CHAPTER 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 on 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.

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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to dinitrocresols, but may not be inclusive of the entire body of literature.

Inhalation studies (animals), oral studies (controlled human and animal), and dermal studies (controlled human and animal) are presented in Table 2-1 and Figure 2-2, Table 2-2 and Figure 2-3, and Table 2-3, respectively.

Levels of significant exposure (LSEs) 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 endpoint 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 endpoints. 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.

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

Studies regarding toxic effects of dinitrocresols conducted by the inhalation, oral, or dermal routes of exposure almost exclusively employed 4,6-dinitro-*o*-cresol (DNOC). Therefore, the focus of this profile is on 4,6-dinitro-*o*-cresol. DNOC 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 should be noted that in the United Kingdom, 4,6-dinitro-*o*-cresol (or more correctly 2-methyl-4,6-dinitrophenol) was 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).

As described in detail in Section 2.21 (Mechanisms of Action), DNOC-induced acute toxic effects are related to DNOC acting directly on cell metabolism and interfering with oxidative phosphorylation.

The health effects of DNOC have been evaluated in human and animal studies. As illustrated in Figure 2-1, most of the health effects data come from acute- or intermediate-duration oral studies in animals. In addition to the studies summarized in Figure 2-1, 23 studies examined DNOC lethality following inhalation, oral, or dermal exposure. Only one study evaluated immunological endpoints; developmental endpoints were evaluated in only one other study.

The available human and animal data suggest the following sensitive targets of toxicity:

- **Metabolic Endpoint:** Increased basal metabolic rate and accompanying increases in body temperature and blood sugar, as well as decreased activity of selected enzymes have been reported in humans and animals exposed to DNOC by inhalation or oral routes.

- **Neurological Endpoint:** Lethargy, dizziness, twitching, ataxia, salivation, and/or sluggishness in DNOC-exposed humans and animals

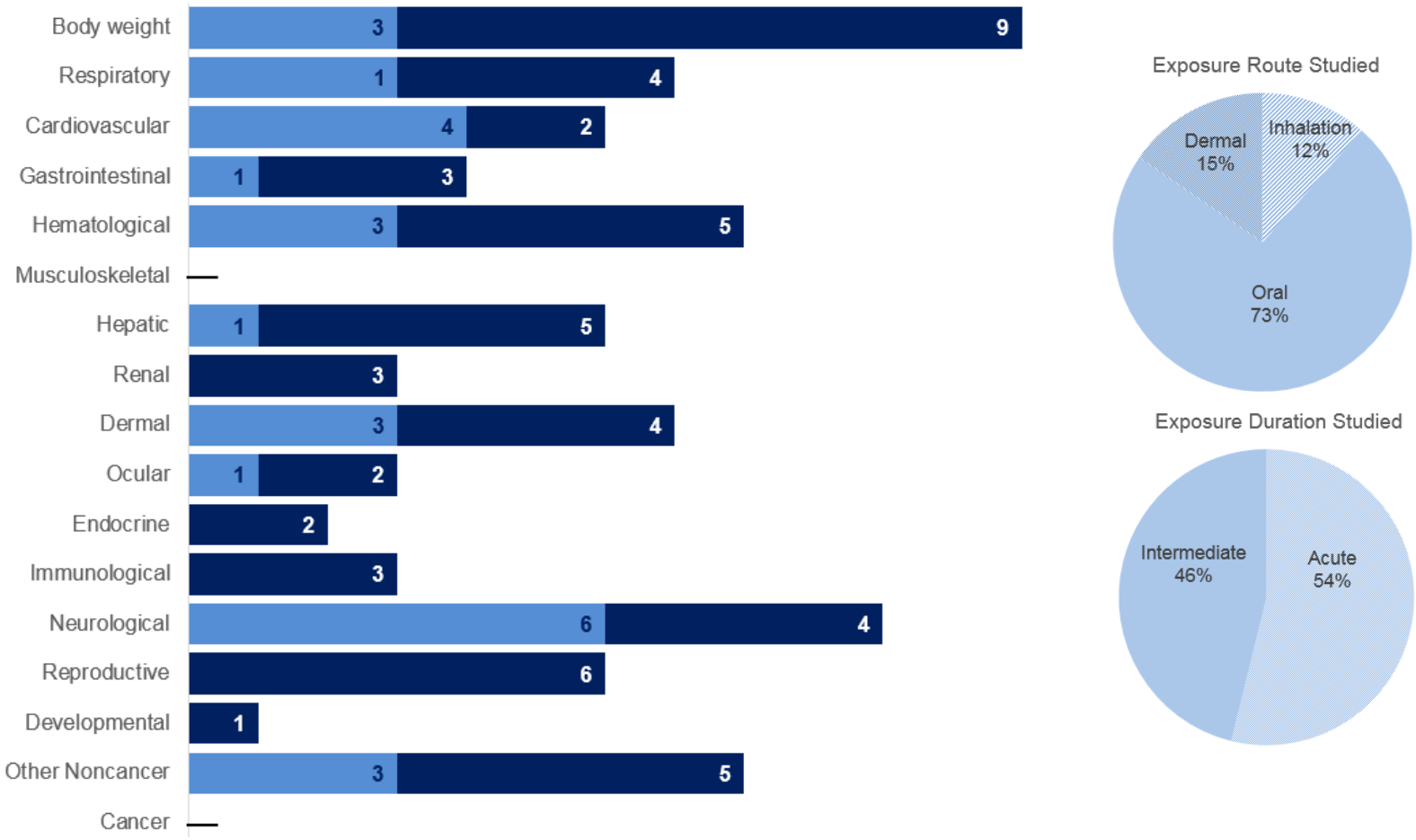
- **Cardiovascular Endpoint:** Increased pulse rate, palpitations, swelling of fingers and hands in humans

- **Immunological Endpoint:** Urticarial eruptions on skin (an allergic response) following oral exposure in humans

Figure 2-1. Overview of the Number of Studies Examining Dinitrocresols Health Effects

Body weight, hematological, neurological, and other noncancer effects of dinitrocresols were the most widely examined potential toxicity outcomes

More studies evaluated health effects in humans than humans (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 28 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to Dinitrocresols – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Exposure levels (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
ACUTE EXPOSURE									
1	Cat (NS) 3 or 6 (sex NS)	4 hours; liquid aerosol (head-only)	0.4, 1.4, 40, 100	BI, BW, CS, HE, LE	Death			40	1/3 cats died at 40 mg/m^3; 2/6 cats died at 100 mg/m^3
					Resp	1.4		40	Dyspnea in 1/3 cats (followed by death) at 40 mg/m^3; dyspnea in 3/6 cats at 100 mg/m^3
					Hemato	1.4		40	Decreases in hemoglobin and RBCs, increased leukocytes
					Neuro	1.4		40	Sluggishness, loss of muscle tone at 40 mg/m^3 in the cat that subsequently died; sluggishness, salivation, loss of appetite, tremors, ataxia at 100 mg/m^3
					Other noncancer (metabolic)	1.4		40	Increased body temperature, decreased catalase and peroxidase activities, increased blood sugar
Burkatskaya 1965b 4,6-DNOC									
2	Cat (NS) 3 or 6 (sex NS)	4 hours; solid aerosol (head-only)	36, 60, 100	BI, BW, CS, HE, LE	Death			100	2/6 died (one each on postexposure days 6 and 11)
					Resp		36		Dyspnea, sneezing, nasal secretions
					Hemato		36		Accelerated erythrocyte sedimentation rate, decreases in hemoglobin and RBCs, increased leukocytes
					Ocular		36		Lacrimation and blepharospasm
					Neuro			36	Twitching, tremors, ataxia, sluggishness, salivation
					Other noncancer (metabolic)	1.4	36		Increased body temperature, increased blood sugar, decreased catalase and peroxidase activities
Burkatskaya 1965b 4,6-DNOC									

Table 2-1. Levels of Significant Exposure to Dinitrocresols – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Exposure levels (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
3	Rat (albino and hooded); Number and sex NS	4–5 hours	100	BI, BW, CS, HE, LE	Resp		100		10% increase in respiration rates 16 hours after exposure
					Neuro		100		Lethargy
					Other noncancer (metabolic)		100		0.7°C increase in body temperatures 16 hours after exposure
King and Harvey 1953a 4,6-DNOC									
INTERMEDIATE EXPOSURE									
4	Cat (NS); 3 (sex NS)	1 month 4 hours/day liquid aerosol (head-only)	2.0	BI, BW, CS, HE, LE	Death			2.0	Death of 2/3 cats
					Bd Wt		2.0		Depressed body weight
					Hemato		2.0		Accelerated erythrocyte sedimentation rate, decreased hemoglobin and RBCs, increased leukocytes, alterations in differential WBC count
					Other noncancer (metabolic)		2.0		Increased body temperature, increased blood sugar, decreased catalase and peroxidase activities
Burkatskaya 1965b 4,6-DNOC									

Table 2-1. Levels of Significant Exposure to Dinitrocresols – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Exposure levels (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
5	Cat (NS); 3 (sex NS)	2–3 months 4 hours/day solid aerosol (head-only)	0.2	BI, BW, CS, HE, LE	Hemato		0.2		Accelerated erythrocyte sedimentation rate, decreased hemoglobin and RBCs, increased leukocytes
					Other noncancer (metabolic)		0.2		Increased body temperature, increased blood sugar, decreased catalase and peroxidase activities
Burkatskaya 1965b 4,6-DNOC									

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

Bd Wt or BW = body weight; BI = biochemical changes; CS = clinical signs; DNOC = dinitrocresol; HE = hematology; Hemato = hematological; LE = lethality; LOAEL= lowest-observed-adverse-effect level; Neuro = neurological NOAEL = no-observed-adverse-effect level; NS = not specified; RBC = red blood cell; Resp = respiratory; WBC = white blood cell

Figure 2-2. Levels of Significant Exposure to Dinitrocresols – Inhalation

Acute (≤14 days)

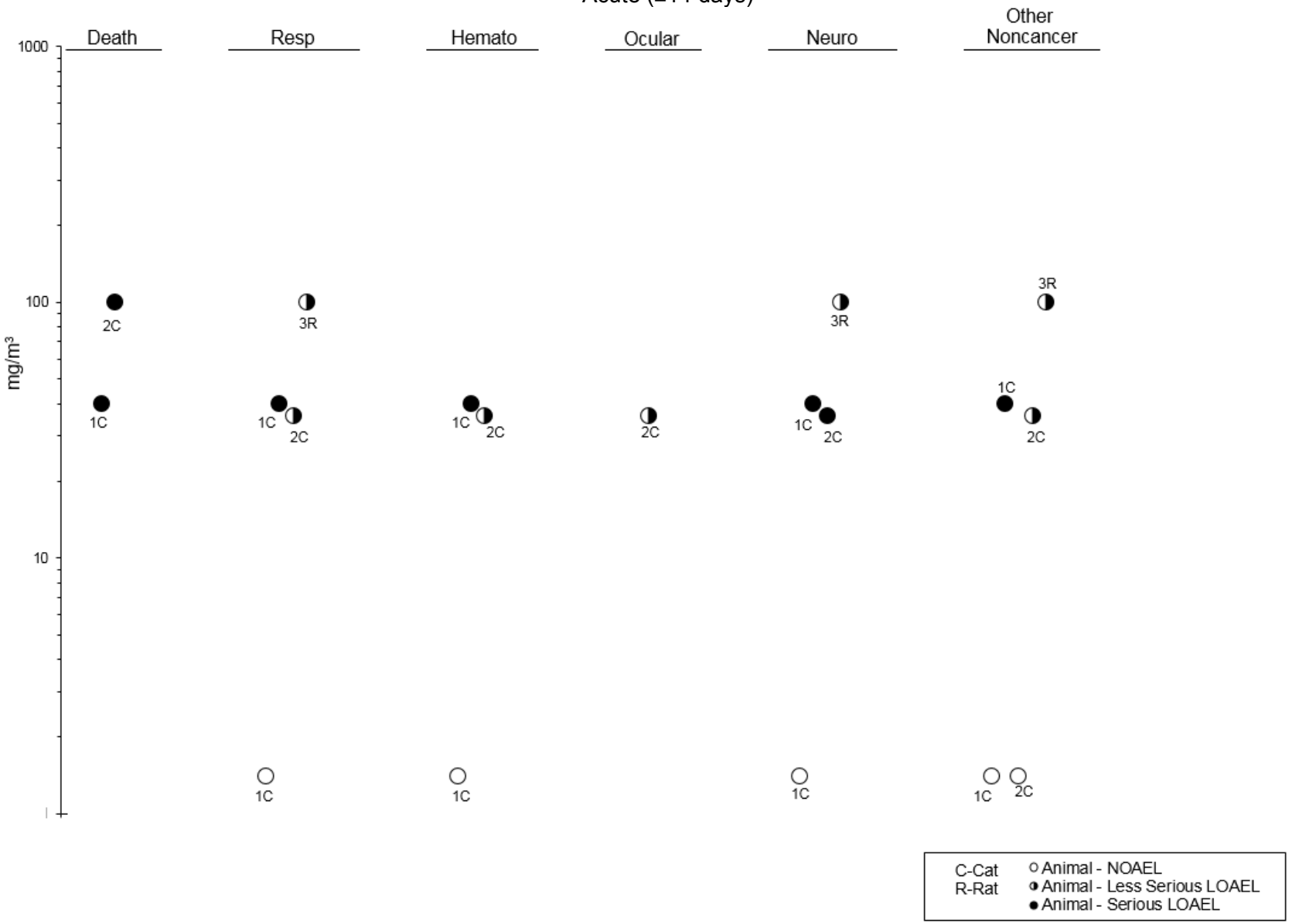


Figure 2-2. Levels of Significant Exposure to Dinitrocresols – Inhalation
Intermediate (15-364)

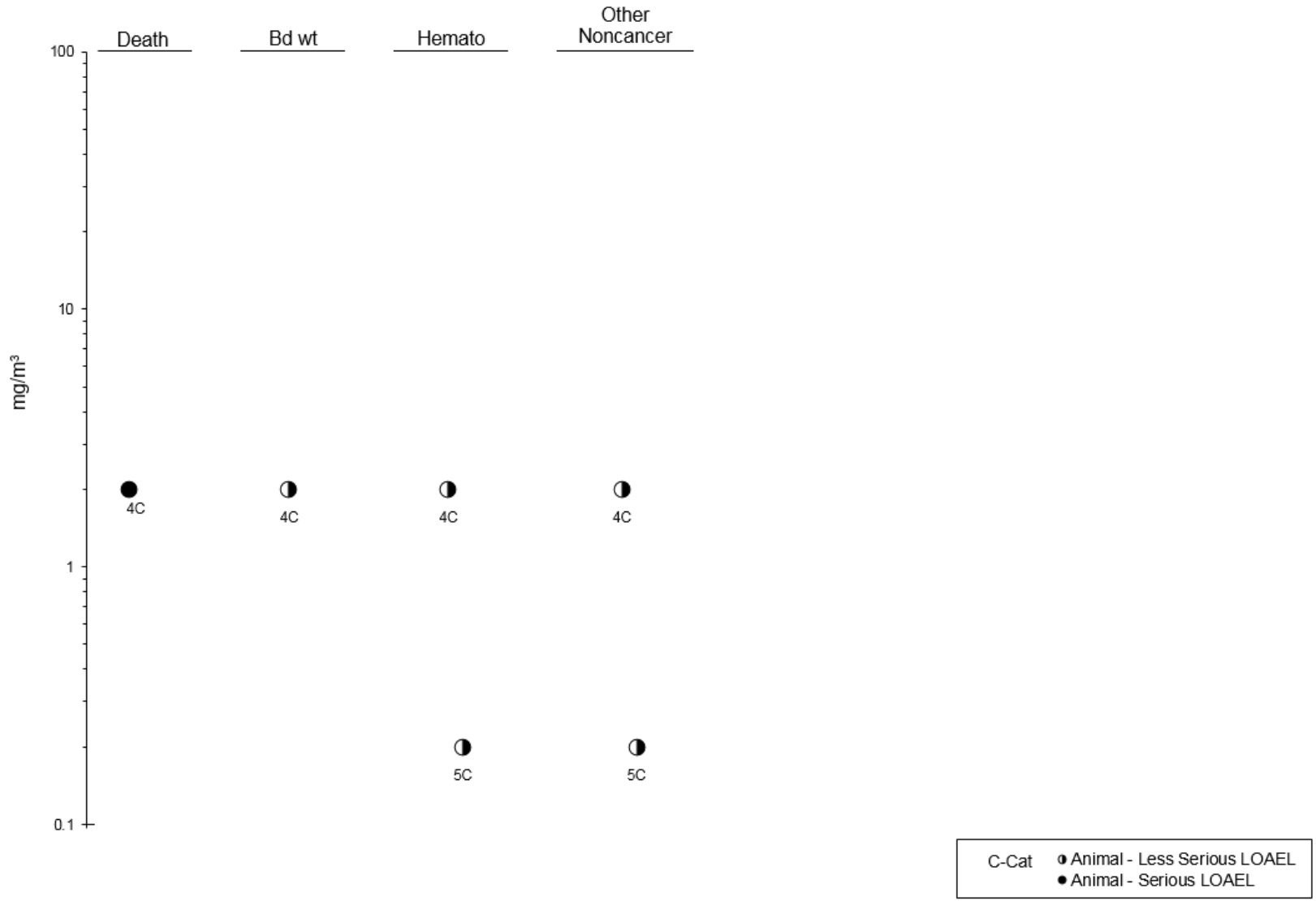


Table 2-2. Levels of Significant Exposure to Dinitrocresols – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Human; number and sex NS	1–4 days 1 time/day (C)	3	CS, HE	Neuro		3		Lethargy, headache, loss of appetite
					Other noncancer (metabolic			3	>50% increase in basal metabolic rate, sweating
Dodds and Robertson 1933 4,6-DNOC									
2	Human 1 F	11 d 1 time/day (C)	2.27 (TWA)	CS, HE	Cardio		2.27 F		Pulse rate 90/minute; swelling of fingers and hands
					Gastro		2.27 F		Nausea, vomiting
					Hemato	2.27 F			
					Hepatic	2.27 F			
					Immuno		2.27 F		Maculopapular eruption on skin
					Neuro		2.27 F		Drowsiness, headache, ringing in ears
Gordon and Wallfield 1935 4,6-DNOC									
3	Human 5 M	5–7 days 1 time/day (C)	0.92–1.27	BW, CS, HE	Bd Wt	1.27 M			
					Resp	1.27 M			
					Cardio	1.27 M			
					Hemato	1.27 M			
					Immuno	1.27 M			
					Neuro		1.27		Malaise, lassitude, headache
Harvey et al. 1951 4,6-DNOC									

Table 2-2. Levels of Significant Exposure to Dinitrocresols – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
4	Human 1 M	3–5 days 1 time/day (C)		BI, CS	Neuro		0.35[b] M		Fatigue, dizziness
					Other noncancer (metabolic)		0.35 M		Increased perspiration and fatigue, elevated temperature (38.2°C)
Plotz 1936 4,6-DNOC									
5	Rat (NS) 10–30 M	Once (G)	18, 27, 36, 45, 90	CS, LE	Death			27 M	3/10 died, 100% mortality at higher doses; dose adjusted for DNOC content in sodium DNOC
					Resp	27 M		36 M	Dyspnea, asphyxia convulsions
					Hemato	27 M		36 M	Cyanosis
					Neuro	18 M		27 M	Depression
Ambrose 1942 Sodium 3,5-DNOC									
6	Rat (white)	Once (G)	NS	LE	Death			25	LD_{50}
Ben-Dyke et al. 1970; Jones et al. 1968 4,6-DNOC									
7	Rat (white)	Once (GO)	NS	LE	Death			30	LD_{50}
Dow Chemical Co. 1950 4,6-DNOC									
8	Rat (NS)	Once (GO)	NS	LE	Death			50	100% mortality
Dow Chemical Co. 1940 4,6-DNOC									

Table 2-2. Levels of Significant Exposure to Dinitrocresols – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
9	Rat (NS) 10–12 (sex NS)	Once (GW)	5, 10, 20, 30, 40, 50, 60, 70	LE	Death			20 40	6/12 deaths at 37–40°C 2/12 deaths at 20–22°C
King and Harvey 1953a 4,6-DNOC									
10	Rat (NS) 6 (sex NS)	10 days 1 time/day (GW)	5, 10, 25	BW, CS, LE	Death			25	3/6 deaths
					Bd Wt	25			
King and Harvey 1953a 4,6-DNOC									
11	Rat (albino) 12 (sex NS)	4–10 days (F)		LE	Death			60	4/12 died
Parker et al. 1951 2,4-DNOC									
12	Rat (NS) 20 (sex NS)	Once (GO)	10, 20, 30, 40, 50	LE	Death			20	Deaths at 20, 30, 40, and 50 mg/kg/day were 3/20, 9/20, 15/20, and 20/20, respectively
Spencer et al. 1948 4,6-DNOC									
13	Rat (Jcl:SD) 6 M	5 days 1 time/days	0, 4, 7.5. 15	BW, CS, HP, LE, OF, OW	Death			15	5/12 died prior to cessation of dosing
					Bd Wt	15			
					Repro	7.5		15	At 14 days following cessation of dosing, 21% decreased sperm motility and 31% decreased percentage of normal sperm
Takahashi et al. 2004 4-6-DNOC									

Table 2-2. Levels of Significant Exposure to Dinitrocresols – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
14	Rat (Jcl:SD) 24 or 36 M	5 days 1 time/day	0, 10, 15	BW, CS, HP, LE, OF, OW	Bd Wt	15			
					Repro	10	15		Up to 35% decreased percentage of normal sperm
Takahashi et al. 2006 4-6-DNOC									
15	Mouse (white) 12, 20, or 30 (sex NS)	Once (GW)	10, 15, 20, 25, 30, 35	CS, GN, LE	Death			10	Deaths among 10, 15, 20, 25, 30, and 35 mg/kg/day dose groups were 3/30, 6/12, 16/30, 9/12, 29/30, and 20/20, respectively
					Resp			10	Dyspnea, hemothorax
					Gastro			10	Coagulative necrosis in stomach mucosa; catarrhal inflammation of small intestine
					Hepatic			10	Enlarged liver with foci of hemorrhage and necrosis
					Neuro			10	Severe agitation, muscle twitches, prostration
Arustamyan 1972 4,6-DNOC									
16	Mouse (white) number and sex NS	Once (GW)	NS	LE	Death			16.4	LD_{50}
Arustamyan 1972 4,6-DNOC									

Table 2-2. Levels of Significant Exposure to Dinitrocresols – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
17	Mouse (C3H, C57BL/6) 6 M	5 days 1 time/day (GW)	0, 3, 6, 12	HP, OF, OW	Repro	12 M			No effects on sperm parameters or testicular weights at 35 days following cessation of dosing
Quinto et al. 1989 4,6-DNOC									
18	Mouse (DBA and CFLP) 13–20 F	GDs 11–14 1 time/day (GW)	0, 8	DX	Develop	8			
Nehez et al. 1987 4,6-DNOC									
19	Chicken	Once (GO)	2.48–59.45	CS, OP	Ocular		2.48		Cataract formation in 4–5 hours posttreatment; earlier onset of cataracts at higher dose levels
					Other noncancer (metabolic)		2.48		Decreased body temperature; at 4 mg/kg, increased oxygen uptake
Buschke 1947 4,6-DNOC									

Table 2-2. Levels of Significant Exposure to Dinitrocresols – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
INTERMEDIATE EXPOSURE									
20	Human	14–63 days 1 time/day (C)	0.8–1.42 (average 1.05)	BI, BW, CS	Bd Wt		1.05		Weight loss of 0.45 kg/week
					Cardio	1.05			
					Neuro		1.05		Lethargy, depression
					Other noncancer (metabolic)			1.05	34–77% increase in basal metabolic rate, excessive thirst and perspiration, 40°C body temperature
Ibrahim et al. 1934 4,6-DNOC									
21	Human 2 M, 1 F, 1 NS	4–11 weeks 1 time/day (C)	0.58, 0.75, 1.0, 1.5	BI, BW, CS	Bd Wt		0.75		Decrease in body weight of 0.6 kg/week
					Cardio		0.75		Palpitations
					Immuno		0.75		Urticarial eruptions
					Neuro		0.75		Headache, lassitude
					Other noncancer (metabolic)		0.58		2°F average increase in body temperature
Plotz 1936 4,6-DNOC									
22	Rat (Wistar) 5–10 M	105 days (F)	0, 0.36, 0.81, 1.62, 3.6, 7.6, 18	BW, FI, GN, HP	Bd Wt	7.6 M	18 M		15% depressed body weight gain
Ambrose 1942 Sodium 3,5-DNOC									

Table 2-2. Levels of Significant Exposure to Dinitrocresols – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
23	Rat (Wistar) 10 M, 10 F	90 days (F)	0, 2.5, 5, 10, 20	BC, BI, BW, CS, FI, HE, HP, LE, OW, UR	Death			20	5/20 deaths at highest dose level
					Cardio	20			
					Gastro	10	20		Histopathologic alterations in salivary glands and fundus
					Hemato	2.5	5		Increases in hemoglobin, hematocrit, and MCH/MCV
					Hepatic	10	20		Increased serum ALT
					Renal	2.5	5		Increased blood urea nitrogen, decreased urinary creatinine
					Endocr		2.5		Decreased thyroid hormones at 2.5 mg/kg; histopathologic adrenal and pancreatic lesions at 20 mg/kg
					Immuno	10		20	Atrophy or under-development of thymus, spleen, lymph nodes; decreased circulating lymphocytes
					Neuro	5	10		Increased relative brain weight (magnitude not specified)
					Repro	10		20	No corpora lutea in ovaries; juvenile uteri; aspermatogenesis
					Other noncancer (metabolic)		2.5		Decreased carbohydrate and increased fat metabolism (magnitude not specified)

Table 2-2. Levels of Significant Exposure to Dinitrocresols – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
Den Tonkelaar et al. 1983 **4,6-DNOC**									
24	Rat (white) 20-22 M	77–182 days (F)	0, 1, 2.5, 5, 10, 25	BC, BW, FI, HE, HP, LE, OW	Bd Wt	10 M	25 M		18% decreased body weight, depletion of body fat
					Resp	25 M			
					Cardio	25 M			
					Gastro	25 M			
					Hepatic	25 M			
					Renal	10 M	25 M		Increased blood urea nitrogen
					Ocular	25 M			
					Immuno	10 M	25 M		Hemosiderosis and congestion of the spleen
					Repro	25 M			
Spencer et al. 1948 **4,6-DNOC**									
25	Rat (white) NS F	6 months 1 time/day (G)	0, 2, 5, 10	BI, BW, CS, GN	Bd Wt	5 F	10 F		10–18% reduced body weight gain
					Hepatic	5 F	10 F		Fatty degeneration
Vashakidze 1967 **4,6-DNOC**									
26	Rat (white) 6 M	3 weeks (F)	1.25, 5, 20	BW, FI, GN, HE, HP, LE, OW	Hemato	20 M			
					Hepatic	20 M			
					Renal	20 M			
					Endocr	20 M			
					Immuno	20 M			
					Repro	20 M			
Vos et al. 1983 **4,6-DNOC**									

Table 2-2. Levels of Significant Exposure to Dinitrocresols – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
27	Mouse (white) 6 NS	32 days 1 time/day (GW)	3	BW, CS, LE	Death			3	100% mortality
Arustamyan 1972 4,6-DNOC									

[a]The number corresponds to entries in Figure 2-3.
[b]Used to derive both an acute and an intermediate 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).

ALT = alanine transaminase; BC = serum (blood) chemistry; BI = biochemical changes; Bd Wt or BW = body weight; (C) = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DNOC = dinitrocresol; DX = developmental; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; (G) = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; (GO) = gavage in oil vehicle; (GW) = gavage in water vehicle; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; MRL = minimal risk level; Neuro = neurological; NOAEL = no-observed- adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; Resp = respiratory; Repro = reproductive; TWA = time-weighted average; UR = urinalysis

Figure 2-3. Levels of Significant Exposure to Dinitrocresols – Oral
Acute (≤14 days)

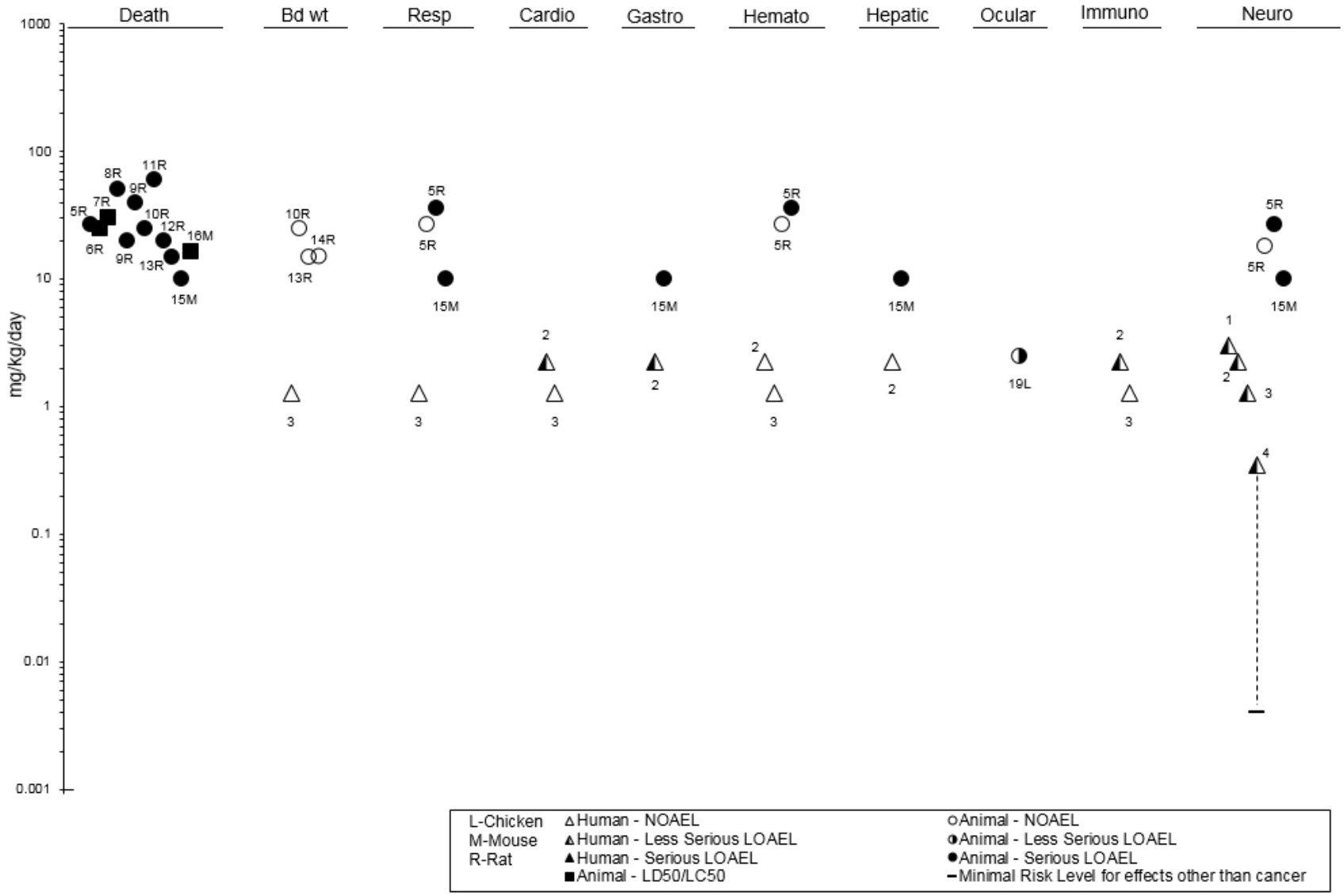


Figure 2-3. Levels of Significant Exposure to Dinitrocresols – Oral
Acute (≤14 days)

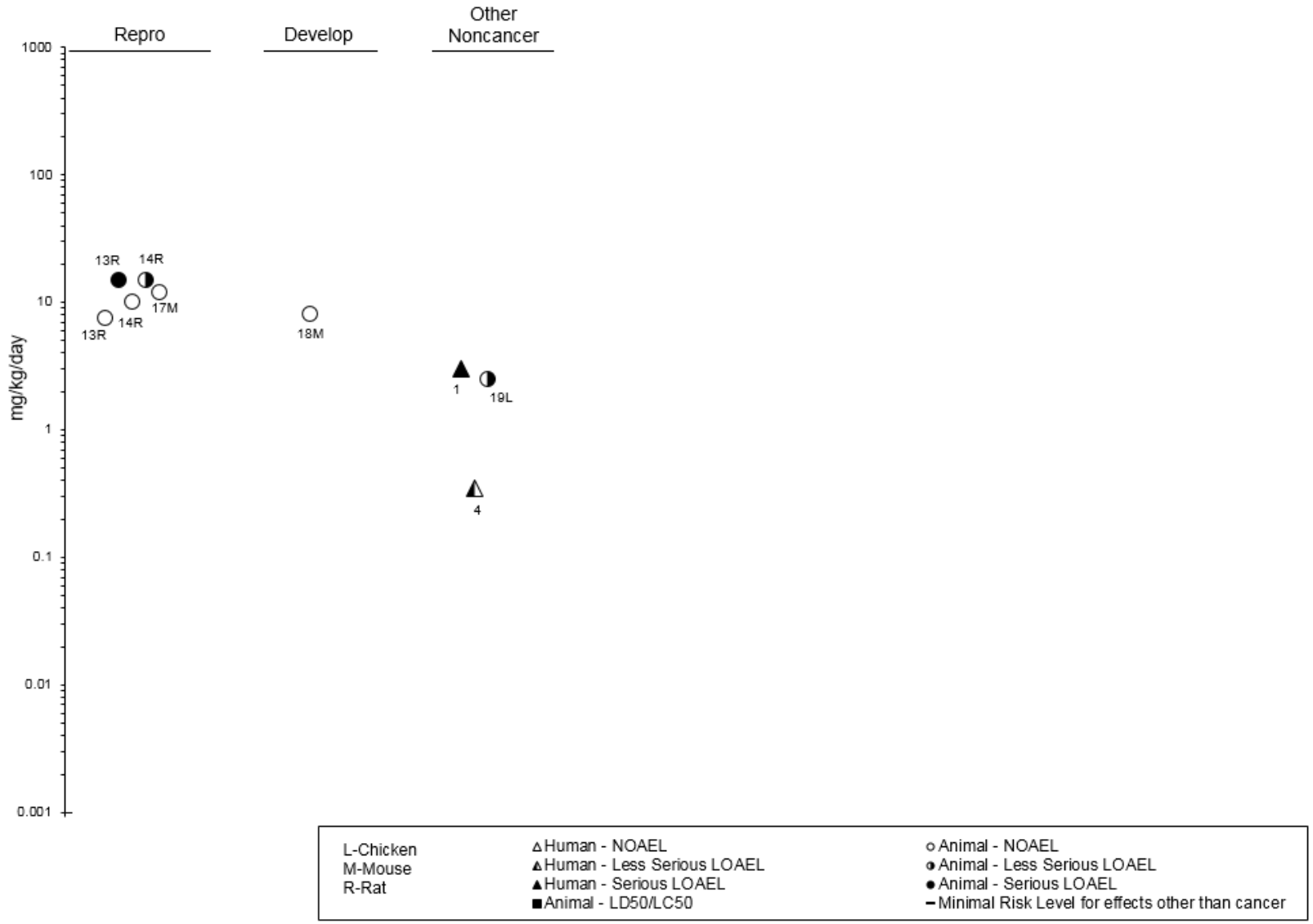


Figure 2-3. Levels of Significant Exposure to Dinitrocresols – Oral
Intermediate (15-364 days)

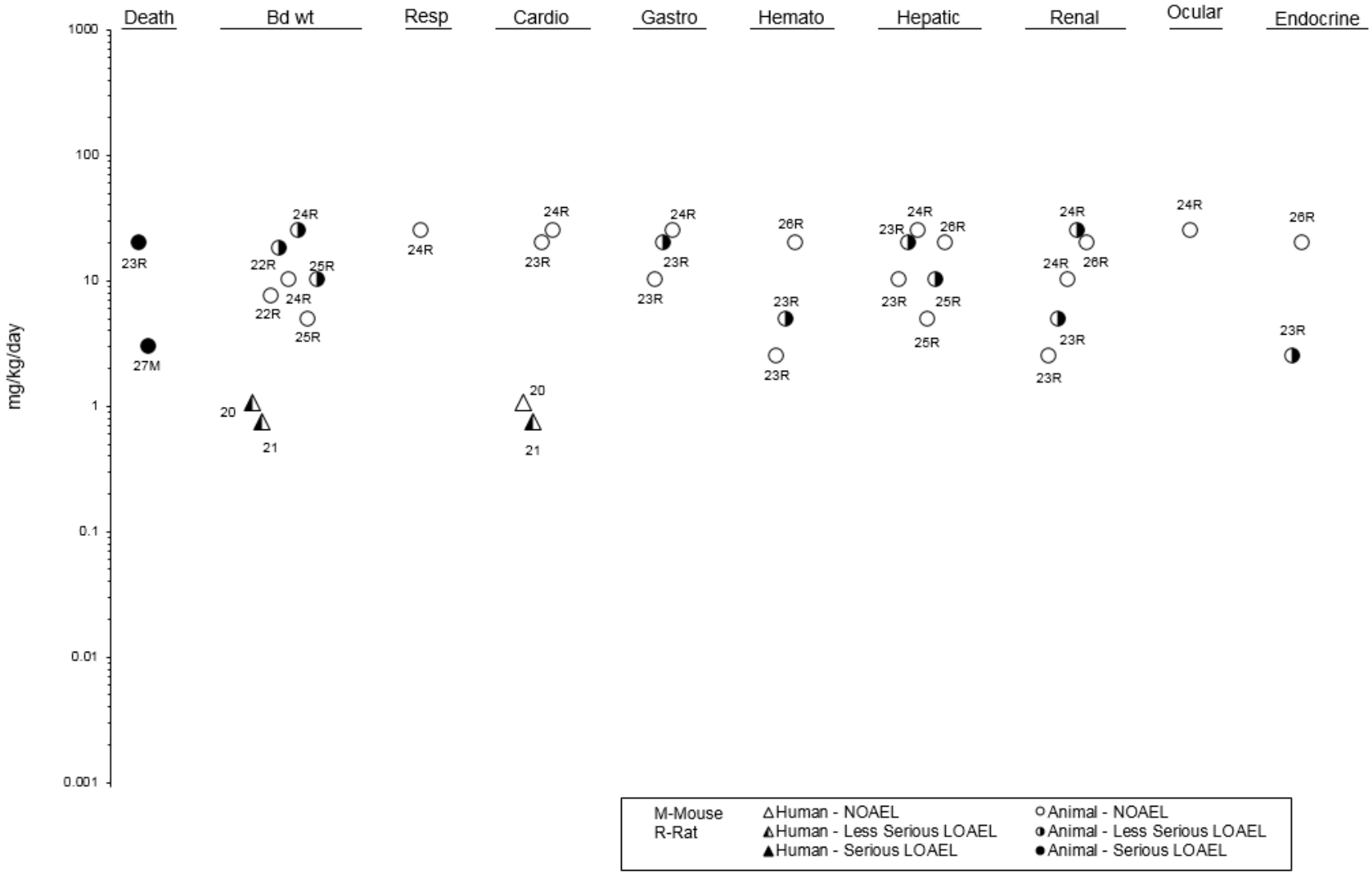


Figure 2-3. Levels of Significant Exposure to Dinitrocresols – Oral
Intermediate (15-364 days)

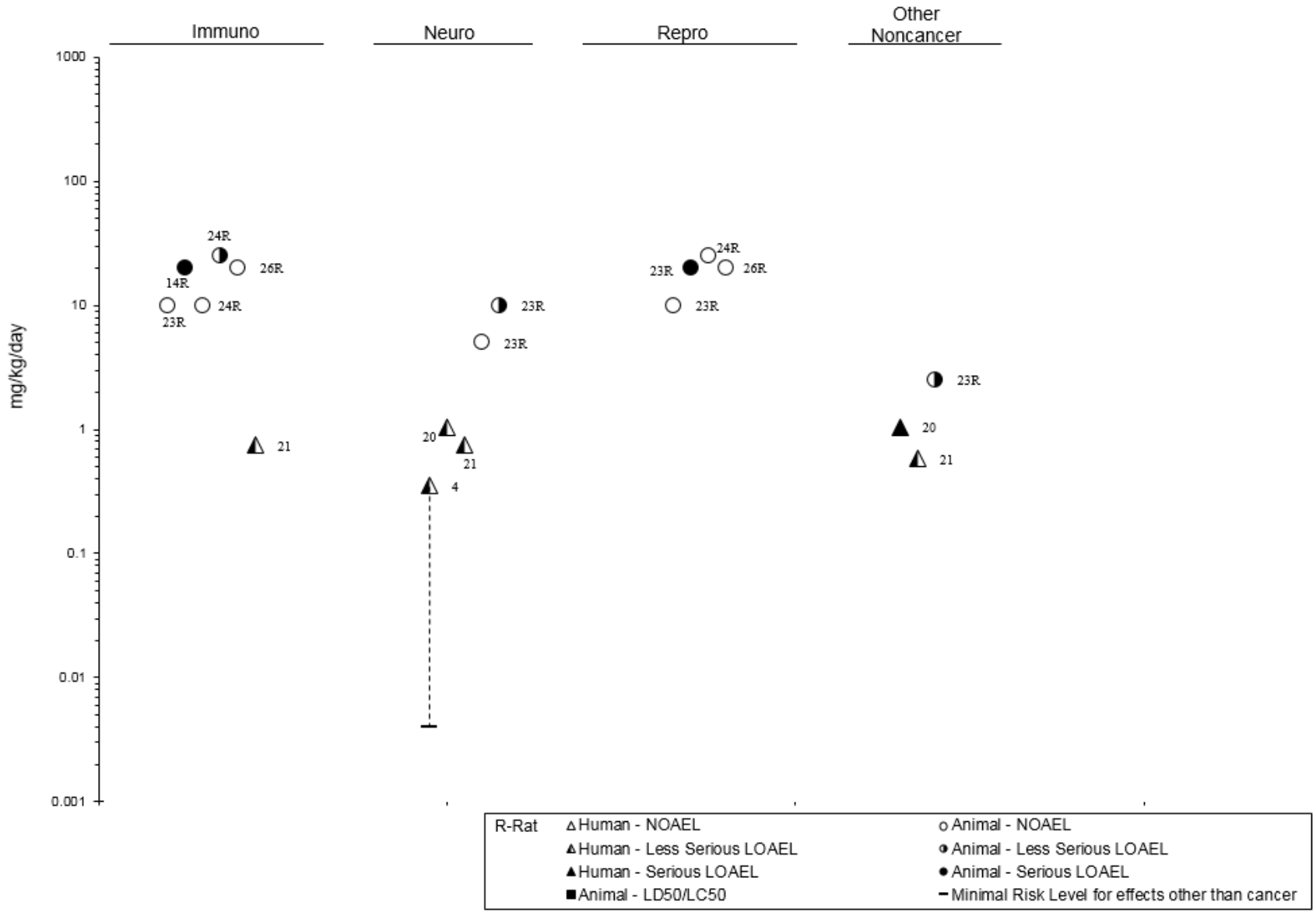


Table 2-3. Levels of Significant Exposure to Dinitrocresols – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
ACUTE EXPOSURE								
Human 443–492	Once	0.5 or 1%	CS	Dermal	1%			No evidence of dermal irritation
Lisi et al. 1987								
Rat (NS); number and sex NS	Once	NS	LE	Death			200 mg/kg	LD_{50}
Ben-Dyke et al. 1970; Jones et al. 1968 4,6-DNOC								
Mouse (white); number and sex NS	Once	NS	CS, GN, LE	Death			186.7 mg/kg	LD_{50}
Arustamyan 1972 4,6-DNOC								
Rabbit (NS); number and sex NS	Once	NS	LE	Death			1,000 mg/kg	LD_{50}
Burkatskaya 1965b 4,6-DNOC								
Rabbit (NS); number and sex NS	Once	NS	LE	Death			1,671 mg/kg	LD_{50}
ERM Program Management Co. 1992 4,6-DNOC								
Rabbit (NS); number and sex NS	Once	NS	LE	Death			1,732 mg/kg	LD_{50}
ERM Program Management Co. 1992 2,6-DNPC								
Rabbit (white); number and sex NS	Up to 7 days, 1 time/day	3 or 4%	CS, LE	Death			3%	Death of unspecified number of animals
				Dermal		3%		Slight skin irritation
Spencer et al. 1948 4,6-DNOC								

Table 2-3. Levels of Significant Exposure to Dinitrocresols – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
Guinea pig 5 (sex NS)	Once	0, 100, 200, 300, 400, 500 mg/kg	LE	Death			300 mg/kg	Deaths were 0/5, 0/5, 1/5, 3/5, and 5/5 at 100, 200, 300, 400, and 500 mg/kg, respectively
Spencer et al. 1948 **4,6-DNOC**								
INTERMEDIATE EXPOSURE								
Human	30 days, 1 time/day			Dermal	1.8%			
Ambrose 1942 **Sodium 3,5-DNOC**								
Rat	30 days, 1 time/day		BW	Bd Wt	1.8%			
				Dermal	1.8%			No signs of dermal irritation
Ambrose 1942 **Sodium 3,5-DNOC**								
11 Rabbit	30 days, 1 time/day	0, 1.8%	BW, CS	Bd Wt	1.8%			
				Dermal	1.8%			No signs of dermal irritation
Ambrose 1942 **Sodium 3,5-DNOC**								
12 Rabbit	4 weeks, 5 days/week, 1 time/day	5%	CS, LE	Dermal		5%		Slight skin irritation
Spencer et al. 1948 **4,6-DNOC**								

Bd Wt or BW = body weight; CS = clinical signs; DNOC = dinitrocresol; DNPC = dinitro-*p*-cresol; GN = gross necropsy; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified

2.2 DEATH

Information regarding death of humans is limited to case reports of suspected inhalation, oral, and/or dermal exposure to DNOC. A spray operator who inhaled a dense DNOC mist for an unspecified time 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/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. Another spray operator was found dead after drinking an unknown amount of DNOC in water from a contaminated fresh water tank (Bidstrup and Payne 1951). The worker had also been exposed to DNOC aerosols for 3 weeks prior to death. A patient died <14 hours after admission to a hospital and <48 hours after the onset of signs and symptoms of DNOC toxicity (Steer 1951). The patient had sprayed DNOC for an unspecified, but apparently acute, time period. 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. 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 2 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 five 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.

Deaths were reported in cats exposed (head only) to DNOC liquid or solid aerosols for 4 hours at 40 and/or 100 mg/m^3, and in other cats exposed to DNOC liquid aerosol 4 hours/day for 1 month at 2 mg/m^3 (Burkatskaya 1965a). Mortality was reported following single or repeated oral dosing of rats or mice in the range of 10–50 mg/kg (Ambrose 1942; Arustamyan 1972; Ben-Dyke et al. 1970; Den Tonkelaar et al. 1983; Dow Chemical Co. 1940, 1950; Jones et al. 1968; King and Harvey 1953a; Spencer et al. 1948; Takahashi et al. 2004). 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). 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 37–40ºC, while only 2 of 12 rats died after receiving twice the dose (40 mg/kg) at almost half the temperature (20–22ºC). Therefore, increased environmental temperatures increased the toxicity of DNOC in rats. Because DNOC uncouples oxidative phosphorylation, an increase in heat production and body temperature occurs. Elevated environmental temperatures lower the rate of heat dissipation and further exacerbate the signs of DNOC toxicity, which may become fatal. 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).

Reported acute dermal LD_{50} values for dinitrocresols are 200–600 mg/kg for rats (Ben-Dyke et al. 1970; Jones et al. 1968), 186.7 mg/kg for mice (Arustamyan 1972), and 1,000–1,732 mg/kg for rabbits (Burkatskaya 1965b; ERM Program Management Co. 1992). Death was noted in 1/5, 3/5, and 5/5 guinea pigs administered DNOC at 300, 400, and 500 mg/kg, respectively to a shaved area of the abdomen (Spencer et al. 1948). An unspecified number of rabbits died after seven applications of 3% solution of DNOC in 95% alcohol to the ear and shaven abdomen (Spencer et al. 1948).

2.3 BODY WEIGHT

DNOC was once used to treat obesity, but this practice has been discontinued due to recognized toxic effects. 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/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 one of four 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). 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). 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) or other rats administered DNOC by gavage for 5 days at up to 15 mg/kg/day and observed for up to 14 days posttreatment (Takahashi et al. 2004, 2006). 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).

2.4 RESPIRATORY

Respiratory effects have been observed in both humans and animals following exposure to DNOC. In most human cases, exposure likely involved inhalation and dermal exposure. Effects such as dyspnea, elevated respiration rate, and shallow breathing have been observed in individuals exposed to DNOC during production, mixing, and/or spray operations (Bidstrup and Payne 1951; Buzzo and Guatelli 1949; Hunter 1950; Pollard and Filbee 1951; Steer 1951; van Noort et al. 1960). 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. 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).

Respiratory rates were increased in rats exposed to 100 mg/m^3 DNOC for 4 hours and remained elevated 20 hours following cessation of exposure (King and Harvey 1953a). Dyspnea, sneezing, and/or nasal secretions were observed in cats exposed to liquid aerosol of DNOC at 36 mg/m^3 or as a dust at 40 mg/m^3 for 4 hours (Burkatskaya 1965a). Signs of respiratory distress (dyspnea and asphyxial convulsions) were observed prior to death in rats given single oral doses of 36–90 mg/kg DNOC (Ambrose 1942) as the sodium salt. Mice that received single oral doses in the range of 10–35 mg/kg DNOC became dyspneic within 60–80 minutes (Arustamyan 1972). Necropsy 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 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).

2.5 CARDIOVASCULAR

Elevated pulse rates have been observed in humans exposed to DNOC by inhalation and/or dermal routes. A male factory worker who had been employed for 17 days pouring DNOC powder had a pulse rate of 130 beats per minute; the employee also reported that he had periodically inhaled DNOC aerosols (Hunter 1950). 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 occasionally spraying DNOC for 5 weeks (Pollard and Filbee 1951). 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. 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 2 consecutive days (Buzzo and Guatelli 1949). One industrial and five agricultural workers, who were likely exposed via inhalation and/or dermal routes to unknown doses of DNOC for 2–8 weeks, had elevated pulse rates and cyanosis at the hospital; all six workers died (Bidstrup and Payne 1951). Increased pulse rate and heart palpitations were also observed in employees who sprayed DNOC for 14 days to 4 months (van Noort et al. 1960).

Cardiovascular effects appear to be secondary to cellular anoxia, but do not appear to be consistent 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 (TWA) 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 oral DNOC doses in the range of 0.5–1.0 mg/kg/day for 40–48 days (Dodds

and Robertson 1933). 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 orally 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 two capsules per day for an unspecified period (Quick 1937). The patient maintained this regimen for about 3 years.

Limited information was located regarding cardiovascular effects in laboratory animals exposed to DNOC. 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). 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.

2.6 GASTROINTESTINAL

Gastrointestinal effects such as nausea and vomiting have been reported among individuals exposed to DNOC by inhalation, oral, and/or dermal routes (Buchinskii 1974; Gordon and Wallfield 1935; van Noort et al. 1960). In cases that culminated in death, lesions in gastric and or intestinal mucosa were noted at autopsy (Bidstrup and Payne 1951; Buchinskii 1974).

Vomiting was reported to occur within 60–80 minutes in mice administered 10–35 mg/kg DNOC by gavage (Arustamyan 1972). Necropsy examination revealed lesions in gastric mucosa. 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; the presence of food may have prevented the irritating effects of DNOC in the stomach (Spencer et al. 1948). 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 receiving DNOC from the diet for 90 days at 20 mg/kg/day (Den Tonkelaar et al. 1983).

2.7 HEMATOLOGICAL

Unspecified hemorrhagic irregularities and irregular bleeding were observed in some field workers exposed to DNOC for about 8 hours (Varnai and Kote 1969). 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 (further described as anoxemic) was found throughout the femoral shaft of an agricultural worker who died after being exposed to unknown levels of DNOC for 2–8 weeks (Bidstrup and Payne 1951). 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). 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 TWA dose of 2.27 mg/kg/day DNOC for 11 days for treatment of obesity (Gordon and Wallfield 1935).

In the only study located regarding hematological effects in animals after inhalation exposure to DNOC, significantly decreased hemoglobin content and red blood cell 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 leukocyte counts were found in the cats exposed to the dust. Accelerated erythrocyte sedimentation rate, decreased hemoglobin and red blood cells, increased leukocytes, and alterations in differential white blood cell count were observed when the cats were exposed to DNOC mist at 2 mg/m^3 for 1 month; these effects (with the exception of differential white blood cell count) were also observed in cats 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.

Increases in hemoglobin, hematocrit, and the ratio of mean corpuscular volume to mean corpuscular hemoglobin (MCV/MCH) were reported in rats given 5, 10, or 20 mg/kg/day DNOC orally for 90 days; the highest dose also resulted in increased erythrocyte count and decreased total leukocyte and lymphocyte counts (Den Tonkelaar et al. 1983). Hemosiderosis and congestion of the spleen were observed in rats fed diets providing 25 mg/kg/day of DNOC for 77–182 days; however, there were no differences in hematological parameters such as erythrocyte count, hemoglobin concentration, total leucocyte count, differential count, or bone marrow counts, and no evidence of histopathologic lesions in bone marrow (Spencer et al. 1948). 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). Cyanosis was observed in rats administered DNOC (as the sodium salt) once by gavage at doses in the range of 36–90 mg/kg (Ambrose 1942). This condition is most probably related to the dyspnea and asphyxial convulsions observed in the affected rats.

2.8 MUSCULOSKELETAL

Limited information was located regarding musculoskeletal effects in humans or animals exposed to DNOC. 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). Shortly before death, muscular rigidity and loss of motor function were observed in two individuals who had sprayed 10% DNOC for 2 consecutive days (Buzzo and Guatelli 1949). Continuous involuntary contraction of leg muscles and pain in calf muscles were observed in a spray operator exposed to 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, which may have been representative of a neurological effect (Burkatskaya 1965a).

2.9 HEPATIC

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 resulted 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.

However, there is some evidence of DNOC-induced hepatotoxicity. 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). 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). Unspecified liver damage and enlarged livers were observed in several agricultural workers who were exposed to DNOC for 8 hours (Varnai and Kote 1969). Congested livers were generally observed in one industrial and five agricultural workers, who died after being exposed to unknown levels of DNOC for 2–8 weeks (Bidstrup and Payne 1951). No evidence of liver damage was observed in a girl who ingested a TWA dose of 2.27 mg/kg/day DNOC for 11 days for treatment of obesity (Gordon and Wallfield 1935).

Results from two animal studies indicated that DNOC may cause hepatic pathology; data from several other studies demonstrated that DNOC may cause changes in liver weight without evidence of histopathology. 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 observed in rats given daily gavage doses of 10 mg/kg/day DNOC for 6 months (Vashakidze 1967). In intermediate-duration feeding studies, no histological evidence of liver pathology was found in rats fed diets providing 25 mg/kg/day DNOC (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 DNOC from the diet at 10–20 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). Two rats had greatly increased levels of serum alanine aminotransferase (ALT) 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 2.21), 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.

2.10 RENAL

Available information regarding DNOC-induced renal effects is limited. An elevated blood urea nitrogen (BUN) level was observed in an individual who mixed DNOC, refilled sprayer tanks, and occasionally sprayed DNOC for 5 weeks (Pollard and Filbee 1951). 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). 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). Unspecified kidney damage was observed in several agricultural workers exposed to DNOC for 8 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 exposed to unknown levels of DNOC for 2–8 weeks (Bidstrup and Payne 1951).

In intermediate-duration feeding studies of rats, absolute kidney weights were decreased (Den Tonkelaar et al. 1983; Spencer et al. 1948) and relative kidney weights were increased at doses of 10 or

20 mg/kg/day, respectively; 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). BUN was increased 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 a 90-day oral study; urinalysis revealed that urinary protein was decreased at 10 and 20 mg/kg/day, urinary glucose increased at 20 mg/kg/day, and urinary creatinine decreased at 5, 10, and 20 mg/kg/day (Den Tonkelaar et al. 1983). The elevated urine glucose was considered 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.

2.11 DERMAL

As noted in Section 2.9, 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. Whereas the yellow staining of the skin may be unsightly, such cosmetic effects are not regarded as adverse. However, oral doses of DNOC may cause urticarial eruptions (allergic reaction) in humans. See Section 2.14 (Immunological) for discussion of urticarial eruptions in humans following oral exposure to DNOC.

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 armpits 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).

2.12 OCULAR

Despite the occurrence of a 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 yellow tinge to the sclera was also observed in a 14-year-old girl who ingested a TWA 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 yellow 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. An 8-hour dermal exposure to DNOC was reported to have caused unspecified visual disturbances in several agricultural workers (Varnai and Kote 1969).

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 six 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 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.

Ingestion of an unspecified dose of DNOC for 3 years was associated with a pearly swollen cataract of the left eye of a woman (Quick 1937). The right eye, which had punctate central lenticular opacity, eventually became blind 1 month after the cataract was diagnosed. 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 l–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). 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.

2.13 ENDOCRINE

Information regarding DNOC-induced endocrine effects is limited. Although DNOC has been described to induce a syndrome similar to hyperthyroidism in humans (Dodds and Robertson 1933), blood triiodothyronine (T3) and thyroxin (T4) 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. 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. Changes in pituitary, thyroid, and adrenal weight and histology were not observed in rats given daily doses of DNOC in the range of 1.25–20 mg/kg/day for 3 weeks (Vos et al. 1983).

2.14 IMMUNOLOGICAL

Urticaria (hives) were reported in one female following ingestion of a TWA DNOC dose of 2.27 mg/kg/day 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 TWA dose of 0.75 mg/kg/day DNOC for 11 weeks for weight reduction (Plotz 1936). 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).

Data regarding immunological effects in animals are conflicting. Decreased absolute thymus weight was observed at 10 and 20 mg/kg/day and decreased relative thymus weight was noted 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 effect on these immunoglobulins.

2.15 NEUROLOGICAL

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

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). 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). No tremors or exophthalmos were observed in a male factory worker who had been employed for 17 days pouring DNOC powder (Hunter 1950). Neurological effects such as mental depression and headaches were observed in two volunteers given 3 mg/kg/day DNOC orally for 4 days (Dodds and Robertson 1933) and in two of five volunteers given 0.92 and 1.27 mg/kg/day orally 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 7th day. Drowsiness, headaches, and ringing of the ears were experienced by a girl who ingested a TWA 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).

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 or as DNOC dust, for 4 hours at concentrations ≤36 mg/m^3 (Burkatskaya 1965a). Clinical signs of depression were observed in rats given single doses 227 mg/kg DNOC as the sodium salt (Ambrose 1942). In another acute rat study, a single oral dose of 19.8 mg/kg DNOC caused a 90–120% increase in brain blood flow within 4 hours posttreatment (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 oral dose of DNOC in the range of l0–35 mg/kg (Arustamyan 1972). The mice also became prostrate for 3–7 hours, approximately 2–3 hours posttreatment. 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; the study was limited by reporting deficiencies regarding experimental details and data (Burkatskaya 1965b). Decreased absolute brain weight was observed at 20 mg/kg/day, and increased relative brain weight was observed at 10 and 20 mg/kg/day in rats given DNOC orally for 90 days; however, no histopathological lesions were observed in the brain (Den Tonkelaar et al. 1983).

2.16 REPRODUCTIVE

Limited information was located regarding potential for DNOC-induced reproductive effects in humans. Among 47 agricultural workers who became ill after dermal exposure 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 women subsequently bore full-term healthy children.

DNOC did not affect either sperm counts, percent abnormal sperm, or testicular weights in mice given single oral doses in the range of 3–12 mg/kg/day for 5 days (Quinto et al. 1989). However, significantly decreased sperm motility and decreased percentage normal sperm were observed in rats at 7 or 14 days following cessation of DNOC oral dosing at 15 mg/kg/day for 5 days (Takahashi et al. 2004, 2006). 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 of 1–25 mg/kg/day 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).

2.17 DEVELOPMENTAL

Limited information was located regarding potential DNOC-induced developmental effects in humans or animals. Among 47 agricultural workers who became ill after being exposed to DNOC for about 8 hours, 3 of the workers were pregnant (Varnai and Kote 1969). The women 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 developmental effects were observed when DBA strains of mice given 8 mg/kg/day DNOC from day 11 to 14 of gestation (Nehez et al. 1981). On the 18th day of gestation, the numbers of corpora lutea, implantations, live embryos, and 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.

2.18 OTHER NONCANCER

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. Uncoupling of oxidative phosphorylation results in heat production that exceeds the organism's capacity to dissipate heat; consequently, fatal hyperthermia may occur. Elevated body temperature, profuse sweating, and/or increased metabolic rate were observed among workers involved in production of DNOC (Hunter 1950) or mixing and/or spraying DNOC (Bidstrup and Payne 1951; Buzzo and Guatelli 1949; Pollard and Filbee 1951; Steer 1950; van Noort et al. 1960). Increased basal metabolic rates, elevated body temperature, and/or excessive perspiration were also observed among individuals administered DNOC orally, typically for the intended purpose of body weight reduction (Dodds and Robertson 1933; Ibrahim et al. 1934; Plotz 1936).

Elevated body temperature was observed in rats exposed to DNOC aerosol at 100 mg/m^3 for 4 hours (King and Harvey 1953a). The body temperature was still elevated 20 hours following cessation of exposure. Increased blood glucose 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 for 4 hours (Burkatskaya 1965a). Body temperatures were increased by 0.6–1.4°C. Elevated body temperature and increased blood glucose were also observed in cats exposed to 2.0 mg/m^3 of DNOC mist for 1 month or 0.2 mg/m^3 of DNOC dust for 2–3 months (Burkatskaya 1965a). These effects were first noted during the first l–2 weeks of exposure. Urinary ketones, an indicator of endogenous fat catabolism, were increased in rats orally dosed at 2.5, 5, and 10 mg/kg/day DNOC for 90 days (Den Tonkelaar et al. 1983). 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.

2.19 CANCER

No studies were located regarding cancer in humans or animals exposed to DNOC via inhalation, oral, or dermal routes.

2.20 GENOTOXICITY

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. As shown in Table 2-5, DNOC tested positive for sex-linked recessive lethal mutations in *Drosophila melanogaster* (Mueller and Haberzettl 1980), DNA damage in rat hepatocytes (Grilli et al. 1991), chromosomal aberrations in bone marrow cells of rats (Hrelia et al. 1990, 1994), chromosomal aberrations in mouse germ cells (Nehéz et al. 1978b) and bone marrow cells (Nehéz et al. 1978a, 1984), and dominant lethal mutations in mice (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). Two studies found negative results for chromosomal aberrations in male germ cells (Nehéz et al. 1982) and bone marrow cells (Kurinnyi et al. 1982) after mice were treated with DNOC.

Table 2-4. Genotoxicity of Dinitrocresols *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Drosophila melanogaster (feed)	Sex-linked recessive lethal	+	Mueller and Haberzettl 1980
Rat (7.5, 15, or 30 mg/kg single gavage dose)	Chromosomal aberrations	+	Hrelia et al. 1994
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 germ cells	+	Nehéz et al. 1982
Mouse (intraperitoneal)	Chromosomal aberrations in male germ 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 F1, F2, and F4 generations	+	Nehéz et al. 1984
Male mouse (intraperitoneal)	Chromosomal aberrations in F1 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 not specified)	Chromosomal aberrations in bone marrow cells	–	Kurinnyi et al. 1982

– = negative result; + = positive result; DNA = deoxyribonucleic acid

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

		Results		
		Activation		
Species (test system)	Endpoint	With	Without	Reference
Salmonella typhimurium (eight histidine-requiring mutants)	Gene mutation	No data	–	Andersen et al. 1972
S. typhimurium (TA98)	Gene mutation	+	–	Nishimura et al. 1982
S. typhimurium (TA100)	Gene mutation	–	–	Nishimura et al. 1982
S. typhimurium (TA98, TA1537, TA2637)	Gene mutation	+[a]	+	Remondelli et al. 1986

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

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference
S. typhimurium (TA92, TA100, TA1535)	Gene mutation	–	–	Remondelli et al. 1986
S. typhimurium (TA98, TA1537)	Gene mutation	No data	–	Somani et al. 1981
S. typhimurium (TA100)	Gene mutation	No data	+	Somani et al. 1981
S. typhimurium (TA97, TA98, TA100, TA102)	Gene mutation	–	–	Hrelia et al. 1990, 1994
S. typhimurium (TA1535, TA1538)	Gene mutation	No data	+	Sundvall et al. 1984
S. typhimurium (TA98, TA100)	Gene mutation	+[a]	+	Sundvall et al. 1984
S. typhimurium (TA98NR)	Gene mutation	No data	(+)	Sundvall et al. 1984
S. typhimurium (TA100NR)	Gene mutation	No data	–	Sundvall et al. 1984
S. typhimurium (TA98)	Gene mutation	–[b]	–[b]	Sundvall et al. 1984
S. typhimurium (TA100)	Gene mutation	+[a,b]	+[b]	Sundvall et al. 1984
S. typhimurium (TA100, TA1535, TA1538)	Gene mutation	–[c]	–[c]	Spanggord et al. 1982b
S. typhimurium (TA98)	Gene mutation	–[c]	+[c]	Spanggord et al. 1982b
S. typhimurium (TA1537)	Gene mutation	+[c]	+[c]	Spanggord et al. 1982b
S. typhimurium (TA98)	Gene mutation	No data	+	Remondelli et al. 1986
Escherichia coli WP29 (hcr^+), WP29 (hcr^-)	Gene mutation	No data	–	Nagy et al. 1975
E. coli T_4 bacteriophage rII mutants	Gene mutation	No data	–	Andersen et al. 1972
E. coli T_4 bacteriophage wildtype	Gene mutation	No data	–	Andersen et al. 1972
Proteus mirabilis PG273 (wildtype), PG713 (rec^- hcr^-)	DNA repair	No data	+	Adler et al. 1976
Saccharomyces cerevisiae D7 strain	Mitotic crossing over	No data	+	Hrelia et al. 1990
Human peripheral blood lymphocytes	Sister chromatid exchange	–	–	Hrelia et al. 1990, 1994
Human blood leukocytes	Chromosomal aberrations	No data	+	Nehéz et al. 1978a
Human blood peripheral lymphocytes	Unscheduled DNA synthesis	–	–	Hrelia et al. 1994

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

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference

[a]Mutagenicity was decreased by the addition of S9.
[b]Test substance was 2,6-dinitro-*p*-cresol.
[c]Test substance was 4,6-dinitro-*m*-cresol.

+ = positive results; (+) = weakly positive results; – = negative results; DNA = deoxyribonucleic acid

Mixed results have been obtained from *in vitro* assays of DNOC. In tests for reverse mutations in *Salmonella typhimurium*, some investigators found consistently negative results with and/or without metabolic activation in several strains (Andersen et al. 1972; Hrelia et al. 1990, 1994; 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* T4 bacteriophage rII mutants and for forward mutation in *E. coli* T4 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, 1994).

2.21 MECHANISMS OF ACTION

DNOC has a relatively low pK_a and K_{ow} (see Chapter 4), but no information was located to indicate whether absorption of DNOC following inhalation, oral, or dermal exposure occurs by passive diffusion or by active transport.

Evidence from one study suggests that DNOC (rather than a metabolite) is the putative toxic agent (Smith et al. 1953). Results of genotoxicity studies indicate that DNOC is more genotoxic in the absence (rather than the presence) of exogenous metabolic activation systems. Acute toxic effects are 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 (Castilho et al. 1997; 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, as well as the relationship between their uncoupling potency and toxicity, were investigated (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. Results from an *in vitro* study of neonatal pig intestinal epithelium indicated DNOC inhibition of active transport by via uptake of gamma-globulin (Lecce 1966).