Continued)



4 days after the exposure. No histopathological lesions were observed in lungs from rats fed diets providing daily doses in the range of l-25 mg/kg/day DNOC for 77-182 days (Spencer et al. 1948).

Cardiovascular Effects. Cardiovascular effects appear to be secondary to cellular anoxia but do not appear to be consistent cardinal signs of DNOC exposure in humans. However, elevated pulse rates, tachycardia, and palpitations were observed in several patients. Although the basal metabolic rate was increased, the cardiovascular system was not affected after volunteers ingested 3 mg/kg/day DNOC for 4 days (Dodds and Robertson 1933) or 0.92-1.27 mg/kg/day for 5-7 days (Harvey et al. 1951). Changes in blood pressure and pulse rate were regarded as not significant. A pulse rate of 90 beats per minute (insignificant increase over the 72-beat norm) was observed in a girl who ingested a time-weighted-average dose of 2.27 mg/kg/day DNOC for 11 days for purposes of weight reduction (Gordon and Wallfield 1935). Edema of the fingers and hands was also observed, possibly suggestive of circulatory dysfunction.

No changes in pulse or blood pressure were observed in two humans who received doses in the range of 0.5-1.0 mg/kg/day for 40-48 days (Dodds and Robertson 1933). Because this dose appeared to cause no other signs of toxicity, the investigators assumed that a dose in this range was safe to administer to humans. The cardiovascular system in 15 patients was not affected after they had ingested an average of 1.05 mg/kg/day DNOC for 14-63 days (Ibrahim et al. 1934). A patient who received 0.75 mg/kg/day DNOC for 8 weeks followed by 1.0 mg/kg/day DNOC experienced marked palpitations (Plotz 1936). Tachycardia was periodically observed in a young woman who had ingested one capsule per day of an unspecified dose of DNOC for the first 6 months for weight reduction therapy, but had periodically ingested 2 capsules per day for an unspecified period (Quick 1937). The patient maintained this regimen for about 3 years.

In intermediate-duration feeding studies, absolute heart weights were significantly ($p<0.05$) decreased in rats given diets providing 210 mg/kg/day (Den Tonkelaar et al. 1983; Spencer et al. 1948). In one study, relative heart weight was increased (Den Tonkelaar et al. 1983). However, no histopathological lesions were observed in heart tissue in either study. The toxicological significance of the heart weight changes is not clear.

Gastrointestinal Effects. Limited data suggest that DNOC may cause pathology of the stomach and salivary glands. Pathology of other regions of the gastrointestinal tract were rarely reported.

2. HEALTH EFFECTS

Hemorrhage of the gastric mucosa was observed in an agricultural worker who had sprayed DNOC for 3 weeks and died after accidentally ingesting an unknown amount of DNOC (Bidsyrup and Payne 1951). Nausea and vomiting were observed in a girl who ingested a time-weighted-average dose of 2.27 mg/kg/day DNOC for 11 days for the treatment of obesity (Gordon and Wallfield 1935).

Vomiting was reported to occur within 60-80 minutes in mice that ingested single doses of 10-35 mg/kg DNOC (Arustamyan 1972). Necropsy examination revealed that the mucosa of the stomach was easily separated in the form of a white, curdIed mass. The small intestine in similarly treated mice also showed catarrhal inflammation over its entire length. No histopathological lesions were observed in the stomach tissue from rats fed diets providing daily doses in the range of 1-25 mg/kg/day DNOC for 77-182 days (Spencer et al. 1948). The presence of food may have prevented the irritating effects of DNOC in the stomach of the rats exposed via diet. However, a reduced number of hydrochloric acid releasing cells in the fundus of the stomach and smaller acini and no granules in the salivary glands were observed in rats given 20 mg/kg/day DNOC for 90 days (Den Tonkelaar et al. 1983). These effects were not seen at 10 mg/kg/day.

Hematological Effects. Reticulocyte numbers were unchanged and Heinz bodies were not observed in volunteers who ingested 0.92-1.27 mg/kg/day for 5-7 days (Harvey et al. 1951). Hematological parameters were also within normal limits in a girl who ingested a time-weighted-Average dose of 2.27 mg/kg/day DNOC for 11 days for treatment of obesity (Gordon and Wallfield 1935).

Cyanosis was observed in rats given single acute doses in the range of 36-90 mg/kg DNOC as the sodium salt, but not at doses ≤27 mg/kg (Ambrose 1942). This condition is most probably related to the dyspnea and asphyxial convulsions observed in the affected rats. Total leukocyte and differential leukocyte counts were not affected in rats given daily doses in the range of 1.25-20 mg/kg/day for 3 weeks (Vos et al. 1983). No differences in hematological parameters such as erythrocyte count, hemoglobin concentration, total leucocyte count, differential count, or bone marrow counts were observed in rats fed diets providing ≤25 mg/kg/day for 77-182 days (Spencer et al. 1948). Furthermore, no histopathological lesions were observed in the bone marrow from these rats. However, hemosiderosis and congestion of the spleen were observed at 25 mg/kg/day. In another intermediate-duration study, hemoglobin, hematocrit, and the ratio of mean corpuscular volume to mean corpuscular hemoglobin (MCV/MCH) were increased in rats given 5, 10, or 20 mg/kg/day

2. HEALTH EFFECTS

DNOC for 90 days (Den Tonkelaar et al. 1983). The highest dose also resulted in increased erythrocyte count and decreased total leukocyte and lymphocyte counts. The reason for the different results in these studies is not known.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans or animals after oral exposure to DNOC.

Hepatic Effects. DNOC is a yellow compound that stains human (Hunter 1950) and animal (Ambrose 1942) skin on contact. Absorption of DNOC by any route and subsequent distribution to tissues results in a characteristic yellow staining of visceral organs and tissues including the conjunctiva and sclera of the eye (Ibrahim et al. 1934; Pollard and Filbee 1951), blood serum, skeletal tissue, and urine (Ambrose 1942). The yellow staining of the skin and sclera of patients exposed to DNOC prompted physicians to test for liver effects. Results for the icteric index and the Van den Bergh tests have been consistently negative (Dodds and Robertson 1933; Gordon and Wallfield 1935; Plotz 1936).

Congestion of the liver was observed in an agricultural worker who had sprayed DNOC for 3 weeks and died after accidentally ingesting an unknown amount of DNOC (Bidstrup and Payne 1951). Based on the icteric index and results of the Van den Bergh test, no evidence of liver damage was observed in a girl who ingested a time-weighted-average dose of 2.27 mg/kg/day DNOC for 11 days for treatment of obesity (Gordon and Wallfield 1935).

Two studies were located that demonstrated that DNOC may cause hepatic pathology, while data from several other animal studies demonstrated that DNOC may cause changes in liver weight with no histological evidence of hepatic pathology. Enlarged dark brown livers with petechial hemorrhages and necrotic foci were observed in mice that received single gavage doses in the range of 10-35 mg/kg DNOC (Arustamyan 1972). Fatty degeneration of unspecified parenchymatous organs was also observed in rats that were given daily gavage doses of 10 mg/kg/day DNOC for 6 months (Vashakidze 1967). Although not indicated in this study, this degenerative change can most likely occur in the liver and may lead to necrosis of hepatocytes. In intermediate-duration feeding studies, no histological evidence of liver pathology was found in rats fed diets providing ≤25 mg/kg/day (Den Tonkelaar et al. 1983; Spencer et al. 1948; Vos et al. 1983). The method of administration (i.e., gavage versus dietary) may partly account for the different results for hepatic pathology in the

intermediate-duration gavage study and the intermediate-duration feeding studies. Absolute liver weights were significantly decreased in rats receiving l0-20 mg/kg/day (Den Tonkelaar et al. 1983; Spencer et al. 1948), and relative liver weights were increased in rats receiving 5-20 mg/kg/day (Den Tonkelaar et al. 1983; Vos et al. 1983). In addition, two rats had greatly increased levels of serum glutamic pyruvic transaminase (SGPT) at 20 mg/kg/day, and liver activity of glucose-6-phosphatase dehydrogenase (G6PDH) was decreased at ≥5 mg/kg/day (Den Tonkelaar et al. 1983). As DNOC is an uncoupler of oxidative phosphorylation (see Section 2.3.5), reduced G6PDH activity can be explained by a decrease in adenosine triphosphate (ATP) formation and the subsequent formation of glucose-6-phosphate during oxidative phosphorylation.

Renal Effects. Cloudy swelling of the kidney was observed at autopsy in a DNOC spray operator who died after accidentally ingesting an unknown amount of DNOC from a water tank (Bidstrup and Payne 1951).

In intermediate-duration feeding studies in rats, no histological evidence of renal pathology was found at doses ≤25 mg/kg/day (Den Tonkelaar et al. 1983; Spencer et al. 1948; Vos et al. 1983). Absolute kidney weights were decreased (Den Tonkelaar et al. 1983; Spencer et al. 1948), and relative kidney weights were increased (Den Tonkelaar et al. 1983) at doses of 10 or 20 mg/kg/day, respectively. BUN was increased from 15.8 mg% in controls to 24-35 mg% in rats fed diets providing daily doses of 25 mg/kg/day for 77-182 days (Spencer et al. 1948). BUN was also increased at doses of 5, 10, and 20 mg/kg/day in the 90-day study (Den Tonkelaar et al. 1983). Urinalysis revealed that urinary protein was decreased at 10 and 20 mg/kg/day, urinary glucose was increased at 20 mg/kg/day, and urinary creatinine was decreased at 5, 10, and 20 mg/kg/day. The elevated urine glucose was due to elevated blood glucose and the inhibitory effect of DNOC on oxidative phosphorylation and subsequent ATP-dependent active transport in the proximal tubules of the kidney.

Endocrine Effects. Although DNOC has been described to induce a syndrome similar to hyperthyroidism in humans (Dodds and Robertson 1933), blood triiodothyronine (T_3) and thyroxin (T_4) levels were decreased at all levels in rats given 2.5-20 mg/kg/day DNOC for 90 days (Den Tonkelaar et al. 1983). Histological examination revealed inactive thyroids. Absolute thyroid weights were decreased at 20 mg/kg/day, while relative thyroid weights were increased at the same dose. Absolute weights were decreased for the pituitary gland at 10 and 20 mg/kg/day and the adrenal gland at 20 mg/kg/day, while the relative weights for both glands were increased at the same dose.

2. HEALTH EFFECTS

Histological examination revealed fewer acidophilic cells in the pituitary gland and vacuolization of acini, no clear zona fasciculata, and swollen medullary cells in the adrenals. Atrophy of the Isle of Langerhans cells in the pancreas was also observed. Many of these effects were attributed to the ability of DNOC to uncouple oxidative phosphorylation, leading to a deficit in ATP (see Section 2.3.5). However, changes in pituitary, thyroid and adrenal weight and histology were not observed in rats given daily doses in the range of 1.25-20 mg/kg/day DNOC for 3 weeks (Vos et al. 1983).

Dermal Effects. Oral doses of DNOC may cause urticarial eruptions in humans. An itching maculopapular eruption appeared in a girl who ingested a time-weighted-average dose of 2.27 mg/kg/day DNOC for 11 days for treatment of obesity (Gordon and Wallfield 1935). Maculopapular urticarial eruptions, slightly reddish in color, involving both deltoids, the upper anterior chest, and both upper axillae were also observed in a female patient who received a time-weighted-average dose of 0.75 mg/kg/day DNOC for 11 weeks for weight reduction (Plotz 1936). A yellow staining of the palms of the hand, soles of the feet, scalp, beard and pubic hair, skin of the thighs and chest, and buccal mucosa was also observed in a DNOC spray operator who had accidentally ingested an unknown dose of DNOC, confirming dermal exposure (Bidstrup and Payne 1951).

As noted above for Hepatic Effects, DNOC is a yellow compound that stains human and animal skin on contact. While the yellow staining of the skin and sclera may be unsightly, such cosmetic effects are not regarded as adverse.

Ocular Effects. As noted above for Hepatic Effects, DNOC is a yellow compound that stains human and animal skin on contact. Contact with the eyes or absorption of DNOC also results in a characteristic yellow staining of the conjunctiva and sclera of the eye (Dodds and Robertson 1933; Gordon and Wallfield 1935; Ibrahim et al. 1934; Plotz 1936; Pollard and Filbee 1951).

Despite the occurrence of a green-yellow pigmentation of the conjunctiva in humans who had ingested 3 mg/kg/day DNOC for 4 days (Dodds and Robertson 1933) or 0.92-1.27 mg/kg/day for 5-7 days (Harvey et al. 1951), no adverse ocular effects were observed. A similar observation was made for a DNOC spray operator who had accidentally ingested an unknown dose of DNOC (Bidstrup and Payne 1951). A greenish-yellow tinge to the sclera was also observed in a 14½year-old girl who ingested a time-weighted-average dose of 2.27 mg/kg/day DNOC for 11 days for the treatment of obesity

(Gordon and Wallfield 1935). A yellow pigmentation of the conjunctiva occurred in all 15 patients who had ingested an average of 1.05 mg/kg/day DNOC for 14-63 days (Ibrahim et al. 1934). A greenish tinge of the sclera was also observed in two of four patients who received 0.75 mg/kg/day DNOC for 6-11 weeks (Plotz 1936). Although the yellow staining of the skin and sclera may be unsightly, such cosmetic effects are not regarded as adverse.

Ingestion of an unspecified dose of DNOC for 3 years was associated with a pearly swollen cataract of the left eye in a woman (Quick 1937). The right eye, which had punctate central lenticular opacity, eventually became blind 1 month after the cataract was diagnosed. A slight yellow pigmentation of the conjunctiva also appeared periodically during the 3-year treatment.

Because dinitrophenolic compounds have been known to be cataractogenic in humans, attempts have been made to a find a suitable animal model to study this phenomenon (Spencer et al. 1948). Cornea1 opacity and cataracts were not observed in rats fed diets providing doses in the range of 1-25 mg/kg/day for 77-182 days. However, cataract formation was observed in ducklings fed a diet of 1,200 ppm DNOC for l-2 days (doses in mg/kg/day were not reported). In addition, administration of a single oral dose of DNOC in the range of 2.48-59.45 mg/kg to chickens produced cataracts within l-5 hours (Buschke 1947). The cataract formation was considered related to interference with oxidative phosphorylation.

Body Weight Effects. DNOC was once used to treat obesity, but this practice has been discontinued because of recognized toxic effects since the 1930s. Body weight was not affected in humans who ingested 0.92-1.27 mg/kg/day for 5-7 days (Harvey et al. 1951). However, a patient's weight was reduced by 15 kg after ingesting an unknown amount of DNOC for 3 years (Quick 1937). The average weight lost by 15 patients was 0.45 kg per week after they had ingested an average of 1.05 mg/kg/day DNOC for 14-63 days (Ibrahim et al. 1934). About 9.1 kg was the maximum weight loss during a 2-month period of DNOC therapy. DNOC did not cause a rise in blood glucose nor did it cause the appearance of ketones in the urine. A decrease in body weight was also observed in only 1 of 4 patients who received 0.75 mg/kg/day DNOC for 6 weeks for weight reduction purposes (Plotz 1936).

DNOC also causes decreases in body weight gain in animals. Significant decreases in body weight gain were observed in rats that received 10 or 25 mg/kg/day for 10 days, but the decrease amounted to

only 2% and 5%, respectively (King and Harvey 1953a). Ten daily doses of 5 mg/kg/day did not appear to alter the growth rate of the animals. No change in body weight gain was observed in rats fed diets providing doses of 15 mg/kg/day for 18 weeks (Parker et al. 1951). However, growth was inhibited by 15% in rats fed a diet providing 18 mg/kg/day DNOC as the sodium salt for 105 days (Ambrose 1942), by 18% in rats fed a diet providing 25 mg/kg/day DNOC for 77-182 days (Spencer et al. 1948), and by 10-18% in rats given 10 mg/kg/day DNOC by gavage for 6 months (Vashakidze 1967). Despite the decrease in growth rate, food consumption was increased in one study (Ambrose 1942). Depletion of adipose tissue was also observed at the end of the 182-day study in rats that received 25 mg/kg/day (Spencer et al. 1948).

Metabolic Effects. The basal metabolic rate was increased by 70-100% within 3 days in two humans who were given 3 mg/kg/day DNOC (Dodds and Robertson 1933). Doses of DNOC that increased the basal metabolic rates by 50% above normal usually resulted in sweating, loss of appetite, depression, headaches, and yellow-green pigmentation of the eye. An overweight man who initially received two doses of 0.75 mg/kg/day DNOC for weight reduction had an elevated body temperature and complained of feeling hot and tired (Plotz 1936). Following a drug withdrawal period of 2 weeks and a subsequent dose of 0.35 mg/kg/day DNOC, the patient complained of profuse perspiration and fatigue on the seventh day.

In two humans who received doses in the range of 0.5-1.0 mg/kg/day for 40-48 days, basal metabolic rate peaked at 35% above normal on day 34 in one individual, while in another individual it was greater than 50% above normal from days 21 to 23 (Dodds and Robertson 1933). Because this dose appeared to cause no other symptoms, the investigators assumed that a dose in this range was safe to be administered to humans. An elevated basal metabolic rate was observed in 6 patients who had ingested an average of 1.05 mg/kg/day DNOC for 14-63 days (Ibrahim et al. 1934). Basal metabolic rate was increased by as much as 77% in one individual. All patients involved with the study had an elevated body temperature accompanied with perfuse perspiration and frequently complained of thirst and fatigue. Food intake was either diminished or remained the same. An elevated body temperature and excessive perspiration were also observed in three of four patients who received 0.58-1.0 mg/kg/day DNOC for 4-11 weeks (Plotz 1936).

Other Systemic Effects. Other systemic effects observed in humans and animals included effects on growth rate and blood sugars, protein, and related metabolic products.

Growth and food efficiency were decreased in a dose-related manner in rats given 5, 10, and 20 mg/kg/day DNOC for 90 days (Den Tonkelaar et al. 1983). Urinary ketones, an indicator of endogenous fat catabolism, were increased at doses of 2.5, 5, and 10 mg/kg/day DNOC, but not at 20 mg/kg/day DNOC. Blood glucose was increased at 10 and 20 mg/kg/day DNOC, while blood protein was decreased only at 20 mg/kg/day DNOC. Blood pyruvate was also decreased at all doses. The increased blood glucose and decreased blood pyruvate were indicative of an inhibitory action of DNOC on glycolysis.

2.2.2.3 Immunological and Lymphoreticular Effects

As discussed in Section 2.2.2.2 for Dermal Effects, oral doses of DNOC have caused urticaria in humans. Whether these dermal effects are immunological is not clear.

Data regarding immunological effects in animals are conflicting. Decreased absolute weight of the thymus was observed at 10 and 20 mg/kg/day and decreased relative weight of thymus was observed at 20 mg/kg/day in rats given DNOC for 90 days (Den Tonkelaar et al. 1983). The relative weight of the spleen was slightly increased at 20 mg/kg/day, while the absolute weight was decreased at 20 mg/kg/day. Upon histological examination, the lymph nodes were underdeveloped, the thymus was atrophied, and the spleen had small follicles at 20 mg/kg/day. Changes in thymus, spleen, and mesenteric and popliteal lymph node weight and histology were not observed in rats given daily doses in the range of 1.25-20 mg/kg/day DNOC for 3 weeks (Vos et al. 1983). When IgM and IgG were further analyzed and quantified, DNOC had no affect on these immunoglobulins. The highest NOAEL values and all LOAEL values from each reliable study for immunological effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.4 Neurological Effects

Oral exposure to DNOC has caused depression and headaches in humans in most cases. Neurological effects such as mental depression and headaches were observed in two volunteers given 3 mg/kg/day DNOC for 4 days (Dodds and Robertson 1933) and in 2 of 5 volunteers given 0.92 and 1.27 mg/kg/day for 7 and 5 days, respectively (Harvey et al. 1951). Hemorrhage of the pia mater was observed in a DNOC spray operator who had accidentally ingested an unknown amount of DNOC and subsequently died (Bidstrup and Payne 1951). Prior to death, no neurological signs were reported for

this worker. An overweight man who initially received two doses of 0.75 mg/kg/day DNOC for purposes of weight reduction complained of feeling dizzy (Plotz 1936). Following a drug withdrawal period of 2 weeks and a subsequent dose of 0.35 mg/kg/day DNOC, the patient complained of fatigue on the seventh day. The LOAEL of 0.35 mg/kg/day was used to derive an acute and an intermediate oral MRL of 0.004 mg/kg/day for DNOC as described in the footnote in Table 2-2. Drowsiness, headaches, and ringing of the ears were experienced by a girl who ingested a time-weighted-average dose of 2.27 mg/kg/day DNOC for 11 days (Gordon and Wallfield 1935).

Lethargy and mental depression were also common complaints of 15 patients who ingested an average of 1.05 mg/kg/day DNOC for 14-63 days (Ibrahim et al. 1934). A slight headache and lassitude were reported by a female patient who received 0.75 mg/kg/day DNOC for 8 weeks (Plotz 1936).

Depression following oral ingestion of DNOC has also been reported in animals. Clinical signs of depression were observed in rats given single doses 227 mg/kg DNOC as the sodium salt (Ambrose 1942). In another acute study, a single oral dose of 19.8 mg/kg DNOC caused a 90-120% increase in brain blood flow in rats in 4 hours (Verschoyle et al. 1987). Brain blood flow returned to normal within 24 hours, while no histopathological changes were observed in the brains of these rats. The authors concluded that the observed increase in brain blood flow was consistent with the expected increased metabolic rate produced by DNOC. Severe agitation and muscle twitches were observed within 60-80 minutes in mice that received a single dose of DNOC in the range of l0-35 mg/kg DNOC (Arustamyan 1972). The mice also became prostrate for 3-7 hours, approximately 2-3 hours after exposure to DNOC. Cats that received a single oral dose of 25 mg/kg DNOC developed ataxia and became sluggish during the first hour, while muscle twitches and weakness developed on the second day after exposure (Burkatskaya 1965b). However, this study was limited by reporting deficiencies regarding experimental details and data.

Decreased absolute brain weight was observed at 20 mg/kg/day DNOC, and increased relative brain weight was observed at 10 and 20 mg/kg/day DNOC in rats given DNOC for 90 days (Den Tonkelaar et al. 1983). However, no histopathological lesions were observed in the brain. The highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to DNOC.

DNOC did not affect either sperm counts or testicular weights in mice given single doses in the range of 3-12 mg/kg/day DNOC for 5 days (Quint0 et al. 1989). In addition, DNOC failed to cause abnormal sperm.

Intermediate-duration studies provided conflicting data regarding reproductive effects in animals after oral exposure to DNOC. No histopathological lesions were observed in testes from rats fed diets that provided daily doses in the range of 1-25 mg/kg/day DNOC for 77-182 days (Spencer et al. 1948) or 1.25-20 mg/kg/day for 3 weeks (Vos et al. 1983). However, absolute and relative weights of the testes/prostate were decreased, and reduced spermatogenesis or aspermatogenesis was observed in rats given 20 mg/kg/day DNOC for 90 days (Den Tonkelaar et al. 1983). The reason for the conflicting data for testicular effects in intermediate-duration studies is not clear. Absolute weight of ovaries was decreased at ≥5 mg/kg/day DNOC, and relative weight of uterus/ovary was decreased at 20 mg/kg/day DNOC (Den Tonkelaar et al. 1983). No corpora lutea were observed in the ovaries, and the uteri appeared juvenile at 20 mg/kg/day DNOC. Damaged ovaries and disrupted estrus cycles were observed in rats given oral doses of 5 mg/kg/day DNOC for 6 months (Vashakidze 1967). The investigators demonstrated that DNOC caused an increase in gonadotrophic hormones in the hypophysis. This change in hormone balance may be the reason for the disruption of the functioning of the reproductive glands. A higher dose of 10 mg/kg/day DNOC also disrupted the reactivity of the vaginal mucosa to estrogenous influences. Further experiments also demonstrated that DNOC caused atrophy of the uterine horns. Because of the poor experimental design and because the data were not clearly presented, it is difficult to substantiate the conclusions made by the author. However, some of the findings from this study support those reported by Den Tonkelaar et al. (1983). The highest NOAEL values and all LOAEL values for reproductive effects in these studies are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to DNOC.

No developmental effects were observed when DBA strains of mice given 8 mg/kg/day DNOC from day 11 to 14 of gestation (NehCz et al. 1981). On the eighteenth day of gestation, the numbers of corpora lutea, implantations, live embryos, resorbed embryos, pre-implantation loss, post-implantation loss, weight of embryos, and number of malformations did not differ significantly from the data obtained from the negative control group. The NOAEL value for developmental effects in mice is recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

Increased incidences of chromosomal aberrations were found in the offspring of mice treated orally with DNOC (Nehéz et al. 1981). CFLP strain female mice (number not specified) were given DNOC in saline at doses of 5 mg/kg/day every other day for a total of 4 treatments during the first trimester of pregnancy or on gestational days 9-12 (second trimester). On days 14-16, the mice were sacrificed, and fetal liver was removed for examination of chromosomal aberrations. Treatment during the second trimester resulted in a significantly increased incidence of chromosomal aberrations in the fetal liver ($p<0.01$) compared with controls. Treatment during the first trimester did not significantly ($p>0.05$) increase the frequency of chromosomal aberrations.

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to DNOC.

2.2.3 Dermal Exposure

2.2.3.1 Death

A patient died less than 14 hours after admission to a hospital and less than 48 hours after the onset of signs and symptoms of DNOC toxicity (Steer 1951). The patient had previously sprayed DNOC for an unspecified, but apparently acute time period. The dose that the patient received was also not reported. Although the yellow staining of the skin suggests dermal exposure, the patient may also have inhaled DNOC aerosols. A 4-year-old boy died 3.5 hours after 12,500 mg of DNOC was

accidentally applied as an ointment to a skin rash (Buchinskii 1974). Because DNOC was applied to the rash rather than intact healthy skin, considerable amounts of DNOC were rapidly absorbed and thus became fatal. No supporting data from human studies regarding dermal exposure to DNOC were located to suggest whether this dose would have been fatal if applied to intact skin. Two of three employees died after spraying 2% DNOC for two consecutive days (Buzzo and Guatelli 1949). Although inhalation may have contributed to total exposure, the yellow staining of the skin and the fact that no appropriate precautions were taken to minimize dermal exposure suggest that exposure was mainly dermal.

One industrial and 5 agricultural workers, who were thought to be dermally exposed to unknown doses of DNOC for 2-8 weeks, died after brief periods of illnesses related to DNOC exposure (Bidstrup and Payne 1951). Because of the intense heat and discomfort, protective clothing was often discarded. This suggests that most of the DNOC was absorbed dermally, although limited amounts of DNOC aerosols may have also been inhaled.

The dermal LD_{50} for DNOC was 200-600 mg/kg for rats (Ben-Dyke et al. 1970; Jones et al. 1968). No other details were provided. A dermal LD_{50} for DNOC in mice was reported to be 186.7 mg/kg (Arustamyan 1972) and in rabbits to be 1,000 mg/kg (Burkatskaya 1965b). Although doses of 100 and 200 mg/kg DNOC were not lethal, 1 of 5, 3 of 5, 5 of 5, and 2 of 2 guinea pigs died after 300, 400, 500, and 1,000 mg/kg DNOC was applied, respectively, to a shaved area on the abdomen (Spencer et al. 1948). Prior to application, DNOC was dissolved in ethanol and the treated area was kept moist with ethanol for 4 hours. In another experiment, an unspecified number of rabbits died after seven applications of 3% solution of DNOC in 95% alcohol to the ear and seven applications were bandaged onto the shaven abdomen. The LD_{50} values and doses resulting in death of animals are recorded in Table 2-3.

2.2.3.2 Systemic Effects

Studies regarding systemic effects in humans and animals after dermal exposure to DNOC are described below. The highest NOAEL values and all LOAEL values from each reliable study for systemic end points in each species and duration category are recorded in Table 2-3.

TABLE 2-3. Levels of Significant Exposure to Dinitrocresol - Dermal

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL			Reference
				Less Serious	Serious		
ACUTE EXPOSURE							
Death							
Human	2 d 8hr/d				10% M	(2 deaths)	Buzzo and Guatelli 1949 (4,6-DNOC)
Rat	once				200 mg/kg	(LD50)	Ben-Dyke et al. 1970; Jones et al. 1968 (4,6-DNOC)
Mouse (white)	once				186.7 mg/kg	(LD50)	Arustamyan 1972 (4,6-DNOC)
Rabbit (NS)	once				1000 mg/kg	(LD50)	Burkatskaya 1965b (4,6-DNOC)
Rabbit (white)	1-7 d 1x/d				3%	(death of unspecified number)	Spencer et al. 1948 (4,6-DNOC)
Gn pig	once				300 mg/kg	(1/5 died)	Spencer et al. 1948 (4,6-DNOC)

TABLE 2-3. Levels of Significant Exposure to Dinitrocresol - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
Systemic						
Human	2 d 8hr/d	Resp			10% M (dyspnea)	Buzzo and Guatelli 1949 (4,6-DNOC)
		Cardio		10% M (elevated pulse rate 120/min in workers who survived)		
		Musc/skel			10% M (muscular rigidity, loss of motor function)	
		Derm	10% M			
		Metabolic		10% M (increased body temperature, perspiration, thirst)		
Human	once	Derm	1.0%			Lisi et al. 1987 (4,6-DNOC)
Rabbit	6 hr 2x/hr	Ocular	0.9%			Ambrose 1942 (sodium 4,6-DNOC)
Rabbit (white)	1-7 d 1x/d	Derm		3% (slight skin irritation)		Spencer et al. 1948 (4,6-DNOC)
Immunological/Lymphoreticular						
Human	once		1.0%			Lisi et al. 1987 (4,6-DNOC)
Neurological						
Human	2 d 8hr/d				10% M (coma, convulsions, loss of motor function)	Buzzo and Guatelli 1949 (4,6-DNOC)

TABLE 2-3. Levels of Significant Exposure to Dinitrocresol - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
INTERMEDIATE EXPOSURE						
Systemic						
Human	30 d 1x/d	Derm	1.8%			Ambrose 1942 (sodium 4,6-DNOC)
Rat	30 d 1x/d	Derm	1.8%			Ambrose 1942 (sodium 4,6-DNOC)
		Bd Wt	1.8%			
Rabbit	30 d 1x/d	Derm	1.8%			Ambrose 1942 (sodium 4,6-DNOC)
		Bd Wt	1.8%			
Rabbit	4 wk 5 d/wk 1x/d	Derm		5% (slight skin irritation)		Spencer et al. 1948 (4,6-DNOC)

d = day(s); Derm = dermal; Gn pig = guinea pig; hr = hour(s); LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; wk = week(s); x = time(s)

2. HEALTH EFFECTS

Respiratory Effects. Dermal exposure to DNOC may result in elevated respiratory rates in humans. Shallow breathing and an elevated respiratory rate were observed in a spray operator exposed to DNOC for an unspecified but apparently short time period (Steer 1951). Two hours after 12,500 mg of DNOC in a fatty ointment was applied to a skin rash, an increase in respiratory rate and moist rales were observed in a young boy who subsequently died (Buchinskii 1974). Autopsy and histological examination revealed severe capillary hyperemia in the lungs and pulmonary edema. Because DNOC was applied to the rash rather than intact healthy skin, absorption of DNOC was facilitated. The amount of DNOC applied to intact skin required to produce these effects is not known. Dyspnea was observed in employees who sprayed 10% DNOC for 2 consecutive days (Buzzo and Guatelli 1949). These employees had yellow-stained skin and did not take the appropriate precautions to minimize dermal exposure to DNOC. Respiratory rates were slightly elevated in a worker involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal. One industrial and 5 agricultural workers, who died after dermal exposure to unknown doses of DNOC for 2-8 weeks, had a rapid rate of respiration and difficulty breathing at the hospital (Bidstrup and Payne 1951). Edematous and hemorrhagic lungs were observed at autopsy. It is not known if these effects were specific to DNOC exposure. Because DNOC aerosols were present in both work environments, inhalation is also a potential route of exposure. Dyspnea and increased respiratory rates were also observed in 4 spray operators after spraying with DNOC for 14 days to 4 months (van Noort et al. 1960). In these employees, exposure may have also been by a combination of inhalation and dermal.

No studies were located regarding respiratory effects in animals after dermal exposure to DNOC.

Cardiovascular Effects. Within 2 hours after 12,500 mg of DNOC in a fatty ointment was applied to a skin rash in a young boy who subsequently died, the pulse rate was elevated and thready and heart sounds were muffled (Buchinskii 1974). Histological examination at autopsy showed severe hemorrhage and capillary hyperemia in the myocardium. Because DNOC was applied to the rash rather than intact healthy skin, absorption of DNOC was facilitated. The amount of DNOC applied to intact skin required to produce these effects is not known. An elevated pulse rate and subsequent cardiac fibrillation were observed in a spray operator exposed to DNOC for an unspecified but apparently short time period (Steer 1951). The pulse was also elevated in three employees who were exposed primarily by the dermal route to 10% DNOC for two consecutive days (Buzzo and Guatelli

1949). A pulse rate of 100 beats per minute, a blood pressure of 155/70 mm Hg, and a normal electrocardiogram were observed in an employee involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal. One industrial and 5 agricultural workers, who were thought to be dermally exposed to unknown doses of DNOC for 2-8 weeks, had elevated pulse rates and cyanosis at the hospital (Bidstrup and Payne 1951). All six workers died. Increased pulse rate and heart palpitations were also observed in employees that sprayed DNOC for 14 days to 4 months (van Noort et al. 1960). Because DNOC aerosols were present in the work environments, inhalation is also a potential route of exposure.

No studies were located regarding cardiovascular effects in animals after dermal exposure to DNOC.

Gastrointestinal Effects. About 1 hour after 12,500 mg of DNOC in a fatty ointment was applied to a skin rash, the 4-year-old male patient vomited (Buchinskii 1974). He subsequently died. Autopsy revealed a hemorrhagic intestinal mucosa, and histological examination revealed severe hyperemia in the intestinal walls. Because DNOC was applied to the rash rather than intact healthy skin, absorption of DNOC was facilitated. The amount of DNOC applied to intact skin required to produce these effects is not known. At autopsy, multiple hemorrhagic erosions also were observed in the mucosa of the stomach of 1 industrial and 5 agricultural workers who died after being dermally exposed to unknown doses of DNOC for 2-8 weeks (Bidstrup and Payne 1951). Vomiting was also observed in employees that sprayed with DNOC for 14 days to 4 months (van Noort et al. 1960). Because DNOC aerosols were present in these work environments, inhalation is also a potential route of exposure.

No studies were located regarding gastrointestinal effects in animals after dermal exposure to DNOC.

Hematological Effects. Unspecified hemorrhagic irregularities and irregular bleeding were observed in some field workers after dermal exposure to DNOC for about 8 hours (Vamai and Kote 1969). Limited human data suggest that dermal exposure to DNOC also may affect the bone marrow. An increased red bone marrow at distal ends of the femur and failure of blood to clot were observed in a spray operator exposed to DNOC for an unspecified, but apparently short time period (Steer 1951). At autopsy, red bone marrow was found throughout the shaft of the femur of an agricultural worker who died after being dermally exposed to unknown doses of DNOC for 2-8 weeks (Bidstrup

and Payne 1951). The bone marrow was further described as anoxemic. Because DNOC aerosols were present in both work environments, inhalation is also a potential route of exposure. No abnormal hematological parameters were observed in an employee involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal.

No studies were located regarding hematological effects in animals after dermal exposure to DNOC.

Musculoskeletal Effects. Only one of four employees complained of pain in the calf muscle after being exposed to a dense DNOC mist for an acute duration (van Noort et al. 1960). Exposure was probably a combination of inhalation and dermal. Muscular rigidity and loss of motor function were also observed in employees who were dermally exposed to 10% DNOC for 2 consecutive days (Buzzo and Guatelli 1949).

No studies were located regarding musculoskeletal effects in animals after dermal exposure to DNOC.

Hepatic Effects. DNOC is a yellow compound that stains human (Hunter 1950) and animal (Ambrose 1942) skin on contact. Absorption of DNOC by any route and subsequent distribution to tissues results in a characteristic yellow staining of visceral organs and tissues including the conjunctiva and sclera of the eye (Ibrahim et al. 1934; Pollard and Filbee 1951), blood serum, skeletal tissue, and urine (Ambrose 1942). The yellow staining of the skin and sclera of patients exposed to DNOC prompted physicians to test for liver effects. Results for the icteric index and the Van den Bergh tests have been consistently negative (Dodds and Robertson 1933; Gordon and Wallfield 1935; Plotz 1936).

Data from several human studies suggest that DNOC may cause liver damage. In these studies, further specific liver function tests were either not performed or not reported. Severe capillary hyperemia was observed in the liver of a young boy who died after 12,500 mg of DNOC was accidentally applied to a skin rash (Buchinskii 1974). Because DNOC was applied to the rash rather than intact healthy skin, absorption of DNOC was facilitated. The amount of DNOC applied to intact skin required to produce these effects is not known. Unspecified liver damage and enlarged livers were also observed in several agricultural workers who were dermally exposed to DNOC for 8 hours

(Vamai and Kote 1969). Congested livers were generally observed in 1 industrial and 5 agricultural workers, who died after being dermally exposed to unknown doses of DNOC for 2-8 weeks (Bidstrup and Payne 1951). Because DNOC aerosols were present in the work environments, inhalation is also a potential route of exposure.

No studies were located regarding hepatic effects in animals after dermal exposure to DNOC.

Renal Effects. Limited data suggest that dermal exposure to DNOC may cause pathological changes in the human kidney. Severe capillary hyperemia was observed in the kidney of a young boy who died after 12,500 mg of DNOC in an ointment was accidentally applied to a skin rash (Buchinskii 1974). Because DNOC was applied to the rash rather than intact healthy skin, absorption of DNOC was facilitated. The amount of DNOC applied to intact skin required to produce these effects is not known. Unspecified kidney damage was also observed in several agricultural workers who were dermally exposed to DNOC for eight hours (Varnai and Kote 1969). Congested kidneys and cloudy swelling of the renal tubules were generally observed in one industrial and five agricultural workers, who died after being dermally exposed to unknown doses of DNOC for 2-8 weeks (Bidstrup and Payne 1951). Because DNOC aerosols were present in these work environments, inhalation is also a potential route of exposure. An elevated BUN was also observed in an employee involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for five weeks (Pollard and Filbee 1951).

No studies were located regarding renal effects in animals after dermal exposure to DNOC.

Dermal Effects. As noted above for Hepatic Effects, DNOC is a yellow compound that stains human and animal skin on contact. While the yellow staining of the skin may be unsightly, such cosmetic effects are not regarded as adverse. The skin was stained yellow in an employee who was involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for five weeks (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal. A generalized yellow staining of the skin was observed in 1 industrial and 5 agricultural workers, who were thought to be dermally exposed to unknown doses of DNOC for 2-8 weeks (Bidstrup and Payne 1951). Because DNOC aerosols were present in both work environments, inhalation is also a potential route of exposure. The hands of two

individuals engaged in cleaning the jets of spray booms of aircraft spraying a 10% solution of DNOC in oil were also stained yellow (Stott 1956).

Dermal exposure to DNOC does not appear to cause local irritation of the skin of humans. DNOC was not a dermal irritant ≤48 and 72 hours after concentrations of 0.5% or 1.0% were diluted in water and applied to the upper back of agricultural workers, former agricultural workers, and other humans (Lisi et al. 1987). No signs of local irritation or evidence of systemic toxicity were observed after 1.8% DNOC as the sodium salt was applied daily to the shaved arm pits and to the anterior cubital surface of each arm of two humans for 30 days (Ambrose 1942).

DNOC is generally not irritating to the skin of animals. No signs of local irritation or evidence of systemic toxicity were observed after 1.8% DNOC as the sodium salt was applied daily to the depilated dorsal surface of 10 rats or 6 rabbits for 30 days (Ambrose 1942). However, slight skin irritation was observed only on the abdomen after DNOC was applied to both the abdomen and the ears of rabbits daily, for 1-7 days or for 5 days/week for 4 weeks (Spencer et al. 1948).

Ocular Effects. Contact with the eyes or absorption of DNOC also results in a characteristic yellow staining of the conjunctiva and sclera of the eye (Dodds and Robertson 1933; Gordon and Wallfield 1935; Ibrahim et al. 1934; Plotz 1936; Pollard and Filbee 1951). While the yellow staining of the sclera may be unsightly, such cosmetic effects are not regarded as adverse. The sclera were stained yellow in an employee who was involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for five weeks (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal.

Dermal exposure to DNOC does not appear to cause local irritation of the eye of humans; however, an g-hour dermal exposure to DNOC was reported to have caused unspecified visual disturbances in several agricultural workers (Vamai and Kote 1969).

DNOC is generally not irritating to the eyes of animals. DNOC did not cause any signs of ocular irritation ≤24 hours after 5 drops of 0.9% DNOC as the sodium salt was instilled into the conjunctival sac of 6 rabbits (Ambrose 1942). Blepharospasm and excessive lacrimation were observed in cats exposed to 36 or 60 mg/m^3 DNOC dust for 4 hours (Burkatskaya 1965a). Since these effects were not

reported in the cats similarly exposed to a mist of DNOC in solution, they were probably due to a direct irritating effect of the dust particles on the eyes, rather than to DNOC.

Metabolic Effects. Metabolic effects observed in humans include elevated body temperature, profuse sweating, and increased basal metabolic rate. These clinical signs are related to the uncoupling of oxidative phosphorylation by DNOC (see Section 2.3.5). Uncoupling of oxidative phosphorylation results in heat production that exceeds the organism's capacity to dissipate heat. Consequently, fatal hyperthermia may occur. These clinical signs were not observed or reported in animals dermally exposed to DNOC.

An elevated body temperature as well as profuse sweating were observed in spray operators (Buzzo and Guatelli 1949; Steer 1950) and agricultural workers (Vamai and Kote 1969) who were exposed to DNOC for acute durations. In one case, the temperature was ≤40.4 ºC (Steer 1950). An elevated body temperature (38.9 ºC), increased basal metabolic rate, and profuse sweating were observed in an employee who was involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951). The patient's clinical history also suggested that exposure was probably a combination of inhalation and dermal. High amounts of nitrogen (≈4 mg urea/100 mL urine) were also excreted in the urine. An elevated body temperature, increased thirst, and profuse perspiration were also observed in 1 industrial and 5 agricultural workers, who subsequently died after being dermally exposed to unknown doses of DNOC for 2-8 weeks (Bidstrup and Payne 1951). Basal metabolic rates were elevated by 52.5% and 17% in 2 of 4 employees and body temperatures were elevated by approximately 2 ºF in most employees that sprayed DNOC for 14 days to 4 months (van Noort et al. 1960). Because DNOC aerosols were present in both work environments, inhalation is also a potential route of exposure.

Other Systemic Effects. Other systemic effects observed in humans include changes in body weight.

No changes in body weight were observed after 1.8% DNOC as the sodium salt was applied daily to the depilated dorsal surface of 10 rats or 6 rabbits for 30 days (Ambrose 1942).

2.2.3.3 Immunological and Lymphoreticular Effects

DNOC did not cause allergic reactions ≤48 and 72 hours after concentrations of 0.5% or 1.0% were diluted in water and applied to the upper back of agricultural workers, former agricultural workers, and other human subjects (Lisi et al. 1987). However, a petechial rash was observed on the right shoulder of an individual engaged in cleaning the jets of spray booms of aircraft spraying a 10% solution of DNOC in oil (Stott 1956). The exposure period was estimated to be 17 days.

No studies were located regarding immunological or lymphoreticular effects in animals after dermal exposure to DNOC.

2.2.3.4 Neurological Effects

Severe neurological effects such as coma and convulsions were associated primarily with the agonal phase of the toxicosis. One hour after 12,500 mg of DNOC in an ointment was accidentally applied to a skin rash, a 4-year-old boy complained of headaches (Buchinskii 1974). The boy later developed convulsions and he subsequently died. Histopathological changes included severe capillary hyperemia in the brain as well as brain edema. Because DNOC was applied to the rash rather than intact healthy skin, absorption of DNOC was facilitated. The amount of DNOC applied to intact skin required to produce these effects is not known. A spray operator who lapsed into a coma and developed convulsions prior to death was exposed to DNOC for an unspecified, but apparently short time period (Steer 1951). An unspecified number of field workers dermally exposed to DNOC for about 8 hours became unconscious (Vamai and Kote 1969). These patients subsequently recovered with no other apparent neurological effects. Loss of motor function in the lower limbs, convulsions, and coma and subsequent death occurred in 1 or 2 employees who sprayed 10% DNOC for 2 consecutive days (Buzzo and Guatelli 1949). Because these employees did not take appropriate precautions to minimize dermal exposure to DNOC, the author assumed that significant amounts of DNOC were absorbed through the skin to eventually cause death. An agricultural worker, who was thought to be dermally exposed to unknown doses of DNOC for 2-8 weeks, developed convulsions and lapsed into a coma prior to death (Bidstrup and Payne 1951). Because DNOC aerosols were present in the work environment, inhalation is also a potential route of exposure. An employee involved with mixing DNOC, refilling sprayer tanks with DNOC, and occasionally spraying DNOC for 5 weeks complained of headache and lassitude prior to hospital admission (Pollard and Filbee 1951). The patient's clinical

history also suggested that exposure was probably a combination of inhalation and dermal. Depression, headache, confusion, and delirium were experienced by spray operators after spraying with DNOC for 14 days to 4 months (van Noort et al. 1960). In these patients, exposure could also have been a combination of inhalation and dermal.

Peripheral neuritis was reported in two human cases to be an early sign of dermal exposure to DNOC. This effect disappeared soon after the patients were removed from the chemical. The two individuals, who were engaged in cleaning the jets of spray booms of aircraft spraying a 10% solution of DNOC in oil, developed peripheral neuritis (Stott 1956). In one individual, this symptom was described as a sensation of "pins and needles" on the backs of his hands, fingers, and legs, and occurred after one month of exposure. This patient also had a loss of sensation to pin prick or cotton-wool on the back of his fingers and toes. The second individual noted similar symptoms such as tingling sensation on backs of fingers and leg numbness after 17 days of exposure to DNOC; however, there was no loss of sensation to pin prick or cotton-wool. These neurological symptoms occurred before any other systemic effects of DNOC poisoning. The authors speculated that the symptoms could be due to a local action of DNOC on the skin since the areas most affected are those most likely to come into contact; that is the fingers and arms.

No studies were located regarding neurological effects in animals after dermal exposure to DNOC.

2.2.3.5 Reproductive Effects

Among 47 agricultural workers who became ill after dermal exposure to DNOC for about 8 hours, 3 were pregnant (Vamai and Kote 1969). One of these women gave birth to a full-term healthy child 3 days after exposure to DNOC. The investigators believed that DNOC induced labor in this woman. The other two women subsequently had full-term healthy children as well.

No studies were located regarding reproductive effects in animals after dermal exposure to DNOC.

2.2.3.6 Developmental Effects

Among 47 agricultural workers who became ill after dermal exposure exposed to DNOC for about 8 hours, 3 were pregnant (Varnai and Kote 1969). One of these women gave birth to a full-term

healthy child 3 days after exposure to DNOC. The investigators believed that DNOC induced labor in this woman. The other two women eventually also gave birth to healthy children, suggesting that DNOC was not fetotoxic in these cases. None of these workers were exposed to DNOC during the period of organogenesis and, thus, no conclusions can be drawn from these cases regarding the embryotoxicity of DNOC.

No studies were located regarding developmental effects in animals after dermal exposure to DNOC.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after dermal exposure to DNOC.

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to DNOC.

2.3 TOXICOKINETICS

The toxicokinetics of DNOC in humans and animals is dependent on its physicochemical characteristics and metabolism. Because DNOC is moderately nonpolar, it should be easily absorbed by oral, inhalation, and dermal routes. Although its distribution in human tissues is not well documented, animal data suggest that DNOC is distributed to most tissues including the lungs, heart, liver, kidney, brain, spleen, and muscle. DNOC and its metabolites are eliminated primarily via the urine in humans and animals, and elimination is slower in humans than in animals.

DNOC appears to be metabolized to less toxic metabolites readily eliminated via the urine. Although small quantities of DNOC may be conjugated, most of the dose appears to be reduced to mono amino derivatives and then subsequently conjugated prior to excretion. These relatively harmless metabolites have been found in the urine and kidney of humans and animals exposed to DNOC.

2. HEALTH EFFECTS

DNOC is an uncoupler of oxidative phosphorylation. In DNOC exposed humans or animals, a portion of the energy formed from the Krebs cycle is therefore not stored as ATP, but is given off as heat. This usually results in signs and symptoms, such as hyperthermia, perspiration, and fatigue, in humans exposed to DNOC. High doses of DNOC, elevated environmental temperatures, or physical exercise tends to exaggerate these effects and can result in death.

2.3.1 Absorption

DNOC has a relatively low pK_a and K_{ow} (see Chapter 3), but no information was located whether absorption of DNOC following inhalation, oral, or dermal exposure occurs by passive diffusion or by active transport.

2.3.1.1 Inhalation Exposure

DNOC is rapidly absorbed by the respiratory tract in humans and animals. A serum DNOC concentration of 1,000 µg/mL was detected in a spray operator 24-36 hours after inhaling a dense DNOC mist for an acute duration (van Noort et al. 1960). The worker subsequently died. Because the spray operator had previous dermal exposure to DNOC, the acute inhalation of dense DNOC mist probably caused the serum DNOC level to spike to lethal levels. A blood DNOC concentration of 60 µg/g was detected in a spray operator who had periodically inhaled an unknown amount of DNOC for 5 weeks (Pollard and Filbee 1951). The blood sample was collected after a 2-day period of no exposure. In addition, a DNOC peak urinary level of 22 mg was detected on the third day after the patient was admitted to the hospital, and a total of 89.9 mg DNOC was eliminated in the urine over 20 days. While these data indicate absorption after inhalation exposure, there was also possible dermal absorption. In an occupational exposure study involving DNOC manufacturers, winter-washer sprayers, and cereal-crop sprayers, a correlation between blood DNOC levels and the symptoms and signs of poisoning was observed (Bidstrup et al. 1952). Blood DNOC levels ≤10-20 µg/g were not generally associated with signs of toxicity, while concentrations greater than 44 µg/g resulted in several illnesses.

Limited studies in rats also show that DNOC is absorbed after inhalation exposure. DNOC was absorbed into the blood of rats exposed to DNOC aerosols for 4 or 5 hours (King and Harvey 1953a, 1954). Exposure to 0.1 mg/m^3 DNOC caused increases in the blood concentration with time. During

the 5 hours of exposure, peak blood concentrations did not exceed 60 μg/g (King and Harvey 1953a). At the end of the exposure period, 16-28 μg/g of DNOC were found in the lungs (method unspecified). In a separate experiment, exposure of rats to 100 mg/m^3 for 4 hours caused incremental increases in blood DNOC concentrations; peak blood concentrations ranged from 21 to 64 μg/g in 5 rats.

2.3.1.2 Oral Exposure

DNOC is readily absorbed by the gastrointestinal tract in humans and animals. Although doses of DNOC and blood DNOC levels were not reported, the detection of DNOC in liver, stomach, kidney, heart, and brain of two humans who committed suicide by ingesting DNOC provides evidence of gastrointestinal absorption (Sovljanski et al. 1971). DNOC was readily absorbed when 75 mg DNOC/day was given to 5 volunteers for 5 days (Harvey et al. 1951; King and Harvey 1953b). Blood DNOC levels, which ranged between 15 and 20 μg/g during the first 3 or 4 days, increased gradually during this period of dosing, and peaked from 2 to 4 hours after ingestion on each day (Harvey et al. 1951). In one individual who received the highest dose on a mg/kg/day basis, blood DNOC levels peaked at 40 μg/g after the fifth dose. Subsequent doses for 2 additional days caused peak blood levels of 40-50 μg/g in another individual. The authors suggested that this marked temporary increase in blood DNOC following administration of higher or additional doses was due to saturated mechanisms of metabolism. Although this study was limited by small sample size, the authors further suggested that blood concentrations approaching 40-48 μg/g may be associated with adverse effects. Exercise on the seventh day of the study caused an increase in blood DNOC levels, while neither alcoholic nor nonalcoholic beverages had an effect on blood DNOC concentration. Further analysis of the data for these volunteers revealed that they excreted ≈7% of the total DNOC dose in the urine over 13 days from the first dosing days (King and Harvey 1953b). Only 0.016% of the dose was excreted in the first 5 hours and 1.3% in 24 hours after dosing. In the first 24 hours after a single dose of 75 mg, an average of 39.2% of the dose could be accounted for by blood levels and 1.3% by urinary levels. Thus, an average of 59.5% of the oral dose could not be accounted for in these compartments.

Studies in animals reveal differences among species and between animals and humans. Maximum blood DNOC concentrations of 72.2 μg/g at 6 hours after the last dose of 20 mg/kg/day for 9 days and 105 μg/g at 3.5 hours after a single dose of 30 mg/kg DNOC were found in rats (King and Harvey 1953b). When rabbits were similarly treated, peak values were 54.7 μg/g at 4.5 hours after multiple

doses of 25 mg/kg/day DNOC and 49.5 μg/g at 6 hours after a single dose of 30 mg/kg. Blood DNOC levels of 25, 34, and 50 μg/g were detected in rabbits given single oral doses of 10, 15, or 18 mg/kg DNOC, respectively (Truhaut and De Lavaur 1967). Urinary excretion of DNOC and its metabolite, 6-amino-4-nitro-*o*-cresol, accounted for 25-38% of the l0-15 mg/kg/day doses in 3 days. Of this, 87-97% was excreted in the first day.

In an attempt to determine the extent and rate of gastrointestinal absorption, the concentrations of DNOC in blood and in the stomach and intestinal tissues were summed, and the concentration in the contents of the gastrointestinal tract was subtracted at various intervals after rats were dosed with 30 mg/kg (King and Harvey 1953a). The absorption of DNOC was ≈20, 10, and 5% of the dose at 1, 2, and 7 hours after dosing, respectively. The effect of environmental temperature on the absorption of DNOC was also studied. Mean DNOC blood levels were 97.4-100.3 and 93.9-100.8 μg/g in rats maintained at low temperatures (20-22 ºC) and high temperatures (37-40 ºC), respectively, in surviving rats 6 hours after an oral dose of 40 mg/kg was given. Although blood levels were not altered by environmental temperature changes, higher temperatures caused an increase in the mortality rate of rats dosed orally, but not dermally. It is probable that the greater gastrointestinal absorption of DNOC compared with dermal dosing, along with a hot environment, would have increased the chances of death due to hyperthermia.

Comparison of human and animal absorption, accumulation, and tissue saturation is difficult because of limited human data and significant differences in exposure levels. Humans exposed orally to 0.92-1.27 mg/kg/day for 5-6 days showed increasing body levels over the dosing period with blood DNOC level peaks from 2 to 4 hours after ingestion (Harvey et al. 1951). Blood DNOC levels drop slowly over several days and excretion in urine support the data that humans slowly reduce body DNOC burdens. Rats and rabbits have been exposed to much higher amounts of DNOC and at these higher levels appear to reach saturation within a day or two. In rats given oral doses of 1-100 mg/kg/day DNOC for l-8 days, the blood levels of DNOC generally reached maximum levels 2-4 hours after dosing (King and Harvey 1953a). Two daily doses of DNOC resulted in significantly higher blood levels, but continuation of doses beyond the second day maintained the 2-day level at more or less constant values, suggesting saturated blood levels. In rabbits, blood DNOC levels peaked at 8, 4, and 4-6 hours after administration of 5, 10, and 20 mg/kg DNOC, respectively (King and Harvey 1953a). Blood DNOC levels were in the range of 2.5-7.4 μg/g 24 hours after the doses were

administered. Unlike the case with rats, blood DNOC levels did not increase significantly on the second day or subsequent days when a daily dose of 25 mg/kg DNOC was given for 8 days.

In two rats given oral doses of 0.4 mg/kg ^{14}C-DNOC, 60% of the radioactive dose was accounted for in blood, urine, and tissues from one rat that was killed 1 day later and the other rat that was killed 3 days later (Leegwater et al. 1982). In the rat killed 1 day later, 15% of the radioactive dose was detected in the blood, while 28.7% was accounted for in the urine and ≈41% was distributed to other body tissues. In the rat killed 3 days after dosing, 5.5% of the radioactive dose was detected in the blood, while 41% was accounted for in the urine and ≈20% was distributed to other body organs.

As part of a study to determine the influence of dietary fats on the absorption of DNOC, mean blood levels of 50.7, 71.0, 81.0, 76.3, 61.4, 42.6, 28.3, and 19.1 μg/mL DNOC were detected at 15 minutes, 1, 3, 6, 12, 24, 30, and 48 hours, respectively, after rats were given a single dose of 15 mg/kg DNOC in saline (Starek and Lepiarz 1974). Gavage administration of olive oil, rape seed oil, or castor oil immediately after DNOC resulted in some alteration of these blood levels, indicating that the influence of fats on the amount of DNOC absorbed from the gastrointestinal tract depends on the type and dose of the fat. In general, readily digested olive oil was associated with little change in DNOC blood levels, the more slowly digested rape seed oil slightly inhibited DNOC absorption, and castor oil decreased the absorption considerably. This latter result probably reflects castor oil's cathartic effect. These interactions are further discussed in Section 2.6 of this profile.

2.3.1.3 Dermal Exposure

DNOC is rapidly absorbed by the skin in small quantities by humans (Batchelor et al. 1956; Harvey et al. 1951; Steer 1951) and rabbits (King and Harvey 1953a). Blood DNOC levels were increased by l-3 μg/g within less than 6 hours in 3 male volunteers who had an aqueous solution of DNOC dermally applied to the forearms (Harvey et al. 1951). In an experimental study, two volunteers initially placed one foot and subsequently both feet in a pail containing a 1% solution of DNOC (van Noort et al. 196'0). Serum DNOC levels were 2-4, 3-4, 7.5-8, and 27 μg/mL (roughly equivalent to μg/g) at 1, 2, 5.5, and 6.5 hours, respectively, after exposure. The data suggest that DNOC accumulated during the exposure period and probably very little was eliminated within this time. DNOC has also been detected in the blood of spray operators following dermal occupational exposure of 63.2 mg/hour for 548 hours over 5 days (Batchelor et al. 1956). DNOC serum levels did not

exceed 4.3 μg/g in 6 of these spray operators, and no correlation was apparent between total hours of exposure and serum levels. In a separate case study, 75 μg/g DNOC was recovered from the blood of a spray operator who died after dermal exposure to DNOC for an unspecified period (Steer 1951). A blood DNOC concentration of 60 μg/g was detected in another spray operator after being dermally exposed to an unknown amount of DNOC for 5 weeks (Pollard and Filbee 1951).

Dermal absorption of DNOC has been studied in rabbits. Blood DNOC levels peaked at 10-40 μg/g within l-2 hours in rabbits dermally exposed to 1 or 2 mg/cm^2 of DNOC (King and Harvey 1953a). A second dose of DNOC caused another increase in the blood DNOC values. Detection of blood DNOC 48, hours after exposure at levels higher with dermal exposure than for other routes suggests that skin acts as a reservoir for DNOC. Increased environmental temperatures appeared to have caused a sometimes delayed, but significant increase in dermal absorption of DNOC in rabbits.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

Information regarding distribution of DNOC in humans and animals after inhalation exposure is limited. About 0.9 μg/g of DNOC was recovered from the cerebrospinal fluid of an employee exposed dermally by inhalation to an unknown amount of DNOC over a 5-week period (Pollard and Filbee 1951).

Concentrations of 16, 20, 31, and 28 μg/g were recovered from the lungs of 4 rats exposed to 0.1 mg/m^3 DNOC for 4 hours (King and Harvey 1953a). The concentrations of DNOC in the alimentary tract and contents were 2.5, 3.1, 2.8, and 2.2 μg/g. The recovery of DNOC from the alimentary tract probably resulted from enterohepatic circulation and/or impaction of the aerosol along the trachea and bronchi and subsequent mucocilliary action to bring it up to the epiglottis to be swallowed.

2.3.2.2 Oral Exposure

DNOC was detected in several organs of two humans who had committed suicide after ingesting unknown quantities of DNOC (Sovljanski et al. 1971). The following levels in each respective

individual were: 13 and 400 mg/100 g in the stomach, 0.75 and 10 mg/100 g in the intestines, 0.3 and 4.72 mg/100 g in the liver, 0.125 and 2.0 mg/100 g in the kidneys, 0.3 and 2.42 mg/100 g in the heart, and 0.125 and 1.2 mg/100 g in the brain. The low levels in the one individual should cause one to question the relationship between DNOC exposure and suicide.

In two rats given oral doses of 0.4 mg/kg ^{14}C-DNOC, ≈20-41% of the radioactive dose was distributed to other body tissues (Leegwater et al. 1982). In the rat killed 1 day after the dose, 15% of the dose was detected in the blood, 5.0% in the liver, 0.94% in the kidney, 0.08% in the spleen, 6.67% in the gastrointestinal tract, and 28% in the residual carcass. In the rat killed 3 days after dosing, 5.5% was detected in blood, 2.3% in liver, 0.9% in kidneys, 0.04% in spleen, 4.0% in gastrointestinal tract, and 12.6% in residual carcass.

No information was located regarding distribution of DNOC per se in animals after oral exposure, but the metabolite, 6-amino-4-nitro-*o*-cresol was detected in the liver, kidney, and brain of rabbits given single doses of DNOC (Truhaut and De Lavaur 1967). In addition, the ratio of 6-amino-4-nitroo-cresol to DNOC increased from 0.42 to 5.29 in the kidney when the dose increased from 10 to 20 mg/kg DNOC.

2.3.2.3 Dermal Exposure

DNOC was detected in unspecified tissues of a spray operator who died after dermal exposure to an unknown amount of DNOC (Steer 1951). About 0.9 μg/g of DNOC was recovered from the cerebrospinal fluid of a spray operator thought to have been exposed dermally and by inhalation to an unknown amount of DNOC over a 5-week period (Pollard and Filbee 1951). The blood level was ≈37 μg/g on the same day, indicating a relatively smaller distribution to the cerebrospinal fluid. No studies were located regarding the distribution of DNOC in animals after dermal exposure to DNOC.

2.3.2.4 Other Routes of Exposure

DNOC was measured in the serum, brain, spleen, kidney, liver, muscle, lung, and heart of rats at 30 minutes, 1, 2, 3, 4, 5, and 6 hours after a subcutaneous dose of 10 mg/kg (Parker et al. 1951).

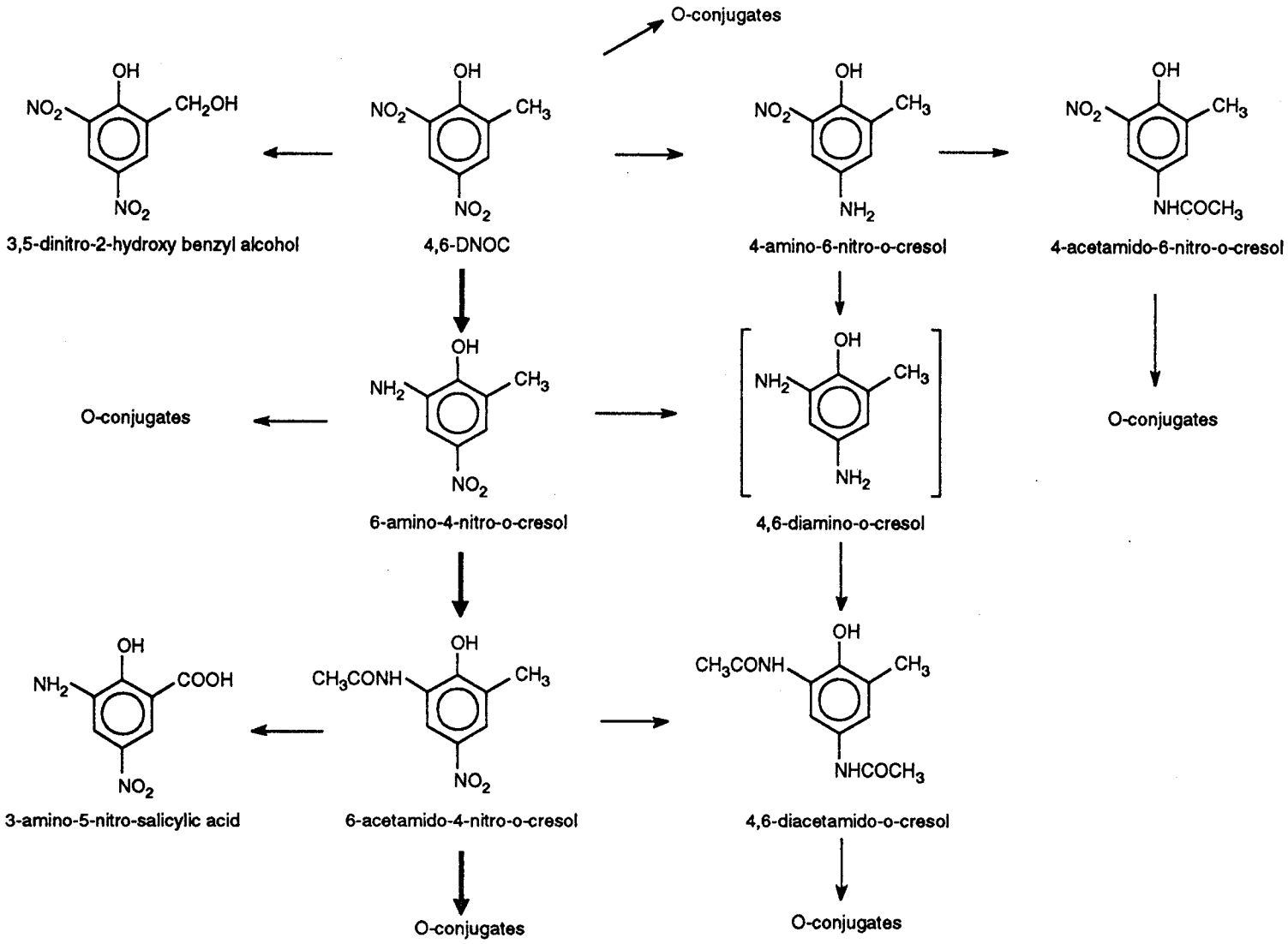
2. HEALTH EFFECTS

Except for liver and lung tissues, tissue DNOC increased from levels of 0.5-8.0 μg/g to 3.5-19 μg/g during the first 3 hours, but declined to levels of 1.5-10.5 μg/g during the next 3 hours. Liver levels fell from 14 μg/g at 30 minutes to 8 μg/g at 6 hours, while lung levels increased from 18 μg/g at 30 minutes to 20.5 μg/g at 2 hours and 30 μg/g at 6 hours. More DNOC was distributed to the lungs, heart, liver, and kidneys than to other tissues analyzed at the end of 6 hours. This can be attributed to increased blood supply to these organs and their relative affinity for DNOC. A single dose of 20 mg/kg DNOC resulted in DNOC tissue levels of 8, 7, and 45 μg/g in liver, kidney, and serum, respectively, 24 hours after the injection. A subcutaneous dose of 20 mg/kg/day for 40 days resulted in DNOC tissue levels of 7, 7, and 38 μg/g in liver, kidney, and serum, respectively, 24 hours after the last injection. The data therefore suggest that there was no tendency for DNOC to accumulate in these body tissues. In addition, there was no difference in these tissue levels when the levels were compared 24 or 48 hours after the last injection (either single or multiple dose injections).

2.3.3 Metabolism

The metabolic fate of DNOC has been determined from a limited number of in viva metabolic studies in experimental animals(Leegwater et al. 1982; Parker et al. 1951; Smith et al. 1953; Truhaut and De Lavaur 1967). Studies have reported the detection of a urinary metabolite in humans (WHO 1975). In one study, no amino-nitrophenol, glucuronides, or ethereal sulfates were detected in urine from dogs or rabbits that received 10 mg/kg DNOC subcutaneously (Parker et al. 1951). Only DNOC was detected in the urine. The data from two other studies suggest that DNOC is biotransformed to less toxic metabolites in rats (Leegwater et al. 1982) and in rabbits (Smith et al. 1953; Truhaut and De Lavaur 1967) (see Figure 2-3). Unchanged DNOC and conjugated and unconjugated metabolites of DNOC were recovered from urine 2 days after rabbits received oral doses of 20-30 mg/kg DNOC (Smith et al. 1953). Less than 20% of the dose was excreted as metabolites; almost 5% of the dose was excreted as unchanged DNOC and 1% as conjugated DNOC. Therefore, the conjugation of DNOC represents a minor pathway. The metabolites were derivatives of 6-amino-4-nitro-*o*-cresol (≈11-12% of the dose). 6-Acetamido-4-nitro-*o*-cresol represented 1-1.5% of the dose and O-conjugates of this compound ≈10% of the dose. Small amounts of 3-amino-5-nitrosalicylic acid and derivations of 4-amino-6-nitro-*o*-cresol were also excreted. The 6-nitro group, therefore, appears to be more readily reduced than the 4-nitro group. According to the pathway, the acetylated metabolite of 6-amino-4-nitro-*o*-cresol, 6-acetamido-4-nitro-*o*-cresol, is further metabolized to traces of 3-amino-5-nitrosalicylic acid and larger amounts of conjugates of 6-acetamido-4-nitro-*o*-cresol. 6-Amino-

FIGURE 2-3. Metabolic Pathways for 4,6-Dinitro-o-cresol*



* Adapted from Leegwater et al. 1982; Smith et al. 1953; Truhaut and DeLavaur 1967

2. HEALTH EFFECTS

4-nitro-*o*-cresol and 6-acetamido-4-nitro-*o*-cresol were far less toxic than DNOC when oral doses were given to rabbits, suggesting that the major detoxification pathway in rabbits occurs via reduction at the 6-nitro group rather than via conjugation of the hydroxyl group of DNOC alone.

No amino derivatives of DNOC were detected in the blood, bone marrow, or adipose tissue, but 6-amino-4-nitro-*o*-cresol was detected in the liver, kidneys, and brain of rabbits that received an oral dose of 18 mg/kg DNOC (Truhaut and De Lavaur 1967). No 4-amino-6-nitro-*o*-cresol was detected in these tissues. Both DNOC and 6-amino-4-nitro-*o*-cresol were recovered from the urine as 25-38% of the dose. Smaller amounts of 4-amino-6-nitro-*o*-cresol were also detected in the urine. Further experiments demonstrated that as the dose of DNOC increased, the ratio of 6-amino-4-nitro-*o*-cresol to DNOC in urine increased. The data from this study support the findings of Smith et al. (1953) by demonstrating that the metabolic reduction of DNOC to 6-amino-4-nitro-*o*-cresol was the major detoxification pathway and that this pathway becomes more important at higher doses.

The following urinary metabolites were identified and quantitated in a rat given 0.4 or 6.0 mg/kg ^{14}C-DNOC: 6-amino-4-nitro-*o*-cresol (l-2%); 6-acetamido-4-nitro-*o*-cresol (2-3%); 3,5dinitro-2-hydroxybenzyl alcohol (4-5%); 4,6-diacetamido-*o*-cresol (18%); 4-acetamido-6-nitro-*o*-cresol (l-2%) (Leegwater et al. 1982). In addition, the urine contained several unknown metabolites and conjugates. In another experiment, the metabolites 6-amino-4-nitro-*o*-cresol, 6-acetamido-4-nitro-*o*-cresol, and 4,6-diacetamido-*o*-cresol were also identified in a 24-hour urine sample from rabbits given 20 mg/kg. This study confirms findings of King and Harvey (1953a, 1953b), Smith et al. (1953), and Truhaut and De Lavaur (1967) showing slow elimination of DNOC and reduction as the major metabolic pathway. The metabolites 3,5-dinitro-2-hydroxybenzyl alcohol and 4,6-diacetamido-*o*-cresol had not been previously found in rats.

Rat cecal contents were incubated with DNOC to determine whether the compound is metabolized in the large intestine (Ingebrigtsen and Froslie 1979). About 80% of DNOC was metabolized to 6-amino-4-nitro-*o*-cresol within 1 hour. Within the next 12 hours, 90% of this metabolite was further reduced to 2-methyl-4,6-diaminophenol. The authors determined that the cecal microorganisms in rats were responsible for the reduction of DNOC and its subsequent metabolites to diamino derivatives. Although not detected in humans or other monogastrics, these diamino derivatives are formed in sufficient quantities in ruminants to cause methemoglobinernia, which can be fatal in these species (Froslie 1973).

2. HEALTH EFFECTS

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

Based on measured DNOC blood levels of a worker exposed to DNOC by a combination of inhalation and dermal routes (Pollard and Filbee 1951), an elimination rate constant of 0.002 $hour^{-1}$ and a half-life of 153.6 hours were determined (King and Harvey 1953b). A peak urinary quantity of 22 mg DNOC was found on the third day after the employee was admitted to the hospital and 5 weeks after his initial exposure (Pollard and Filbee 1951). About 89.9 mg of DNOC was eliminated via the urine over the 20 days after admission. The data suggest that humans have a relatively inefficient mechanism for eliminating DNOC and this may be due to slow detoxification and excretion or storage of DNOC in the body.

In the only inhalation study located for animals, an elimination rate constant of 0.01 $hour^{-1}$ was determined for female hooded rats exposed to 2 mg/m^3 of DNOC aerosols for 5 hours (King and Harvey 1954). This was determined to correspond to an initial blood level of 60 µg/g that would result in essentially complete elimination of DNOC in 182 hours.

2.3.4.2 Oral Exposure

Urinary excretion data from 5 humans who each ingested 75 mg DNOC/day for 5 days suggested that at least 7% of the dose was eliminated via the urine over a 13-day period (King and Harvey 1953b). Only 0.016% and 0.8-2.0% of the dose were excreted in the first 5 and 24 hours, respectively, after dosing (Harvey et al. 1951; King and Harvey 1953b). In addition, the amount of DNOC excreted in the urine was independent of the concentration of DNOC in the blood of three humans. The data from both studies suggested that humans metabolized DNOC less efficiently than rats and rabbits. The apparent accumulation of DNOC in humans could be due to slow metabolism and excretion and/or storage of DNOC in the body. Because DNOC binds to albumin, the authors suggested that the chief internal stores were extracellular fluids containing albumin.

Species differences in elimination have been found among animals administered DNOC orally. Elimination rate constants were 0.0105 and 0.0112 $hour^{-1}$ in rats given 9 daily doses of 20 mg/kg/day DNOC and a single dose of 30 mg/kg DNOC, respectively (King and Harvey 1953b). The half-lives

for DNOC were 26.8 and 28.5 hours for the multiple dose study and the single dose study, respectively. These data suggested that rats eliminated DNOC faster than humans. An average of only 1.9% of total DNOC ingested was recovered in the urine over 4 days after 4 rats had received total doses in the range of 5.75-7.0 mg DNOC per rat. The rats excreted no DNOC in the urine in the first 5 hours and very little during the next 96 hours. Although rats appeared to eliminate DNOC in the urine at a similar rate as humans, the authors concluded that repeated daily dosing with DNOC did not appear to affect the rats' capacity for eliminating DNOC and that the degree of detoxification is probably greater in rats than in humans. The sex of the rats, the magnitude of the dose, and the frequency of dosing were found to have little effect on the elimination of DNOC (King and Harvey 1954). Higher elimination rate constants were obtained for rabbits compared to rats; that is, 0.0448 $hours^{-1}$ for the multiple dose study and 0.0454 $hours^{-1}$ for the single dose study (King and Harvey 195313). The half-lives were also shorter (6.6-6.7 hours) than those obtained for rats. An average of 7.7% of the dose was recovered from the urine within 3 days after (34.4-44.6 mg per rabbit) DNOC was given orally. Most of the excreted amount (average 6.4% of the dose) was eliminated through the urine in the first 5 hours. After comparing the data from humans, rats, and rabbits, the authors concluded that the rabbit is most efficient in detoxifying and eliminating DNOC.

In another study, the elimination rate constants for DNOC in rats, rabbits, guinea pigs, mice, and monkeys given single unspecified oral doses of DNOC were 0.01, 0.045, 0.032, 0.036, and 0.01 hours-l, respectively (Lawford et al. 1954). Additional toxicokinetic data were not reported.

DNOC and its metabolite, 6-amino-4-nitro-*o*-cresol, which were detected in the urine of rabbits, made up 25-38% of the 10-15 mg/kg DNOC dose (Truhaut and De Lavaur 1967). Of this amount, 82-97% was eliminated within 1 day, and the rest was excreted within 2-3 days. As the dose of DNOC increased from 10 to 20 mg/kg, the ratio of 6-amino-4-nitro-*o*-cresol to DNOC in urine increased from 0.66 to 1.47 when measured at 2.5-3.75 hours after the dose. These ratios may be useful biomarkers of exposure to DNOC if the same phenomenon occurs in humans.

Within 2 days after receiving a single dose of 20-30 mg/kg DNOC, Chinchilla rabbits excreted <20% of the dose as metabolites (Smith et al. 1953). Unchanged DNOC accounted for ≈5% of the dose and conjugated DNOC accounted for ≈1%. Derivatives of 6-amino-4-nitro-*o*-cresol comprised ≈11-12% of the dose, including 6-acetoamido-4-nitro-*o*-cresol (1-1 .5% of the dose), O-conjugates of this metabolite

(10% of the dose), and unspecified amounts of 3-amino-5-nitro-salicylic acid and derivatives of 4-amino-6-nitro-*o*-cresol that were also excreted in the urine.

In 2 rats given oral doses of 0.4 mg/kg ^{14}C-DNOC, about 29-41% of the radioactive dose was excreted in urine and l0-23% was excreted in the feces (Leegwater et al. 1982). The half-life for elimination of radioactivity was 1-1.5 days. In the rat killed 1 day after the dose, 28.7% of the dose was excreted in the urine and 28% in feces. In the rat killed 3 days after dosing, excretion amounted to 23% of the dose in the first 24 hours, 16.4% in the next 24 hours, and 11.5% in the third 24 hours. Fecal excretion amounted to 7.1% in the first 24 hours, 9.3% in the second 24 hours, and 6.2% in the third 24-hour period. The following urinary metabolites were determined in a rat given 0.4 or 6.0 mg/kg ^{14}C-DNOC: DNOC (3-4%); 6-amino-4-nitro-*o*-cresol (l-2%); 6-acetamido-4-nitro-*o*-cresol (2-3%); 3,5-dinitro-2-hydroxybenzyl alcohol (4-5%); 4,6-diacetamido-*o*-cresol (18%); and 4-acetamido-6-nitro-*o*-cresol (l-2%). In addition, the urine contained several unknown metabolites and conjugates. The dose of DNOC had little affect on the distribution pattern of metabolites. In another experiment, the metabolites 6-amino-4-nitro-*o*-cresol, 6-acetamido-4-nitro-*o*-cresol, and 4,6-diacetamido-*o*-cresol were identified in a 24-hour urine sample from rabbits given 20 mg/kg DNOC. This study confirms findings of King and Harvey (1953a, 1953b) and Smith et al. (1953), showing slow elimination of DNOC and reduction as the major metabolic pathway.

DNOC and its cresolic metabolites were not detected in the urine from rats given 0.029 or 0.293 mg/kg DNOC for 3 days (Shafik et al. 1973). However, these were very low doses, and the detection limits ranged from 0.01 to 0.05 μg/g. The elimination rate constants for DNOC in rats exposed to 9 daily doses of DNOC averaged 0.0124 hour^{-1} (King and Harvey 1954). The authors calculated that an initial blood level of 60 μg/g DNOC will be eliminated almost completely from the blood within 182 hours.

2.3.4.3 Dermal Exposure

An average concentration of 0.8 ug/g DNOC with a range of 0.6-1.3 μg/g was detected in the urine from spray operators exposed dermally to 63.2 mg DNOC/hour (Batchelor et al. 1956). Of the 183 urine samples obtained from the spray workers, only 5 contained ≥0.5 μg/g DNOC as the sodium salt (limit of detection). Based on measured DNOC blood levels of a worker exposed to DNOC by a combination of inhalation and dermal routes (Pollard and Filbee 1951), an elimination rate constant of

0.002 $hours^{-1}$ and a half-life of 153.6 hours were determined (King and Harvey 1953b). Three of 4 spray operators, who were exposed to DNOC primarily by the dermal route for 14 days to 4 months, had initial serum DNOC levels of <5-100 μg/mL at the time of hospitalization (van Noort et al. 1960). In 2 of these patients, serum levels decreased from 60 to 40 μg/mL in 1 week and from 100 to 5 μg/mL in 3 weeks, respectively. Although the initial serum DNOC level was not determined in the fourth patient, 10 μg/mL DNOC was detected in the serum 1 month after exposure, suggesting that the initial serum level was extremely high. Thus DNOC was eliminated slowly and at similar rates in these humans. A peak urinary DNOC excretion of 22 mg was observed on the third day after the employee was admitted to the hospital and 5 weeks after his initial combined dermal and inhalation exposure to DNOC (Pollard and Filbee 1951). A total of 89.9 mg of DNOC was eliminated via the urine over the 20 days after admission. The data suggest that humans have a relatively inefficient mechanism for eliminating DNOC and this may be due to slow metabolism and excretion and/or storage of DNOC in the body.

No studies were located regarding the rate and extent of excretion of DNOC following dermal exposure in animals.

2.3.4.4 Other Routes of Exposure

Urinary DNOC accounted for 10% of the total dose of 0.5-80 mg/animal over 3 days after the last, daily, subcutaneous injection of DNOC in rabbits and dogs (Parker et al. 1951). Further specific details regarding the amount and rate of excretion were not provided. The determined elimination rate constants for DNOC were 0.02, 0.077, 0.021, 0.04, and 0.02 $hour^{-1}$ in rats, rabbits, guinea pigs, mice, and monkeys, respectively, following single intraperitoneal doses of DNOC (Lawford et al. 1954). Additional excretion data were not reported for this study. Neither the sex of the test species nor the magnitude of the DNOC dose had a marked effect on the elimination of DNOC in rats given various intraperitoneal doses of DNOC (King and Harvey 1954). Elimination rate constants ranged from 0.013 to 0.019 $hours^{-1}$ for the 20 mg/kg dose and 0.01-0.018 $hours^{-1}$ for the 5, 10, and 15 mg/kg dose groups. The determined mean elimination rate constant was 0.015 $hour^{-1}$ for this study. The investigators also observed significantly higher elimination rate constants for blood obtained by cardiopuncture than those obtained by tail bleeding. In addition, higher elimination rate constant values were observed in rats acclimatized to high temperatures, compared to those suddenly exposed to high temperatures.

2. HEALTH EFFECTS

2.3.5 Mechanisms of Action

DNOC is readily absorbed from the gastrointestinal and respiratory tract (Bidstrup et al. 1952; Harvey et al. 1951; King and Harvey 1953a, 1954) and less readily by the skin (Batchelor et al. 1956; King and Harvey 1953a). No information was located whether DNOC is absorbed by passive diffusion or by active transport after inhalation, oral, or dermal exposure.

Available data from one study suggest that more DNOC is distributed in decreasing order to the lungs, heart, liver, kidney, spleen, brain, and muscle of rats (Parker et al. 1951). As DNOC binds to albumin, the main internal stores of DNOC may be extracellular fluids containing albumin (King and Harvey 1953b).

DNOC is generally metabolized in animals to less toxic metabolites that are mostly eliminated in the urine (Leegwater et al. 1982; Smith et al. 1953; Truhaut and De Lavaur 1967). No studies have reported the detection of DNOC metabolites in the urine from humans exposed to DNOC.

No information was located regarding the mechanism of excretion of DNOC or any of its isomers. Since DNOC, its metabolites, and its isomers are relatively lipophilic, excretion by passive diffusion is the probable mechanism.

Evidence from one study suggests that DNOC, rather than a metabolite is the putative toxic agent (Smith et al. 1953). In addition, results of genotoxicity studies indicated that DNOC is more genotoxic in the absence, rather than the presence, of metabolic activation systems (see Genotoxic Effects in Section 2.4). Acute toxic effects are therefore related to DNOC acting directly on cell metabolism and interfering with oxidative phosphorylation. DNOC is believed to cause an acceleration of metabolic processes that are part of the tricarboxylic acid (TCA) cycle (Parker et al. 1951). During the TCA cycle, the energy produced from the catabolism of glucose is stored in the form of ATP. DNOC produces its accelerative effect by interrupting the phosphate transfer to adenosine diphosphate (ADP) to form ATP. Uncoupling allows electron transport to proceed unchecked even when ATP synthesis is inhibited. As a consequence, more ADP and inorganic phosphate are available to drive the TCA cycle, and most of the energy produced from catabolism of glucose is not stored in high energy phosphate bonds as ATP but is given off as heat (Parker et al. 1951). If heat production exceeds the capacity for heat loss, fatal hyperthermia may result (Murphy

1986). Signs of DNOC toxicity such as hyperthermia, tachycardia, increased respiration and basal metabolic rates, perspiration, cataractogenesis, and death in humans and animals are related to the uncoupling of oxidative phosphorylation. Several case reports have described the occurrence of elevated body temperatures and complaints of excessive perspiration from employees and patients exposed to DNOC (Bidstrup et al. 1952; Plotz 1936; Pollard and Filbee 1951; Stott 1956).

Several *in vitro* studies have further demonstrated the ability of DNOC to uncouple oxidative phosphorylation (Ilivicky and Casida 1969; Muscatello et al. 1975; Verschoyle et al. 1987; Williamson and Metcalf 1967). In one study, the uncoupling action of DNOC and other dinitrophenol derivatives were investigated as well as the relationship between their uncoupling potency and toxicity (Ilivicky and Casida 1969). Mitochondria from mouse liver and brain were equally sensitive to the uncoupling action of DNOC. Isolated brain and liver mitochondria from mice treated with dinitrophenols derivatives other than DNOC were completely uncoupled or inhibited only when the dose resulted in severe symptoms of poisoning (Ilivicky and Casida 1969). DNOC was not tested in this experiment, but the data from these studies suggest that a relationship between the severity of DNOC toxicity and the extent of uncoupling by DNOC may exist.

In another *in vitro* study, the effect of uncoupling by DNOC on the structure of rat liver mitochondria was investigated using electron microscopy (Muscatello et al. 1975). When the mitochondria were placed in the uncoupled state, the rate of oxygen uptake was increased and the mitochondria appeared condensed with deep invaginations of the inner membrane, compared to its expanded configuration when DNOC was not present. The authors also determined that the ultrastructural modification was as rapid as the functional one.

Active transport is required for the absorption and movement of biologically important molecules across a membrane against a concentration gradient. This process, which requires ATP, can be inhibited if DNOC is present. An *in vitro* study in the pig demonstrated DNOC inhibition of active transport by observing the uptake of gamma-globulin by neonatal intestinal epithelium (Lecce 1966).

2.4 RELEVANCE TO PUBLIC HEALTH

Because DNOC is moderately nonpolar, it should be rapidly absorbed by the lungs, gastrointestinal tract, and the skin. Animal data suggest that DNOC is distributed to most tissues including the lungs,

heart, liver, and kidney. Results from animal studies suggest that most of the dose is metabolized to water soluble conjugates that are mostly eliminated via the urine. Elimination of DNOC from the blood is more prolonged in humans than in laboratory animals; therefore, there is a potential for accumulation.

The most significant and sensitive effects resulting from acute, intermediate, or chronic exposure are related to increased basal metabolic rates in humans. Despite insufficient data regarding inhalation and dermal routes, these effects are not likely to be route-dependent.

DNOC uncouples oxidative phosphorylation resulting in energy being given off as heat and manifested as hyperthermia. In an attempt to reduce body temperature, the body increases respiratory rate and heart rate as part of a compensatory mechanism. As a result, increased pulse rate, respiratory rate, and profuse sweating were commonly seen in humans and animals exposed to DNOC. Neurological signs such as lethargy, depression, and peripheral neuritis have occurred in humans exposed to DNOC. Maculopapular urticarial eruptions were also observed in humans after oral exposure; this effect was not seen in animals.

DNOC has been associated with cataract formation in humans. Cataract formation is an important reason why the government and the medical community stopped use of DNOC and dinitrophenol for weight-loss in humans.

No reliable data were located regarding reproductive or developmental effects in humans, although one study suggested that DNOC may have induced labor in a pregnant agricultural worker. However, DNOC was reported to cause aspermatogenesis and a lack of corpora lutea in rats.

DNOC has been tested for genotoxicity in a variety of assays with mostly positive results for *in vivo* test systems and both positive and negative results for the *in vitro* test systems. These results indicate genotoxic potential. No cancer studies were available.

Very little information on other dinitrocresol isomers was located, but toxicity studies in animals conducted by parenteral routes and genotoxicity studies in bacteria indicate that 2,6-dinitro-*p*-cresol is similar in potency and action to 4,6-DNOC, but that 4,6-dinitro-m-cresol is less toxic.

2. HEALTH EFFECTS

Employees at hazardous waste sites, employees at pesticide manufacturing plants and formulating plants, and farm workers are more likely to be exposed to DNOC than the general population. DNOC was once used as a weight reduction drug, but this practice has been discontinued since the toxic effects have been recognized. Although high atmospheric concentrations of DNOC have been detected at manufacturing plants that make DNOC or at farms that use DNOC, data regarding concentrations of DNOC in ambient air were not located. DNOC has been detected in the waste water from chemical and pest control production plants that manufacture DNOC and infrequently in ground and surface water from areas where DNOC has been used as a pesticide. However, DNOC has not been detected in drinking water. Humans can be exposed to DNOC by ingesting food that has been sprayed with DNOC; however, DNOC levels in this media are also unknown.

Minimal Risk Levels for DNOC

Inhalation MRLs.

No MRLs have been derived for inhalation exposure to DNOC because data for all durations are insufficient. Although health effects have occurred in humans occupationally exposed to DNOC, exposure probably involved both the inhalation and dermal routes, and exposure concentrations were not known. Only one study was located regarding health effects in animals after inhalation exposure to DNOC. In this study, rats exposed to 0.1 or 100 mg/m^3 DNOC for 4-5 hours were lethargic, and rats exposed to 100 mg/m^3 had increased respiratory rates and body temperatures (King and Harvey 1953a). No NOAEL was identified, and other end points were not evaluated. Therefore, data are insufficient to derive MRLs for inhalation exposure to DNOC.

Oral MRLs

- An MRL of 0.004 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) and for intermediate-duration oral exposure (15-364 days) to DNOC.

The MRL was based on LOAEL of 0.35 mg/kg/day for neurological effects in a human who took DNOC for the purpose of weight reduction (Plotz 1936). In this report, three other individuals also took DNOC for the purpose of weight reduction, and the investigator took DNOC in a self-experiment. The average doses taken by the other patients and the investigator were 0.58-1.0 mg/kg/day for

periods of 4-11 weeks. Basal metabolic rate, pulse, blood pressure, body temperature, and body weight were monitored. The patient who received the LOAEL of 0.35 mg/kg/day initially received 0.75 mg/kg/day for 3 days, but experienced elevated body temperature, fatigue, and dizziness after 2 days of taking 0.75 mg/kg/day. After a 2-week period of taking no DNOC, treatment was resumed at 0.35 mg/kg/day, and he complained of excessive perspiration, fatigue, and dizziness on the 7th day. Signs of toxicity observed in the other patients included marked palpitations, elevated pulse rate, elevated body temperature, excessive perspiration, fatigue, lassitude, headache, a greenish tinge to the sclerae, and maculopapular, urticarial eruptions. Thus, the other cases in the report by Plotz (1936) support the LOAEL of 0.35 mg/kg/day.

Other studies in humans also support the LOAEL. Five healthy male volunteers weighing 59-81.4 kg who received 75 mg/day DNOC (0.92-1.27 mg/kg/day) on 5 consecutive days experienced lassitude, headache, and malaise (Harvey et al. 1951). Two of an unspecified number of human subjects who received 3 mg/kg/day DNOC had a 70-100% increase in metabolic rate within 3 days, a slight increase in pulse rate, sweating, lethargy, headache, loss of appetite, and definite greenish-yellow pigmentation of the conjunctivae (Dodds and Robertson 1933).

In a case report of 15 patients who received 50 mg DNOC/day (average dose consumed equalled 1.05 mg/kg/day for 14-63 days), the average amount of weight loss was 0.45 kg/week (Ibrahim et al. 1934). DNOC caused an increase in basal metabolic rate, excessive perspiration, thirst, and fatigue. Yellow pigmentation of the conjunctivae occurred in all cases. Thus, it appears that some individuals were able to tolerate higher doses of DNOC for longer periods of time before developing symptoms. For this reason, the acute oral LOAEL of 0.35 mg/kg/day was considered to be an appropriate basis for the intermediate-duration MRL as well as for the acute-duration MRL. Animal studies all used higher doses of DNOC than human studies. Toxicokinetic studies indicate that humans tend to accumulate DNOC to a greater extent and eliminate DNOC more slowly than animals do (King and Harvey 1953b). Furthermore, the use of DNOC as a weight-reducing drug in humans was carried out under medical supervision, and information on actual doses and durations were available.

No MRL has been derived for chronic-duration oral exposure to DNOC because no studies of chronic duration were located.

2. HEALTH EFFECTS

Death. Humans exposed occupationally to DNOC for acute- (Steer 1951; van Noort et al. 1960) or intermediate- (Bidstrup and Payne 1951; Bidstrup et al. 1952) durations have died. Occupational exposure usually involves a combination of inhalation and dermal exposure. A blood level of 75 µg/g DNOC was found in one worker who died (Bidstrup et al. 1952). In addition, a worker died after he drank water contaminated with DNOC (Bidstrup and Payne 1951). The dose or the amount of DNOC was not specified in case reports regarding death in humans after occupational or oral exposure to DNOC. However, in one case report, the application of a fat-based ointment containing 12,500 mg of DNOC to a skin rash resulted in death in a 4-year-old boy (Buchinskii 1974). Because DNOC was applied to the rash rather than intact healthy skin, absorption of DNOC was facilitated.

Only one study was located regarding the death of animals after inhalation of DNOC aerosols (Burkatskaya 1965a). In this study, cats died after they were exposed to concentrations of 40 or 100 mg/m^3 DNOC for 4 hours or 2.0 mg/m^3 DNOC for 1 month. The dermal LD_{50} for DNOC was 200-600 mg/kg for rats and the oral LD_{50} ranged from 25 to 40 mg/kg in rats (Ben-Dyke et al. 1970; Jones et al. 1968). Intraperitoneal LD_{50} values for rats were also within the same range as those obtained for the oral LD_{50} studies (Parker et al. 1951; Stoner 1969). There were no appreciable differences between oral and intraperitoneal LD_{50} values in rats, and intraperitoneal LD_{50} values among rats (Stoner 1969), rabbits, mice, and guinea pigs (Lawford et al. 1954). Furthermore, the intraperitoneal LD_{50} of 2,6-dinitro-*p*-cresol (24.8 mg/kg) in mice was no different from that of DNOC (24.2 mg/kg) (Harvey 1953). Elevated environmental temperatures increased the toxicity of DNOC, as evidenced by reduced oral LD_{50} values in rats and mice and exacerbated clinical signs (Harvey 1959).

Because DNOC is eliminated more slowly in humans (Harvey et al. 1951) than in most laboratory animals (King and Harvey 1953b), single exposures to high enough doses or repeated exposure to DNOC by any route can possibly result in accumulation of sufficient amounts to cause death. This becomes more important with employees working in hot environments.

Systemic Effects.

Respiratory Effects. Occupational exposure to DNOC aerosols has caused dyspnea and increased respiratory rates in humans (Hunter 1950; Pollard and Filbee 1951; Steer 1951; van Noort et al. 1960). However, no signs of respiratory effects were seen in volunteers that ingested 0.92-1.27 mg/kg/day for 5-7 days (Harvey et al. 1951). In an agricultural worker who died after drinking water contaminated

with DNOC, respiratory distress and pulmonary pathology were more associated with the agonal phase of the toxicosis (Bidstrup and Payne 1951). Dermal exposure to DNOC caused increased respiratory rates and pulmonary edema in one individual (Buchinskii 1974) and dyspnea in spray operators (Buzzo and Guatelli 1949).

Respiratory rates were also increased in rats exposed to 100 mg/m^3 DNOC aerosols for 4 hours (King and Harvey 1953a). A concentration of 40 or 100 mg/m^3 DNOC caused dyspnea as well as sneezing and/or nasal secretions in cats after a 4-hour exposure (Burkatskaya 1965a). The data suggest that DNOC aerosols may also irritate the upper respiratory tract in humans. Although a lethal oral dose of 36-90 mg/kg DNOC as the sodium salt may have caused severe acute respiratory signs in rats (Ambrose 1942) similar to those described in a human (Bidstrup and Payne 1951), doses of DNOC in the range of l-25 mg/kg/day for 77-182 days did not result in either signs of respiratory distress or histopathology of the respiratory tract in rats (Spencer et al. 1948). However, oral doses in the range of 10-35 mg/kg DNOC caused dyspnea in mice within 60-80 minutes (Arustamyan 1972), and an oral dose of 25 mg/kg DNOC caused dyspnea and heavy breathing in cats within the first hour (Burkatskaya 1965b). The latter study is limited by inadequate reporting of experimental details and data. No animal data were located to provide supportive evidence of respiratory effects after dermal exposure.

Dyspnea and increased respiratory rates appear to be related to the ability of DNOC to uncouple oxidative phosphorylation, resulting in energy loss as heat rather than the energy being stored as ATP. This is manifested as hyperthermia. Respiratory rates may increase as a compensatory mechanism to reduce body heat. Pulmonary edema, which was observed in both humans and animals, was more likely related to agonal heart failure, rather than a direct effect of DNOC on the respiratory system. The results from both human and animal studies suggest that the respiratory effects in humans may be mild in low-dose acute or long-term exposures, but severe and possibly fatal in acute high-dose exposures.

Cardiovascular Effects. Agricultural workers and factory workers died after occupational exposure to unknown amounts of DNOC for acute (Steer 1951) or intermediate durations (Bidstrup and Payne 1951). The elevated pulse rate deteriorated to cardiac fibrillation and death in an acute dermal exposure (Steer 1951) and to cyanosis and death in workers exposed to DNOC aerosols (Bidstrup and Payne 1951). Occupational exposure to DNOC for acute (Buzzo and Guatelli 1949; Hunter 1950) and

intermediate (Pollard and Filbee 1951; van Noort et al. 1960) durations also resulted in elevated pulse rates without causing death. Although the basal metabolic rate was elevated, no effect on heart rate or pulse was found in volunteers who had ingested acute doses of 0.92-3.0 mg/kg/day for 4-7 days (Dodds and Robertson 1933; Harvey et al. 1951). However, pulse rate was elevated in a patient taking DNOC as a weight-reducing drug (Gordon and Wallfield 1935). Although the cardiovascular system was not affected in volunteers who ingested 3 mg/kg/day for 4 days or in some patients who ingested 0.5-1.05 mg/kg/day DNOC for intermediate durations (Dodds and Robertson 1933; Ibrahim et al. 1934), marked palpitations, tachycardia, or elevated pulse rates were observed in some patients after ingesting ≈0.75-1.0 mg/kg/day for 2 weeks to >6 months (Plotz 1936; Quick 1937). An acute dermal exposure to DNOC (12 grams in a 4-year-old) caused elevated pulse rates within 2 hours of exposure as well as severe capillary hyperemia in the myocardium (Buchinskii 1974). The effects of DNOC on heart rate and pulse may also be related to uncoupling of oxidative phosphorylation by DNOC.

No histological evidence of cardiac lesions were found in rats exposed to DNOC in the diet for intermediate durations (Den Tonkelaar et al. 1983; Spencer et al. 1948).

The human case reports strongly suggest that some human subpopulations may develop mild to severe cardiovascular effects after either acute or intermediate-duration exposure to DNOC.

Gastrointestinal Effects. DNOC appears to target the gastric mucosa in humans after oral and occupational exposure. Hemorrhagic gastritis occurred in an agricultural worker who died after drinking water contaminated with DNOC (Bidstrup and Payne 1951), and a girl who took 2.27 mg/kg/day DNOC for 11 days became nauseated and vomited (Gordon and Wallfield 1935). Multiple hemorrhagic erosions were also observed in the gastric mucosa of 6 workers who died after occupational exposure to DNOC (Bidstrup and Payne 1951). DNOC caused nausea and vomiting in spray operators after occupational exposure (van Noort et al. 1960) and vomiting in a boy who had died after DNOC was applied to a skin rash (Buchinskii 1974). In the latter case report, a hemorrhagic intestinal mucosa and severe hyperemia of the intestinal walls were observed at autopsy. Data from one animal study support the human data by demonstrating that the hydrochloric acid releasing cells in the fundus of the stomach of rats were reduced in number after a 90-day oral exposure to DNOC (Den Tonkelaar et al. 1983). Cells in the salivary gland were similarly affected. Another oral study in mice demonstrated that DNOC caused catarrhal inflammation in the small intestine as well as the coagulative necrosis of the gastric mucosa (Arustamyan 1972). The human and

animal data suggest that the cells of the gastric mucosa may be targeted by DNOC and that necrosis and/or degeneration of the gastric mucosa occurs in humans exposed DNOC.

Hematological Effects. DNOC did not result in changes in hematological parameters in humans following occupational exposure for 5 weeks (Pollard and Filbee 1951) or following oral exposure to 2.27 mg/kg/day for 11 days (Gordon and Wallfield 1935) or 0.97-1.27 mg/kg/day for 5-7 days (Harvey et al. 1951). However, an unspecified and poorly defined bleeding disorder was observed in employees after an acute-duration occupational exposure to DNOC (Vamai and Kote 1969). The bone marrow from one worker who died was histologically characteristic of anoxemia (Bidstrup and Payne 1951).

Inhalation of DNOC aerosols for acute or intermediate durations significantly decreased erythrocyte counts and hemoglobin content, accelerated the erythrocyte sedimentation rate and/or significantly increased the leukocyte count in cats (Burkatskaya 1965a). Data regarding hematological effects in animals after dietary exposure to DNOC for intermediate durations are conflicting. While no effects on hematological parameters were found in rats exposed orally at l-25 mg/kg/day DNOC in 2 studies (Spencer et al. 1948; Vos et al. 1983), hemosiderosis and congestion of the spleen were seen at 25 mg/kg/day (Spencer et al. 1948). In addition, similar oral doses of DNOC caused changes in the hematocrit, hemoglobin, red and white blood cell count, and leukocyte differential count in rats exposed for 90 days in another study (Den Tonkelaar et al. 1983). Therefore, the potential for DNOC to cause hematological effects in humans cannot be ruled out.

Musculoskeletal Effects. Limited human data suggest that DNOC causes muscular pain, involuntary contraction (van Noort et al. 1960), muscular rigidity, and loss of motor function (Buzzo and Guatelli 1949) after acute occupational exposure. Data from one animal study suggest that inhalation of DNOC also causes loss of muscle tone in cats (Burkatskaya 1965a). Because no detailed muscular examinations were performed, whether these were effects on the muscles or on the neurons innervating the muscles is not clear.

Hepatic Effects. Although exposure to DNOC usually results in an icteric appearance in humans, the yellow color in tissues is due to the yellow color of DNOC. Negative results for the icteric index and the Van den Bergh test have been consistently found (Dodds and Robertson 1933; Gordon and Wallfield 1935; Plotz 1936). However, data from several human case reports suggest that DNOC

directly affects the liver. Congestion of the liver was observed in an agricultural worker who died after accidentally drinking water contaminated with DNOC and in five other workers who died after occupational exposure to DNOC (Bidstrup and Payne 1951). However, the occurrence of severe capillary hyperemia of the liver in a young boy who died after DNOC in an ointment was applied to a skin rash (Buchinskii 1974) and unspecified liver damage and enlarged livers in several employees after acute dermal exposure (Vamai and Kote 1969) is further evidence that DNOC may have direct effects on the liver.

No studies were located regarding hepatic effects in animals after inhalation or dermal exposure to DNOC. In mice exposed orally to DNOC, enlarged livers with petechial hemorrhages and necrotic foci were seen after single doses of 10-35 mg/kg (Arustamyan 1972), and fatty degeneration of the liver was observed after repeated doses of 10 mg/kg/day for 6 months (Vashakidze 1967). Despite observed changes in liver weight in some animal studies, no histopathological changes were observed in the liver from rats in intermediate-duration feeding studies (Den Tonkelaar et al. 1983; Spencer et al. 1948; Vos et al. 1983). However, hepatic damage was evident from increased SGPT and decreased liver glucose-6-phosphate dehydrogenase activity in rats given 20 and ≥5 mg/kg/day DNOC, respectively, for 90 days (Den Tonkelaar et al. 1983). DNOC also caused choleresis in dogs given intravenous doses of DNOC (Pugh and Stone 1968). Because further details were not provided, the toxicological significance of this effect is not clear.

In a comparison of hepatotoxicity *in vitro*, DNOC reduced the growth of hepatic cells (Parent-Massin and Thouvenot 1993). The authors tested several pesticides in animal and human cell cultures and found, in general, that human cells were more sensitive to hepatotoxins than were other mammalian cells. However, they did not test for DNOC effects on human cells, so no comparisons could specifically be drawn for DNOC.

Results from human and animal studies suggest that, compared to other effects, hepatic effects in humans would be mild if they were exposed to DNOC in the environment or at hazardous waste sites. It is possible that in cases of chronic or high level exposures, hepatic injury may become irreversible and further compromise hepatic function in exposed individuals.

***Renal* Effects**. DNOC caused elevated BUN levels in a spray operator exposed to DNOC aerosols for 5 weeks (Pollard and Filbee 1951) and cloudy swelling of the kidney in one spray operator who died

after drinking water contaminated with DNOC (Bidstrup and Payne 1951) and 6 workers occupationally exposed to DNOC for 2-8 weeks (Bidstrup and Payne 1951). DNOC also caused severe capillary hyperemia in the kidneys of a boy who died after an extremely large quantity of DNOC was accidentally applied to a skin rash (Buchinskii 1974). Therefore, the human data suggest that inhalation, oral, or dermal exposure to DNOC may cause renal effects.

No studies were located regarding renal effects in animals after inhalation or dermal exposure to DNOC. Dietary exposure of rats to DNOC for intermediate durations also resulted in elevated BUN levels (Spencer et al. 1948; Den Tonkelaar et al. 1983). Elevated urinary glucose and urinary ketones in rats were associated with the inhibitory effect of DNOC on oxidative phosphorylation and subsequent decrease in ATP-dependent absorption of glucose by proximal kidney tubules and subsequent increased fat catabolism, respectively (Den Tonkelaar et al. 1983). However, no histopathological changes were observed in kidneys from rats given DNOC in the diet for intermediate durations (Den Tonkelaar et al. 1983; Spencer et al. 1948; Vos et al. 1983). Despite the lack of supporting histopathological evidence of DNOC related effects in animals, the occurrence of elevated BUN levels in humans and animals suggest that the possibility of nephrotoxicity effects in humans exposed to DNOC cannot be ruled out.

Dermal Effects. Maculopapular urticarial eruptions were observed in a $14^{1}/_{2}$-year-old female after oral exposure to 2.27 mg/kg/day DNOC for 11 days (Gordon and Wallfield 1935) and a 36-year-old woman who received 0.75 mg/kg/day DNOC for 11 weeks (Plotz 1936). In both cases, the urticaria occurred within 1-4 days after the dose was increased. DNOC is a yellow staining compound that stains human (Hunter 1950) and animal (Ambrose 1942) skin on contact. While the yellowish appearance of the skin may be unsightly, such cosmetic effects are not considered adverse.

Ocular Effects. Both 2,4-dinitrophenol and DNOC are believed to be cataractogenic in humans; however, the case for 2,4-dinitrophenol is better documented (Homer 1941). Cataract formation with corneal opacity was diagnosed in a human who ingested an unspecified dose of DNOC for 3 years (Quick 1937). The condition became so severe that one eye became blind shortly after the diagnosis. Five other patients with cataracts were identified in case reports involving the consumption of capsules containing either 2,4-dinitrophenol or DNOC for weight reduction (Anonymous 1938). The actual drug contained in the capsules was not clearly identified. In a study designed to identify a suitable animal model to investigate this phenomenon, cataract formation did not occur in rats given

l-25 mg/kg/day for 77-182 days, but did occur in ducklings fed 1,200 ppm DNOC for l-2 days (Spencer et al. 1948). In addition, chickens developed cataracts within 5 hours after single gavage doses of DNOC ≥2.48 mg/kg (Buschke 1947).

No reliable animal studies have demonstrated that DNOC causes cataracts in mammals. However, case reports of the coincidental occurrence of cataracts after ingestion of DNOC and the structural similarity between dinitrophenol and DNOC, both of which are uncouplers of oxidative phosphorylation, suggest that this effect may occur in humans exposed to DNOC.

Absorption of DNOC by any route can also cause a yellow staining of the conjunctiva and/or the sclera (Dodds and Robertson 1933; Gordon and Wallfield 1935; Ibrahim et al. 1934; Plotz 1936; Pollard and Filbee 1951). While the yellowish appearance of the eyes may be unsightly, such cosmetic effects are not considered adverse.

Other Systemic Effects. Elevated body temperature, increased basal metabolic rate, and/or profuse sweating were the most common signs of DNOC toxicity in exposed workers or individuals taking DNOC for weight reduction (Dodds and Robertson 1933; Hunter 1950; Ibrahim et al. 1934; Plotz 1936; Pollard and Filbee 1951; Steer 1951). These effects were not dependent on duration and route of exposure. Basal metabolic rates increased approximately 50% or more in one study and appear to be associated with DNOC toxicity (Dodds and Robertson 1933). Weight loss was not a consistent finding among humans who ingested DNOC for acute periods (Harvey et al. 1951), but significant weight losses were observed in humans who ingested DNOC for intermediate (Ibrahim et al. 1934; Plotz 1936) or chronic (Quick 1937) durations.

One animal study associated elevated body temperatures in rats with inhalation of DNOC aerosols (King and Harvey 1953a). Whereas subcutaneous injections of DNOC increased body temperatures in dogs, subcutaneous injections of dinitro-*m*-cresol did not have this effect in dogs, rats, and pigeons (Tainter et al. 1935). Several studies demonstrated decreased growth rates in rats that ingested DNOC for acute (King and Harvey 1953a) or intermediate durations (Ambrose 1942; Den Tonkelaar et al. 1983; Spencer et al. 1948; Vashakidze 1967). However, no change in growth rate was observed in rabbits after dermal exposure to 2% DNOC for 30 days (Ambrose 1942). Comparison of human and animal data suggest that weight loss may not be a consistent effect.

2. HEALTH EFFECTS

Oral doses of DNOC for 90 days decreased thyroid hormone levels in rats with associated changes in thyroid morphology and histology (Den Tonkelaar et al. 1983). These effects may be related to a 70-100% competition of DNOC for T_4 binding sites of a carrier protein, transthyethrin (Van den Berg et al. 1991). Endocrine effects have been observed in a 90-day rat study with reduced absolute and relative weights of the thymus gland at 10 mg/kg/day, atrophy of the Islets of Langerhans at 20 mg/kg/day, and changes in the adrenal glands at 20 mg/kg/day (Den Tonkelaar et al. 1983). However, no effects were observed in these 3 glands in rats given similar doses for 3 weeks (Vos et al. 1983).

Increased body temperature, increased basal metabolic rate, and/or profuse perspiration may be regarded as cardinal signs of DNOC toxicosis in humans because they are directly related to the mechanism of action of DNOC. and occur irrespective of the route of exposure. Based on results from human studies, case reports, and limited animal studies, weight loss and changes in the endocrine system also may be also expected to occur in humans exposed to DNOC.

Immunological Effects. No studies were located regarding immunological effects in humans or animals after inhalation exposure to DNOC. As discussed above for Derrnal Effects, oral exposure to DNOC may cause urticaria in humans, but whether this is a manifestation of immunological effects is not clear. However, allergic reactions did not result from acute dermal exposure to DNOC in volunteers (Lisi et al. 1987). In a 90-day study, dietary exposure of rats to DNOC resulted in thymic atrophy, underdeveloped lymph nodes and spleens, changes in thymus and spleen weights, and decreased numbers of circulating lymphocytes (Den Tonkelaar et al. 1983), while dietary exposure at similar doses for shorter periods (21 days) did not result in these immunological effects (Vos et al. 1983). Changes in leukocyte count and differential leukocyte count (Den Tonkelaar et al. 1983) may suggest pathological changes in lymphatic tissue, spleen, and thymus. Based on the limited human and animal data, the potential for DNOC to cause immunological effects in humans cannot be ruled out.

Neurological Effects. Exposure to DNOC aerosols may cause headaches, lethargy, and depression in humans. These effects were observed in a spray operator who inhaled an unknown amount of DNOC aerosols for 5 weeks (Pollard and Filbee 1951), 2 volunteers who ingested 3 mg/kg/day DNOC for 4 days (Dodds and Robertson 1933), 2 of 5 volunteers who ingested 0.92 and 1.27 mg.kg/day for 5 and 7 days, respectively (Harvey et al. 1951), individuals who ingested 0.35 mg/kg/day for 7 days or

0.75 mg/kg/day for 8 weeks (Plotz 1936), and 15 individuals who ingested an average of 1.05 mgfkg/day DNOC for 14-63 days (Ibrahim et al. 1934). Two cases of peripheral neuritis were observed in workers after dermal exposure to ≈20% DNOC solution in oil for 17 days to 1 month (Stott 1956). More severe neurological signs, such as coma and convulsions, occurred in humans whc died (Bidstrup and Payne 1951; Buchinskii 1974; Steer 1951; van Noort et al. 1960). Animals exposed to DNOC developed neurological effects similar to those observed in humans. Lethargy was observed in rats exposed to 0.1 or 100 mg/m^3 DNOC for 4-5 hours (King and Harvey 1953a) and in rats that ingested single doses ≥27 mg/kg DNOC in the sodium salt (Ambrose 1942). Muscle twitches, tremors, ataxia, and sluggishness were observed in cats that inhaled concentrations ≥36 mg/m^3 DNOC aerosols for 4 hours (Burkatskaya 1965a) or that received a single oral dose of 25 mg/kg (Burkatskaya 1965b). Muscle twitches and agitation were also seen in mice that ingested a single dose of 10-35 mg/kg DNOC (Arustamyan 1972). Intraperitoneal injection of either DNOC or 2,6-dinitro-*p*-cresol caused thirst, stretching, decreased activity, and marked rigor in mice (Harvey 1953). The acute toxicity of the two isomers were not significantly different. Increased brain blood flow (Verschoyle et al. 1987), decreased absolute brain weights, and increased relative brain weights (Den Tonkelaar et al. 1983) were observed in rats exposed to a single dose of 19.8 and l0-20 mg/kg/day DNOC for 90 days, respectively. No histological lesions were observed in the latter study, and it is questionable whether changes in brain weight and brain blood flow observed in animals are related to the clinical effects observed in humans and animals. Neurological effects have been observed in humans after exposure to DNOC by any route and at relatively low doses; therefore, it is possible that humans could develop neurological effects after any exposure scenario.

Reproductive Effects. DNOC was thought to have induced labor in 1 of 3 pregnant agricultural workers who became ill after they were dermally exposed to DNOC for about eight hours (Vamai and Kote 1969). All three women gave birth to full-term healthy children. Data from a 5-day oral study in mice (Quint0 et al. 1989) and 3-week and 77-182-day studies in rats (Spencer et al. 1948; Vos et al. 1983) suggested that DNOC does not affect spermatogenesis, while data from another study (90-day) in rats suggested that DNOC causes aspermatogenesis (Den Tonkelaar et al. 1983). In one study, no effects on sperm morphology, sperm counts, or testicular weights were found, in mice given DNOC by gavage or by intraperitoneal injection at doses of 3-12 mg/kg/day for 5 days (Quinto et al. 1989). However, intraperitoneal injection of male mice with DNOC increased the frequency of chromosomal aberrations in male germinal cells (Nehéz et al. 1978b). No histopathological lesions were found in the testes of rats fed diets that provided doses of DNOC ≤25 mg/kg/day for 3 weeks

(Vos et al. 1983) or 6 months (Spencer et al. 1948). However, aspermatogenesis was observed in a 90-day feeding study, in which rats were exposed to 20 mg/kg/day DNOC (Den Tonkelaar et al. 1983). Because rats in the 3 intermediate-duration feeding studies were exposed to similar doses of DNOC, it is difficult to explain why aspermatogenesis occurred after a 90-day exposure, but not after a 6-month exposure.

The toxic effect of DNOC on the female reproductive system was described in only two studies. Absolute and relative ovary and uterus weights were found, and histopathological examination of the ovaries and uteri revealed a lack of corpora lutea in the ovaries and the juvenile appearance of the uteri (Den Tonkelaar et al. 1983). The absolute weight of the ovaries and relative weight of uterus/ovary were also decreased. DNOC also appeared to have caused damage to ovaries, atrophy to uterine horns, and disruption of the estrus cycle in rats that were exposed orally to DNOC for 6 months (Vashakidze 1967). Although the data for testicular effects from animal studies are conflicting, the possibility for reproductive effects in human males or females exposed to DNOC cannot be ruled out.

Developmental Effects. No developmental effects were observed in the offspring of three pregnant agricultural workers who were dermally exposed to DNOC for 8 hours (Vashakidze 1967). Exposure was not during the period of organogenesis. No developmental effects were observed in the offspring of mice given DNOC at 8 mg/kg/day by gavage or 15 mg/kg/day by intraperitoneal injection during gestation (Nehéz et al. 1981). However, intraperitoneal injection of male mice with DNOC before mating with untreated females resulted in chromosomal aberrations in several subsequent filial generations (Nehez et al. 1978a, 1984). When pregnant mice were given DNOC by gavage during the second trimester of pregnancy, but not during the first trimester, an increased frequency of chromosomal aberrations was found in the embryos (Nehéz et al. 1978a). The data are too limited to draw any conclusion regarding the potential for development effects in the offspring of humans exposed to DNOC.

Genotoxic Effects. DNOC has been tested for genotoxicity in a variety of *in vivo* and *in vitro* test systems (see Tables 2-4 and 2-5). Mostly positive results have been obtained in *in vivo* tests. DNOC tested positive for sex-linked recessive lethal mutations in *Drosophila melanogaster* exposed via food (Mueller and Haberzettl 1980). In addition, positive results have been obtained for DNA damage in hepatocytes (Grilli et al. 1991) and for chromosomal aberrations in bone marrow cells (Hrelia et al.

TABLE 2-4. Genotoxicity of DNOC *In Vivo*

Species (test system)	End point	Results	Reference
Drosophila melanogaster (feed)	Sex-linked recessive lethal	+	Mueller and Haberzettl 1980
Rat (intraperitoneal)	DNA damage (unwinding rate) in hepatocytes	+	Grilli et al. 1991
Rat (intraperitoneal)	Chromosomal aberrations in bone marrow cells	+	Hrelia et al. 1990
Mouse (intraperitoneal)	Chromosomal aberrations in male germinal cells	–	Nehéz et al. 1982
Mouse (intraperitoneal)	Chromosomal aberrations in male germinal cells	+	Nehéz et al. 1978b
Male mouse (intraperitoneal)	Dominant lethality	+	Nehéz et al. 1978a
Mouse (intraperitoneal)	Chromosomal aberrations in bone marrow cells	+	Nehéz et al. 1978a
Mouse (intraperitoneal)	Chromosomal aberrations in bone marrow cells	+	Nehéz et al. 1984
Mouse (subcutaneous)	Chromosomal aberrations in bone marrow cells	+	Nehéz et al. 1984
Male mouse (intraperitoneal)	Chromosomal aberrations in F_1, F_2, and F_4 generations	+	Nehéz et al. 1984
Male mouse (intraperitoneal)	Chromosomal aberrations in F_1 generation	+	Nehéz et al. 1978a
Female Mouse (oral during first trimester of pregnancy)	Chromosomal aberrations in embryos	–	Nehéz et al. 1981
Female Mouse (oral during second trimester of pregnancy)	Chromosomal aberrations in embryos	+	Nehéz et al. 1981
Mouse (route NS)	Chromosomal aberrations in bone marrow cells	–	Kurinnyi et al. 1982

DNA = deoxyribonucleic acid; DNOC = 4,6-Dinitro-o-cresols; NS = not specified; – = negative result; + = positive result

TABLE 2-5. Genotoxicity of Dinitrocresols *In Vitro*

Species (test system)	End point	Result: With activation	Result: Without activation	Reference	Isomer
Prokaryotic organisms:					
Salmonella typhimurium (8 strains NOS)	Reverse mutation	No data	–	Andersen et al. 1972	4,6-DNOC
S. typhimurium	Reverse mutation			Nishimura et al. 1982	4,6-DNOC
TA100		–	–		
TA98		+	–		
S. typhimurium	Reverse mutation			Remondelli et al. 1986	4,6-DNOC
TA98		a	+		
TA100		–	–		
TA1535		–	–		
TA1537		a	+		
TA2637		a	+		
TA92		–	–		
S. typhimurium	Reverse mutation			Somani et al. 1981	4,6-DNOC
TA98		No data	–		
TA100		No data	+		
TA1537		No data	–		
S. typhimurium	Reverse mutation			Hrelia et al. 1990	4,6-DNOC
TA98		–	–		
TA97		–	–		
TA100		–	–		
TA102		–	–		

TABLE 2-5. Genotoxicity of Dinitrocresols *In Vitro* (continued)

Species (test system)	End point	Result: With activation	Result: Without activation	Reference	Isomer
S. typhimurium	Reverse mutation			Sundvall et al. 1984	4,6-DNOC
TA1538		No data	+		
TA98NR		No data	(+)		
TA1535		No data	+		
TA98		a	+		
TA100		a	+		
TA100NR		No data	–		
S. typhimurium	Reverse mutation			Sundvall et al. 1984	2,6-DNPC
TA98		–	–		
TA100		a	+		
S. typhimurium	Reverse mutation			Spanggord et al. 1982b	4,6-DNMC
TA1535		–	–		
TA1537		+	+		
TA1538		–	–		
TA98		–	+		
TA100		–	–		
S. typhimurium	Forward mutation			Remondelli et al. 1986	4,6-DNOC
TA98		No data	+		
Escherichia coli	Reverse mutation			Nagy et al. 1975	4,6-DNOC
WP2(hcr^+)		No data	–		
WP2 (hcr^-)		No data	–		
E. coli T_4 bacteriophage rII mutants	Reverse mutation	No data	–	Andersen et al. 1972	4,6-DNOC
E. coli T_4 bacteriophage wildtype	Forward mutation	No data	–	Andersen et al. 1972	4,6-DNOC

TABLE 2-5. Genotoxicity of Dinitrocresols *In Vitro* (continued)

Species (test system)	End point	Result: With activation	Result: Without activation	Reference	Isomer
Proteus mirabilis PG273 (wildtype); PG713 (rec^- hcr^-)	DNA repair	No data	+	Adler et al. 1976	4,6-DNOC
Eukaryotic organisms:					
Saccharomyces cerevisiae	Mitotic crossing over	No data	+	Hrelia et al. 1990	4,6-DNOC
Human peripheral lymphocytes	Unscheduled DNA synthesis	–	–	Hrelia et al. 1990	4,6-DNOC
Human peripheral lymphocytes	Sister chromatid exchange	–	–	Hrelia et al. 1990	4,6-DNOC
Human blood leukocytes	Chromosomal aberrations	No data	+	Nehéz et al. 1978a	4,6-DNOC

[a]The mutagenicity was decreased by the addition of S9

DNA = Deoxyribonucleic acid; DNMC = dinitro-m-cresol; DNOC = dinitro-o-cresol; DNPC = dinitro-p-cresol; NOS = not otherwise specified; – = negative result; + = positive result; (+) = weakly positive result

1990) of rats injected intraperitoneally with DNOC. Intraperitoneal injection of mice with DNOC increased the frequency of chromosomal aberrations in male germinal cells (Nehéz et al. 1978b) and in bone marrow cells (Nehéz et al. 1978a, 1984). Increased frequencies of chromosomal aberrations in bone marrow cells were also found in mice after subcutaneous injection with DNOC (Nehéz et al. 1984). Intraperitoneal injection of male mice with DNOC before mating with untreated females resulted in chromosomal aberrations in several subsequent filial generations (Nehéz et al. 1978a, 1984). DNOC was also positive for dominant lethal mutations when male mice were injected intraperitoneally (Nehéz et al. 1978a). When pregnant mice were administered DNOC by gavage during the second trimester of pregnancy, an increased frequency of chromosomal aberrations was found in the embryos (Nehéz et al. 1981). However, the frequency of chromosomal aberrations was not increased in the embryos when the mice were given DNOC during the first trimester. Two studies found negative results for chromosomal aberrations in male germinal cells (Nehéz et al. 1982) and bone marrow cells (Kurinnyi et al. 1982) after mice were treated with DNOC.

When DNOC was tested for reverse mutations in *Salmonella typhimurium,* mixed results were obtained. While some investigators found consistently negative results for reverse mutations with and/or without metabolic activation in several strains (Andersen et al. 1972; Hrelia et al. 1990; Nishimura et al. 1982), others found some positive results without metabolic activation in S. *typhimurium* strains TA98, TA1537, TA2637 (Remondelli et al. 1986), TA100 (Somani et al. 1981; Sundvall et al. 1984), TA1538, TA98NR, and TA1535 (Sundvall et al. 1984). When a metabolic activation system was used, the frequency of reverse mutations caused by DNOC was generally decreased (Remondelli et al. 1986; Sundvall et al. 1984). However, some investigators found negative results in the same strains for which other investigators found positive results (see Table 2-5). The reason for these inconsistent results is not clear. A positive result without activation was also found for forward mutations in *S. typhimurium* strain TA98 (Remondelli et al. 1986). DNOC was consistently negative for reverse mutation in *Escherichia coli* (Nagy et al. 1975) and *E. coli* T_4 bacteriophage rII mutants and for forward mutation in *E. coli* T_4 bacteriophage wildtype (Andersen et al. 1972). DNOC was positive in a DNA repair assay in *Proteus mirabilis* (Adler et al. 1976). In eukaryotic systems, positive results were found for mitotic crossing over in *Saccharomyces cerevisiae* (Hrelia et al. 1990) and for chromosomal aberrations in cultured human blood leukocytes (Nehéz et al. 1978a). However, negative results were obtained for unscheduled DNA synthesis and sister chromatid exchange in human peripheral lymphocytes (Hrelia et al. 1990).

When 2,6-dinitro-*p*-cresol was tested for reverse mutation in S. *typhimurium,* positive results were obtained in TAl00 without activation, but negative results were obtained in TA98 with or without metabolic activation (Sundvall et al. 1984). The frequency of mutations produced in TAl00 by 2,6-dinitro-*p*-cresol decreased in the presence of a metabolic activation system. 4,6-Dinitro-m-cresol was negative for reverse mutation in *S. typhimurium* in TA1535, TA1538, and TA100 with and without metabolic activation, but it was positive in TA1537 with and without activation and in TA98 only without activation (Spanggord et al. 1982a).

The weight of evidence indicates that DNOC and other dinitrocresols are genotoxic. Therefore, the potential for dinitrocresols to cause genotoxic effects in humans exposed occupationally, in the ambient environment, or at hazardous waste sites cannot be ruled out.

Cancer. No studies were located regarding cancer in humans or animals after exposure to DNOC by any route. However, as discussed above, DNOC appears to be genotoxic, and potentially carcinogenic.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are

commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to DNOC are discussed in Section 25.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by DNOC are discussed in Section 25.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic, or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, Populations That Are Unusually Susceptible.

2.5.1 Biomarkers Used to Identify or Quantify Exposure to DNOC

DNOC and/or its metabolites have been measured in various body fluids and tissues such as blood, urine, liver, stomach, intestine, brain, and heart of humans (Harvey et al. 1951; King and Harvey 1953b; Sovljanski et al. 1971) and animals (King and Harvey 1953a; Leegwater et al. 1982; Truhaut and De Lavaur 1967). Detection of DNOC in body fluids or tissues, therefore, can serve as a qualitative indication that exposure to DNOC occurred.

Detectable blood and urinary levels of DNOC have been found in humans exposed occupationally by the inhalation and dermal routes (Batchelor et al. 1956; Bidstrup et al. 1952; Pollard and Filbee 1951; Steer 1951) or experimentally by the oral and dermal routes (Harvey et al. 1951; King and Harvey 1953b). While the exposure concentrations in occupational studies were not known, the experiments in volunteers provided information on doses and durations.

2. HEALTH EFFECTS

In 5 volunteers given oral doses of DNOC of 0.92-1.27 mg/kg/day for 5-7 days, the level of DNOC in the blood gradually increased for the first 3 or 4 days and was maximal for 24 hours after ingestion on each day (Harvey et al. 1951). The blood levels on these days ranged from 15-20 μg/g. In one volunteer, the blood level peaked at 40 μg/g after the fifth dose. Subsequent dosing on days 6 and 7 caused profound peak blood levels of 40-50 μg/g in another volunteer. Generally, blood levels of DNOC began to decrease gradually within 2 days of exposure. As much as 1-5 μg/g DNOC were still detected in the blood 40 days after the last dose. Thus, the measurement of DNOC in blood is a useful indicator of exposure; however, since DNOC is still detectable in the blood 40 days after exposure, it may not be a reliable indicator of the magnitude or the time of exposure.

The urinary excretion of DNOC was also studied in these volunteers (King and Harvey 1953b). The 5 volunteers excreted about 7% of the total DNOC dose in the urine over 13 days after exposure. However, only 0.016% of the dose was excreted within 5 hours after exposure and 1.3% within 24 hours after exposure. In the first 24 hours after a single exposure of 75 mg per person, 35.2-46.6% of the dose could be accounted for by blood levels and 0.8-2.0% could be accounted for by urinary levels. Thus, 51.7-64.0% of the oral dose was unaccounted for. These data suggest that DNOC is stored longer in the human body than in the animal body. Since DNOC binds to albumin, the chief internal stores may be extracellular fluids containing albumin. Therefore, urinary levels of DNOC may not be useful biomarkers to quantitate exposure.

Metabolites of DNOC are more likely than DNOC to be detected in the urine of animals. The following metabolites were detected in the urine from rats and rabbits exposed to DNOC: 6-amino-4-nitro-*o*-cresol, 6-acetamido-4-nitro-*o*-cresol, 4-acetamido-6-nitro-*o*-cresol, 4-amino-6-nitro-*o*-cresol, 4,6-diacetamide-*o*-cresol, 3-amino-5-nitro-salicylic acid, and 3,5-dinitro-2-hydroxybenzyl alcohol (Leegwater et al. 1982; Smith et al. 1953; Tmhaut and De Lavaur 1967). About 15% of the dose was excreted as 4,6-diacetamido-*o*-cresol (Leegwater et al. 1982), 10% as O-conjugates of 6-acetamido-4-nitro-*o*-cresol (Smith et al. 1953), and 25-38% as DNOC and 6-amino-4-nitro-*o*-cresol (Tmhaut and De Lavaur 1967). Metabolite ratios may be useful biomarkers of exposure to DNOC (Truhaut and De Lavaur 1967). The ratio of urinary DNOC to 6-amino-4-nitro-*o*-cresol in rabbits increased from 0.66 to 1.47 as the dose of DNOC was increased from 10 to 20 mg/kg. However, no studies were located regarding urinary metabolites of DNOC in humans, and toxicokinetic data show that humans eliminate DNOC more slowly than animals (King and Harvey 1953b). It is possible that humans metabolize DNOC more slowly than animals and by different pathways.

2. HEALTH EFFECTS

Yellow staining of skin, sclera, or conjunctiva may alert a physician to the possibility of DNOC exposure. This yellow staining is not a sign of icterus, but is due to the yellow color of DNOC. However, yellow staining of the skin can also suggest a diagnosis of exposure to other nitrophenolic compounds. The yellow staining does not appear to be associated with blood level, exposure route, or the severity of effects (Ellenhom and Barceloux 1988).

2.5.2 Biomarkers Used to Characterize Effects Caused by DNOC

DNOC exposure results in a hypermetabolic state that resembles heat exhaustion and heat stroke. The basal metabolic rate was increased by 70-100% within 3 days in 2 humans given 3 mg/kg/day DNOC (Dodds and Robertson 1933). Headaches, hyperthermia, profuse sweating, increased pulse rate, and dyspnea are other common signs and symptoms associated with DNOC exposure. In severe cases, tachycardia, delirium, coma, and convulsions are usually observed in humans. An increased basal metabolic rate may, therefore, indicate profound metabolic disturbances.

Attempts have been made to correlate blood levels with the onset of DNOC toxicity. In workers engaged in the manufacture of DNOC or with spraying DNOC as a pesticide, blood levels <10-20 μg/g were not associated with signs of DNOC toxicity (Bidstrup et al. 1952). In 4 cases of acute poisoning, a worker with a blood level of 75 μg/g died, 2 workers who became seriously ill had blood levels of 55 and 60 μg/g, and a moderately ill worker had a blood level of 44 μg/g. The investigators concluded that workers with blood levels ≥20 μg/g should have no further contact with DNOC, while blood levels ≥40 μg/g will probably result in signs and symptoms of toxicity. Other case reports describe individuals with blood levels of 60-75 μg/g who became seriously ill or died (Pollard and Filbee 1951; Steer 1951).

DNOC blood levels were measured in volunteers who ingested an average of 75 mg/day DNOC (0.92-1.27 mg/kg/day) (Harvey et al. 1951; King and Harvey 1953b). Although blood levels did not usually exceed 40-48 μg/g, less serious neurological effects such as headaches and depression were observed. These authors also suggested that blood levels approaching 4048 μg/g may be indicators of DNOC toxicity.

Data from hematological, clinical chemistry, and urine analyses of animals exposed to DNOC suggest that DNOC may alter several hematological, biochemical, hepatic, and renal parameters. These

parameters are not unique to DNOC, but may be measured to determine disease states caused by DNOC. An oral dose of 20 mg/kg/day of DNOC for 90 days caused an increase in erythrocyte count and a decrease in total leucocyte and lymphocyte counts in rats (Den Tonkelaar et al. 1983). Doses of 5 or 10 mg/kg of DNOC also caused a decrease in liver glucose-6-phosphate dehydrogenase in rats, while a dose of 20 mg/kg/day caused an increase in SGPT. The changes in these enzyme levels are indicative of some degree of hepatic injury and/or interference with carbohydrate metabolism. Urinary ketones, which are good indicators of fat metabolism, were also elevated in rats given 2.5-10 mg/kg/day DNOC. BUN was increased in rats given 20-25 mg/kg/day DNOC for 90-182 days (Den Tonkelaar et al. 1983; Spencer et al. 1948). Elevated BUN is associated with renal effects. Increased blood glucose and decreased blood pyruvate may also be indicative of metabolic disturbances caused by DNOC. Additional information regarding the effects of exposure to DNOC can be found in OTA (1990) and CDC/ATSDR (1990). For a more detailed discussion of the health effects caused by DNOC see Section 2.2 of Chapter 2.

2.6 INTERACTIONS WITH OTHER SUBSTANCES

Very little information was located regarding interactions of DNOC with other chemicals, but the toxicity of DNOC is influenced by several physical and environmental factors.

Environmental temperatures influenced the mortality rate among rats after oral exposure to DNOC (King and Harvey 1953a). Six out of 12 rats died after being given 20 mg/kg at 37-40 ºC, while only 2 of 12 rats died after being given twice the dose (40 mg/kg) at almost half the temperature (20-22 ºC). It appears that increased environmental temperatures increased the toxicity of DNOC in rats. The authors further proposed that the increase in environmental temperature exacerbated the increased metabolic effect of DNOC, but did not appear to initiate or stimulate any reactions affecting the linkage of DNOC to any intracellular or extracellular substances. Environmental temperatures could also alter normal body functions so that the rate of absorption, diffusion, distribution, or metabolism of a compound would be changed. A similar observation was made in another study after rats given intraperitoneal doses of DNOC were exposed to 8, 26, or 36 ºC (Keplinger et al. 1959). The approximate lethal dose was 42 mg/kg at 8 ºC, 28 mg/kg at 26 ºC, and 18 mg/kg at 36 ºC. In mice given 22 mg/kg DNOC subcutaneously, the mean time of death (LT_{50}) values decreased as environmental temperature increased (Tesic et al. 1972). Hence, DNOC was most toxic at high temperatures and least toxic at cold temperatures.

2. HEALTH EFFECTS

An attempt was made to determine the best treatment regimen for rats and mice exposed to intraperitoneal doses of 2.5-30 mg/kg DNOC (Harvey 1959). Fifty percent mortality was observed at 2.5 mg/kg DNOC and 100% mortality was observed at ≥5.0 mg/kg when rats were exposed to 39-41 °C. Sponging with water within 1 hour of exposure to DNOC completely protected all rats given doses of 2.5-10 mg/kg. This protective effect was not observed at 20 or 30 mg/kg DNOC. Removal of the rats to a cold room completely protected the rats treated with 10 mg/kg, but had no affect on mortality at 20 mg/kg. The authors concluded that cooling of the skin may be beneficial in reducing the toxicity of DNOC in humans. Because rats eliminate DNOC more rapidly than humans, sponging and cooling treatment would have to be prolonged and efficient. In the same study, administration of 4-methyl-2-thiouracil, an inhibitor of the thyroid gland, 1 hour after injection of DNOC reduced mortality to 50% at 5.0 mg/kg, but had no effect on mortality at 10.0 mg/kg. No mechanism was proposed for this interaction between DNOC and 4-methyl-2-thiouracil. Similar results of sponging with water or treatment with 4-methyl-2-thiouracil were found with mice.

The mean time to death (LT_{50}) values were prolonged in mice that were pretreated with vitamin E, Vitamin A, and/or glucose 30 minutes before dosing with DNOC (Tesic et al. 1972). In addition, thiamazole increased the LT_{50} value by a factor of 2.72, while chlorpromazine had a greater influence on LT_{50} values and was more protective than thiamazole in DNOC treated mice. Doses of 8 and 12 mg/kg chlorpromazine were more protective than doses <6 mg/kg, which had no protective effect. The authors proposed that larger doses of chlorpromazine may cause a significant reduction in oxidative processes and decrease in body temperature, while the protective effect of thiamazole may be associated with its ability to decrease basal metabolic rate.

The effect of nonfatal injuries such as a 2-hour period of bilateral hind-limb ischemia or a full thickness scald of 20% of skin surface on the LD_{50} of DNOC and its hyperthermic effect were evaluated in male rats (Stoner 1969). The intraperitoneal LD_{50} of DNOC was significantly ($p<0.00l$) reduced from 24.8 to 26.2 mg/kg to 14 mg/kg DNOC when DNOC was given 1.5-24 hours after either type of nonfatal injury. The authors concluded that the toxicity of DNOC was increased by previous trauma. These investigators proposed that this interaction was associated with sequential blocking of the tricarboxylic acid cycle with inhibition of citrate synthetase reaction during the early part of the response to the injury. Because DNOC acts as an uncoupler of oxidative phosphorylation, less ATP is produced. Therefore, the effects of trauma will be enhanced by an uncoupling agent such as DNOC.

Impurities in DNOC mixtures did not appear to cause an additive or synergistic effect in mice given intraperitoneal doses of DNOC (Harvey 1953). The intraperitoneal LD_{50} values for pure DNOC, 80% DNOC, and 33% DNOC were 24.2, 22.9, and 32.5 mg/kg, respectively. The 33% DNOC and 80% DNOC were contaminated with trinitro-*o*-cresol, which alone had an intraperitoneal LD_{50} of 168 mg/kg. Clinical signs of DNOC toxicity were similar for all treatments. The authors suggested that contamination by trinitro-*o*-cresol did not lower the effective total toxicity of pure DNOC.

Gavage administration of olive oil, rape oil, or castor oil to rats immediately after DNOC resulted in some alteration of blood DNOC levels, indicating that the influence of fats on the amount of DNOC absorbed from the gastrointestinal tract depends on the type and dose of the fat (Starek and Lepiarz 1974). In general, readily digested olive oil had little effect on blood levels, the more slowly digested rape oil slightly inhibited DNOC absorption, and castor oil decreased the absorption considerably. A nonpurgative dose of 0.2 mL of castor oil inhibited DNOC absorption from the alimentary tract, while a purgative dose of 1.0 mL first inhibited absorption for the first 6 hours and then increased blood DNOC levels in the next few hours approaching control values. In some instances, castor oil inhibited DNOC absorption by as much as 43-49% 6 hours after the oil was given. Aspirin enhances uncoupling of oxidative phosphorylation and therefore increases DNOC toxicity (Ellenhom and Barceloux 1988).

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to DNOC than will most persons exposed to the same level of DNOC in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

No data identifying subpopulations of humans inherently more susceptible to the toxic effects of DNOC were located. Animal studies did not indicate that there were sex or age differences in the susceptibility to DNOC toxicosis.

Several human studies suggest that populations living in tropical or warm climates are more susceptible to DNOC toxicity than those persons in cooler climates (Bidstrup and Payne 1951; Pollard and Filbee 1951; Stott 1951). This phenomenon is supported by studies in rats and mice that indicate that environmental temperature increases the toxicity of DNOC (Harvey 1959; King and Harvey 1953a). Some human subpopulations that are predisposed to a syndrome known as malignant hyperthermia, may be more likely to develop fatal hyperthermia following DNOC exposure. Malignant hyperthermia is an inherited disease of skeletal muscle characterized by a drug-induced hyperpyrexia (Schroeder and McPhee 1990). Human populations with this inherited disease are predisposed to acute hyperthermic reactions triggered by stress or drugs, such as, inhalation anesthetic agents, skeletal muscle relaxant and amide local anesthetics (Britt 1979). Although no data were located linking DNOC with malignant hyperthermia, persons with the genetic predisposition may be more susceptible to the hyperthermic effects of DNOC.

DNOC is an uncoupler of oxidative phosphorylation and causes metabolic disturbances. Therefore, people with already compromised metabolic rates may be more susceptible; however, no studies were located that demonstrate such a population exists.

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and experimental research concerning methods for reducing toxic effects of exposure to DNOC. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposure to DNOC. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

DNOC is absorbed rapidly by the respiratory and gastrointestinal tracts. Methods to reduce its absorption require that the amount of time prior to treatment be minimized. Although absorption

through skin is slower than via inhalation and oral routes, reducing dermal exposure is important because DNOC is not modified in the skin and systemic effects can occur.

If DNOC is inhaled, the victim should be removed to a fresh air environment. Artificial respiration and the use of oxygen by non-rebreather mask have been recommended (Bronstein and Currance 1988; Haddad and Winchester 1990). Because DNOC is rapidly absorbed by the respiratory tract, very little can be done to reduce its absorption. If DNOC is ingested, water is recommended for its dilution (Bronstein and Currance 1988). Emetics were contraindicated by these authors. However, some authors (Ellenhorn and Barceloux 1988; Stutz and Janusz 1988) recommend syrup of ipecac followed by activated charcoal and a cathartic such as magnesium sulfate following exposure to aromatic nitro compounds. In rats, gavage administration of olive oil, rape oil, or castor oil to rats immediately after DNOC resulted in some alteration of blood DNOC levels, indicating that the influence of fats on the amount of DNOC absorbed from the gastrointestinal tract depends on the type and dose of the fat (Starek and Lepiarz 1974). In general, readily digested olive oil had little affect on blood levels, the more slowly digested rape oil slightly inhibited DNOC absorption, and castor oil decreased the absorption considerably. A nonpurgative dose of castor oil inhibited DNOC absorption from the alimentary tract, likely associated with increased gastrointestinal motility, while a purgative dose initially inhibited absorption for the first 6 hours and then increased blood DNOC levels in the next few hours approaching control values. In some instances, castor oil inhibited DNOC absorption by as much as 43-49% 6 hours after the oil was given.

In the event of dermal exposure, contaminated clothing should be moved and the patient washed with copious amounts of water (Stutz and Janusz 1988). The eyes should be flushed with water if they are contaminated with DNOC.

2.8.2 Reducing Body Burden

Limited information is available regarding methods for specifically reducing the body burden of DNOC. Although no effective methods of elimination enhancement were documented by Ellenhom and ,Barceloux (1988), dialysis has been used with mixed results (Locket 1970). Because DNOC has a relatively long half-life in humans (King and Harvey 1953b), procedures such as diuresis, dialysis, and hemoperfusion may be effective. Repeated treatment with activated charcoal may be useful in

preventing resorption following biliary excretion. Ellenhom and Barceloux (1988) also recommends correcting fluid acidosis and electrolyte imbalance.

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism of action of DNOC depends on its ability to uncouple oxidative phosphorylation and consequently cause elevated metabolic rates and hyperthermia. There is no antidote to arrest or reverse the metabolic disturbances in humans exposed to DNOC. However, 4-methyl-2-thiouracil has been given to DNOC-poisoned animals to reduce high metabolic rates and other toxic effects of DNOC toxicity (Harvey 1959). This compound reduced mortality among rats and mice exposed to DNOC. No mechanism was proposed for this interaction between DNOC and 4-methyl-2-thiouracil, but it has been suggested that this agent might be effective in reducing the basal metabolic rate (Clarke et al. 1981). Data from one animal study suggest that thiamazole may also be effective in decreasing the basal metabolic rate (Tesic et al. 1972).

Because there is no approved antidote for DNOC poisoning in humans, rest and general supportive measures are usually recommended (Anonymous 1951; Clarke et al. 1981). Individuals who recover do so rapidly and completely, with no permanent damage. Other supportive measures include rapid cooling of the body in cases of hyperthermia (Bronstein and Currance 1988; Ellenhom and Barceloux 1988). The protective effect of cooling with water following DNOC exposure is well documented in animals (Harvey 1959; King and Harvey 1953a) and humans (Pollard and Filbee 1951).

Correction of fluid acidosis and electrolyte imbalance may be required (Ellenhorn and Barceloux 1988; Stutz and Janusz 1988). At the same time, drug therapy for pulmonary edema should be considered (Bronstein and Currance 1988). Diazepam may be necessary to control seizures (Bronstein and Currance 1988; Haddad and Winchester 1990). However, sedatives such as chlorpromazine may potentiate the action of DNOC (Clarke et al. 1981). However, a study in mice indicated that the effect of chlorpromazine may be dose-dependent. Mice that were pretreated with 8 and 12 mg/kg chlorpromazine were protected against DNOC toxicity, but a dose of 6 mg/kg potentiated the toxicity of DNOC (Tesic et al. 1972). However, the efficacy of chlorpromazine administered after intoxication with DNOC was not evaluated. In the same study, pretreatment with vitamin E, vitamin A and/or glucose 30 minutes before dosing with DNOC prolonged the mean time to death in mice.

Unfortunately the study did not evaluate the efficacy of vitamin E or vitamin A after DNOC intoxication in mice.

Salicylates and anticholinergics are contraindicated in the management of DNOC-poisoned individuals because they may also potentiate the action of DNOC (Haddad and Winchester 1990). Because of the limited animal studies and understanding regarding the interaction between DNOC and proposed antidotes such as 4-methyl-2-thiouracil, chlorpromazine, and vitamins; further animal testing would be useful before these dosages are used in cases of human DNOC toxicosis.

2.9 ADEQUACY OF THE DATABASE

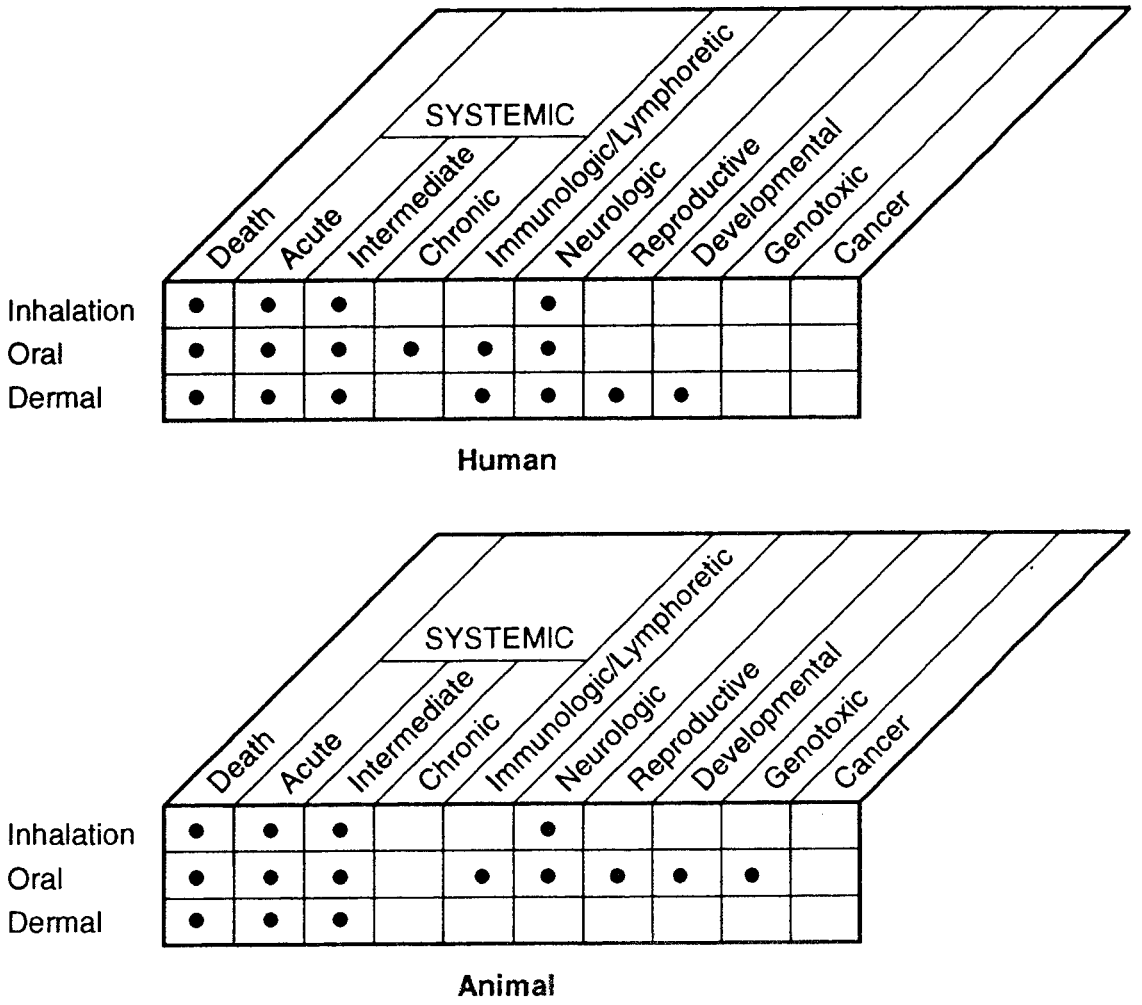
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of DNOC is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of DNOC.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of DNOC

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to DNOC are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of DNOC. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as “data needs.” A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989), is substance-specific information necessary to

FIGURE 2-4. Existing Information on Health Effects of 4,6-Dinitro-o-cresol



● Existing Studies

conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As seen from Figure 2-4, data exist regarding death, systemic effects, neurological, and reproductive effects in humans after occupational exposure to DNOC, which usually involved a combination of inhalation and dermal exposure, for acute and intermediate durations. Systemic effects consisted of respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal and ocular effects, and increases in body temperature and basal metabolic rate and changes in body weight. Data also exist regarding death, systemic effects of acute-, intermediate-, and chronic-duration exposure, immunological, and neurological effects in humans after oral exposure to DNOC. Systemic effects consisted of respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, dermal and ocular effects, and changes in body weight and increased basal metabolic rate. Ocular effects consisted of cataract formation after chronic oral exposure to an unknown dose to DNOC.

There are animal data regarding death, systemic, and neurological effects after acute-duration inhalation exposure to DNOC. Systemic effects consisted of respiratory, hematological, and musculoskeletal effects and elevated body temperatures. Data exist regarding death, systemic effects of acute- and intermediate-duration exposure, immunological, neurological, developmental, reproductive, and genotoxic effects in animals after oral exposure to DNOC. Systemic effects consisted of respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal and ocular effects, and effects on the thyroid, pituitary, adrenal, and pancreatic glands and growth rate. Data also exist regarding ocular irritation after acute exposure of the eyes, skin irritation after acute and intermediate-duration dermal exposure, and body weight changes after intermediate dermal exposure.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. Several studies were located regarding death, systemic, and neurological effects in humans after occupational exposure. Exposure usually involved a combination of inhalation and dermal exposure to an unknown amount of DNOC for a few days (Buzzo and Guatelli 1949; Steer 1951; van Noort et al. 1960). The workers had respiratory, cardiovascular, gastrointestinal, hematological, and musculoskeletal effects, and/or increased body temperature with profuse sweating. Acute dermal exposure has also resulted in musculoskeletal effects (van Noort et al.

1960) and renal and hepatic effects (Buchinskii 1974; Varnai and Kote 1969). A worker who died after drinking water contaminated with an unknown amount of DNOC had respiratory and gastric effects, while pathology of the lung, kidney, and liver were associated with agonal changes (Bidstrup and Payne 1951). The heart, stomach, and skin were also identified as target organs in humans after acute oral exposure to DNOC (Bidstrup and Payne 1951; Dodds and Robertson 1933; Gordon and Wallfield 1935; Harvey et al. 1951; Plotz 1936). Some of the information regarding human exposure comes from controlled laboratory experiments using volunteers and from individuals who ingested DNOC as a weight reduction drug under medical supervision, so doses and durations were known. DNOC uncouples oxidative phosphorylation; therefore, the compound increased basal metabolic rates and body temperatures and caused profuse perspiration in humans after acute oral exposure (Dodds and Robertson 1933; Plotz 1936). DNOC was not a dermal irritant in several agricultural workers experimentally exposed to the compound (Lisi et al. 1987).

In addition to elevating body temperatures, acute inhalation of DNOC aerosols caused respiratory effects in rats (King and Harvey 1953a) as well as respiratory, hematological, and musculoskeletal effects and hyperglycemia in cats (Burkatskaya 1965a). Acute oral exposure studies in animals have identified the respiratory tract, the liver, the gastrointestinal tract, and the cardiovascular system as possible target organs (Ambrose 1942; Arustamyan 1972; Spencer et al. 1948). DNOC did not cause ocular irritation in rabbits after acute intraocular application (Ambrose 1942), but caused cataracts in ducklings (Spencer et al. 1948) and chickens (Buschke 1947) after acute oral exposure.

Data from case reports identified the respiratory tract, heart, musculoskeletal system, liver, kidneys, skin, eyes, and stomach as possible target organs after acute exposure to DNOC. In most cases, these organs were affected in animals after acute exposure to DNOC. An acute oral MRL of 0.004 mg/kg/day was derived from human data for fatigue and dizziness in humans who ingested DNOC to lose weight (Plotz 1936). Acute-duration inhalation studies in animals that involve identification of specific target organs and sensitive effects and use several concentrations might provide data from which an acute inhalation MRL can be derived. Additional acute dermal studies in animals might provide more information on systemic target organs, since DNOC does appear to be absorbed dermally. This information is important because there are populations residing near hazardous waste sites that might be exposed to DNOC for brief periods. Humans appear to be more sensitive than animals to the effects of oral exposure to DNOC, with increases in basal metabolic rates and resultant effects on body temperature, respiratory rates, and the central nervous system being the

most sensitive end points. In addition, toxicokinetic data indicate that humans accumulate DNOC and eliminate it much more slowly than animals (Ring and Harvey 1953b); therefore, additional acute oral studies in animals may not provide information relevant to public health.

Intermediate-Duration Exposure. The respiratory tract, heart, bone marrow, gastric mucosa, liver, and kidney were target organs in humans after occupational exposure that involved a combination of inhalation and dermal exposures to DNOC for intermediate durations (Bidstrup and Payne 1951; Hunter 1950; Pollard and Filbee 1951). Increased basal metabolic rates and body temperatures were also observed in humans after occupational exposure to DNOC for intermediate durations (Hunter 1950; Pollard and Filbee 1951). Elevated pulse rates, weight loss, and increased basal metabolic rates and body temperatures were observed in humans after ingestion of DNOC for intermediate durations (Dodds and Robertson 1933; Ibrahim et al. 1934; Plotz 1936).

The stomach, the hematological and hematopoietic systems, and the kidney are possible target organs and systems in animals after oral exposure to DNOC for intermediate durations (Ambrose 1942; Den Tonkelaar et al. 1983; Spencer et al. 1948; Vos et al. 1983). The liver may also be a potential target organ (Vashakidze 1967). Cornea1 opacity and cataracts were not observed in rats after oral exposure to DNOC for intermediate durations (Spencer et al. 1948). Other possible target organs include the thyroid, pituitary, adrenal, and pancreas (Den Tonkelaar et al. 1983). Weight loss was also observed in rats after oral exposure to DNOC for intermediate durations (Ambrose 1942; Den Tonkelaar et al. 1983; Spencer et al. 1948). The skin was also identified as a potential target organ in animals after dermal exposure to DNOC for intermediate durations (Spencer et al. 1948). This was indicated by slight skin irritation in rabbits. An intermediate-duration inhalation MRL was not derived because exposure concentrations to which humans were exposed occupationally were not known and no intermediate-duration inhalation studies in animals were located. An intermediate-duration oral MRL was not derived from intermediate-duration exposure, because there appears to be tolerance for some individuals for higher exposure levels than the level eliciting acute response; however, the acuteduration MRL was used as the intermediate-duration oral MRL. Intermediate-duration inhalation studies in animals that involve identification of specific target organs and sensitive effects and provide dose-response data might provide data from which an intermediate-duration inhalation MRL can be derived. Additional intermediate-duration dermal studies in animals might provide more information on systemic target organs, since DNOC does appear to be absorbed dermally. This information is important because there are populations residing near hazardous waste sites that might be exposed to

2. HEALTH EFFECTS

DNOC for intermediate durations. A number of intermediate-duration oral studies in animals have provided information on target organs and dose-response relationship in animals. Humans appear to be more sensitive to the effects of oral exposure to DNOC, and toxicokinetic data indicate that humans accumulate DNOC and eliminate it much more slowly than animals (King and Harvey 1953b), indicating that additional intermediate-duration oral studies in animals would be of little value for purposes of deriving an intermediate oral MRL to protect humans.

Chronic-Duration Exposure and Cancer. No studies were located regarding systemic effects in humans after inhalation or dermal exposure or in animals after inhalation, oral, or dermal exposure to DNOC for chronic durations. DNOC caused marked palpitations, bilateral cataracts, blindness in one eye, and a non-icteric yellow discoloration of the eyes in a woman who ingested an unknown amount of DNOC for 3 years (Quick 1937). Derivations of chronic-duration inhalation and oral MRLs are, therefore, precluded by the lack of data. Chronic-duration inhalation, oral, and dermal studies in laboratory animals that use several dose levels and examine several end points might identify systemic target organs. This information is important because there are populations residing near hazardous waste sites that might be exposed to DNOC for long periods.

No studies were located regarding cancer in humans or animals after inhalation, oral, or dermal exposure to DNOC, but some positive genotoxic studies in animals were found (see below). There are no known populations that are presently exposed to significant levels of DNOC and, thus, no urgency to investigating cancer effects. However, it would be useful to follow up on genotoxic results with study of DNOC carcinogenicity.

Genotoxicity. Negative results were obtained for mitotic crossing in yeast and for unscheduled DNA synthesis and sister chromatid exchange in human lymphocytes (Hrelia et al. 1990), but DNOC increased the frequency of chromosomal aberrations in cultured human blood leukocytes (Nehez et al. 1978a). DNOC was clearly clastogenic, producing chromosomal aberrations in bone marrow cells, male germinal cells, and in several filial generations after treated males were mated to untreated females, in a number of in viva studies in which animals were injected intraperitoneally (Grilli et al. 1991; Hrelia et al. 1990; Nehez et al. 1978a, 1978b, 1984). Treatment of female mice by gavage with DNOC during the second trimester of pregnancy resulted in chromosomal aberrations in the embryonic livers (Nehez et al. 1981). DNOC also produced sex-linked recessive lethal mutations in *D. melanogaster* fed DNOC (Mueller and Haberzettl 1980) and dominant lethal mutations in male

mice injected intraperitoneally (Nehez et al. 1978a). However, conflicting results were obtained in *in vitro* studies in bacteria, even with the same strains (Andersen et al. 1972; Hrelia et al. 1990; Nishimura et al. 1982; Remondelli et al. 1986; Somani et al. 1981; Sundvall et al. 1984). In bacterial systems where positive results were obtained without metabolic activation, however, the presence of a metabolic activation system had the effect of reducing the genotoxic response of DNOC (Remondelli et al. 1986). Thus DNOC is clearly clastogenic in animals and humans. It is doubtful that additional genotoxicity testing in bacteria would shed light on the conflicting data, but dominant lethal testing in mice would provide additional useful information.

Reproductive Toxicity. No studies were located regarding reproductive effects in humans after inhalation or oral exposure or in animals after inhalation or dermal exposure to DNOC. Although acute dermal exposure to DNOC may have induced labor in one of three pregnant agricultural workers (Vamai and Kote 1969), this was mere speculation on the part of the investigators and has not been verified. Intermediate-duration feeding (Den Tonkelaar et al. 1983) and gavage (Vashakidze 1967) studies in rats suggest that the ovaries and the uterus are target organs of DNOC. In addition, male rats fed DNOC for 90 days had aspermatogenesis (Den Tonkelaar et al. 1983). However, no histological evidence of testicular lesions was found in other intermediate-duration feeding studies in rats using similar doses (Spencer et al. 1948; Vos et al. 1983). An intermediate-duration inhalation study conducted in two species of animals should examine reproductive end points, including organ pathology. An oral study could be specifically designed to address the conflicting data regarding reproductive effects in male rats and confirm the reproductive effects in female rats. An oral study in mice would provide information pertaining to reproductive effects for another species. If reproductive effects are confirmed, multigeneration studies by both routes would provide information on reproductive function.

Developmental Toxicity. No studies were located regarding developmental effects in humans after inhalation or oral exposure or in animals after inhalation or dermal exposure to DNOC. Three pregnant agricultural workers exposed dermally to DNOC eventually delivered healthy children (Vamai and Kote 1969), but this information is insufficient to conclude that DNOC does not cause developmental or fetotoxic effects in humans. In the only available animal study, no developmental effects were observed in the offspring of mice after oral or intraperitoneal exposure to DNOC during gestation (Nehez et al. 1981). However, when pregnant mice were administered DNOC by gavage during the second trimester of pregnancy, the frequency of chromosomal aberrations in the embryos

increased (Nehéz et al. 1981). The frequency of chromosomal aberrations in the embryos did not increase when the mice were given DNOC during the first trimester. The finding of chromosomal aberrations in the embryos raises a concern for developmental effects. Additional developmental studies involving inhalation, oral, or dermal exposure might indicate whether DNOC causes developmental effects.

Immunotoxicity. No studies were located regarding immunological effects in humans after inhalation exposure or in animals after inhalation or dermal exposure to DNOC. Maculopapular urticarial eruptions were observed in humans after ingestion of DNOC for acute (Gordon and Wallfield 1935) or intermediate durations (Plotz 1936), and a petechial rash was observed in an individual after dermal exposure to DNOC for an intermediate-duration (Stott 1956). Whether these dermal lesions represent immunological effects is not known. No histopathology of the spleen and mesenteric and popliteal lymph node, no changes in leukocyte and differential leukocyte counts, and no changes in quantities of IgM and IgG were observed in rats after oral exposure to DNOC for intermediate durations (Vos et al. 1983). The limited animal data suggest that a battery of immune function tests may be useful in confirming whether the immune system is affected by exposure to DNOC.

Neurotoxicity. No studies were located regarding neurological effects in animals after dermal exposure to DNOC. DNOC caused lethargy, depression, fatigue, dizziness, headaches, or loss of appetite in humans after occupational or oral exposure to DNOC for acute (Dodds and Robertson 1933; Gordon and Wallfield 1935; Harvey et al. 1951) or intermediate durations (Ibrahim et al. 1934; Plotz 1936). In contrast, convulsions, coma, and hemorrhage in the pia mater have been associated with agonal changes in workers who subsequently died after occupational exposure to DNOC for acute durations (Bidstrup and Payne 1951; Buzzo and Guatelli 1949; Steer 1951; van Noort et al. 1960), peripheral neuritis has been reported as an early sign of neurotoxicity in workers primarily after dermal exposure to DNOC for intermediate-durations (Stott 1956). Lethargy and depression were observed in animals after inhalation (King and Harvey 1953a) and oral (Ambrose 1942) exposure to DNOC for acute durations. Twitching, tremors, and ataxia occurred in cats after acute inhalation exposure to DNOC (Burkatskaya 1965a), and twitching, agitation, and prostration occurred in mice after acute oral dosing with DNOC (Arustamyan 1972). Clinical signs in humans and animals suggest that the cerebral cortex and/or the hind brain may be affected by DNOC (Bidstmp and Payne 1951; Harvey 1953; Harvey et al. 1951). However, there are insufficient animal studies and related histopathological data to confirm what components of the nervous system are most sensitive to DNOC. Additional

animal studies could be designed to elucidate neurological responses that would reflect the mechanisms of action, such as oxidative phosphorylation uncoupling or synaptic changes.

Epidemiological and Human Dosimetry Studies. No epidemiological studies of workers or other populations exposed to DNOC were located; however, a survey of workers (Bidstrup et al. 1952) and case reports involving occupational exposure (Bidstrup and Payne 1951; Hunter 1950; Pollard and Filbee 1951; Steer 1951) or oral use of DNOC as a weight-reducing drug (Gordon and Wallfield 1935; Ibrahim et al. 1934; Plotz 1936) are available. In addition, some experimental studies in humans were conducted (Harvey et al. 1951; Dodds and Robertson 1933). The main limitation of the studies involving workers is that exposure concentrations were not known; however, the individuals who took DNOC as a weight-reducing drug did so under medical supervision, so doses and durations are known. Similarly, the experimental studies in humans provide information on doses and durations. The available studies in humans have shown that DNOC increases basal metabolic rate, body temperature, pulse, heart rate, and respiratory rate and causes profuse perspiration, excessive thirst, lethargy, dizziness, and fatigue. These end points appear to be the most sensitive. Studies in animals have shown that the toxicity of DNOC is exacerbated in hot environments (King and Harvey 1953a). This suggests that people who live and work in tropical climates, particularly agricultural workers who use pesticides, may be more susceptible to the adverse effects of DNOC. Therefore, agricultural workers in the tropics or people who live or work near hazardous waste sites anywhere, but particularly in tropical climates, could be studied to establish cause and effect relationships.

Biomarkers of Exposure and Effect.

Exposure. DNOC and/or its metabolites have been measured in various body fluids and tissues, such as blood, urine, feces, liver, stomach, intestine, spleen, brain, and heart, of humans (Harvey et al. 1951; King and Harvey 1953a, 1953b; Sovljanski et al. 1971) and animals (King and Harvey 1953a; Leegwater et al. 1982; Truhaut and De Lavaur 1967). Detection of DNOC in body fluids or tissues, therefore, can serve as a qualitative indication that exposure to DNOC occurred. Because DNOC persists in the human body for long periods, it is difficult to determine from urine or blood levels whether there was short-term, intermediate-term, or long-term exposure to DNOC.

For this reason, however, blood and urine levels are suitable biological materials that should be monitored for DNOC exposure. The measurement of DNOC in blood is a useful indicator of

exposure, but since DNOC is still detectable in the blood 40 days after exposure, it may not be a reliable indicator of the magnitude or the time of exposure (Harvey et al. 1951). Since DNOC binds to albumin, the chief internal stores may be extracellular fluids containing albumin. Therefore, urinary levels of DNOC may not be useful biomarkers to quantitate exposure.

Metabolites of DNOC are more likely to be detected in the urine than in blood. The ratio of urinary DNOC to 6-amino-4-nitro-*o*-cresol may be a useful biomarker in humans (Truhaut and De Lavaur 1967). However, no studies were located regarding urinary metabolites of DNOC in humans, and toxicokinetic data show that humans eliminate DNOC more slowly than animals (King and Harvey 1953b).

Yellow staining of skin, sclera, or conjunctiva may alert a physician to the possibility of exposure to DNOC. This yellow staining is not a sign of icterus, but is due to the yellow color of DNOC. Yellow staining of the skin can also suggest exposure to other nitrophenolic compounds; therefore, it is not specific for DNOC. The yellow staining does not appear to correlate with blood level, exposure route, or the severity of effects (Ellenhorn and Barceloux 1988). As discussed in the preceding paragraphs, there are no reliable biomarkers that correlate well with levels of DNOC exposure. If there are identifiable individuals who continue to be exposed to DNOC, research to develop a more reliable biomarker would facilitate future medical surveillance.

Effect. Reliable biomarkers of effect in DNOC exposure include headaches, hyperthermia, profuse sweating, increased pulse rate, and dyspnea are other common signs associated with DNOC exposure. In severe cases, tachycardia, delirium, coma, and convulsions are usually observed in humans. Blood levels ≤40 μg DNOC/g in workers have been correlated with signs and symptoms of toxicity and/or death (Bidstrup et al. 1952; Pollard and Filbee 1951; Steer 1951). Other authors have also suggested that blood levels approaching 40-48 μg DNOC/g may be indicators of DNOC toxicity (Harvey et al. 1951; King and Harvey 1953b).

Although hematological, biochemical, hepatic, and renal parameters are not specific for DNOC exposure, these parameters may be measured to deterrnine disease states caused by DNOC. One animal study demonstrated changes in these parameters following oral exposure to DNOC for intermediate-duration (Den Tonkelaar et al. 1983). Based on DNOC's mechanism (uncoupling of

exudative phosphorylation), urinary ketone levels and urine and blood glucose levels may be monitored for effects on carbohydrate metabolism.

Absorption, Distribution, Metabolism, and Excretion. DNOC is readily absorbed by the respiratory and gastrointestinal tracts, and more slowly by the skin in both humans (Batchelor et al. 1956; Harvey et al. 1951; King and Harvey 1953b; Pollard and Filbee 1951) and animals (King and Harvey 1953a, 1954). An experimental study indicated that DNOC tends to be cleared from blood more slowly in humans than in animals (Harvey et al. 1951). Inhalation of DNOC has resulted in detectable levels in the cerebrospinal fluid of a man after occupational exposure (Pollard and Filbee 1951) and in the lungs of rats (King and Harvey 1954). While DNOC can be distributed to liver, kidneys, heart, and brain in humans following oral exposure (Sovljanski et al. 1971), metabolites of DNOC have been detected in the liver, kidney, and brain of rabbits (Truhaut and De Lavaur 1967). In rats and rabbits, DNOC is metabolized to less toxic metabolites that are relatively polar and readily excreted in the urine (Leegwater et al. 1982; Smith et al. 1953; Truhaut and De Lavaur 1967). No studies were located that investigated the presence of these metabolites in the urine of humans exposed to DNOC. Human data suggest that DNOC is excreted slowly in the urine after inhalation, oral, or dermal exposure (Batchelor et al. 1956; Harvey et al. 1951; King and Harvey 1953b; Pollard and Filbee 1951). Small amounts of DNOC may be excreted in the urine for as many as 20 days (Pollard and Filbee 1951). DNOC is eliminated much more rapidly in animals (King and Harvey 1954) than in humans (King and Harvey 1953b) after oral or inhalation exposure, on the order of 5 times faster. No studies were located regarding the rate and extent of excretion of DNOC in animals after dermal exposure. There are insufficient data regarding the distribution and metabolism of DNOC in humans. Because DNOC is excreted more slowly in humans than in animals, additional studies in animals would not be useful in predicting toxicokinetics of DNOC in humans. Additional experimental studies in humans would be unethical, but the urine of workers with known exposure to DNOC could be examined for metabolites.

Comparative Toxicokinetics. The target organs of DNOC appear to be similar in both animals and humans because the mechanism of toxicity (i.e., uncoupling of oxidative phosphorylation) occurs in every species. Toxicokinetic studies have been performed in rats, rabbits, and humans, and the data indicate that humans eliminate DNOC much more slowly than animals (King and Harvey 1953b). The difference in rate of elimination may be related to species differences in the metabolism of DNOC. Therefore, rats or rabbits are not a good model. Metabolites of DNOC have been identified in rats

(Leegwater et al. 1982) and rabbits (Smith et al. 1953; Truhaut and De Lavaur 1967) and appear to be similar in these species. However, no studies were located that investigated the presence of urinary metabolites in humans or primates. Since further experimental studies in humans would be unethical, the urine of workers with known exposure to DNOC could be examined to determine whether humans metabolize DNOC similarly to rats and rabbits. Toxicokinetic studies in other species of animals, especially primates, would be useful to determine the best animal model for extrapolating results to humans.

Methods for Reducing Toxic Effects. DNOC is a moderately nonpolar molecule and readily absorbed after inhalation, oral, or dermal exposure. Very little can be done to reduce absorption after inhalation exposure. However, administration of water to dilute DNOC in the gastrointestinal tract and thorough washing of the skin are standard recommendations to reduce absorption after oral and dermal exposures, respectively (Bronstein and Currance 1988; Stutz and Janusz 1988). Studies in rats have shown that administration of castor oil after oral exposure decreased the gastrointestinal absorption of DNOC substantially (Starek and Lepiarz 1974). This could be tried in humans who are known to have ingested DNOC. DNOC is distributed to the tissues of the body via the blood, possibly facilitated by binding to albumin (King and Harvey 1953b). Limited information is available regarding methods for specifically reducing the body burden of DNOC. Dialysis has been used with mixed results (Locket 1970). Because DNOC has a relatively long half-life in humans, on the order of several days (King and Harvey 1953b), procedures such as diuresis, dialysis, and hemoperfusion may be effective. Repeated treatment with activated charcoal may be useful in preventing resorption following enterohepatic circulation. The mechanism of action of DNOC depends on its ability to uncouple oxidative phosphorylation and consequently cause elevated metabolic rates and hyperthermia. There is no human antidote to arrest or reverse the metabolic disturbances in humans exposed to DNOC. However, 4-methyl-2-thiouracil has been given to DNOC-poisoned animals to reduce high metabolic rates and other effects of DNOC toxicity (Harvey 1959). It has been suggested that this agent might be effective in reducing the basal metabolic rate (Clarke et al. 1981), since it inhibits the function of the thyroid gland. General supportive measures are usually recommended for humans exposed to DNOC (Anonymous 1951; Clarke et al. 1981). These measures include rapid cooling of the body in cases of hyperthermia (Bronstein and Currance 1988; Ellenhom and Barceloux 1988). In addition, correction of fluid acidosis and electrolyte imbalance may be required (Ellenhorn and Barceloux 1988; Stutz and Janusz 1988). Research on enzyme inducers would open up the possibility of increasing

metabolism of DNOC and, thus, reducing its effects since its metabolic products are less toxic. Other approaches of disrupting its mechanism of action would open additional treatment protocols.

2.9.3 Ongoing Studies

No ongoing studies of dinitrocresols were identified.

3. CHEMICAL AND PHYSICAL INFORMATION

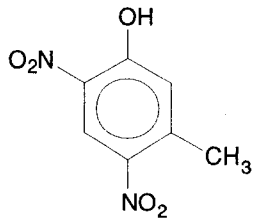
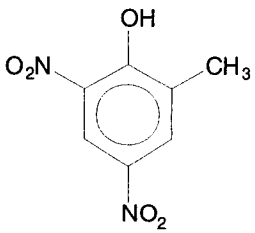
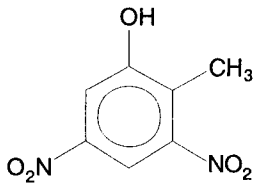
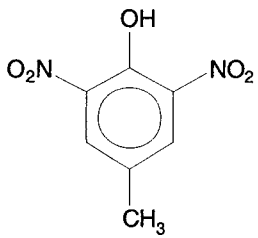
3.1 CHEMICAL IDENTITY

Information regarding the chemical identity of the selected dinitrocresols is given in Table 3-l. According to the current numbering system used in the United States, the phenolic OH substituent to the benzene ring is assigned the number one position. In the older literature, the methyl group is sometimes assigned the number one position. As a result, the compound referred to as 3,.5dinitroo-cresol is synonymous with 4,6-dinitro-*o*-cresol or DNOC (Bailey and White 1965).

3.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of the selected dinitrocresols is located in Table 3-2. Like dinitrophenols, the dinitrocresols are pseudoacids and readily form water soluble sodium, potassium, ammonium, and calcium salts (HSDB 1994; Metcalf 1978). Of the theoretically possible 18 isomers of dinitrocresols (Harvey 1953), the isomer 4,6-dinitro-*o*-cresol is the most commercially important (HSDB 1994). At a pH of 4.4, ≈50% of the DNOC in water exists as the dissociated compound (see pK_a value in Table 3-2). The concentration of the ionized form increases as the pH increases. Essentially, 100% of the DNOC at pH 7 or above will be in the ionized form. Thus, in a whole animal all of the DNOC exists in the ionized form or is associated with a macromolecule such as albumin (King and Harvey 1953b).

TABLE 3-1. Chemical Identity of Selected Isomers of Dinitrocresols

Characteristic	4,6-Dinitro-*m*-cresol	4,6-Dinitro-*o*-cresol[a]	3,5-Dinitro-*o*-cresol	2,6-Dinitro-*p*-cresol[a]
Synonym(s)	4,6-Dinitro-*m*-cresol	4,6-Dinitro-*o*-cresol; DNOC; DNC; 3,5-dinitro-2-hydroxy-toluene, 2-methyl-4,6-dinitrophenol[b]	3,5-Dinitro-*o*-cresol	2,6-Dinitro-*p*-cresol; DNPC; 3,5-Dinitro-4-hydroxy toluene
Registered trade name(s)	No data	Antinonnin; Detal; Dinitrol; Effusan; Selinon; others[b]	No data	Victoria Orange; Victoria Yellow
Chemical formula	$C_7H_6N_2O_5$	$C_7H_6N_2O_5$	$C_7H_6N_2O_5$	$C_7H_6N_2O_5$
Chemical structure				
Identification numbers:				
CAS registry	616–73–9	534–52–1	497–56–3	609–93–8
NIOSH RTECS[c]	G09400000	G09625000	G09500000	G09800000
EPA hazardous waste	No data	P047	No data	No data
OHM/TADS	No data	8100073	No data	No data
DOT/UN/NA/IMCO shipping	No data	UN 1598; IMO 6.1	No data	No data
HSDB	No data	1596	No data	No data
NCI	No data	No data	No data	No data

[a]All information obtained from ChemID 1993 and HSDB 1994 except where noted
[b]Merck 1989
[c]NIOSH 1987

CAS = Chemical Abstracts Services; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry to Toxic Effects of Chemical Substances

TABLE 3-2. Physical and Chemical Properties of Selected Isomers of Dinitrocresols

Property	4,6-Dinitro-*m*-cresol	4,6-Dinitro-*o*-cresol[a]	3,5-Dinitro-*o*-cresol[b]	2,6-Dinitro-*p*-cresol[c]
Molecular weight	198.13	198.13	198.13	198.13
Color	No data	Yellow	Yellow	Yellow
Physical state	No data	Solid	Solid	Solid
Melting point	No data	87.5 °C; 86.5 °C[d]	85.8 °C	80–81 °C; 85 °C[d]
Boiling point	No data	312 °C[e]	No data	No data
Density	No data	No data	1.49 g/cm^3 [f]	No data
Odor	No data	Odorless[g]	No data	No data
Odor threshold:				
Water	No data	No data	No data	No data
Air	No data	No data	No data	No data
pK_a	No data	4.46[h]; 4.38[i]; 4.35[j]	No data	No data
Solubility:				
Water	No data	130 mg/L at 15 °C[k]	No data	290 mg/L
Organic solvent(s)	No data	Soluble in ethanol (4.3g/100g), acetone (100g/100g), and benzene (37g/100g)[g]	Soluble in ether, ethanol, and acetone	Soluble in ether, ethanol, and acetone[d]
Partition coefficients:				
Log K_{ow}	No data	2.12[l], 2.56[m], 2.16[i], 2.85[n]	No data	No data
Log K_{oc}	No data	2.35–2.77[a,o]	No data	No data
Vapor pressure	No data	1.05x10^{-4} mmHg at 25 °C[p] 3.6x10^{-4} mmHg at 35 °C[r,s]	5.2x10^{-5} mmHg at 20 °C[q]	No data
Henry's law constant	No data	1.4x10^{-6} atm-m^3/mol at 25 °C[t,u]	No data	No data
Autoignition temperature	No data	No data	No data	No data
Flashpoint	No data	No data	No data	No data
Flammability limits	No data	No data	No data	No data
Conversion factors at 25 °C	1 mg/m^3 = 0.12 ppm	1 mg/m^3 = 0.12 ppm	1 mg/m^3 = 0.12 ppm	1 mg/m^3 = 0.12 ppm
Explosive limits	No data	No data	No data	No data

[a]All information obtained from Merck 1989 unless otherwise noted
[b]All information obtained from Lide 1993 unless otherwise noted
[c]All information obtained from EPA 1988a unless otherwise noted
[d]Lide 1993
[e]ACGIH 1986
[f]Bailey and White 1965 (no temperature value given)
[g]Metcalf 1978
[h]Cessna and Grover 1978
[i]Jafvert et al. 1990
[j]Weber 1972
[k]Meister 1991
[l]Schwarzanbach et al. 1988
[m]GEMS 1986
[n]Loehr and Krishnamoorthy 1988
[o]Kenaga 1980
[p]Suntio et al. 1988
[q]EPA 1979
[r]Plimmer 1976
[s]Hamaker and Kerlinger 1969
[t]Shen 1982a
[u]Shen 1982b

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

4.1 PRODUCTION

DNOC is prepared by sulfonating *o*-cresol with excess sulfuric acid at 80-100 ºC and subsequently nitrating 4,6-disulfonic-*o*-cresol (produced by the sulfuric acid) with nitric acid or nitrous fumes (Harvey 1953). 2,6-Dinitro-*p*-cresol is prepared by nitrating *p*-cresol with nitric acid in acetic acid or a nitric acid-sulfuric acid mixture (Harvey 1953). Neither 4,6-dinitro-*m*-cresol nor 3,5-dinitro-*o*-cresol can be produced on a commercial level by the simple nitration of *o*- or *m*-cresol (Harvey 1953). The Sea Lion Company of Texas City, Texas, is currently the only manufacturer of DNOC as an end product (SRI 1994; TR192 1994). The current production volume of this compound is not known, but the production volume was between 0.1 and 1.0 million pounds in 1977 (EPA 1988a). PMC Inc. of Chicago, Illinois, is a current manufacturer of 2,6-dinitro-*p*-cresol, and the Sandoz Chemicals Corporation of Charlotte, North Carolina, manufactures another form of dinitro-*p*-cresol (SRI 1994). The current production volume for 2,6-dinitro-*p*-cresol is not known, but 10,000-100,000 pounds were produced in the United States in 1977 (EPA 1988a). No information on the manufacturer or the production volume for 4,6-dinitro-*m*-cresol or 3,5-dinitro-*o*-cresol was located in the literature. There is no indication in the literature that these two compounds are manufactured in the United States since they are not in either *1992 Directory of Chemical Producers* (SRI 1992) or the *1992-1993 Aldrich Catalog Handbook of Fine Chemicals,* which lists over 31,000 chemicals. Table 4-l reports the number of facilities in the United States that manufacture and process DNOC, the intended use of the products, and the range of maximum amounts that are stored on site. The data reported in Table 4-l are derived from the Toxic Release Inventory (TRI) of EPA (TR192 1994). The TRI data should be used with caution since only certain types of facilities were required to report. Hence, Table 4-l is not an exhaustive list.

4.2 IMPORT/EXPORT

Comprehensive current data on the import/export of the dinitrocresols were not located in the literature. However, three U.S. companies imported small amounts (<100,000 pounds or 45,300 kg.) of DNOC in 1977 (EPA 1988a). Two tariff categories are defined covering a variety of meta- and ortho- forms of dinitrocresols. During 1992, tariffs were collected from imports totaling 10,719 kg of dinitrocresols; during 1993, tariffs were collected from imports totaling 800 kg of dinitrocresols

Table 4-1. Facilities that Manufacture or Process 4,6-dinitro-*o*-cresol

Facility	Location[a]	Range of maximum amounts on site in pounds	Activities and uses
CHEVRON CHEMICAL CO.	SAINT JAMES, LA	100-999	As a formulation component
FIRST CHEMICAL CORP.	PASCAGOULA, MS	1,000-9,999	Produce; As a by-product
STERLING CHEMICALS INC.	TEXAS CITY, TX	10,000-99,999	As a chemical processing aid
SEA LION TECH. INC.	TEXAS CITY, TX	100,000-999,999	Produce
AIR PRODUCTS MFG. CORP.	PASADENA, TX	100-999	Produce; As a by-product
HUNTSMAN CHEMICAL CORP.	PASADENA, TX	10,000-99,999	As a chemical processing aid

Source: TRI92 1994

[a] Post office state abbreviation used

(NTDB 1994). The available information suggests a decrease in the import volumes since the late 1970s.

4.3 USE

DNOC is a nonsystemic stomach poison and contact insecticide. In the United States, the EPA canceled its registration as a pesticide agent starting in 1991 (EPA 1993b; Farm Chemicals Handbook 1993; HSDB 1994). It is strongly phytotoxic for broad-leaved plants, and its use as an insecticide in the United States has been limited to dormant sprays, especially for such fruit trees as apples or peaches. As a contact herbicide, it was used to control broad-leaved weeds in cereals and to desiccate potato and leguminous seed crops before harvesting (Worthing 1987). 2,4-Dinitro-6-sec-butylphenol, which is less expensive and a more effective herbicide, had begun to replace DNOC by the late 1980s (EPA 1988a). 4,6-Dinitro-*o*-cresol has been used as a free radical polymerization inhibitor (EPA 1988a). 2,6-Dinitro-*p*-cresol is used as an intermediate for synthesis of fungicides and biologically active compounds, dyes and pharmaceuticals, and as a polymerization inhibitor for vinyl aromatic compounds (EPA 1988a; Hawley 1981).

4.4 DISPOSAL

Rotary kiln incineration at a temperature range of 820-1,000 ºC and residence times of seconds for liquid and gaseous wastes and hours for solids can totally destroy dinitrocresols. Fluidized bed incineration at a temperature range of 450-980 ºC and residence times of seconds for liquid and gaseous wastes and longer for solid wastes can also destroy dinitrocresols. Mixing dinitrocresols with a more flammable solvent may facilitate incineration. Containers used for dinitrocresols that are not to be reused can be disposed by burial in a designated landfill (HSDB 1994).

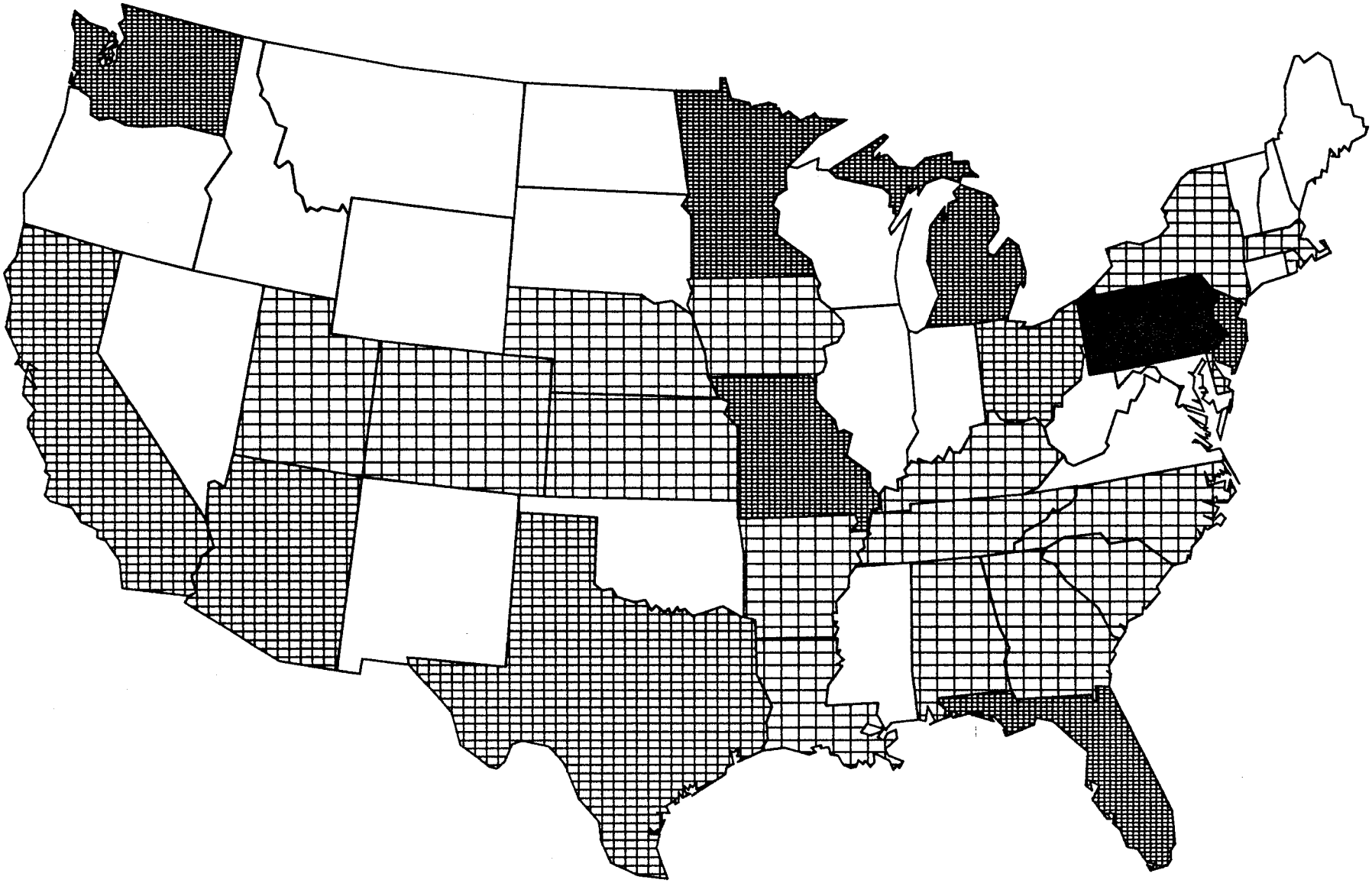
5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

DNOC can be released to the environment when it is manufactured, formulated, used as a pesticide, and disposed (EPA 1988a; Hauser and Bromberg 1982; Leuenberger et al. 1988; Spanggord et al. 1982a, 1982b). In the United States, the EPA canceled its registration as a pesticide agent starting in 1991 (EPA 1993b; Farm Chemicals Handbook 1993; HSDB 1994). DNOC also forms in the atmosphere when 2-methylphenol reacts with NO_x present in ambient air (Leuenberger et al. 1988; Tremp et al. 1986). Significant destruction of DNOC in the atmosphere due to photochemical or other chemical reactions is not likely. Dry and wet deposition of particulate DNOC are the two significant removal processes in the air (Alber et al. 1989; Leuenberger et al. 1988; Tremp et al. 1986). Neither photochemical nor other chemical processes have been identified that would be significant for the transformation/degradation of DNOC in natural waters (Capel et al. 1988; EPA 1979; Tratnyek and Holgne 1991). The loss of DNOC from water due to volatilization is negligible (EPA 1979). Adsorption will transport moderate amounts of DNOC from water to sediment and suspended solids (EPA 1979). Following an accidental spill, the estimated time for one-half the initial DNOC levels to disappear was 30 days (Capel et al. 1988). As in the case of water, no chemical process has been identified that would be significant for the removal of DNOC from soil. Biodegradation may be the most significant process for the removal of DNOC from soil (Kincannon and Lin 1985). The loss of DNOC from soil due to volatilization would not be significant. Although the mobility of DNOC in most soils will be low (Ammon 1985), it has been infrequently detected in groundwater from treated fields (Holden 1986). Therefore, loss of DNOC due to leaching may occur in some soils (e.g., sandy soils). The estimated persistence times of DNOC in soil ranged from 14 days to >l month (Ammon 1985; Goring et al. 1975; Jensen and Lautrup-Larsen 1967; Loehr 1989).

Other than in workplace air, DNOC has not been detected in ambient air in the United States. In Europe, where DNOC has been extensively used as an herbicide, concentrations in ambient air or in rain or fog may be much higher. Tree foliage exposure to DNOC has been suggested as an important contributor to the phenomenon known as forest decline (Kloepffer 1992). DNOC has been detected in effluents from chemical industries (EPA 1976b), but it has not been detected in drinking water. DNOC has been identified in 50 of the 1,350 waste sites in the NPL (HazDat 1994). The frequency of these sites within the United States is summarized in Figure 5-l. No data are available regarding

FIGURE 5-1. FREQUENCY OF NPL SITES WITH DINITROCRESOLS CONTAMINATION *



*Derived from HazDat 1994

the levels of DNOC in food or total diet samples of the U.S. population. Applicators of DNOC pesticide are one group of the population formerly exposed to DNOC by dermal and inhalation routes (Batchelor et al. 1956; Durham and Wolfe 1962; Wolfe 1976).

5.2 RELEASES TO THE ENVIRONMENT

5.2.1 Air

DNOC can enter the atmosphere during mixing and use as a pesticide on plants and soil (Leuenberger et al. 1988). In the United States, the EPA canceled the registration of DNOC as a pesticide starting in 1991 (EPA 1993b; Farm Chemicals Handbook 1993; HSDB 1994). Some DNOC can enter the atmosphere during its manufacture and formulation (EPA 1988a). DNOC releases to air from U.S. facilities that manufactured or processed this compound during 1990 are reported in Table 5-l (TR192 1994). According to TR192 (TR192 1994), an estimated 33 pounds (14.9 kg) were released to the air from manufacturing and processing facilities in 1990. The TRI data should be used with caution since only certain types of facilities were required to report. Table 5-l is not an exhaustive list. DNOC also is formed in the atmosphere as a secondary pollutant via the reaction of toluene and 2-methylphenol with NO, and OH radicals (Leuenberger et al. 1988; Tremp et al. 1986) although such mechanisms are not expected to be a significant contribution. Atmospheric reactions may be a more significant source of DNOC in the air than pesticide application (Leuenberger et al. 1988; Tremp et al. 1986).

5.2.2 Water

Dinitrocresols are released to water as effluents from chemical manufacturing industries, including pesticide and nitrotoluene manufacturing plants (EPA 1988a; Mhalas et al. 1989; Spanggord et al. 1982a, 1982b). DNOC releases in waste water from a pesticide plant and unspecified chemical manufacturing plants have been reported (EPA 1976b, 1988). The releases of DNOC to water from facilities that manufactured or processed this compound in the United States during 1990 are reported in Table 5-l (TR192 1994). According to TRI, an estimated 4,910 pounds (2,224 kg) of DNOC were transferred to publicly owned treatment works (POTW); an additional 20 pounds (9 kg) were released to surface waters from direct industrial discharges. In addition, DNOC enters surface water and groundwater through runoff and leaching from fields and agricultural lands that have been treated with

Table 5-1. Releases to the Environment from Facilities that Manufacture or Process 4,6-dinitro-*o*-cresol

			Reported amounts released in pounds per year						
State[a]	City	Facility	Air	Water	Land	Underground Injection	Total Environment[b]	POTW Transfer	Offsite Waste Transfer
LA	SAINT JAMES	CHEVRON CHEMICAL CO.		15			15		
MS	PASCAGOULA	FIRST CHEMICAL CORP.	3				3		1
TX	PASADENA	AIR PRODUCTS MFG. CORP.						4,900	1,600
TX	PASADENA	HUNTSMAN CHEMICAL CORP.						5	5
TX	TEXAS CITY	STERLING CHEMICALS INC.	30				30	5	770
TX	TEXAS CITY	SEA LION TECH. INC.		5			5		5,550
		Totals	33	20			53	4,910	7,926

Source: TRI92 1994

[a] Post office state abbreviations used
[b] The sum of all releases of the chemical to air, land, water, and underground injection wells by a given facility
POTW = Publically Owned Treatment Works

the pesticide; similar processes can transport DNOC from waste disposal sites that contain the compound (Cohen 1986; Hauser and Bromberg 1982). In the United States, the EPA canceled the registration of DNOC as a pesticide agent starting in 1991 (EPA 1993b; Farm Chemicals Handbook 1993; HSDB 1994). Small amounts enter water via deposition of atmospheric dinitrocresols (Leuenberger et al. 1988; Tremp et al. 1986).

5.2.3 Soil

DNOC is expected to be released to soil from the manufacture and formulation of the pesticide. More importantly, the pesticide would enter soil during its use in agricultural application for pest control. The release of DNOC to land from facilities that manufactured or processed the compound in the United States in 1990 is reported in Table 5-l (TR192 1994). According to TRI, an estimated 7,926 pounds (3,590 kg) of wastes containing DNOC, amounting to 82% of the total environmental release of DNOC (see Table 5-1) from the manufacturing and processing facilities in the United States in 1990, was transported out of these facilities for disposal. Some may have been disposed in landfills and some may have been incinerated. Therefore, disposal of wastes containing the nitrocresols into landfills is another important source of these compounds in soil. Deposition of atmospheric dinitrophenols introduces small amounts to soil.

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

In laboratory experiments, photolysis of o-cresol in the presence of nitrogen oxides produced dinitrocresols in the aerosol phase (Grosjean 1984, 1985). It was, therefore, presumed that dinitrocresols would be present in the ambient atmosphere in aerosol form (Grosjean 1984, 1985). The distance of atmospheric transport for DNOC depends on the half-life and the physical state of the compound in air. Residence times during atmospheric transport can be sufficiently long (see Section 5.3.2.1) so that such physical removal processes as wet or dry deposition may be important. The efficiency of both wet and dry precipitation is higher for particulate matter than for compounds that exist in the gas phase in the air (Schroeder et al. 1987). Therefore, atmospheric dinitrocresols, which exist predominantly in the particulate phase, may be removed by rain and snow, and these compounds may not be transported long distances from their source of emission. The detection of DNOC in rain and

snow, and the observation that the ratio of concentrations of DNOC in rainwater to concentrations in air during a rain event was $5.6x10^4$, confirm the importance of these removal processes (Alber et al. 1989; Leuenberger et al. 1988; Tremp et al. 1986). Precipitation of atmospheric dinitrocresols transports the compound from air to land and water.

The pK_a value of 4.4 (see Table 3-2) for DNOC suggests that in natural waters with a pH 5-9, >50% of the compound exists in the ionic state at pH 5 and the percent of ionic forms increases as the pH increases. In addition to this dissociation effect, DNOC may form H-bonds in water (EPA 1979), reducing its vapor pressure and chances of volatility from water. Using a Henry's law constant value of $1.4x10^{-6}$ atm-m^3/mole (Shen et al. 1982a, 1982b) and an estimation method (Thomas 1990), the estimated volatilization half-life of DNOC from a typical river 1 meter deep, with a current speed of 1 m/second, and an overhead wind speed of 3 m/second, is 36 days. Therefore, direct volatilization from water will not be significant for DNOC.

The partitioning of DNOC from water to solids present in water transports the compound from the water phase to suspended solids and sediment. The adsorption of DNOC from water by suspended solids and sediment is pH dependent, and the adsorption increases as the pH of the solution decreases (Frissel and Bolt 1962; Jafvert 1990). The adsorption of DNOC also depends on the clay and organic carbon content of the suspended solids and sediment; an increase in either value increases adsorption (EPA 1979; Frissel and Bolt 1962; Jafvert 1990). This adsorption will decrease the concentration of DNOC in water. DNOC's adsorption coefficient (a Freudlich sorption parameter, K_p) of 590 mg/g (Dobbs et al. 1987; Dobbs et al. 1989) indicates that the compound moderately sorbs to suspended solids and sediment in water. However, in Rhine River water with a pH of 7.9 (Wanner et al. 1989), only an estimated 9.3% of DNOC accumulated in bottom sediment (Halfon and Bruggemann 1989). This low adsorption may be due to high water pH, lack of clay, or a low organic carbon content of the sediment, or a combination of these factors.

No experimental data regarding the bioconcentration potential of DNOC in aquatic organisms were located. Based on an estimated bioconcentration factor (BCF) of 40 (Kenaga 1980), the bioconcentration of DNOC in aquatic organisms may not be significant; however, based on an estimated log octanol/water partition coefficient [$\log(K_{ow})$] value of 2.85, DNOC may bioaccumulate in aquatic organisms (Loehr and Krishnamoorthy 1988). Given that DNOC exists predominantly in ionic

forms in most natural waters (pH 5-9) and that the compound is markedly toxic to fish, bioconcentration is not expected to be important (EPA 1979).

Given the low values for vapor pressure ($1.05x10^{-4}$ mm Hg) (see Table 3-2) and Henry's law constant ($1.4x10^{-6}$ atm·m^3/mol) (see Table 3-2), and the consideration that the majority of the compound will be either in an ionic state or tied up through H-bonds, volatilization as a significant transport process for DNOC from soil to the air. However, some loss of DNOC by volatilization via co-distillation with water may occur, as observed (Kaufman 1976) in the case of dinoseb, with its active ingredient 2,3-dinitro-6-sec-butyl-phenol. Volatilization is expected to occur more readily with an increase in soil acidity (which facilitates the formation of undissociated species DNOC), moisture content, and temperature (Kaufman 1976); however, a laboratory study of two types of soil found no loss of DNOC by volatilization in 65 days (Loehr 1989). The adsorption of DNOC to soil increases with a decrease in soil pH and an increase in clay and organic carbon contents of soil. The estimated soil sorption (K_{oc}) values ranging from 2.35 to 2.77 indicate that this compound is moderately adsorbed in soil. Therefore, DNOC shows moderate mobility in soil, but because of its short persistence in soil (<1 month), the compound may not leach beyond 5 cm (Ammon 1985). Similar conclusions were reached by other investigators. In spite of moderate mobility, DNOC leaching was not observed from soil columns with at least 16 bed volumes of leachate (Pohland et al. 1987). On the other hand, the water soluble salts of DNOC (sodium, potassium, calcium, and ammonium) might be expected to leach into soil. Although no experimental data were located, it seems likely that DNOC will transfer to adjacent surface water or land via runoff water from treated fields or waste sites.

5.3.2 Transformation and Degradation

5.3.2.1 Air

The two processes likely to remove dinitrocresols from the atmosphere are reactions with hydroxyl and nitrate radicals (Atkinson et al. 1992). No experimental kinetic data are available for these two reactions (Grosjean 1991). The rate constant for the gas phase reaction of dinitrocresols with OH radicals is $3.0x10^{-14}$ cm^3/molecule-second (Grosjean 1991). Using the method of Atkinson (1988), the estimated rate constant for this reaction is $2.1x10^{-13}$ cm^3/molecule-second. Based on an average ambient atmospheric concentration of OH radicals in the northern hemisphere of $5x10^5$ radicals/cm^3 (Atkinson 1988) and either of the rate constant values, the estimated half-life of the DNOC reaction

with OH radicals is >77 days. Since dinitrocresols are expected to be present predominantly in the particulate phase in the atmosphere, the reaction rate will be even slower compared to the gas phase reaction rate (Grosjean 1991). The reactions of phenol and cresols with NO_3 radicals may be significant processes in the air (Atkinson et al. 1992). However, the products of these reactions with phenol or cresols are *o*- and possibly *p*-substituted nitrophenol and cresol compounds (Atkinson et al. 1992). Since both *o* and *p* positions are already occupied by nitro substituents, the reaction of DNOC with NO_3 radicals does not seem to be a significant atmospheric process.

Photolysis of dinitrocresols is another reaction that can be significant for the destruction of these compounds in the air. In water, the neutral DNOC species has a light absorption spectrum with a shoulder at 305 nm (Schwarzenbach et al. 1988). Therefore, it is possible that atmospheric DNOC will absorb sunlight and undergo a reaction such as nucleophilic displacement of the nitro group by a hydroxyl group. Experimental evidence of such transformation reactions is not available (Kaufman 1976). Photolysis of a structurally similar compound, dinoseb (which has a sec-butyl group in place of the o-methyl group), produced side-chain hydroxylation or unsaturation (of the set-butyl group), but no nucleophilic substitution of nitro groups (Kaufman 1976).

5.3.2.2 Water

Both neutral and anionic species of DNOC show absorption shoulders at wavelengths >300 nm (Schwarzenbach et al. 1988). However, photolysis of DNOC in water involving nucleophilic displacement of the nitro group by the hydroxyl group does not seem likely (EPA 1979). The photochemical reduction of the nitro group in DNOC is possible in water in the presence of a reducing agent (e.g., ascorbic acid or ferrous ions) and a sensitizer, such as chlorophyll (EPA 1979). However, there is no experimental evidence of the photochemical reduction of DNOC in water.

The estimated rate constants for the reaction of DNOC and 2,6-dinitro-*p*-cresol with singlet oxygen in water at pH ≈7 are 1.25×10^5/molecule-second and 1.43×10^7/molecule-second, respectively (Tratnyek and Holgne 1991). Based on an average concentration of singlet oxygen in eutrophic freshwater of 4×10^{-14} M (Tratnyek and Holgne 1991) and the above reaction rate constants, this reaction may be insignificant for DNOC. The estimated half-life for 2,6-dinitro-*p*-cresol due to this reaction is 14 days, and it may be a significant process for the destruction of 2,6-dinitro-*p*-cresol in eutrophic freshwater.

5. POTENTIAL FOR HUMAN EXPOSURE

Several pure cultures of microorganisms isolated from soil or sediment, such as *Corynebacterium simplex* (Gundersen and Jensen 1956; Jensen and Gundersen 1955), *Rhizobium leguminosarum* (Hamdi and Tewfik 1970), *Veillonella alkalescens* (McCormick et al. 1976), unadapted or phenol-adapted *Pseudomonas* sp. (Chambers and Kabler 1964; Tewfik and Evans 1966), and *Azotobacter* sp. (Wallnoefer et al. 1978), can biodegrade DNOC. Above a certain concentration, DNOC may be toxic to organisms. For example, at concentrations >500 mg/L, DNOC may be toxic to *C. simplex* (Bollen 1961). The degradation pathway will depend on the microorganism. It has been shown that *C. simplex* releases nitro groups of DNOC as nitrite ions (Gundersen and Jensen 1956). *Pseudomonas* sp. may biodegrade DNOC by ring cleavage. Successive replacement of nitro groups with hydroxyl groups can also occur, forming trihydroxytoluene (Golovleva et al. 1992; Tewfik and Evans 1966). The biodegradation may proceed by the successive reduction of nitro groups to amino groups by *V. alkalescens* and a *Pseudomonas* sp. (McCormick et al. 1976; Williams 1977). The metabolites that have been isolated as biodegradation products are 6-amino-4-nitro-*o*-cresol; 6-acetamido-4-nitro-*o*-cresol; 2-methyl-6-nitro-catechol; 2-methyl-6-amino-catechol and 2,3,5-trihydroxytoluene (Tewfik and Evans 1966; Wallnoefer et al. 1978). Although these studies with pure cultures of microorganisms are important to establish degradative pathways, their relevance to environmental situations is uncertain.

The biodegradation of DNOC was also tested with a mixture of microorganisms in activated sludge (Thorn and Agg 1975; Zahn and Wellens 1980), garden soil, compost, river mud, sediment of a waste lagoon (Tabak et al. 1964), and in settled domestic waste water (Tabak et al. 1981). These biodegradation studies with mixtures of microorganisms concluded that DNOC does not rapidly degrade under these conditions.

A patented waste treatment process that used activated sludge with added powdered activated carbon removed 99% of DNOC from influent that contained 11 µg/L of the compound (Patterson and Kodukala 1981). However, it is difficult to separate the contribution of the biological process from the adsorption effect of the activated carbon in removing DNOC from the influent. DNOC was resistant to anaerobic biodegradation under methanogenic conditions (O'Connor and Young 1989). Both laboratory die-away tests and experiments with natural marine plankton communities showed that DNOC was resistant to anaerobic biodegradation (Kuiper and Hanstveit 1988). Based on observations following a pesticide spill on the Rhine River involving DNOC, it was estimated that one-half the

initial DNOC had disappeared (due to a combination of biotic and abiotic processes) within 30 days (Capel et al. 1988).

5.3.2.3 Sediment and Soil

The transformation and degradation pathways of DNOC in soil and sediment have not been studied thoroughly. The photolysis of DNOC in soil below the surface layer and in sediment is not significant due to the lack of available sunlight. DNOC does not contain any functional groups amenable to hydrolysis (EPA 1988a). It has been speculated that adsorbed DNOC may undergo hydrolysis on clay surfaces under acidic conditions (EPA 1979), but there is no experimental evidence. Biodegradation may be the most significant process for the transformation and of DNOC in soil.

Work reported in Bruinsma (1960) documents that above certain dosage levels, DNOC may be toxic to many types of soil microorganisms. These findings help explain a pattern in the available literature where the biodegradability of DNOC is widely taken for granted but where the results of different empirical studies on the persistence of DNOC in soils may show widely differing results. The effects of DNOC toxicity to soil flora also make it hard to interpret persistent (or disappearance) findings in terms of chemical kinetics and such concepts as half-lives.

In a soil column experiment, the estimated time for degradation on one-half the original amount of DNOC was 14 days (Kincannon and Lin 1985). These results are in line with findings from field plot analyses in Germany (Hurle and Rademacher 1970), where the disappearance of one-half the initial DNOC levels took 15 days. Other investigators have estimated that the persistence of DNOC in soil ranges from 14 days to >l month (Ammon 1985; Goring et al. 1975; Jensen and Lautrup-Larsen 1967). However, in a study to determine the treatability potential of waste sludge from explosives production, no loss of DNOC was observed in two soil samples in 65 days (Loehr 1989). The soil in this experiment was not previously exposed to industrial chemicals, wastes, or any pretreatment to acclimate the microorganisms to the chemicals.

5. POTENTIAL FOR HUMAN EXPOSURE

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.1 Air

Other than in workplace air (see Section 5.5), no data regarding the concentrations of DNOC in ambient air in the United States were located. From the concentration of DNOC in rainwater during a rain event in Dubendorf, Switzerland, the concentration of DNOC in air was estimated to be (from rainwater to air partition ratio) 0.05 μg/m^3 (0.06 ppb) (Leuenberger et al. 1988).

5.4.2 Water

DNOC was detected at a concentration range of 8-400 μg/L in waste water resulting from the production and purification of trinitrotoluene (Spanggord et al. 1982a). DNOC was also qualitatively detected in the waste waters of a plant in England that produced pest control chemicals (EPA 1988a) and in two effluents from unspecified chemical plants in the United States (EPA 1976b). In a project that monitored pollutant levels in urban runoff water of 1.5 cities in the United States, DNOC was not detected (detection limit unspecified) in any runoff water (Cole et al. 1984). The Great Lakes Water Quality Board has not viewed DNOC as a toxic substance of critical concern based on levels typically encountered in water from lakes Erie and Michigan (Great Lakes Water Quality Board 1983). DNOC was detected at a concentration of <10 μg/L in water from the Potomac River near Quantico, Virginia (Hall et al. 1987). Following an accidental spill in 1986, the estimated concentration of DNOC in Rhine River water in Nauf, Switzerland, was 100430 μg/L (Capel et al. 1988). In California, where DNOC had been used as a pesticide, DNOC was detected in five groundwater samples at a maximum concentration of 35 μg/L (Cohen 1986; Holden 1986). In 1985, DNOC was detected in rainwater from Dubendorf, Switzerland, at concentrations ranging from 0.95 to 2.9 μg/L (Leuenberger et al. 1988; Tremp et al. 1986).

5.4.3 Sediment and Soil

DNOC is expected to be found in soil near plants where the pesticide is produced and formulated, near disposal sites, and in agricultural and waste lands to which the pesticide was applied. No quantitative data regarding the levels of DNOC in soil were located. Similarly, DNOC is expected to be found in the sludge of waste treatment plants, such as pesticide manufacturing plants, trinitrotoluene production

plants, and in the sediment of rivers where the pesticide has been discharged from manufacturing plants or carried by runoff water from treated lands or waste disposal sites. However, no quantitative data regarding the levels of DNOC in sludge were located. Studies on the lower Grand Calumet River around the Indian Harbor area (Hoke et al. 1993) has documented sediment concentration of 4,6-dinitro-*o*-cresol ranging from 0.24 to 2.08 mg/kg (dry weight).

5.4.4 Other Environmental Media

DNOC was not found in fish collected between 1980 and 1981 from Great Lakes harbors and tributaries (Devault 1985). DNOC was detected below the tolerance level on Rumanian plums at harvest time and in potatoes from treated fields in what was formerly East Germany (HSDB 1994).

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The general population could be exposed to DNOC from inhaling air or ingesting food and drinking water. To estimate the daily intake of DNOC by the general population from inhaling ambient air or ingesting drinking water and food, the levels of DNOC in these media must be known, and these values were not located in the literature. There was no indication in the literature that DNOC is used in any consumer products that could lead to dermal exposure.

Workers involved in manufacturing and formulating, incinerating, or spraying the pesticide on agricultural products and waste lands, and possibly workers involved in remediating Superfund sites containing this pesticide could have been or might be occupationally exposed to DNOC. Of all the possible exposure scenarios, the level of dermal exposure of workers during spraying of DNOC in the field has actually been measured. During spray-thinning of apples with liquid sprays, the estimated average dermal exposure may range from 22.5 to 63.2 mg/hour, and the corresponding average inhalation exposure may range from <0.05 to 0.4 mg/hour (Batchelor et al. 1956; Durham and Wolfe 1962; Wolfe 1976). The DNOC levels in the urine of sprayers before, during, and after the exposure period were also determined, and DNOC was detected in 5 of 183 spray operators. The DNOC concentrations in urine in these 5 samples ranged from 0.6 to 1.3 mg/L, with an average of 0.8 mg/L (Batchelor et al. 1956). The concentration of DNOC in plasma of spray operators following a total exposure period of 5-48 hours ranged from <l to 4.3 mg/kg (Batchelor et al. 1956).

5. POTENTIAL FOR HUMAN EXPOSURE

5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

As discussed (see Section 5.5), spray operators are one group of people that could have experienced potentially high exposures (higher than background levels) to DNOC. Other occupational groups discussed in Section 5.5 have the potential to be exposed to DNOC at higher levels than the general population, but no experimental evidence of higher exposure among these occupational groups was located.

Within the general population, people who live near incinerators burning DNOC, DNOC disposal facilities, and DNOC manufacturing and formulating plants are potentially exposed to higher than background concentrations of DNOC. However, no study located in the literature provided evidence of higher than background exposure to DNOC among these groups of the population. Moreover, no study demonstrated the potential for higher than background exposure to DNOC from consuming excessive amounts of certain foods (e.g., sprayed apples or contaminated fish).

5.7 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of dinitrocresols is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of dinitrocresols.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

5. POTENTIAL FOR HUMAN EXPOSURE

5.7.1 Identification of Data Needs

Physical and Chemical Properties. Some of the physical and chemical properties (e.g., K_{ow} Henry's law constant), often useful in estimating environmental fate and transport processes, are available for DNOC but not for other isomers of dinitrocresols (see Table 3-2). Although not as important as DNOC, it would still be useful to develop such data for other commercially available isomers of dinitrocresols.

Production, Import/Export, Use, Release, and Disposal. The production and import/export data in recent years for the different isomers of dinitrocresols including DNOC are not available. These data are important for assessing the trend in use for these chemicals. It is known that exposure to DNOC primarily occurs in the workplace (Batchelor et al. 1956; Durham and Wolfe 1962; Wolfe 1976). Since DNOC has been used as a pesticide on certain trees and to control broad-leaved weeds (Worthing 1987), it may have entered certain foods (e.g., apples, cereals). Since DNOC has been primarily used as a pesticide for agricultural products and on land for locust control (Worthing 1987) and is inefficiently transported from soil to other media (Ammon 1985; Kaufman 1976; Loehr 1989), soil is the environmental medium in which DNOC is expected to be found most frequently. Although some data on the methods of DNOC disposal are available (HSDB 1994), more information on disposal methods and their efficiency in destroying DNOC would be helpful. EPA has regulations governing the disposal of DNOC wastes (HSDB 1994).

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to the EPA. The Toxics Release Inventory (TR192 1994), which contains this information for 1988, became available in May of 1990. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

Environmental Fate. The partition of DNOC from water to soil and sediment depends on the pH and organic carbon and clay content of soil and sediment (Frissel and Bolt 1962; Jafvert 1990). When soil and sediment have a low pH and high organic carbon and clay content, DNOC partitions from water to soil and sediment. DNOC has been detected infrequently in groundwater (Holden 1986), indicating that only under certain conditions (e.g., when sandy soil is treated with DNOC), will it transport from soil to groundwater. The abiotic reactions that may degrade/transform DNOC in air,

water, and soil are not known with certainty. Therefore, studies of the natural chemical processes (e.g., photolysis, oxidation/reduction) that may degrade/transform DNOC would be helpful.

Bioavailability from Environmental Media. Available information regarding the absorption rate of DNOC after inhalation, oral, or dermal exposure is discussed in the Toxicokinetics Section (Section 2.3). No quantitative data regarding the bioavailability of DNOC from inhalation of, ingestion of, and dermal contact with contaminated water, or inhalation of and dermal contact with contaminated soil are available. It will be helpful to develop quantitative data for bioavailability of DNOC from environmental media. However, the bioavailability from these routes of exposure are expected to be <l00%, because the compound may be present partially in the sorbed state in air, water, and soil.

Food Chain Bioaccumulation. No experimental data for the bioaccumulation potential of DNOC from water to aquatic organisms were located. However, according to one group of investigators, DNOC may bioaccumulate in terrestrial and aquatic organisms (Loehr and Krishnamoorthy 1988). An experimental determination of the bioaccumulation potential for DNOC in terrestrial or aquatic organisms would be helpful. Biomagnification potential for DNOC is unknown.

Exposure Levels in Environmental Media. Other than in workplace air, no data regarding the ambient level of DNOC in air were located. Similarly, no data regarding the levels of DNOC in drinking water and total diet sample were available that would permit an estimation of the daily intake of DNOC from these routes of exposure. Data regarding the levels of DNOC in air, drinking water, and total diet would be useful for estimating daily DNOC intake by the general population from the various environmental media.

Reliable monitoring data for the levels of dinitrocresols in contaminated media at hazardous waste sites are needed so that the information obtained on levels of dinitrocresols in the environment can be used in combination with the known body burden of dinitrocresols to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. Other than in a few instances arising from occupational exposure (Batchelor et al. 1956; Durham and Wolfe 1962; Wolfe 1976), the levels of DNOC in body tissues and fluids of humans are not available. To assess the severity of occupational exposure, it may be

useful to determine the background levels of DNOC in the different tissues and body fluids of the general population. This information is necessary for assessing the need to conduct health studies on these populations.

Exposure Registries. No exposure registries for DNOC were located. This substance is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

5.7.2 Ongoing Studies

The United States Department of Agriculture's Cooperative State Research Service is funding studies at the Rutgers University Department of Biochemistry and Microbiology on the biodegradation of 13 mononitroaromatic compounds as well as DNOC (FEDRIP 1994). In addition to isolating natural or mutant strains of microorganisms capable of degrading nitroaromatic compounds, key controls on metabolic pathways and mechanisms will be explored along with determinations of the major degradation products.

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring DNOC, its metabolites, and other biomarkers of exposure and effect to DNOC. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

In the design of a study and the selection of an analytical method, it is very important that adequate attention be paid to the extent of validation and field applicability of a particular method. Not all of the methods have been validated to the same extent. It is the analyst's responsibility to determine the data quality needed before initiating the application of a particular method.

The analytical methods used to quantify dinitrocresols, usually dinitro-*o*-cresol, in biological and environmental samples are summarized below. Table 6-1 lists the applicable analytical methods for determining dinitrocresols in biological fluids and tissues, and Table 6-2 lists the methods used for determining dinitrocresols in environmental samples.

A survey of literature revealed that DNOC in environmental and biological samples can be quantified following several separatory steps to isolate the DNOC from the sample matrix. The separatory steps for biological samples generally use liquid-liquid extraction followed by a spectrophotometric method for quantitation of relatively large concentrations of DNOC.

Extractions are commonly used to recover DNOC from environmental matrices; the exact form depends on the matrix (see below) and could include liquid-liquid extraction, solid phase extraction (SPE), or solid phase microextraction (SPME). In SPME, a small silica fiber coated with an organic layer is equilibrated with the sample, either in solution or in a headspace. The compound, DNOC in this application, is recovered by heating the fiber to desorb the organic compound into a gas

TABLE 6-1. Analytical Methods for Determining Dinitrocresols in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Animal tissue	Extract sample mixed with methanol, sulfuric acid, and potassium oxalate with petroleum ether; clean up by gel permeation chromatography, methylate, and clean up by Florisil	GC-NPD	No data	45–50	Hopper et al. 1992
Urine, kidney, liver, brain (DNOC and metabolite 4-amino-2-methyl-6-nitrophenol)	Hydrolyze sample directly or after acetone extraction; extract with petroleum ether	Spectrophotometric	No data	No data	Truhaut and De Lavaur 1967
Serum	Dilute with water; add sodium chloride and sodium carbonate and extract with methyl ethyl ketone	Spectrophotometric	<0.5 mg/L	No data	Parker 1949
Tissue	Dilute homogenized tissue with water; add sodium chloride and sodium carbonate; extract with methyl ethyl ketone	Spectrophotometric	No data	No data	Parker 1949
Urine (DNOC and metabolite 4-amino-2-methyl-6-nitrophenol)	Acidify and subject to continuous extraction with diethyl ether	Spectrophotometric	No data	No data	Smith et al. 1953
Urine	Add sodium chloride and sodium carbonate; extract with methyl ethyl ketone	Spectrophotometric	<0.5 mg/L	No data	Parker 1949

GC = gas chromatography; NPD = nitrogen phosphorous detector

TABLE 6-2. Analytical Methods for Determining Dinitrocresols in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Technical and formulated products	Dissolve sample in methanol or acetone	HPLC/UV	2 ng[a]	No data	Farrington et al. 1982; Yao et al. 1991
Technical products	Dissolve sample in methanol	HPLC/ELCD	0.1 ng[a] (oxidative) 0.4 ng[a] (reductive)	No data	Yao et al. 1991
Air	Draw air through filter and a midget bubbler in series. DNOC extracted into ethylene glycol and 2-propanol added before analysis	HPLC/UV (Method S166)	0.070 mg/m^3 (8 ppb) for 180 L sample	104 for 0.07 mg loaded onto filter	NIOSH 1984
Water	Sample adjusted to pH 6.1 by buffer	HPLC/AdSV HPLC/DPP	0.1 µg/L (AdSV) 1.5 µg/L (DPP)	No data	Benadikova and Kalvoda 1984
Water	Extract reconstituted in methanol-acetonitrile acetic acid (20:78.5:1.5 v/v)	HPLC/UV	No data	97	Tripathi et al. 1989
Drinking water, atmospheric water	Acidify sample, add salt, and extract continuously with methylene chloride. Dry, reduce volume, and solvent exchange to hexane. Derivatize with acetic anhydride	GC/NPD	0.20 µg/L (0.2 ppm)	102 (5.5% RSD)	Herterich 1991
Drinking water, groundwater	Acidify water, add sodium sulfite, and pass through SPE cartridge of Carbopak. Elute with methanol/ methylene chloride; reduce volume	HPLC/UV	0.009 µg/L (9 ppb)	96	Di Corcia and Marchetti 1992
Drinking water, river water	Acidify sample and pass through SPE disk (Teflon with acetyl-polystyrene-divinylbenzene); elute with three aliquots of tetrahydrofuran	HPLC/UV	No data	95 (2% RSD) at 100 µg/L (0.1 ppm)	Schmidt et al. 1993

TABLE 6-2. Analytical Methods for Determining Dinitrocresols in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater	Acidify to pH=2, saturate with salt, and extract using SPME	GC/MS	0.070 µg/L (0.07 ppm) (5.6% RSD)	No data	Buchholz and Pawliszyn 1993
Groundwater, sediment	Extract acidified water with methylene chloride, reduce volume and solvent-exchange to 2-propanol	GC/FID (Method 8040)	160 µg/L	0.84C–1.01 where C= true value of concentration in µg/L	EPA 1986a
Groundwater, soil, solid waste	Extract acidified water with methylene chloride, reduce volume and exchange into 2-propanol. For other matrices, mix with anhydrous sodium sulfate and extract (soxhlet or sonication) with methylene chloride. Reduce volume. Clean-up with silica gel or GPC if needed	GC/MS (Method 8270)	50 µg/L (50 ppm water); 3.3 mg/kg (ppm soil/sediment)	1.04C–28.04 where C= true value of concentration in µg/L	EPA 1986b
Waste water	Extract acidified sample with methylene chloride; concentrate and exchange solvent to 2-propanol	GC/FID (Method 604)	16 µg/L (16 ppm)	83 at 100 µg/L	EPA 1984a
Waste water	Extract acidified sample with methylene chloride; concentrate	GC/MS (Method 625)	24 µg/L (24 ppm)	93 at 100 µg/L	EPA 1984b
Waste water	Extract acidified sample with methylene chloride, dry, and reduce volume. Add deuterated standards	GC/MS isotope dilution (Method 1625)	20 µg/L (20 ppm)	77–133 at 100 µg/L	EPA 1984c
Rain and snow	Extract acidified sample with methylene chloride; concentrate	HPLC/PDD	No data	No data	Alber et al. 1989

TABLE 6-2. Analytical Methods for Determining Dinitrocresols in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Extract with methylene chloride; evaporate to dryness and dissolve residue in alkaline methanol/water	HPLC/UV	0.005 mg/kg (5 ppb)	85–105	Roseboom et al. 1981
Soil	Soxtec extraction of clay loam using hexane:acetone (1:1). Reduce volume	GC/MS	No data	63.4 at 6 mg/kg	Lopez-Avilla et al. 1993
Variety of crops	Extract macerated or homogenized sample with methylene chloride; evaporate to dryness and dissolve in potassium carbonate/methanol mixture	HPLC/UV	0.005 mg/kg (5 ppb)	82–105 at 0.05 mg/kg. %RSD range from 4 to 13%	Roseboom et al. 1981
Various crops	Homogenize sample in blender, adding distilled water as needed. Add Florisil to form free flowing mixture and pack into a column with a sodium sulfate layer at bottom. Elute with methylene chloride-acetone (1:1) or ethyl acetate. Reduce volume	GC/ECD	0.001 mg/kg (1 ppb)	69–79 at 0.01–0.5 mg/kg	Kadenczki et al. 1992
Fatty and nonfat foods	Mix fatty sample with methanol, sulfuric acid, and potassium oxalate and nonfat samples with sulfuric acid and methanol; extract both with petroleum ether or methylene chloride; clean-up by gel permeation chromatography, methylate, and clean-up by Florisil	GC/NPD	No data	45–50 (fatty foods) >80 (nonfat foods)	Hopper et al. 1992

[a] These are absolute detection limits

AdSV = adsorptive stripping voltametric detector; DPP = differential pulse polarographic detector; ELCD = electrochemical detector; FID = flame ionization detection; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; NPD = nitrogen phosphorus detector; PDD = photodiode array detector; RSD = relative standard deviation; SPE = solid phase extraction; SPME = solid phase microextraction; UV = ultraviolet detector; v/v = volume per volume.

chromatograph. Both high performance liquid chromatographic (HPLC) and gas chromatographic (GC) methods have been used as final separator-y methods for environmental samples. A variety of detection methods, including flame ionization detection (FID), electron capture (ECD), thermal energy analyzer, mass spectrometric, nitrogen-phosphorus (NPD), ultraviolet (UV), photodiode detection, and electrochemical methods have been used to detect and quantify DNOC.

Some GC methods recommend the derivatization of dinitrocresols to increase their volatility to enhance elution from the GC column (Mhalas et al. 1989; Roseboom et al. 1981). Derivatization is not used in other GC methods (Buchholz and Pawliszyn 1993; EPA 1984a, 1984b, 1984c, 1986a, 1986b) and good results are obtained. If used, the relative efficiencies of the various derivatization methods should be evaluated. HPLC is advantageous for DNOC determination because derivatization is not required (Alber et al. 1989). A comprehensive study that compares the sensitivities among the detection methods is not available. However, when HPLC was used, an electrochemical detection method was 20 times more sensitive than the UV method (spectrophotometric) (Yao et al. 1991). When a GC method was used, NPD was more suitable than either ECD or a FID for determining DNOC (EPA 1982a; Hopper et al. 1992). On the other hand, GC/FID was more sensitive than GC/MS (EI mode) and HPLCKJV (at 254 nm) (James et al. 1984). The sensitivity of the overall method can be highly dependent on the extraction and clean-up steps. Exceptional selectivity and recovery during sample preparation can result in a very sensitive method even when the determinative step utilizes a technique not generally considered to provide the highest sensitivity, such as HPLC/UV (Di Corcia and Marchetti 1992).

Although not used in any of the overall methods found, Fourier transform-infrared spectroscopy for detection after GC can supplement MS to verify the presence of DNOC in samples (Budzinski et al. 1992; Gurka et al. 1991; Schneider et al. 1991). Alternative separation methods have also been shown to be applicable to nitrophenols, including DNOC, but have not yet become routine. These methods include supercritical fluid chromatography (Ong et al. 1992; Pospisil et al. 1992), capillary zone electrophoresis (Chao and Whang 1994), and micellar electrokinetic chromatography (Ong et al. 1991).

6.1 BIOLOGICAL SAMPLES

Analytical methods used for determining DNOC in biological samples are listed in Table 6-l. With the exception of the method of Hopper et al. (1982), the methods found rely on spectrophotometry for

identification or quantitation in extracts (Smith et al. 1953) or after paper chromatography (Parker 1949). It should be possible to modify Parker's method (1949) for use with modern thin layer chromatographic techniques (Sherma 1991). A quantitative method for monitoring human exposure to DNOC and other genotoxic agents in urine is also available (San et al. 1989). In this method, urine extracts were assayed for their capacity to induce chromosome and chromatid damage in cultured Chinese hamster ovary fibroblasts. This method is not specific for DNOC and additional analysis is required to verify that the effect was due specifically to DNOC.

6.2 ENVIRONMENTAL SAMPLES

Analytical methods used for determining DNOC in environmental samples are given in Table 6-2. Most of the methods for products, waters, soils, and sludges rely on extraction of DNOC from an acidified matrix; acidification minimizes dissociation of the phenolic hydrogen and thus facilitates extraction into an organic solvent or adsorption onto a solid phase extraction medium. The influence of pH on the adsorption of DNOC to humic materials in coal waste waters has been studied (Pbrschmann and Stottmeister 1993) and significant adsorption was found to occur at pH 7 but not at pH=2. In general, methods utilizing MS or selective detectors were less subject to interferences from complex samples than those methods based on the less selective FID detection. The method for DNOC in air (NIOSH 1984) recovers both DNOC vapor and DNOC adsorbed to particulates. DNOC is present in air both as a vapor and adsorbed to both solid (dust) and liquid (rain, fog) particulate matter (Perez and Soderholm 1991). The distribution between the vapor and particulate phase was not measured.

Dinitrocresols present in water samples, especially when present at low concentrations, can be lost via oxidation by hypochlorite (Di Corcia and Marchetti 1992). This can be eliminated through the addition of sodium sulfite prior to extraction. In the multiresidue method of Chen et al. (1991), Mn^{2+} dissolved in the sample would be oxidized to manganese(III,IV) oxides during base extraction, which in turn would oxidize the phenols during acid extraction. The oxidation of phenols can be eliminated by adding sodium thiosulfate to the sample prior to extraction or extraction at acidic pH as the first step, providing that manganese(III,IV) oxides are not present in the sample before extraction.

6. ANALYTICAL METHODS

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of dinitrocresols is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of dinitrocresols.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. In humans, a significant portion of absorbed DNOC appears in the urine as the metabolite 4-amino-2-methyl-6-nitrophenol (WHO 1975). The measurement of this metabolite may be an indicator for DNOC exposure (WHO 1975). Analytical methods for determining DNOC and its urinary metabolite are available (Smith et al. 1953; Truhaut and De Lavaur 1967), although the limits of detection for these methods have not been documented. Harvey et al. (1951) and King and Harvey (1953b) used paper chromatography to study DNOC in blood after exposures of 0.9-1.3 mg/kg/day and the methods were adequate to detect DNOC for many days after exposure. It is not clear, however, if the methods would be adequate to detect DNOC in blood after an exposure at the oral MRL of 0.004 mg/kg/day (calculated in Chapter 2). If a 70 kg person is assumed, an MRL dose of 0.28 mg/day can be calculated. It seems likely that the methods would be sensitive enough to detect DNOC in blood, at least shortly after the exposure, but this has not been shown. The metabolites 4,6-dinitro-2-hydroxymethylphenol and 4,6-diacetamidoo-cresol have also been determined in urine using TLC in conjunction with field desorption mass spectrometry (van der Greel and Leegwater 1983). The limits of detection were not reported for this method either. However, neither blood nor urinary levels of DNOC are reliable indicators for magnitude or the time of exposure to DNOC (Harvey et al. 1951; King and Harvey 1953b). The

6. ANALYTICAL METHODS

DNOC levels in any other tissue or body fluid of humans have not been correlated with the magnitude and duration of exposure to DNOC (see Section 2.5.1). The identification of a biomarker that can be correlated with the level of exposure to DNOC would be helpful and is needed. The analytical methods should be updated and validated.

Although elevated levels of blood urea nitrogen and blood glucose, and decreased blood pyruvate indicate metabolic disturbances caused by DNOC (Den Tonkelaar et al. 1983; Spencer et al. 1948) (see Section 2.5.2), these effects are not unique to DNOC exposure (see Section 2.5.2). Therefore, it would be useful to identify an effect that may be uniquely and quantitatively associated with exposure to DNOC.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The analytical methods presently available are capable of determining DNOC in fatty and nonfat foods at levels well below the tolerance limit (Hopper et al. 1992; Roseboom et al. 1981). Methods for DNOC in water are sufficiently sensitive to monitor concentrations well below the MRL for a 70 kg individual (Buchholz and Pawliszyn 1993; Di Corcia and Marchetti 1992). The method for DNOC in air is sensitive to concentrations below the OSHA standard of 0.2 mg/m^3 (NIOSH 1984) but is inconvenient for personal monitoring because of the liquid contained in the bubbler. Methods are currently available for determining degradation products obtained as a result of DNOC biodegradation by pure cultures of microorganisms (Gundersen and Jensen 1956; McCormick et al. 1976; Tewfik and Evans 1966). The limits of detection have not been established for degradation products. If the degradation products are of interest, methods need to be refined and validated.

6.3.2 Ongoing Studies

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control, is developing methods for the analysis of dinitrocresols and other phenolic compounds in urine. These methods use high resolution gas chromatography and magnetic sector mass spectrometry which gives detection limits in the low parts per trillion (ppt) range.

No other ongoing studies that would improve upon the methods to determine the levels of DNOC or its metabolites in biological samples were located. An ongoing study at Rutgers University designed

to study the degradation of nitroaromatic compounds by microorganisms was found. This work is sponsored by the USDA. It appears that the researchers involved are developing or modifying the analytical methods used to identify metabolic intermediates. The analytical techniques being used include TIC, GC, GC/MS, and infrared absorbance.

7. REGULATIONS AND ADVISORIES

The national and state regulations and guidelines regarding dinitrocresols (DNOC) in air, water, and other media are summarized in Table 7- 1.

An acute-duration and an intermediate-duration oral MRL of 0.004 mg/kg/day were derived for DNOC. The MRLs are based on a LOAEL value of 0.36 mg/kg/day for neurological effects in humans in case reports by Plotz (1936), in which humans were taking DNOC for the purpose for weight reduction.

The reference concentration for dinitrocresols is undergoing review by an EPA Workgroup. No reference dose exists for the compound.

Dinitrocresols are on the list of chemicals appearing in The Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA) (EPA 1988b). Section 313 of Title III of EPCRA requires owners and operators of certain facilities that manufacture, import, process, or otherwise use the chemicals on this list to report annually their release of those chemicals to any environmental medium.

OSHA requires employers of workers who are occupationally exposed to dinitrocresols to institute engineering controls and work practices to reduce and maintain employee exposure at or below permissible exposure limits (PEL). The employer must use engineering and work practice controls, if feasible, to reduce exposure to or below an 8-hour time-weighted average (TWA) of 0.2 ppm. Respirators must be provided and used during the time period necessary to install or implement feasible engineering and work practice controls (OSHA 1993). In 1989, the TWA was promulgated at 0.25 mg/m^3. However, that limit and limits over 400 other chemicals were revoked based on a 1992 Court of Appeals Decision, and the pre-1989 limit of 0.2 mg/m^3 was reinstated. Approximately 13 states continue to enforce the 0.25 mg/m^3 limit.

Dinitrocresols are regulated by the Clean Water Effluent Guidelines as stated in Title 40, Sections 400-475, of the Code of Federal Regulations. The point source categories for which dinitrocresols are controlled as a Total Toxic Organic include electroplating, steam electric power generation, and metal finishing.

7. REGULATIONS AND ADVISORIES

The Resource Conservation and Recovery Act (RCRA) identifies dinitrocresols as hazardous waste when discarded as a commercial product, off-specification materials, (i.e., does not meet a company's specifications on chemical composition), container residue, or spill residue (EPA 1980a).

Table 7-1. Regulations and Guidelines Applicable to Dinitrocresols

Agency	Description	Information	Reference
NATIONAL			
Regulations:			
a. Air:			
EPA	Listed as Hazardous Air Pollutant (dinitro-o-cresols and salts)	Yes	U.S. Congress 1990 (Clean Air Act)
OSHA	PEL TWA (dinitro-o-cresol)	0.2 mg/m^3 (skin)	29 CFR 1910.1000 OSHA 1993
b. Water:			
EPA OW	Appendix D—NPDES Permit Application Testing Requirements (122.21)	Yes	40 CFR 122 EPA 1983a
	Additional Conditions Applicable to Specified Categories of NPDES Permits—Notification Levels	500 µg/L	40 CFR 122.42 EPA 1983b
	Method 604—Phenols	Yes	40 CFR 136 EPA 1973a
	Method 625—Base/Neutrals and Acids	Yes	40 CFR 136 EPA 1973a
	Method 1625—Semivolatile Organic Compounds by Isotope Dilution GC/MS		40 CFR 136 EPA 1973a
	Guidelines Establishing Test Procedures for the Analysis of Pollutants	Yes	40 CFR 136.3 EPA 1973b
	Effluent Guidelines and Standards: Electroplating—Definition of Total Toxic Organic	>10 µg/L	40 CFR 413.02 EPA 1981
	Toxic Pollutant Effluent Limitation and Standards for Direct Discharge Point Sources That Use End-of-pipe Biological Treatment		40 CFR 414.91 EPA 1987a
	- Maximum for any one day - Maximum for monthly average	277 µg/L 78 µg/L	
	Toxic Pollutant Effluent Limitation and Standards for Direct Discharge Point Sources That Do Not Use End-of-pipe Biological Treatment		40 CFR 414.101 EPA 1987b
	- Maximum for any one day - Maximum for monthly average	277 µg/L 78 µg/L	
	Toxic Pollutant Standards for Indirect Discharge Point Sources		40 CFR 414.111 EPA 1993a
	Effluent Guidelines and Standards: Steam Electric Power Generation: Appendix A—126 Priority Pollutants	Yes	40 CFR 423 EPA 1982b
	Effluent Guidelines and Standards: Metal Finishing—Definition of Total Toxic Organic	>10 µg/L	40 CFR 433.11 EPA 1983c

Table 7-1. Regulations and Guidelines Applicable to Dinitrocresols (continued)

Agency	Description	Information	Reference
NATIONAL (cont.)			
EPA OW	Applicability; Description of the Organic Pesticide Chemicals Manufacturing Subcategory	Yes	40 CFR 455.20 EPA 1978
c. Other			
EPA OERR	Reportable Quantity (dinitro-o-cresols and salts)	10 lb	40 CFR 302.4 EPA 1992c
	Appendix A—Extremely Hazardous Substance TPQ (dinitrocresol)	10/10,000 lb	40 CFR 355 EPA 1987c
EPA OPTS	Intent to Cancel, Restrict, or Require Reregistration of Pesticide Products Containing Dinitrocresol	Yes	HSDB 1992
	Toxic Chemical Release Reporting: Community Right-to-Know—Specific Toxic Chemical Listing (4,6-dinitro-o-cresol)	Yes	40 CFR 372.65 EPA 1988b
EPA OSW	Appendix II—Municipal Solid Waste Landfills Method 8040 Method 8270	 150 µg/L 50 µg/L (Practical Quantitation Limit)	40 CFR 258 EPA 1991b
	Listing as Hazardous Waste: Discarded Commercial Chemical Products Off-specification Species, Container Residues, and Spill Residues Thereof	Yes	40 CFR 261.33 EPA 1980a
	Appendix VIII—Listing as Hazardous Waste Constituent (P047)	Yes	40 CFR 261 EPA 1980b
	Appendix IX—Wastes Excluded Under §260.22—Delisting Level	200 mg/L	40 CFR 261 EPA 1984d
	Appendix IX—Groundwater Monitoring Requirement	Yes	40 CFR 264 EPA 1980c
	Treatment Standards Expressed as Specified Technologies	Yes	40 CFR 268.42 EPA 1986d
	Constituent Concentrations in Waste: Waste Waters Concentration Nonwaste Waters	 0.28 mg/L 160 mg/L	40 CFR 268.43 EPA 1988c
Guidelines:			
a. Air			
ACGIH	TLV TWA (dinitro-o-cresol)	0.2 mg/m^3 (skin)	ACGIH 1994
NIOSH	REL - TWA (dinitro-o-cresol)	1.5 mg/m^3 (skin)	NIOSH 1992
	IDLH (dinitrocresol)	5 mg/m^3	NIOSH 1990

Table 7-1. Regulations and Guidelines Applicable to Dinitrocresols (continued)

Agency	Description	Information	Reference
STATE			
Regulations and Guidelines:			
a. Air:			
	Acceptable Ambient Concentration Guidelines or Standards (4,6-dinitro-o-cresol)		NATICH 1992
CT	8 hr avg. time	4 μg/m^3	
FL- Pinella	8 hr avg. time	2 μg/m^3	
FL-Pinella	24 hr avg. time	0.48 μg/m^3	
ND	8 hr avg. time	2 μg/m^3	
NV	8 hr avg. time	5 μg/m^3	
OK	24 hr avg. time	2 μg/m^3	
PA-Phil	24 hr avg. time (Also applies to 3,5-dinitro-o-cresol)	.75 μg/m^3	
TX	30 min avg. time (Also applies to 3,5-dinitro-o-cresol)	2 μg/m^3	
TX	Annual avg. time (Also applies to 3,5-dinitro-o-cresol)	2 μg/m^3	
VA	24 hr avg. time	3.3 μg/m^3	
WA-SW	24 hr avg. time	.7 μg/m^3	
WI	Hazardous air contaminant Acceptable ambient air concentration		CELDs 1992
	<25 feet (emission point)	0.01656 lb/hr	
	≥25 feet (emission point)	0.0672 lb/hr	
b. Water			
	Water Quality: Human Health		CELDs 1994
AZ	(2-methyl - 2,4-dinitrophenol)		
	Domestic water source	2.7 μg/L	
	Fish consumption	120 μg/L	
	Full body contact	550 μg/L	
CA	30 day average (4,6-dinitro-2-methylphenol)	220 μg/L	

Table 7-1. Regulations and Guidelines Applicable to Dinitrocresols (continued)

Agency	Description	Information	Reference
STATE (Cont.)			
CT	(2-methyl - 2,6-dinitrophenol)		
	Fish consumption	765[a]	
	Fish and water consumption	13.4[a]	
HI	Fish consumption (dinitro-o-cresol (2,4))	250 µg/L	
IN	(2,4-dinitro-o-cresol)		
	Outside mixing zone (4-day avg.)	765 µg/L	
	Point of water intake (4-day avg.)	13.4 µg/L	
	Domestic water supply source	13.4 µg/L	
KY	(2,4-dinitro-o-cresol)		
	Fish consumption	765 µg/L	
	Domestic water supply source	13.4 µg/L	
MO	(2-methyl - 4,6-dinitrophenol)		
	Fish consumption	765 µg/L	
	Drinking water supply	13 µg/L	
NJ	24-hour maximum - potable water (2,4-dinitro-o-cresol)	13.4[a]	
OH	Outside mixing zone (30-day avg.) (4,6-dinitro-o-cresol)	765 µg/L	
OR	(4,6-dinitro-o-cresol)		
	Water and fish consumption	13.4 µg/L	
	Fish consumption	765 µg/L	
SD	Domestic water	13.4 µg/L	
	All other uses	765 µg/L	
TN	All categories (2-methyl - 4,6-dinitrophenol)	765 µg/L	
VT	Class A or B waters	13.4 µg/L	
	Class C Waters	765 µg/L	
	Water Quality: Aquatic Life		CELDs 1994
AZ	(2-methyl - 4,6-dinitrophenol)		
	Acute-cold water fishery	310 µg/L	
	Acute-warm water fishery	310 µg/L	
	Acute-effluent dominated water	310 µg/L	
	Chronic-warm water fishery	24 µg/L	
	Chronic-effluent dominated water	24 µg/L	
NJ	(nitrophenols)		
	24 hr maximum-freshwater	230[a]	
	24 hr maximum-saltwater	4,850[a]	

Table 7-1. Regulations and Guidelines Applicable to Dinitrocresols (continued)

Agency	Description	Information	Reference
STATE (Cont.)			
	Water Quality: Agricultural Uses		CELDs 1994
MO	Irrigation (2-methyl - 4,6-dinitrophenol)	13 µg/L	
OH	(4,6-dinitro-o-cresol)	13.4 µg/L	
	Water Quality: Recreational Uses		CELDs 1994
TN		765 µg/L	
	Groundwater Quality Standards		CELDs 1994
MO	Groundwater (2-methyl - 4,6-dinitrophenol)	13 µg/L	
	Groundwater Quality Monitoring Parameters (4,6-dinitro-o-cresol)		CELDs 1994
CO		Yes	
IL		Yes	
KY		Yes	
LA		Yes	
MN		Yes	
NY		Yes	
SC		Yes	
TN		Yes	
WV		Yes	
WI		Yes	
	Discharge Limits		CELDs 1994
NJ	NPDES Permits: Testing Requirements for Organic Toxic Pollutant (4,6-dinitro-o-cresol)	Yes	
SD	Surface Water Discharge Permit Application Requirements: Test Requirements for Organic Toxic Pollutants (4,6-dinitro-o-cresol)	Yes	
WI	Maximum Allowable Concentration = BAT effluent		
c. Other			
	Hazardous Waste Constituents (4,6-dinitro-o-cresol and salts)		CELDs 1994
CA		Yes	
CO		Yes	
IL		Yes	
KY		Yes	

Table 7-1. Regulations and Guidelines Applicable to Dinitrocresols (continued)

Agency	Description	Information	Reference
STATE (cont.)			
LA		Yes	
MA		Yes	
MD		Yes	
MN		Yes	
MT		Yes	
ND		Yes	
NE		Yes	
NH		Yes	
NJ		Yes	
OH		Yes	
SC		Yes	
VA		Yes	
VT		Yes	
WV		Yes	
WI		Yes	
WY		Yes	
	Restricted Pesticides		
CA	(4,6-dinitro-o-cresol)	Yes	CELDs 1994
HI	(1,6-dinitro-o-cresol and salts)	All concentrations	CELDs 1992
ME	Sinox	>2%	CELDs 1994
NH	(dinitro-o-cresol)	Any concentrations	CELDs 1994
NJ	Designated restricted pesticide	Yes	CELDs 1992
OR	Highly toxic pesticide registration required (dinitro-o-cresol and salts)	In any form (all uses)	

[a] Unit of measure was not provided.

ACGIH = American Conference of Governmental and Industrial Hygienists; BAT = Best Available Technology; CFR = Code of Federal Regulations; EPA = Environmental Protection Agency; HSDB = Hazardous Substance Data Bank; GC/MS = Gas Chromatogram/Mass Spectrometry; IDLH = Immediately Dangerous to Life and Health; NATICH = National Air Toxics Information Clearinghouse; NIOSH = National Institute of Occupational Safety and Health; NPDES = National Pollutant Discharge Elimination System; OERR = Office of Emergency and Remedial Response; OPP = Office of Pesticide Programs; OSHA = Occupational Safety and Health Administration; OSW = Office of Solid Waste; OTS = Office of Toxic Substances; OW = Office of Water; OPTS = Office of Pesticides and Toxic Substances; PEL = Permissible Exposure Limit; REL = Recommended Exposure Level; TLV = Threshold Limit Value; TPQ = Threshold Planning Quality; TWA = Time Weighted Average

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9. GLOSSARY

Acute Exposure-Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption Coefficient (K_{oc})-The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)-The amount of a chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Bioconcentration Factor (BCF)-The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Cancer Effect Level (CEL)-The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen-A chemical capable of inducing cancer.

Ceiling Value - A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure-Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Developmental Toxicity-The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Embryotoxicity and Fetotoxicity-Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

EPA Health Advisory-An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)-The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Intermediate Exposure-Exposure to a chemical for a duration of 15-364 days, as specified in the Toxicological Profiles.

9. GLOSSARY

Immunologic Toxicity-The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

***In vitro*-**Isolated from the living organism and artificially maintained, as in a test tube.

***In vivo*-**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})-The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})-A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{LO})-The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})-The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time $_{(50)}$ (LT_{50})-A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)-The lowest dose of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Malformations-Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level-An estimate of daily human exposure to a dose of a chemical that is likely to be without an appreciable risk of adverse noncancerous effects over a specified duration of exposure.

Mutagen-A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Neurotoxicity- The occurrence of adverse effects on the nervous system following exposure to chemical.

No-Observed-Adverse-Effect Level (NOAEL)-The dose of chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})-The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

Permissible Exposure Limit (PEL)-An allowable exposure level in workplace air averaged over an 8-hour shift.

9. GLOSSARY

q_1^* -The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1^* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and $\mu g/m^3$ for air).

Reference Dose (RfD)-An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)-The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity-The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Short-Term Exposure Limit (STEL)-The maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily TLV-TWA may not be exceeded.

Target Organ Toxicity-This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen-A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)-A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-Weighted Average (TWA)-An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose (TD_{50})-A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Uncertainty Factor (UF)-A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIX A

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (2-1, 2-2, and 2-3) and figures (2-l and 2-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer endpoints, and EPA's estimated range associated with an upper-bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed- Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs) .

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 2-l and Figure 2-l are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 2-1

(1) Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exists, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 2-1, 2-2, and 2-3, respectively). LSE figures are limited to the inhalation (LSE Figure 2-l) and oral (LSE Figure 2-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.

APPENDIX A

(2) Exposure Period Three exposure periods - acute (less than 15 days), intermediate (15-364 days), and chronic (365 days or more) are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the 2 "18r" data points in Figure 2-l).

(5) Species The test species, whether animal or human, are identified in this column. Section 2.4, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 2.3, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to toxaphene via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 1981.

(7) System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.0006 ppm (see footnote "c").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference The complete reference citation is given in chapter 8 of the profile.

APPENDIX A

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "c" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.0006 ppm.

LEGEND

See Figure 2-1

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14) Health Effect These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, 18r NOAEL is the critical endpoint for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates to a NOAEL for the test species-rat. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.0006 ppm (see footnote "c" in the LSE table).

(17) CEL Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (ql*).

(19) Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

1 → **TABLE 2-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
2 → INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 → Systemic	↓	↓	↓	↓	↓		↓
4 → 18	Rat	13 wk 5d/wk 6hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
					11		
Cancer					↓		
38	Rat	18 mo 5d/wk 7hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5d/wk 6hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5d/wk 6hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

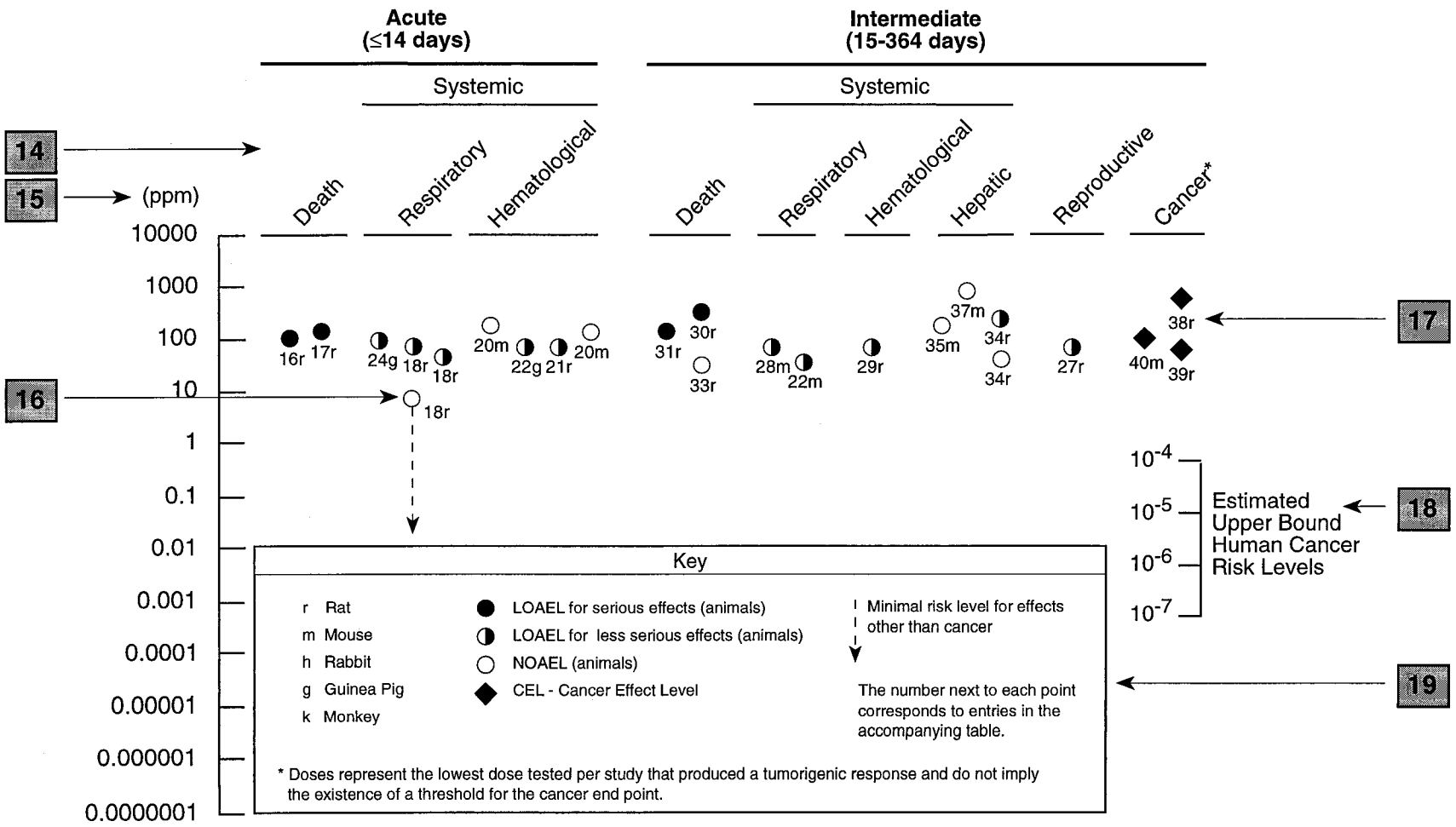
[a] The number corresponds to entries in Figure 2-1.

12 → [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x 10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

CEL = cancer effect level; d = days(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

SAMPLE

13 → **Figure 2-1. Levels of Significant Exposure to [Chemical X] – Inhalation**



APPENDIX A

Chapter 2 (Section 2.4)

Relevance to Public Health

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions.

1. What effects are known to occur in humans?

2 . What effects observed in animals are likely to be of concern to humans?

3 . What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section covers endpoints in the same order they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). In *vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer endpoints (if derived) and the endpoints from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MI&s) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2.4, “Relevance to Public Health,” contains basic information known about the substance. Other sections such as 2.6, “Interactions with Other Substances,” and 2.7, “Populations that are Unusually Susceptible” provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

APPENDIX A

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

APPENDIX B

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, Distribution, Metabolism, and Excretion
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BSC	Board of Scientific Counselors
C	Centigrade
CDC	Centers for Disease Control
CEL	Cancer Effect Level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CLP	Contract Laboratory Program
cm	centimeter
CNS	central nervous system
d	day
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DOL	Department of Labor
ECG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
EKG	see ECG
F	Fahrenheit
F_1	first filial generation
FAO	Food and Agricultural Organization of the United Nations
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
fpm	feet per minute
ft	foot
FR	Federal Register
g	gram
GC	gas chromatography
gen	generation
HPLC	high-performance liquid chromatography
hr	hour
IDLH	Immediately Dangerous to Life and Health
IARC	International Agency for Research on Cancer
ILO	International Labor Organization
in	inch
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient

L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration, low
LC_{50}	lethal concentration, 50% kill
LD_{Lo}	lethal dose, low
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mm Hg	millimeters of mercury
mmol	millimole
mo	month
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
ng	nanogram
nm	nanometer
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPL	National Priorities List
NRC	National Research Council
NTIS	National Technical Information Service
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	permissible exposure limit
pg	picogram
pmol	picomole
PHS	Public Health Service
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
RfD	Reference Dose
RTECS	Registry of Toxic Effects of Chemical Substances
sec	second
SCE	sister chromatid exchange
SIC	Standard Industrial Classification
SMR	standard mortality ratio

STEL	short term exposure limit
STORET	STORAGE and RETRIEVAL
TLV	threshold limit value
TSCA	Toxic Substances Control Act
TRI	Toxics Release Inventory
TWA	time-weighted average
U.S.	United States
UF	uncertainty factor
yr	year
WHO	World Health Organization
wk	week

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
δ	delta
γ	gamma
μm	micron
μg	microgram

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