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.

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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.

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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

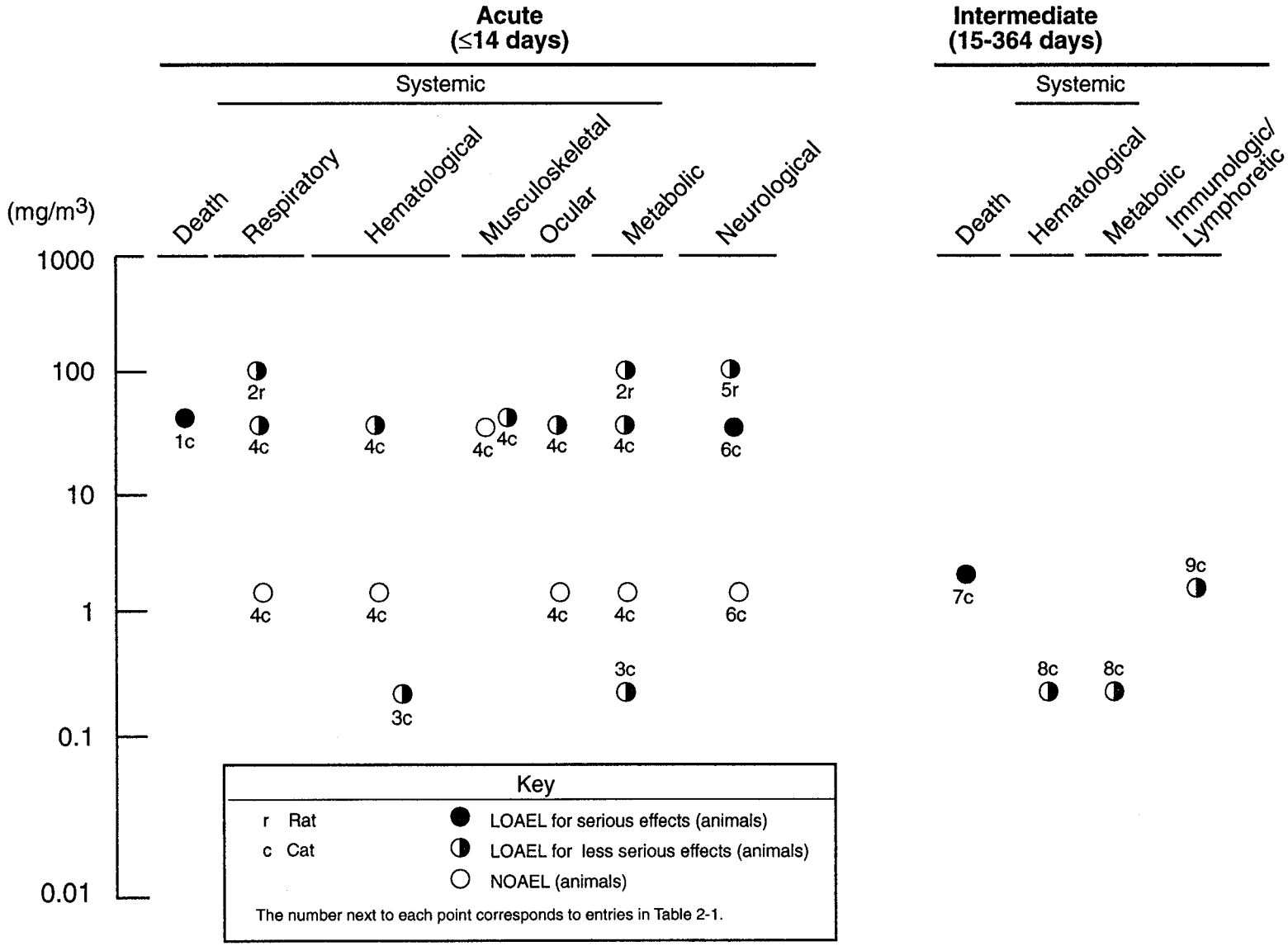
Key[a] to figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
	ACUTE EXPOSURE						
	Death						
1	Cat (NS)	4hr				40 (1/3 died)	Burkatskaya 1965a (4,6-DNOC)
	Systemic						
2	Rat (albino and hooded)	4-5 hr	Resp		100 (10% 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.

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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).

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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.

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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.

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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.

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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

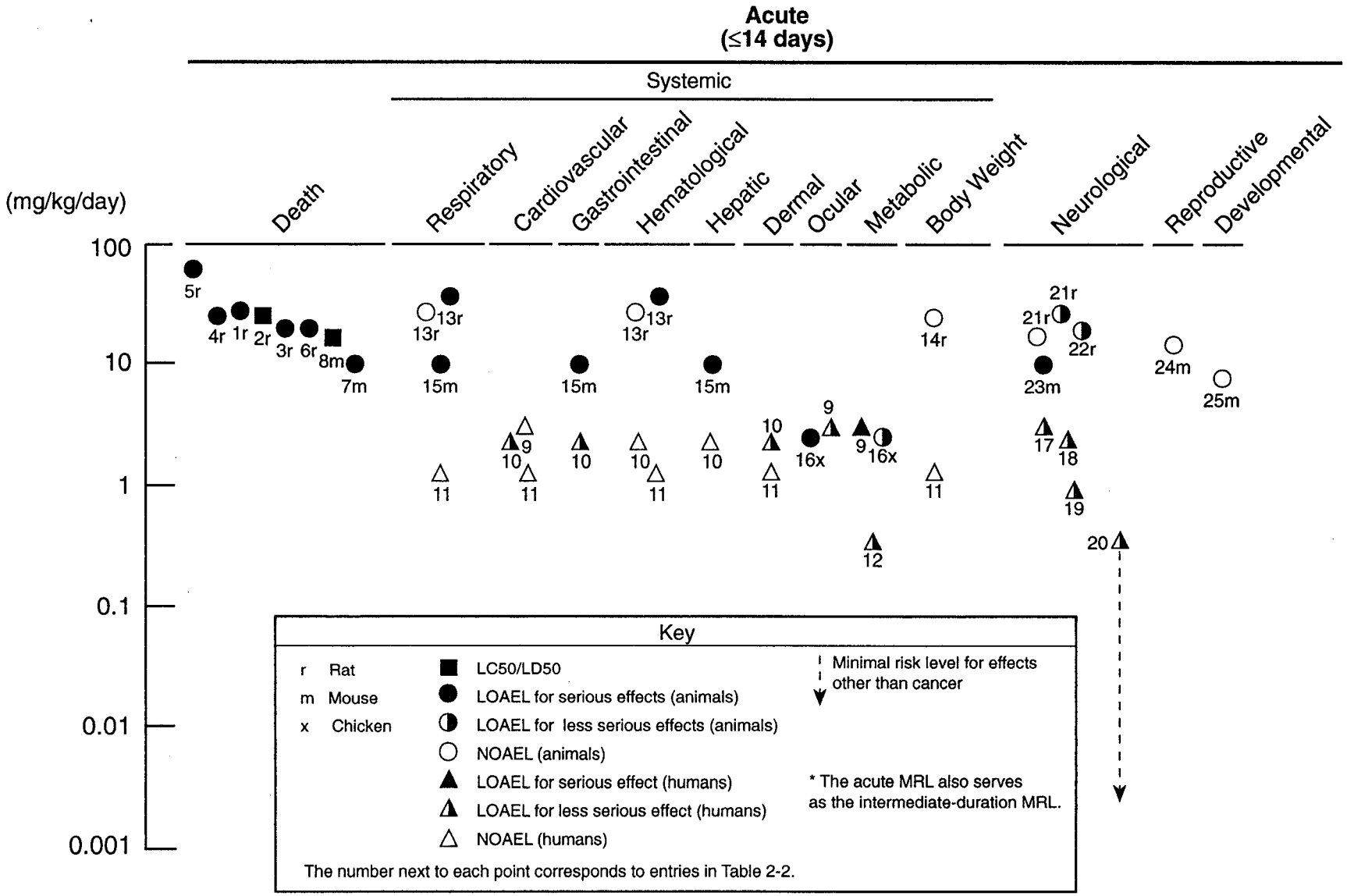
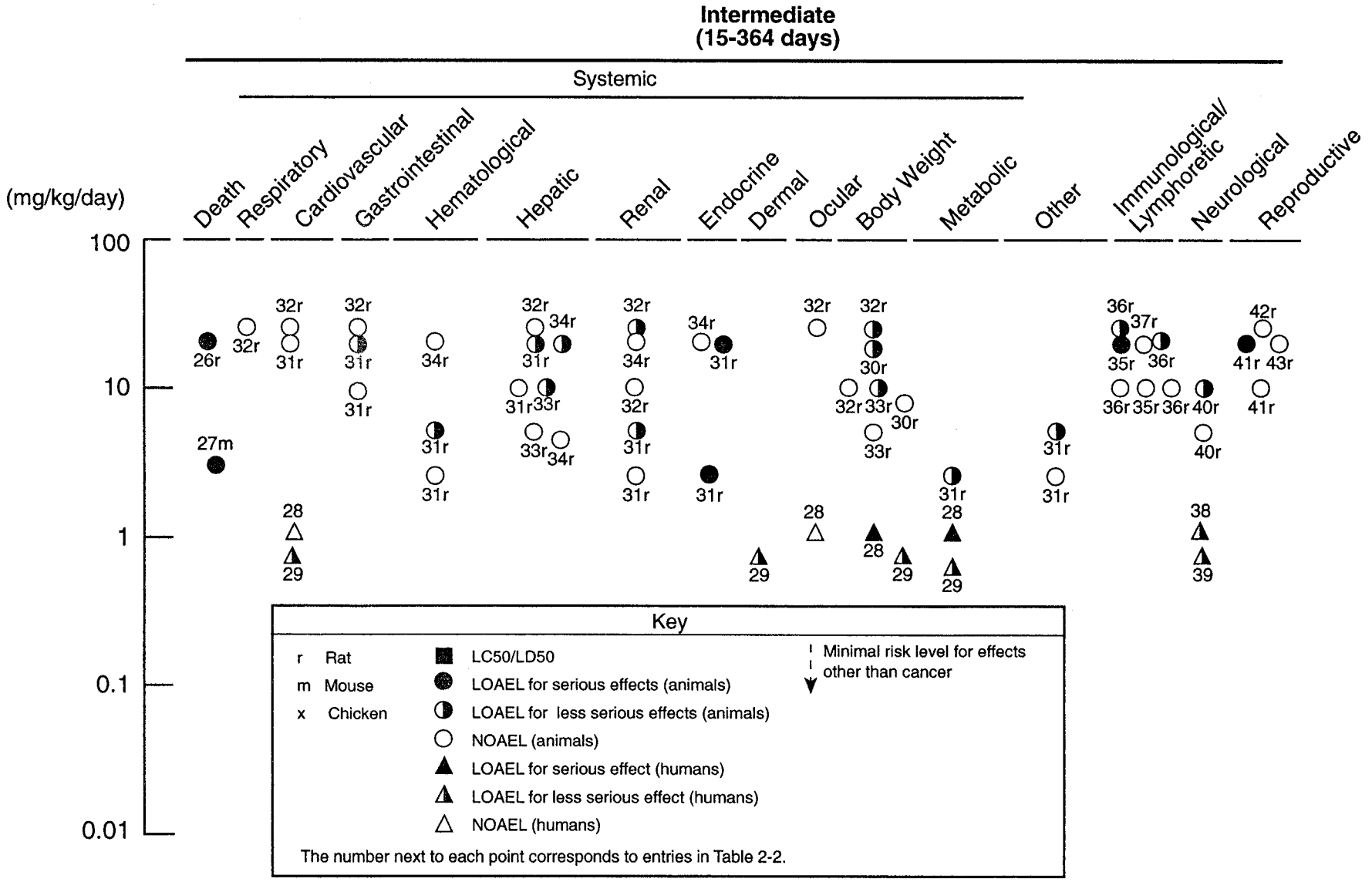


Figure 2-2. Levels of Significant Exposure to 4,6-Dinitro-o-cresol – Oral (Continued)



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.

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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

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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.

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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).

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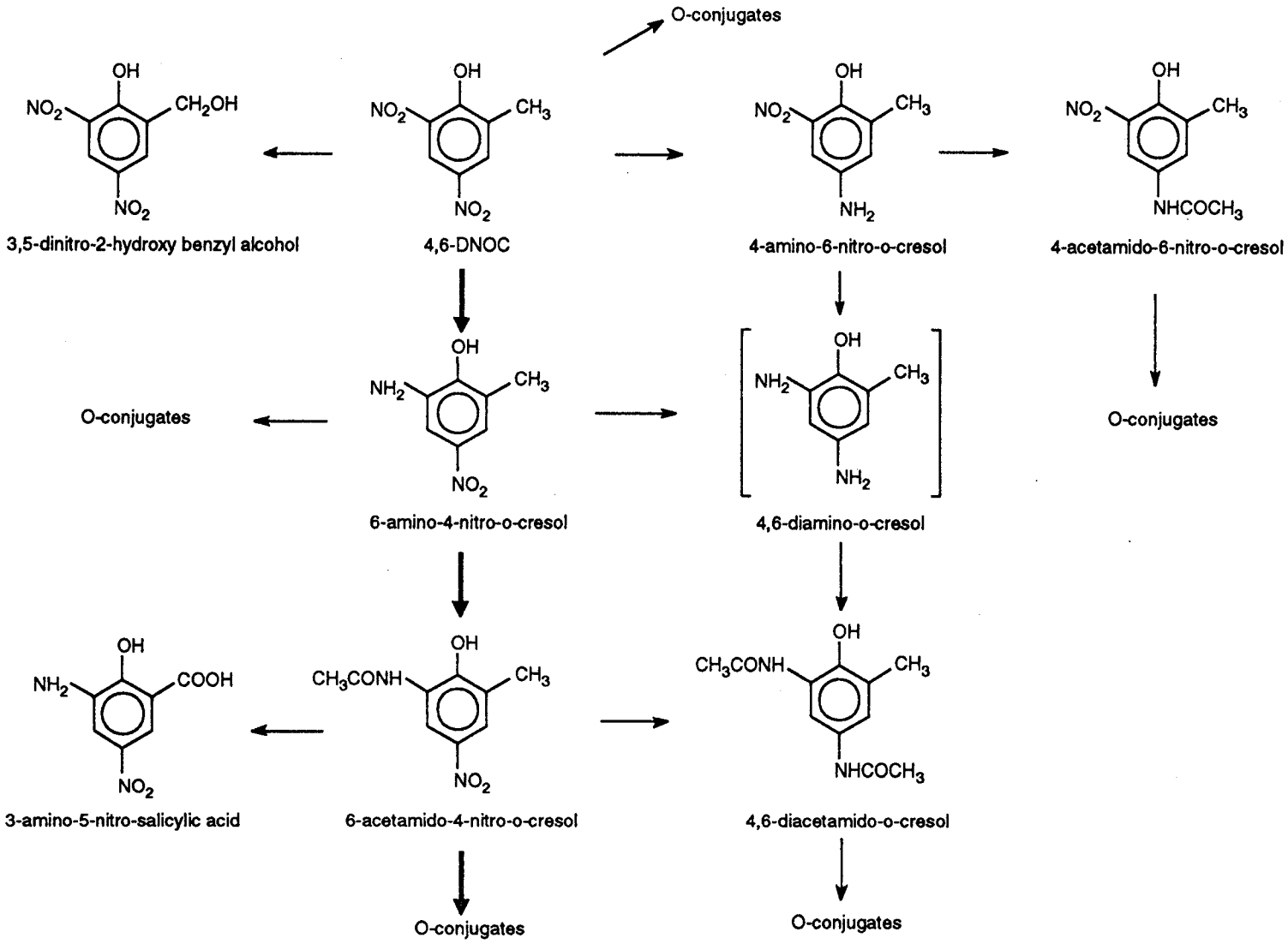
Except for liver and lung tissues, tissue DNOC increased from levels of 0.5-8.0 μg/g to 3.5-19 μg/g during the first 3 hours, but declined to levels of 1.5-10.5 μg/g during the next 3 hours. Liver levels fell from 14 μg/g at 30 minutes to 8 μg/g at 6 hours, while lung levels increased from 18 μg/g at 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

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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).

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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.

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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

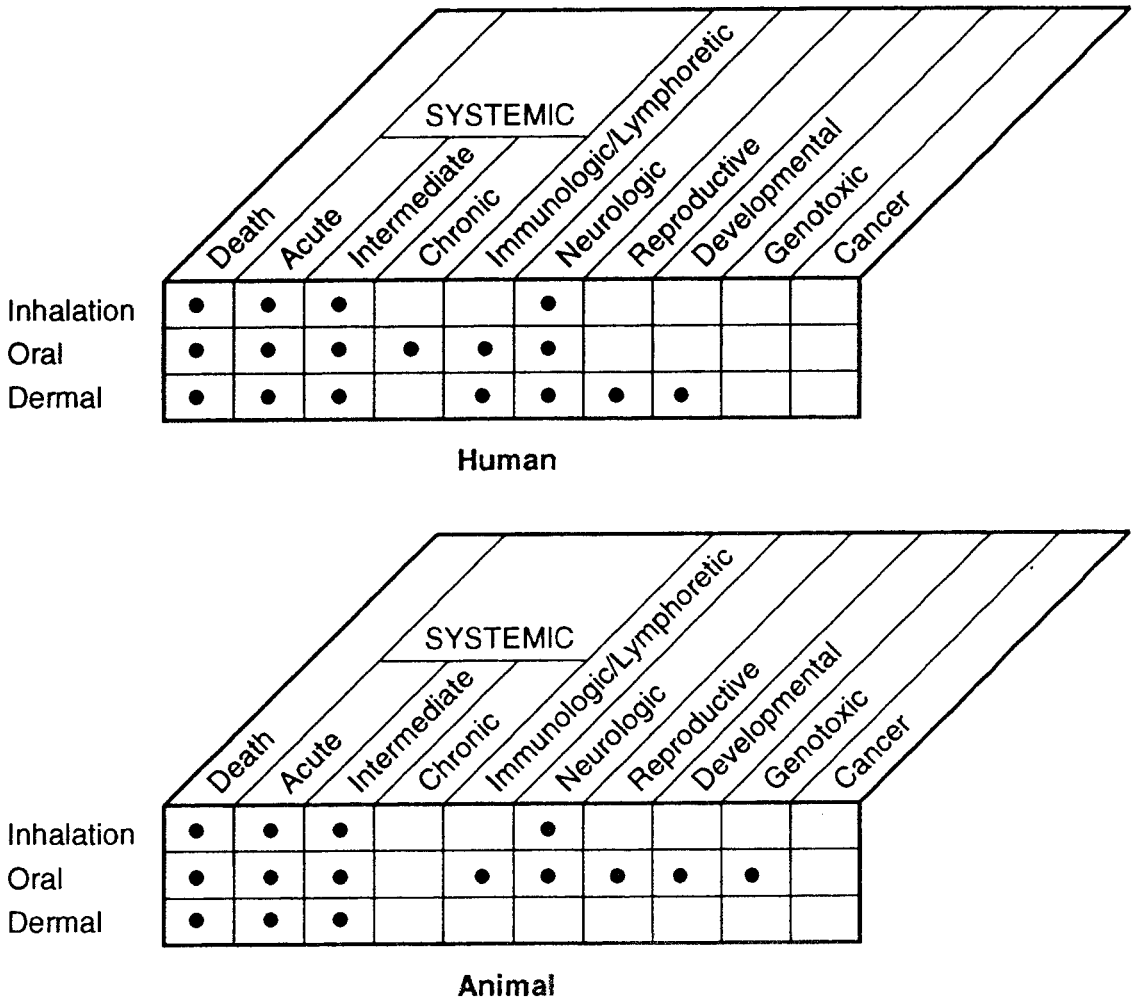
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of DNOC is available. Where adequate information is not available, 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.