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 DNPs. 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 DNPs but may not be inclusive of the entire body of literature.

Oral studies (human case reports and animal studies) are presented in Table 2-1 and Figure 2-2. Animal dermal studies are presented in Table 2-2.

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 (SLOAELs) 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.

There are six isomers of DNP: 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNP. Dinitrophenol (commercial mixture of 2,4-DNP and smaller amounts of 2,3- and 2,6-DNP) is used in the synthesis of dyes, picric acid, picramic acid, wood preservatives, photographic developers, and explosives. 2,5-DNP is also used in the manufacture of dyes and organic chemicals. In the 1930s, 2,4-DNP was prescribed by physicians as a weight-reducing agent; however, the FDA has never approved 2,4-DNP as a pharmaceutical agent (FDA 2016). In 1938, the FDA declared DNP to be extremely dangerous and not fit for human consumption, and use of 2,4-DNP was discontinued due to serious adverse health effects, including fatality (Bartlett et al. 2010; FDA 2020a; NLM 2020). Virtually all of the available information on the toxic effects and toxicokinetics of DNP after inhalation, oral, or dermal exposure is for 2,4-DNP. No studies were located regarding the toxic effects of 2,3-, 2,5-, 3,4-, or 3,5-DNP in humans or animals by these exposure routes. Therefore, the focus of Chapter 2 is on 2,4-DNP.

2,4-DNP exerts its toxic effects via uncoupling of oxidative phosphorylation, resulting in increased metabolic rate and body temperature (e.g., Ilivicky and Casida 1969; Loomis and Lipmann 1948; Lou et al. 2007; Muscatello et al. 1975; Pinchot 1967; Stryer 1988; Weinbach and Garbus 1969). As noted in Section 1.2, for this profile, adverse health effects of 2,4-DNP observed in humans and animals are classified as primary effects and as effects that are secondary to increased metabolic rate and body temperature. Effects classified as primary if they occur in the absence of increased body temperature include skin discoloration and rashes, cataract formation, and developmental effects.
Secondary effects include the following:

- decreased body weight or decreased body weight gain;
- confusion, agitation, delirium, and cerebral edema;
- increased respiratory rates, dyspnea, and respiratory distress;
- nausea, vomiting, and diarrhea;

- increased pulse or heart rate, palpitations, altered blood pressure, and myocardial injury;
- muscle pain or weakness, elevated serum creatine kinase, and rhabdomyolysis;
- acute renal failure;
- hepatic and pancreatic injury;
- hemorrhage, hemorrhagic lesions, and hemorrhagic diseases;
- hematopenia;
- multi-organ system dysfunction and failure; and
- death, typically from cardiac arrest.

The health effects of DNP have been evaluated in human and animal studies. As illustrated in Figure 2-1, most of the health effects data come from case reports in humans. In addition to the studies summarized in Figure 2-1, lethality in humans or animals was examined in 62 oral studies, 2 dermal studies, and 5 studies of humans exposed by both dermal and inhalation routes. Note that in Figure 2-1, secondary effects are not counted as separate effects, but are represented by the "other noncancer" bar.

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

- **Metabolic Endpoint/Death:** Increased basal metabolic rate and accompanying increases in body temperature have been reported in humans and animals exposed to DNP by inhalation or oral routes. These changes trigger widespread physiological sequelae including increases in pulse rate, heart rate, respiratory rate; pulmonary edema; nausea and vomiting; confusion, dizziness, and delirium; muscle pain and weakness; acute renal failure; liver necrosis; and sometimes death.

- **Body Weight Endpoint:** Dose-related decreases in body weight or decreases in body weight gain in humans and animals following oral exposure.

- **Neurological Endpoint:** Peripheral neuritis in humans.

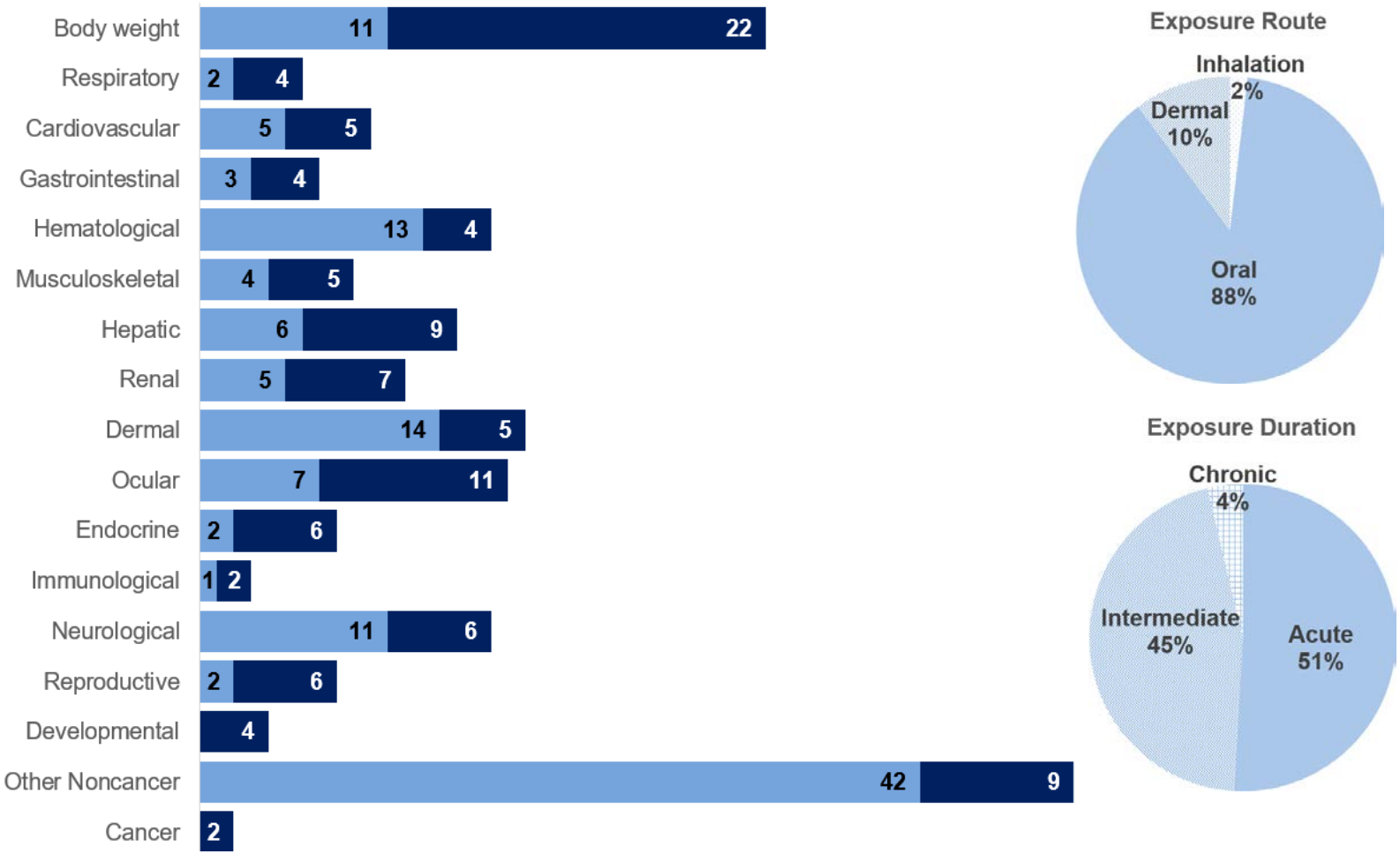
- **Hematological Endpoint:** Agranulocytosis in humans.

- **Dermal Endpoint:** Erythematous and pruritic rashes with maculopapular eruptions in humans.

- **Ocular Endpoint:** Cataracts in humans.

Figure 2-1. Overview of the Number of Studies Examining 2,4-Dinitrophenol Health Effects

Most studies examined the potential body weight, metabolic, and ocular effects of 2,4-dinitrophenol
Fewer studies evaluated health effects in **animals** than in **humans** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 108 studies (46 animal and 62 human, including those finding no effect) have examined toxicity; many studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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 1 F	14 days 3 times/day (C)	2.3	CS	Musc/skel		2.3		Exacerbation of arthritis
					Dermal			2.3	Severe pruritic, edematous, maculopapular eruptions covering most of the body
					Neuro			2.3	Peripheral neuritis, paresthesias
Anderson et al. 1933									
2	Human 1 M	Once (C)	40	CS, LE	Death			40	Death
					Other noncancer (metabolic)			40	Symptoms related to hyperthermia and increased metabolic rate, including renal failure, hyperkalemia, and elevated creatine kinase
Bartlett et al. 2010									
3	Human 1 F	10 days 1 time/day (C)	3.5	BW, CS	Dermal		3.5		Rash, pruritus, urticaria
					Neuro			3.5	Symptoms of peripheral neuritis, tingling and numbness of extremities
Bortz 1934									
4	Human 9 M, 2 F	2 days 3 times/day (C)	3	BC, CS, LE	Other noncancer (metabolic)		3		Increased basal metabolic rate (29%)
Castor and Beierwaltes 1956									
5	Human 8 NS	Once (C)	3–5, 5–10, >10	CS, LE	Other noncancer (metabolic)		3–5	>10	Basal metabolism increased 20–30% (3–5 mg/kg); body temperature increased ≥3°C (>10 mg/kg)
Cutting et al. 1933									

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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
6	Human 14 NS	1–2 weeks 7 days/week 1 times/day (C)	4	CS, LE	Other noncancer (metabolic)			4	Increased basal metabolic rate (38%)
Cutting et al. 1934									
7	Human 4 NS	7–16 days 2 times/day (C)	0, 3.5	BW, CS, UR	Bd wt		3.5		Average weight loss of 0.92 kg
					Other noncancer (metabolic)			3.5	27–55% increase in basal metabolic rate, excessive perspiration
Cutting and Tainter 1933									
8	Human 1 F	3–4 days 1 time/day (C)	4.4	CS	Gastro		4.4		Burning of the throat, inflammation of the pharynx
					Dermal		4.4		Rash on chest
					Other noncancer (metabolic)		4.4		Symptoms related to hyperthermia and increased basal metabolic rate
Dintenfass 1934									
9	Human 3 F	Several days (C)	1–3	BW, BC, CS, LE, UR	Other noncancer (metabolic)		2	3	Increased basal metabolic rates (25–27% at 2 mg/kg; 35–42% at 3 mg/kg); symptoms related to hyperthermia and increased metabolic rate
Dunlop 1934									
10	Human 1 M	Once (C)	36–71	CS, LE	Death			36–71	Death
					Other noncancer (metabolic)			36–71	Symptoms related to hyperthermia and increased basal metabolic rate
Geiger 1933									

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11	Human 1 F	Once (C)	31–38	CS, LE	Death			31–38	Death
					Other noncancer (metabolic)			31–38	Symptoms related to hyperthermia and increased basal metabolic rate
Hsiao et al. 2005									
12	Human 1 F	2 weeks (C)	2	BC, CS, OF, UR, HE	Hemato		2		Slight secondary anemia
					Dermal			2	Severe exfoliating dermatitis over 100% of body surface
					Ocular			2	Cataract formation
					Neuro			2	Polyneuritis
					Other noncancer		2		Temporary hearing impairment due to exudation in the middle ear
Hitch and Schwartz 1936									
13	Human 1 F	2 weeks 7 days/week 4 times/day (C)	6	HE	Hemato			6	Agranulocytosis
Hoffman et al. 1934									
14	Human 1 M	Once (C)	43	CS, LE	Death			43	Death
					Other noncancer (metabolic)			43	Symptoms related to hyperthermia and increased metabolic rate, including pulmonary edema
Holborow et al. 2016									

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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
15	Human 1 M	6 days 1 time/day (C)	4	CS	Dermal		4		Maculopapular rash with edema over thighs, chest, back, face, neck, and arms
					Other noncancer (metabolic)			4	Hyperthermia (40°C)
Le et al. 2015									
16	Human 1 M	10 days 1 time/day (C)	1	CS	Dermal			1	Maculopapular rash over forehead, trunk, limbs, neck, and oral mucosa, with skin sloughing
					Other noncancer (metabolic)			1	Symptoms related to hyperthermia and increased metabolic rate
Lee et al. 2014									
17	Human 15 F	1–8 weeks 7 days/week 3 times/day (C)	4	BW, BC, CS, LE, OF, UR	Cardio			4	Abnormalities on ECGs in 3/6 tested
					Gastro		4		Gastrointestinal disturbances, vomiting in 5/15 subjects
					Musc/skel		4		Loss of strength and endurance on exercise tests in 4/4 subjects
					Hepatic		4		Increased phenol-tetraiodophthalein retention in 3/5 subjects tested at 1–2 weeks
					Renal	4			
					Dermal		4		Severe skin rashes in 3/5 subjects
					Endocr		4		Decreased glucose tolerance in 5/8 subjects
					Neuro		4		Complete loss of taste in 1/15

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
					Other noncancer (metabolic)			4	Increased basal metabolic rate (30–70%), excessive sweating
MacBryde and Taussig 1935									
18	Human 1 F	14 days 1–2 times/day (C)	3	LE, BW, GN, HP, CS, HE, UR	Death			3	Death
					Other noncancer (metabolic)			3	Basal metabolic rate increased by 38%; body temperature of 102°F; symptoms associated with hyperthermia and increased basal metabolic rate
Masserman and Goldsmith 1934									
19	Human 1 M	Once/day for 4 days (C)	6	CS, LE	Death			6	Death
					Other noncancer (metabolic)			6	Symptoms related to hyperthermia and increased basal metabolic rate
McFee et al. 2004									
20	Human 1 F	8 days 1 time/day (C)	0.91, 1.45	BW, CS	Dermal		0.91		Pruritic rash
Nadler 1935									
21	Human 1 F	6 months (C)	≤15.7	CS, OF	Neuro			≤15.7	Axonal sensorimotor polyneuropathy
Phillips and Singer 2013									
22	Human 48 M, F	5 days (C)	7	GN, HP, CS, LE	Death			7	Death
					Other noncancer (metabolic)			7	Symptoms related to hyperthermia and increased metabolic rate
Poole and Haining 1934									

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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	Human 1 F	Once (C)	64	CS, LE	Death			64	Death
					Other noncancer (metabolic)			64	Symptoms related to hyperthermia and increased metabolic rate
Purvine 1936									
24	Human 1 M	Once (C)	40	CS, LE	Death			40	Death
					Other noncancer (metabolic)			40	Symptoms related to hyperthermia and increased metabolic rate
Siegmueller and Narasimhaiah 2010									
25	Human 13 NS	4–12 days 3 times/day (C)	4	CS, HE	Resp	4			
					Cardio			4	Average increase in venous blood pressure of up to 37% and in pulse of up to 12%
					Other noncancer (metabolic)		4		Sensation of warmth, increased perspiration
Stockton and Cutting 1934									
26	Human 1 M	1 week 2 times/week (C)	46	HP, CS, LE	Death			46	Death
					Other noncancer (metabolic)			46	Symptoms related to hyperthermia and increased basal metabolic rate
Tainter and Wood 1934									
27	Human 37 NS	14 days 1 times/day (C)	1	BW, CS	Bd wt		1		Weight loss of 0.43 kg/week
					Other noncancer (metabolic)		1		Sensation of warmth, increased perspiration
Tainter et al. 1935									

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
28	Human 1 F	Once (C)	≥10	CS	Other noncancer (metabolic)			≥10	Symptoms related to hyperthermia and increased basal metabolic rate, including rhabdomyolysis and transient renal failure
van Veenendaal et al. 2011									
29	Rat (Wistar) 8 F	Once (GW)	0, 20	HP	Renal		20		Mild tubular necrosis in 5/16 kidneys
Arnold et al. 1976									
30	Rat (white) NS	Once (GO)	20, 60	LE	Death			60	100% mortality
Dow Chemical Co. 1940									
31	Rat (white) NS	Once (GO)	NS	LE	Death			30	LD_{50}
Dow Chemical Co. 1950									
32	Rat (Harlan Fischer) 4 F	Once (GO)	10, 36.5, 140, 500	BW, CS, LE	Death			500	4/4 died; estimated LD_{50} : 320 mg/kg
					Bd wt	140			No effect on body weight during 14-day observation
					Musc/skel		10		Temporary leg weakness
Eli Lilly and Co. 1992									
33	Rat (NS) 6–36 M	9 days ad lib (F)	0, 350	BW, OW, BI	Bd wt		350		12% decrease in body weight
					Endocr		350		Increased thyroxine secretion
England et al. 1973									
34	Rat (Sherman) NS M	Once (GW)	NS	BW, CS, LE	Death			71	LD_{50}
Kaiser 1964									

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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
35	Rat (NS) 3–12 M	7–14 days ad lib (F)	0, 350	BW, OW, BC, BI, OF	Bd wt			350	24–36% decrease in body weight gain
					Endocr		350		21–35% decrease in absolute thyroid weight, decreased thyroid function, decreased serum protein bound iodine
Maayan 1968									
36	Rat (white) 9–40 M, F	Once (GO)	10, 20, 23, 25, 27, 30, 40, 50, 60, 70, 80, 100	LE	Death			30	11/30 died
Spencer et al. 1948									
37	Rat (Jcl:SD) 6–12 M	5 days (GO)	0, 7.5, 15, 30	CS, BW, OF, OW, HP	Bd wt	30			
					Repro	30			No effect on reproductive organ weights or histopathology, or sperm count, motility, or morphology
Takahashi et al. 2004									
38	Rat (Sprague-Dawley) 4–9 M	2 weeks ad lib (F)	0, 350	BW, OW, BI, OF	Bd wt		350		15% decrease in body weight gain
					Endocr		350		34% decrease in absolute pituitary weight, decreased pituitary function, decreased growth hormone synthesis, decreased thyroid function, decreased serum thyroxin levels
Wilkins et al. 1974									

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
39	Mouse (Yellow adipose and albino) 12 NS	8 hours ad lib (F)	108	LE	Death			108	12/12 died within 8 hours
Bettman 1946									
40	Mouse (Albino) 8 NS	1 week ad lib (F)	325	LE	Death			325	2/8 died
					Ocular	325			
Bettman 1946									
41	Mouse (Swiss-Webster) 7–9 F	3 days GDs 10–12 1 time/day (GW)	0, 25.5, 38.3	CS, FX, MX, DX, TG	Neuro	25.5	38.3		Hyperexcitability of dams
					Develop	38.3			
					Other noncancer (metabolic)	25.5		38.3	Hyperthermia of dams
Gibson 1973									
42	Mouse (C57BL/6J) 8 F	7 days (W)	0,11, 22, 45, 89, 130, 270	BW, FI, WI	Bd wt	45	89		Body weight loss at 30 ºC
Goldgof et al. 2014									
43	Mouse (CF1) NS M	Once (GW)	NS	BW, CS, LE	Death			72	LD_{50}
Kaiser et al. 1964									
44	Mouse (CD-1) 30–40 F	5 days GDs 8–12 1 times/d (GW)	0, 125	BW, FX, MX, DX	Develop	125			
Kavlock et al. 1987									

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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
45	Rabbit (NS) NS	8 hours (F)	41	OP	Ocular	41			
Bettman 1946									
46	Dog (Beagle) 0–1 M, 0–2 F	1–14 days 1 time/day (C)	5, 12.5, 25, 125	CS, OF, LE	Death			25	1/3 deaths
					Other noncancer (metabolic)	12.5		25	“Markedly” increased respiration rate and body temperature
Kaiser 1964									
47	Dog (NS) NS	Once (C)	20, 30	CS	Other noncancer (metabolic)		20		Body temperature increased 0.9°C
Tainter and Cutting 1933a									
48	Chicken (NS) NS	Once (GO)	6, 11, 20, 40, 79	OP	Ocular	6		11	Cataract formation
Buschke 1947									
49	Chicken (NS) 20 F, 32 M	13 days ad lib (F)	0, 16.5, 36.3, 77.9	BW, OW, FI, OF, GN	Bd wt	36.3	77.9		12% decrease in body weight gain
Toyomizu et al. 1992									
50	Quail (Bobwhite) 6–10 F	8 days ad lib	0, 33.6, 56.1	LE, FI, WI, GN	Death			56.1	1/6 died
					Bd wt	33.6	54.1		Mean weight loss of 13%
					Gastro		33.6		Diarrhea
					Other noncancer (metabolic)			33.6	Metabolic rate 23–41% higher than controls
Dominguez et al. 1993									

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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
51	Duck (White Pekin) 3–8 NS	Once (GW)	12, 15, 20, 25, 28, 30	OP	Ocular	15		20	Temporary cataracts in 3/8
Gehring and Buerge 1969a									
INTERMEDIATE EXPOSURE									
52	Human 3–20 NS	51–62 days (C)	2	BW, BC, CS	Other noncancer (metabolic)		2		Symptoms related to hyperthermia and increased metabolic rate
Bayer and Gray 1935									
53	Human 1 F	37 days 1 time/day (C)	2	BW, CS, HE, UR	Bd wt		2		4.5 kg reduction in body weight in 37 days
					Hepatic		2		Palpable and tender liver
					Renal			2	Moderate albuminuria
					Dermal		2		Severe pruritis involving the entire body
Beinhauer 1934									
54	Human 8 NS	3–13 week 7 days/week 1 time/day (C)	1–5	BC, CS, LE	Other noncancer (metabolic)		3		23% increase in basal metabolic rate
Cutting et al. 1934									
55	Human 2 F	42–68 days 1 time/day (C)	2–5	LE, HE, CS	Death			4	Death
					Hemato			4	Agranulocytosis
					Other noncancer (metabolic)			4	Symptoms related to hyperthermia and increased basal metabolic rate
Dameshek and Gargill 1934									
56	Human 1 F	20 days 3 times/day (C)	4	LE, OF, HE, CS	Hemato			4	Agranulocytosis
					Hepatic		4		Impaired liver function on bromsulphalein test
Davidson and Shapiro 1934									

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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
57	Human 1 F	182 days 1 time/day (C)	3	BW, HP, CS, HE, UR, MX	Bd wt			3	Loss of 20% of body weight
					Neuro			3	Peripheral neuritis
					Repro			3	Miscarriage
					Other noncancer (metabolic)			3	Symptoms related to hyperthermia and increased basal metabolic rate
Epstein and Rosenblum 1935									
58	Human 1 F	46 days 1 time/day (C)	1.03	LE, BW, GN, HP, BC, CS, HE, UR	Death			1.0	Death
					Hemato			1.0	Severe agranulocytosis, severe neutropenia
					Other noncancer (metabolic)			1.0	Symptoms related to hyperthermia (105.6°F) and increased basal metabolic rate
Goldman and Haber 1936									
59	Human 1 F	118 days (C)	3	BW, OP	Bd wt		3		Individual weight loss of 9.6 kg
					Ocular			3	Cataracts
Horner et al. 1935									
60	Human 1 M, 2 F	41–49 days (C)	3	BW, CS	Dermal		3 M		Urticaria
					Neuro		3 F		Loss of taste
					Other noncancer (metabolic)		3 M		Excessive perspiration
Hunt 1934									
61	Human 1 F	35 days 2–4 times/day (C)	3.97	HE, BW, CS, UR	Hemato			4	Agranulocytosis
					Renal			4	Albuminuria
					Other noncancer (metabolic)			4	Symptoms related to hyperthermia (102.8°F) and increased basal metabolic rate
Imerman and Imerman 1936									

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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
62	Human 10 NS	7 weeks 7 days/week 1 time/day (C)	3–4	BC, CS, LE, UR, HE	Bd wt		3		Average weight loss of 0.36 kg/week
					Other noncancer (metabolic)			3	50% increase in basal metabolic rate
Looney and Hoskins 1934									
63	Human 15 F	1–8 weeks 7 days/week 3 times/day (C)	4	BW, BC, CS, LE, OF, UR	Cardio			4	Abnormalities on ECGs in 3/6 tested
					Musc/skel		4		Loss of strength and endurance on exercise tests in 4/4 tested
					Hepatic		4		increased phenoltetraiodo-phthalein retention in 3 of 3 tested at 3–8 weeks
					Renal	4			
					Dermal		4		“Quite severe” skin rash in 3/15
					Endocr		4		Decreased glucose tolerance in 4/4 tested at 3–4 weeks
					Neuro		4		Complete loss of taste
					Other noncancer (metabolic)			4	Basal metabolic rate +30 to +70%, excessive sweating
MacBryde and Taussig 1935									
64	Human 3 F	21–112 days 1–3 times/day (C)	4	BW, CS	Bd wt		4	4	16–25% loss of body weight
					Musc/skel		4		Weakness and arthritic pains
					Dermal		4		Pruritic rash
					Neuro			4	Peripheral neuritis
Nadler 1935									

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
65	Human 1 F	2 months 3 times/day (C)	4	BW, CS, OP	Ocular			4	Bilateral cataracts
					Other noncancer (metabolic)		4		Symptoms related to hyperthermia and increased basal metabolic rate
Rank and Waldeck 1936									
66	Human 1 F	41 days 3 times/day (C)	0.6–4	CS, HE, LE	Death			4	Death
					Hemato			4	Agranulocytosis
					Other noncancer (metabolic)			4	Symptoms related to hyperthermia and increased basal metabolic rate
Silver 1934									
67	Human 159 M, F	22–89 days 1 time/day (C)	2.3, 3.0	HE, BW, BC, CS, OF, UR	Bd wt		3		Loss of 0.95 kg/week
					Resp		3		Increased respiratory rate by 10/minute
					Cardio		3		Bradycardia in 2/16, decreased blood pressure in former hypertensive patients
					Gastro		3		Transient diarrhea, vomiting, heartburn
					Hemato	3			
					Hepatic	3			
					Renal			3	Albuminuria
					Dermal		2		Urticaria
					Ocular			3	Cataract formation
					Neuro			3	Peripheral neuritis, weakness, loss of taste
					Repro			3	Altered menstrual cycles, amenorrhea

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
					Other noncancer (metabolic)		3		11% increase in basal metabolic rate per 100 mg daily dose
Simkins 1937a, 1937b									
68	Human 17–45 NS	2–50 weeks 7 days/week 1 time/day (C)	4	BC, LE	Hepatic	4			
Tainter et al. 1934a									
69	Human 20 M, 150 F	88 days 1 time/day (C)	4	BW, CS, LE, HE	Bd wt		4		Weight loss of 0.64 kg/week, total weight loss of 7.8 kg
					Cardio	4			
					Hemato	4			
					Dermal		4		Skin reactions (some severe) in 23/170
					Ocular			4	Cataracts
					Neuro			4	Symptoms of peripheral neuritis (sensory) in 18/100
					Other noncancer (metabolic)			4	38% estimated increase in basal metabolic rate, increased perspiration, which sometimes caused discomfort
Tainter et al. 1935									
70	Human 1 M, 26 F	1-18 months (C)	3.6	BW, CS	Bd wt		3.6		Average weight loss of 17 kg
					Ocular			3.6	Cataracts
Whalman 1936									

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
71	Human 1 M	43 days (C)	1.7–5.4	CS, LE	Death			2.7	Death
					Other noncancer (metabolic)			2.7	Symptoms related to hyperthermia and increased basal metabolic rate
Zack et al. 2016									
72	Rat (Sprague-Dawley) 5-7 M	30 days ad lib (F)	0, 350	BW, OW, CS, BI, HE	Bd wt		350		18% decrease in body weights
					Endocr		350		Decreased thyroid and pituitary weights
					Other noncancer (metabolic)		350		Increased body temperature
Bakke and Lawrence 1965									
73	Rat (Sprague-Dawley CD) 12 M	15 days (G)	0, 20	BW, BC, OW, HP	Bd wt	20			
					Hepatic		20		Increased liver weight (15%); centrilobular hypertrophy, minor necrotic foci, and mitochondrial changes
					Musc/skel		20		Mitochondrial changes (swelling, deformation, decreased matrix density) in skeletal muscle
					Endocr		20		43% increase in blood glucose
Haasio et al. 2002a, 2002b									
74	Rat (Sherman) 6 M, 6 F	4 weeks ad lib (F)	0, 20, 59	BW, FI, CS, LE	Bd wt	59			
Kaiser 1964									

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
75	Rat (Sprague-Dawley) 12–17 M, 12–17 F	18 days 1 time/day (GO)	0, 3, 10, 20, 30	LE, CS, BW, FI, DX, HE, BC, UR, OW, GN, HP	Death			30	4/5 males and 1/5 females died
					Bd wt	10	20		14% decrease in terminal body weight
					Hemato	20			
					Neuro	20	30		Decrease in locomotor activity in 1/4 survivors
					Repro	20			No histopathology changes in testes, epididymides, ovaries, or uterus; small decrease in absolute testes weight
Koizumi et al. 2001, 2002									
76	Rat (Sprague-Dawley) 12 M, 12 F	28 days 1 time/day (GO)	0, 3, 10, 30, 80	LE, CS, BW, FI, HE, BC, UR, OW, GN, HP	Death			80	2/12 males and 6/12 females died
					Hemato	30	80		Decreased red blood cells, hemoglobin, and hematocrit
					Hepatic	30	80		Increased relative liver weight
					Renal	30	80		Mineralization in corticomedullary junction (3/4 males and 2/3 females); increased relative kidney weight
					Neuro	10	30		Decreased locomotor activity
Koizumi et al. 2001, 2002									
77	Rat (Wistar) 8 M	28 days (DW)	0, 30	LE, BW, FI, WI, BI, OF, HP	Bd wt	30			Body weight change of <10% (5.2% at day 28)
					Other noncancer (metabolic)		30		Decreased maximal running speed and running economy (measured on day 21)
Schlagowski et al. 2014									

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
78	Rat (White) 10–20 M	21–24 days ad lib (F)	0, 5, 10, 25, 50, 350	BW, OW, FI, GN, HP, BC, CS, LE, HE, OP	Death			350	4/10 died in 21 days; 6/10 sacrificed as moribund on day 24
Spencer et al. 1948									
79	Rat (NS) 10–20 M	6 months ad lib (F)	0, 5, 10, 25, 50	BW, OW, FI, GN, BC, CS, LE, HE, OP	Bd wt	25	50		17% decrease in body weight gain
					Resp	50			
					Cardio	50			
					Gastro	50			
					Hemato	50			
					Musc/skel	50			
					Hepatic	50			
					Renal	50			
					Ocular	50			
					Immuno	50			
					Repro	50			
Spencer et al. 1948									
80	Rat (White) 6 M	94 days ad lib (F)	0, 14, 28, 42, 56, 84, 420	BW, FI, LE, OP	Death			420	6/6 died within 94 days
					Bd wt	84		420	93% decrease in body weight gain
					Ocular	420			
Tainter 1938									
81	Rat (Albino) 8–9 NS	58–173 days ad lib (F)	0, 50	BW, CS	Bd wt		50		Significant weight loss
					Ocular	50			
Tainter and Borley 1938									

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
82	Rat (Crl:CD) 12 M, 12 F	40–47 days (GO)	0, 3, 10, 30	CS, BW, FI, DX, FX, TG, OW, GN, HP	Bd wt	10	30		Decreased body weight gain in parental males and in parental females during lactation days 0–4
					Cardio	10	30		Increased relative heart weight in females
					Hepatic	10	30		Increased relative liver weights in both sexes
					Renal	10	30		Increased relative kidney weights in both sexes
					Repro	30			No treatment-related effect on length of estrous cycle, fertility, gestation index and length, nursing index, or reproductive organ weights or histopathology
					Devel	10		30	Stillbirths, decreased pup viability, and decreased pup body weight
Takahashi et al. 2009									
83	Mouse (Yellow adipose) 40 NS	6 months ad lib (F)	0, 130	OP	Ocular			130	3/40 developed cataracts
Bettman 1946									
84	Mouse (Albino and black) 40 NS	11 months ad lib (F)	0, 130	OP	Ocular			130	1/20 albino developed cataracts
Bettman 1946									

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
85	Mouse (Swiss) 30 F	50 weeks (W)	0, 0.07	BW, FI, WI, BI	Bd wt		0.07[b]		8–13% decrease in body weight between 20 and 50 weeks of exposure
					Endocr		0.07		Decreased serum glucose, triglycerides, and insulin after 14 weeks of exposure
Caldeira da Silva et al. 2008									
86	Mouse (C57BL/6J) 8 F	8 weeks (W)	0, 89	BW, FI, BI	Bd wt		89		18% decrease in body weight
Goldgof et al. 2014									
87	Guinea pig (NS) 8 NS	21–37 days ad lib (F)	0, 80	CS	Ocular	80			
Tainter and Borley 1938									
88	Dog (NS) 1–2 NS	7–12 times over 45–77 days (C)	5, 10, 15, 17.5, 20	BW, GN, HP, BC, CS, LE, OF	Bd wt	20			
					Resp	20			
					Cardio	20			
					Gastro	20			
					Hepatic	20			
					Renal	20			
					Neuro	20			
					Other noncancer (metabolic)	10	15	20	1°C increase in body temperature at 15 mg/kg; >2°C increase at 20 mg/kg
Tainter and Cutting 1933b									
89	Dog (NS) 3 M	6 months 6 days/week 1 time/day (C)	0.5, 10	BW, GN, HP, BC, LE, HE, UR, OF	Bd wt	10			
					Resp	10			
					Cardio	10			
					Gastro	10			
					Hemato	10			

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
					Musc/skel	10			
					Hepatic	10			
					Renal	10			
					Immuno	10			
					Neuro	10			
					Repro	10			
Tainter et al. 1934b									
CHRONIC EXPOSURE									
90	Human 2 F	16–18 months (C)	2, 3	BW, CS, OP	Bd wt			2	>30% loss of body weight
					Ocular			2	Cataracts
Horner et al. 1935									
91	Rat (white) 6 M	lifetime ad lib (F)	0, 10, 20, 30, 40, 60	BW, FI, WI, GN, HP, LE, OP	Death			60	Approximately 50% decrease in median lifespan
					Bd wt	20		30	25% decrease in body weight gain
					Resp	60			
					Cardio	60			
					Hepatic	60			
					Renal	60			
					Ocular	60			
					Repro	60			
Tainter 1938									

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrophenol – 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
92	Mouse (Swiss) 30 F	Weeks 18 of age up to 140 weeks of age (W)	0, 0.03–0.105	LE	Death				Increased lifespan (controls: 722 days; 2,4-DNP: 771 days)
Caldeira da Silva et al. 2008									

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an intermediate-duration MRL of 0.00007 mg/kg/day; dose divided by an uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for use of a LOAEL, and 10 for human variability). The intermediate-duration MRL is believed to be protective for chronic exposures.

ad lib = *ad libitum*; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BC = biochemistry; Bd wt or BW = body weight; BI = biochemical changes; (C) = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; (DW) = drinking water; DX = developmental toxicity; ECG = electrocardiogram; Endocr = endocrine; (F) = exposure in feed; F = female(s); FI = food intake; FX = fetal toxicity; (G) = gavage, neat; (GO) = gavage in oil vehicle; (GW) = gavage in water vehicle; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; 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); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; Repro = reproductive; Resp = respiratory; TG = teratogenicity; UR = urinalysis; (W) = water; WI = water intake

Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Acute (≤14 days)

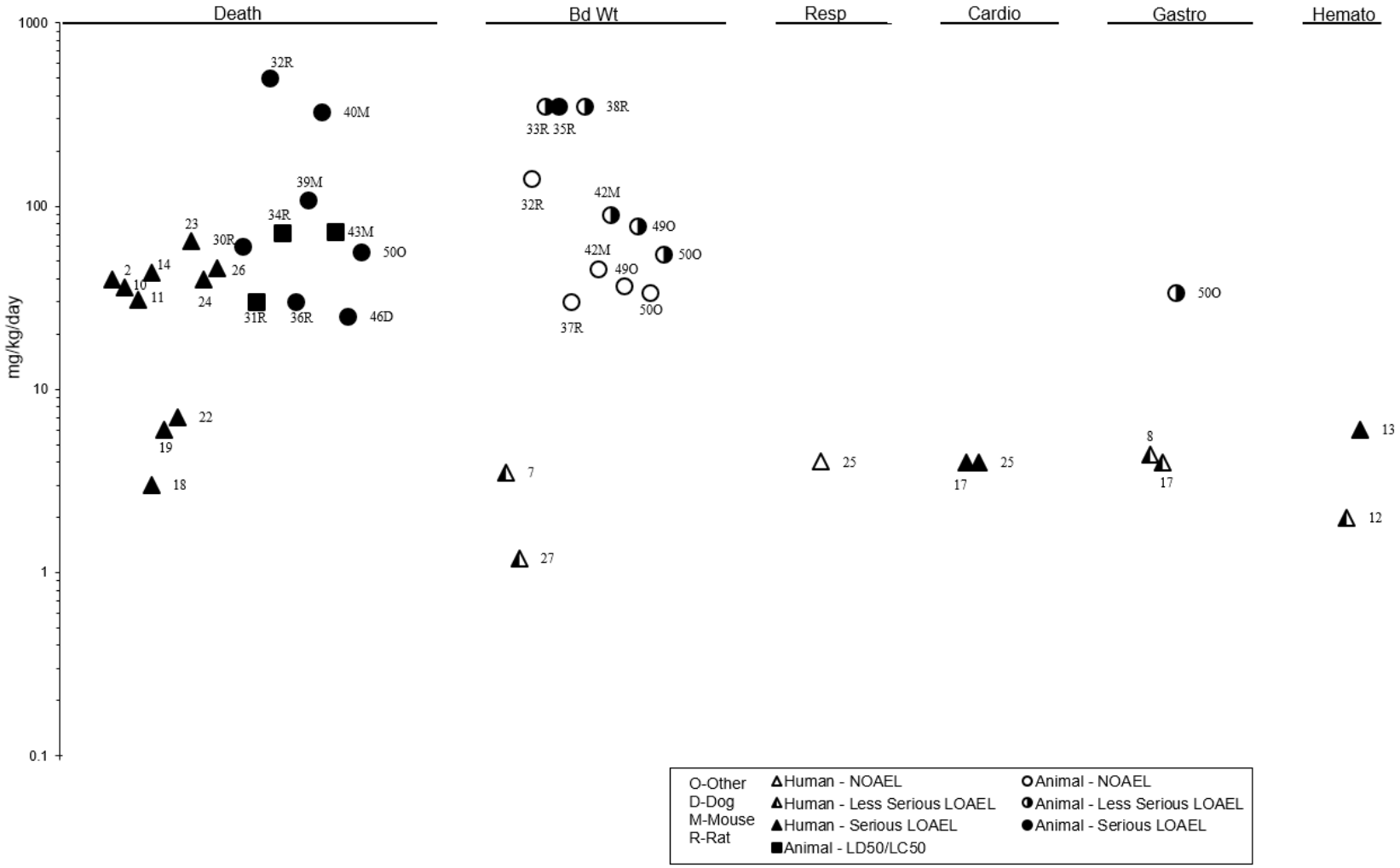

Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Acute (≤14 days)

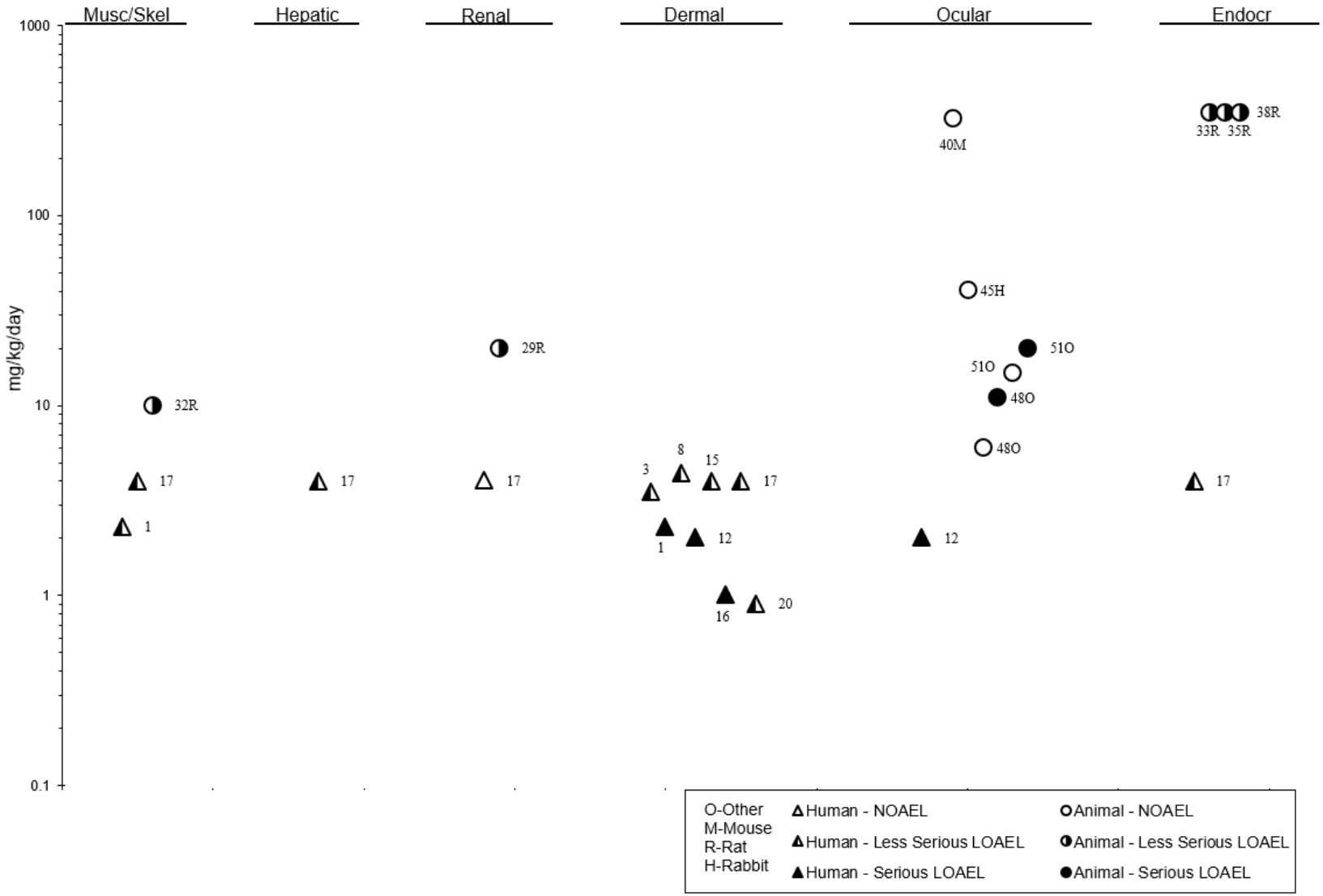

Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Acute (≤14 days)

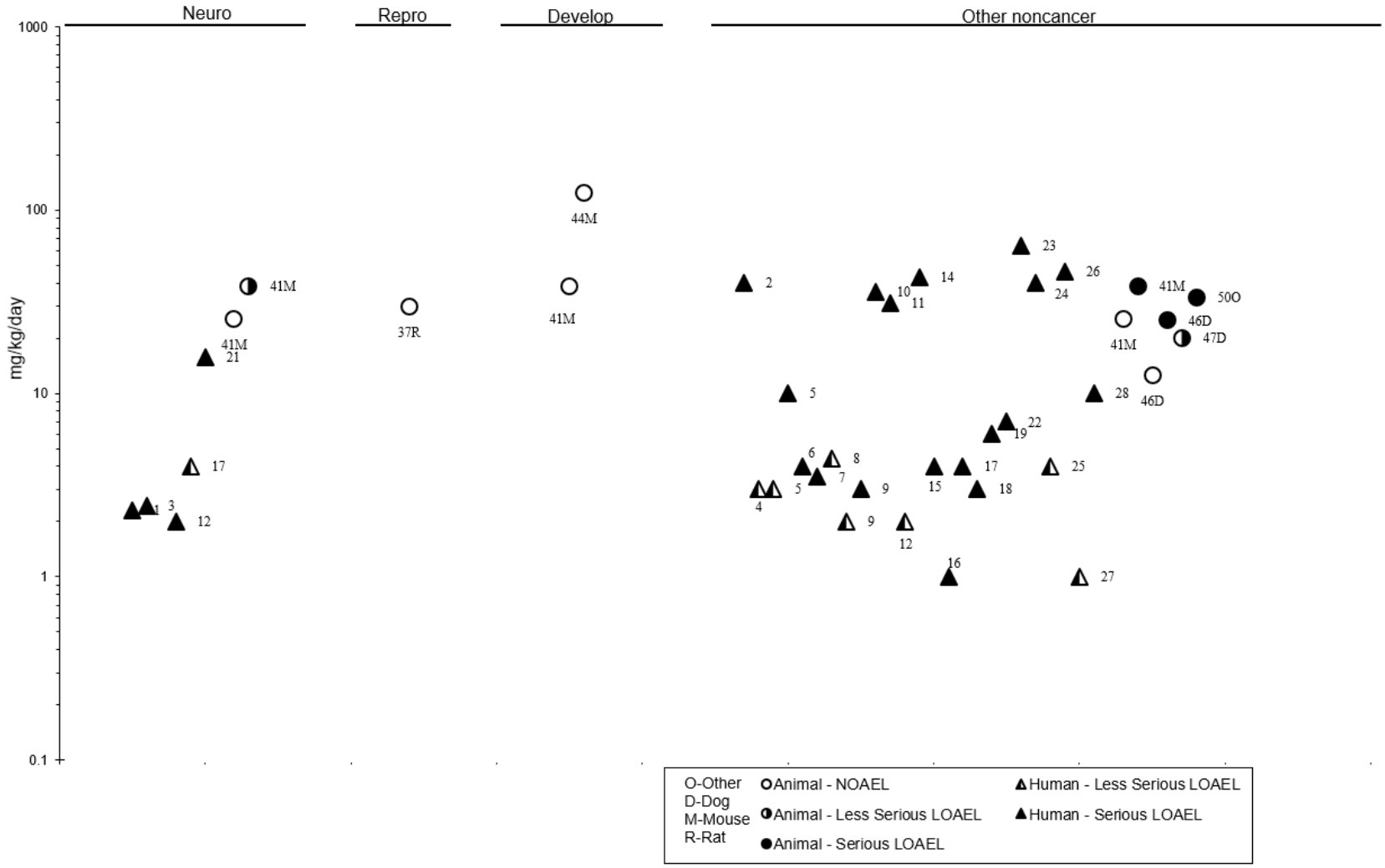

Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral
Intermediate (15-364 days)

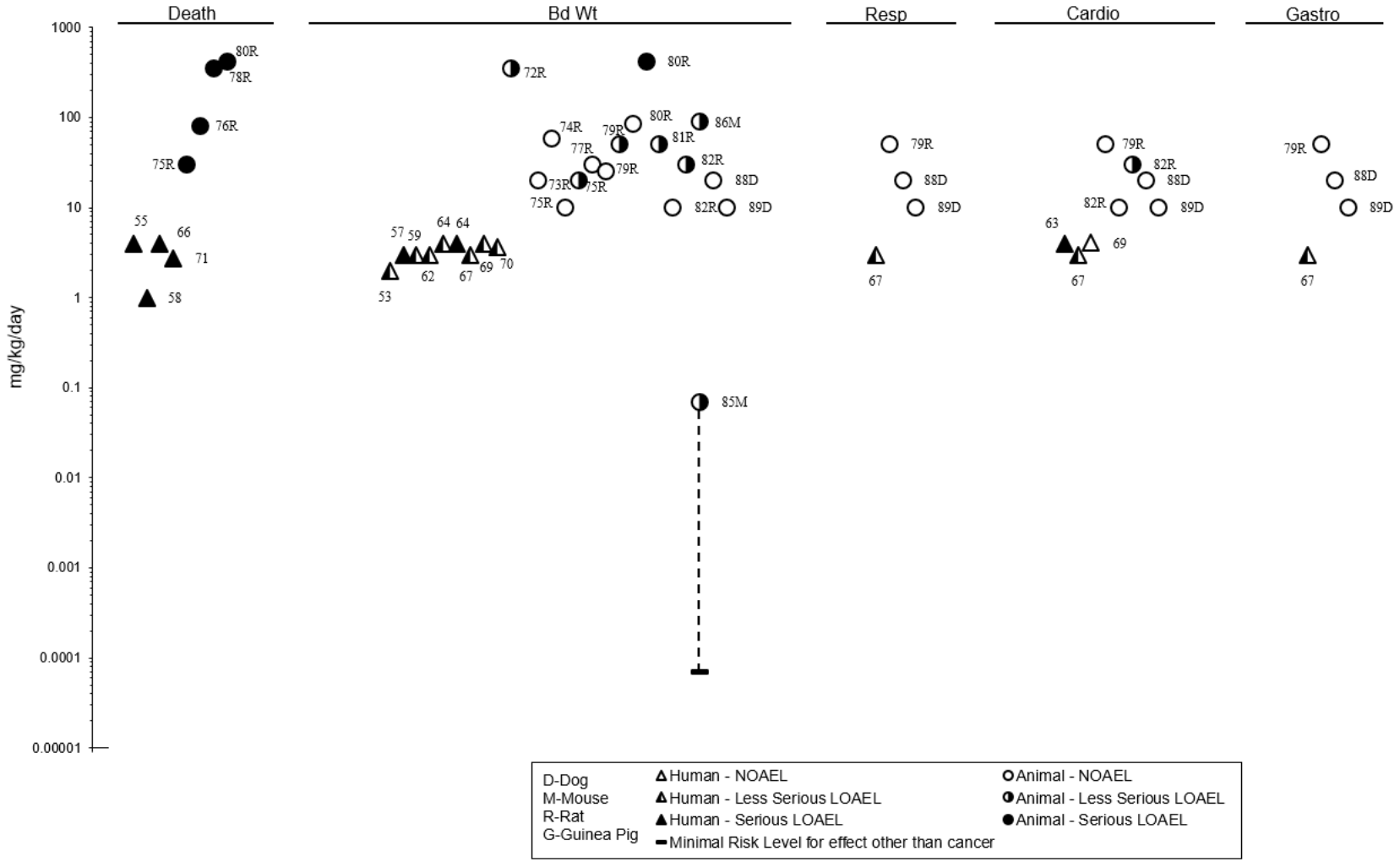

Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Intermediate (15-364 days)

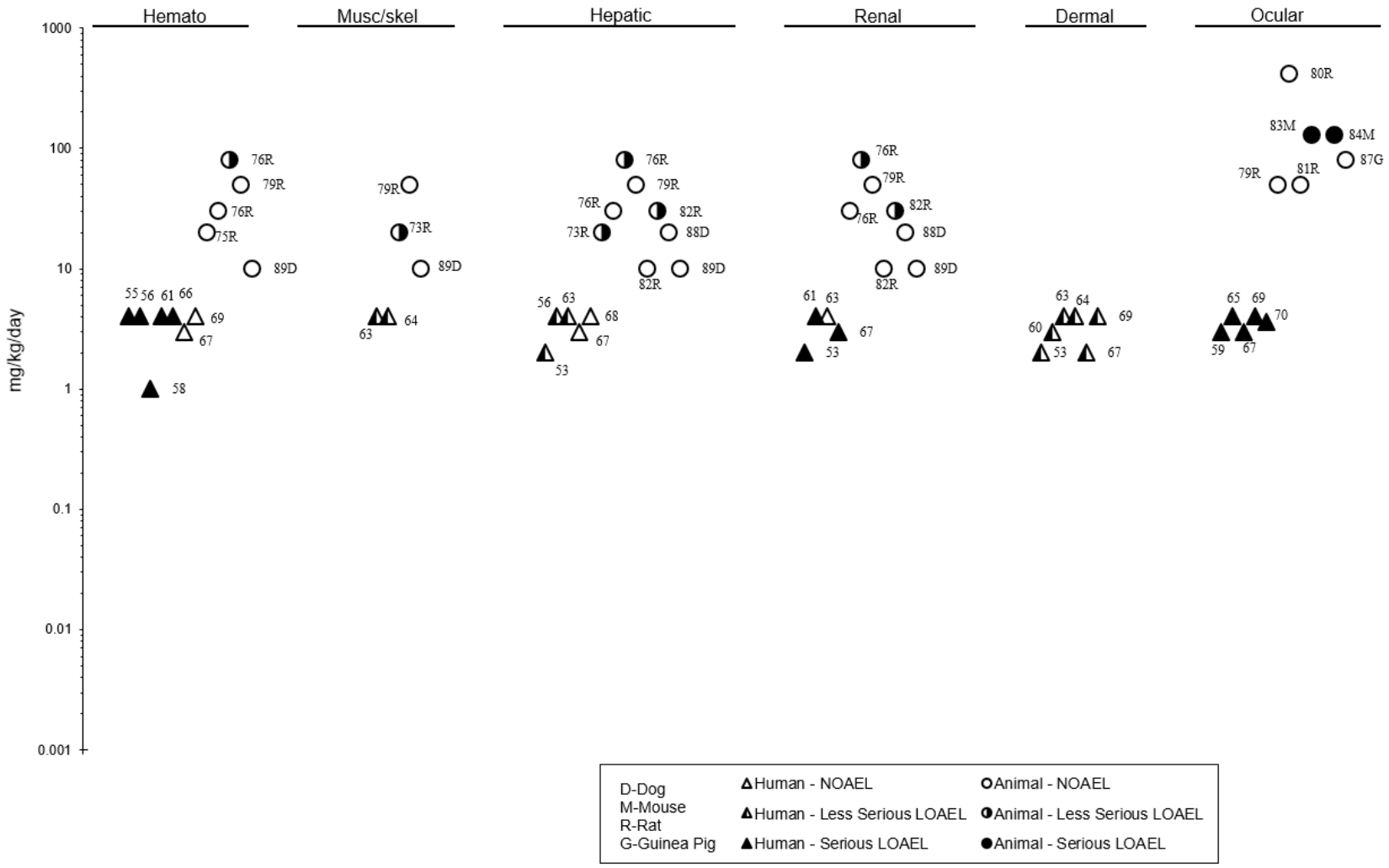

Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Intermediate (15-364 days)

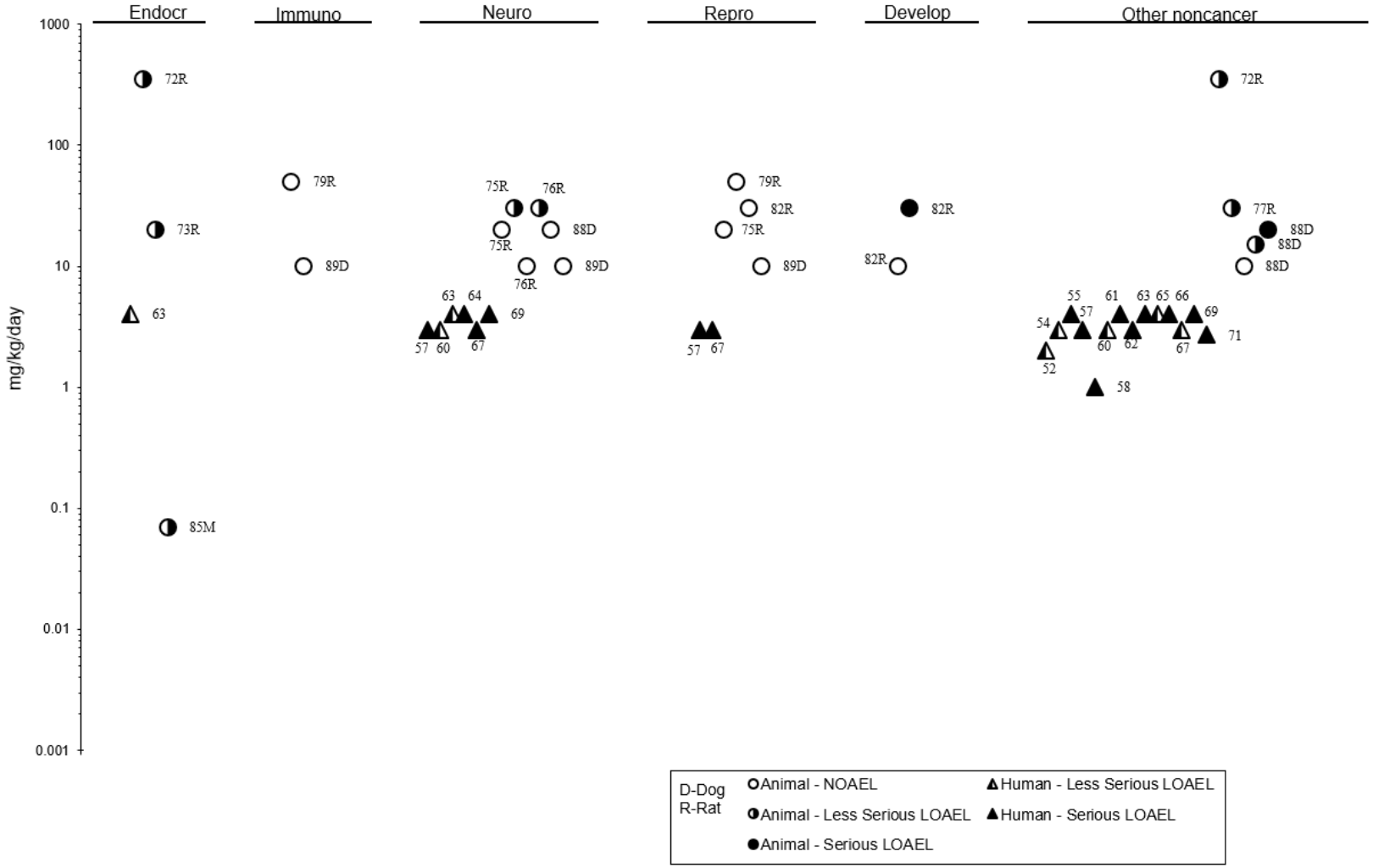

Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Chronic (≥365 days)

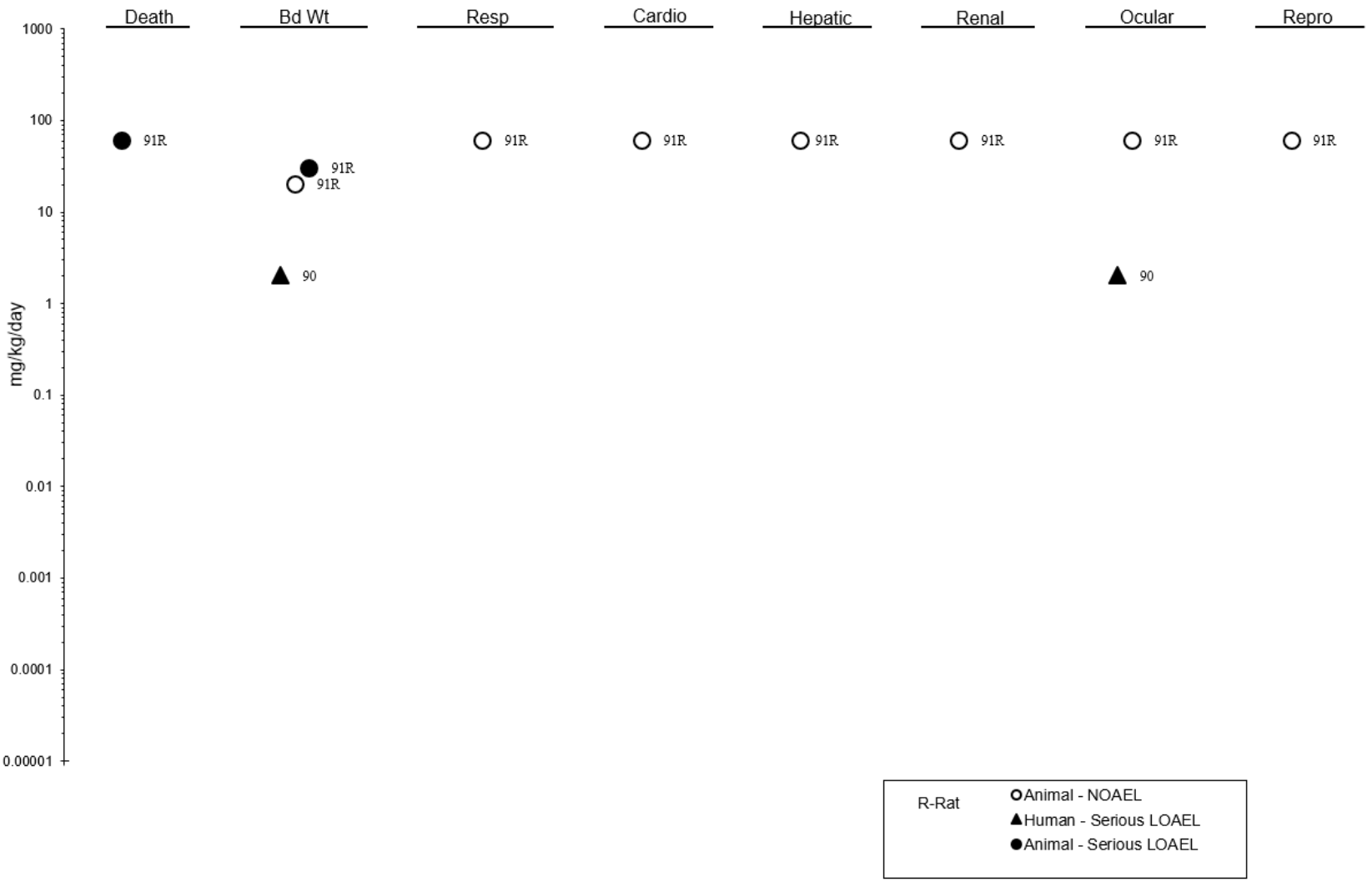

Table 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
ACUTE EXPOSURE								
Guinea pig (NS) 5 M, 5 F	Once 4 hours	100, 200, 300, 400, 500, 700, 1,000 mg/kg	LE	Death			300	1/5 died; 100% mortality at 1,000 mg/kg
Spencer et al. 1948								
Rabbit (NS) NS	6 times	4% in propylene glycol	CS	Dermal		4%		Moderate hyperemia, edema, and denaturation
Dow Chemical Co. 1940								
INTERMEDIATE EXPOSURE								
Rabbit (white) NS	4 weeks 5 days/week	3% in alcohol	CS	Dermal		3%		Mild hyperemia, edema, and exfoliation of skin
Spencer et al. 1948								

CS = clinical signs; F = female(s); LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified

2.2 DEATH

A number of case studies have reported human deaths after acute and intermediate oral exposure to 2,4-DNP consumed as a weight-loss agent (Dameshek and Gargill 1934; Goldman and Haber 1936; Hsiao et al. 2005; Kamour et al. 2015; Lattimore 1934; Masserman and Goldsmith 1934; McFee et al. 2004; Miranda et al. 2006; Pace and Pace 2002; Politi et al. 2007; Poole and Haining 1934; Silver 1934; Suozzi et al. 2005; Tainter and Wood 1934; Tewari et al. 2009; Zack et al. 2016) or with accidental or suicidal intent (Bartlett et al. 2010; Duflou 2019; Holborow et al. 2016; Hsiao et al. 2005; Siegmueller and Narasimhaiah 2010), and after acute and intermediate inhalation and dermal contact in occupational settings (Gisclard and Woodward 1946; Jiang et al. 2011, 2016; Lu et al. 2011; Perkins 1919).

The time course between exposure by ingestion and the onset of serious symptoms and/or death can be very rapid. Symptoms preceding death consisted of fever progressing to hyperthermia, agitation or restlessness, excessive sweating (diaphoresis), increased respiratory rate and gasping (dyspnea and tachypnea), increased heart rate (tachycardia), extreme thirst, nausea, and vomiting. Renal failure was reported in one instance (Bartlett et al. 2010). Muscle rigidity in some cases commenced before death, inhibiting mechanical ventilation (Miranda et al. 2006; Tewari et al. 2009). In most cases, death resulted from cardiac asystole. Autopsy findings typically included hyperemia, edema, and/or hemorrhage of lungs and sometimes other organs (e.g., Hsiao et al. 2005; Poole and Haining 1934; Lattimore 1934; Zack et al. 2016).

Table 2-3 provides a summary of the case reports of fatalities after 2,4-DNP intake. Some studies reported both 2,4-DNP intake and body weight information, while others reported only intake, so approximate doses were calculated using estimated body weights. As the table shows, single oral doses of 2,4-DNP in the range of 30–40 mg/kg were fatal in at least four cases (Bartlett et al. 2010; Holborow et al. 2016; Hsiao et al. 2005; Siegmueller and Narasimhaiah 2010). Deaths also occurred after repeated exposure for 3–5 days to doses in the range of 6–7 mg/kg/day (McFee et al. 2004; Poole and Haining 1934) and 14 days to an average dose of ~3 mg/kg/day (Masserman and Goldsmith 1934). In other clinical and experimental studies in which obese or normal weight subjects were given oral doses of 1–4 mg/kg/day 2,4-DNP for ≤14 days, there were no deaths (Castor and Beierwaltes 1956; Cutting and Tainter 1933; Cutting et al. 1934; MacBryde and Taussig 1935; Stockton and Cutting 1934; Tainter et al. 1935).

Table 2-3. Case Reports of Human Fatalities After Oral Exposure to 2,4-Dinitrophenol

Gender and age	Approximate lethal dose (mg/kg/day)	Exposure duration	Notes	Reference
Acute-duration exposure				
Male, 46 years old	~35	Once	DNP intake with suicidal intent. The patient consumed 2,800 mg. An average male body weight of 80 kg was assumed.	Bartlett et al. 2010
Male, 21 years old	~43	Once	DNP intake with suicidal intent. The patient consumed 4,250 mg. His BMI was 38 kg/m^2; a body weight of 100 kg was assumed for an obese male.	Holborow et al. 2016
Female, 17 years old	~31–38	Once	DNP intake with suicidal intent. The patient consumed 12–15 tablets each containing 192 mg 2,4-DNP. The authors reported the patient's body weight as 75 kg. The concentration of 2,4-DNP in the serum was 315 µg/mL.	Hsiao et al. 2005
Male, adult	~31–62	Once	DNP intake for weight loss. The intake estimated by an expert after death was 2,500–5,000 mg; upon hospital admission, the patient reported consuming 300 mg; the study authors estimated his weight as 80 kg.	Geiger 1933; Tainter and Wood1934
Female, 21 years old	~64	Once	DNP intake with suicidal intent. The patient characterized as obese, consumed 45 capsules each containing 100 mg. A body weight of 70 kg was assumed for an obese female. The concentration of 2,4-DNP in the blood was 12 mg/mL.	Purvine 1936
Male, adult	~35	Once	DNP intake with suicidal intent. The patient consumed 2,800 mg. An average male body weight of 80 kg was assumed.	Siegmueller and Narasimhaiah 2010
Male, 37 years old	46	Twice	DNP intake for weight loss. The patient ingested 3,700 mg 2,4-DNP as the sodium salt on two occasions 1 week apart; the study authors reported his weight as 80 kg.	Tainter and Wood 1934
Male, 22 years old	~6	4 days	DNP intake for weight loss. The patient, characterized as obese, consumed 600 mg 2,4-DNP per day. Body weight was assumed to be 100 kg for an obese male.	McFee et al. 2004
Female, 25 years old	7	5 days	DNP intake for weight loss. The patient ingested 2,880 mg over 5 days; the study authors reported her weight as 66.7 kg.	Poole and Haining 1934
Female, 31 years old	0.8–3.8 (TWA 2.7)	14 days	DNP intake for potential antidepressive effects. The patient ingested a total of 5,820 mg over 14 days; the study authors reported that her body weight ranged between 130 and 127 kg (average of 128.5 kg).	Masserman and Goldsmith 1934

Table 2-3. Case Reports of Human Fatalities After Oral Exposure to 2,4-Dinitrophenol

Gender and age	Approximate lethal dose (mg/kg/day)	Exposure duration	Notes	Reference
Intermediate-duration exposure				
Female, 25 years old	0.6–4	41 days	DNP intake for weight loss. The patient ingested increasing doses from 90 to 540 mg/day 2,4-DNP sodium salt (74–440 mg/day as 2,4-DNP) for 41 days. Her body weight was reported as 120 kg at the beginning of dosing and 117 kg at the end. Agranulocytosis diagnosed.	Silver 1934
Female, 46 years old	3–4	42 days	DNP intake for weight loss. The patient ingested 200 mg/day increasing to 300 mg/day. The patient was characterized as obese; body weight of 70 kg for obese female was assumed. Agranulocytosis diagnosed.	Dameshek and Gargill 1934
Male, 50 years old	1.7–5.4 (TWA 2.7)	43 days	DNP intake for weight loss. Doses were calculated from intakes and body weights reported by study authors.	Zack et al. 2016
Female, "young"	1.0	46 days	DNP intake for weight loss. The patient ingested 5,400 mg 2,4-DNP over 46 days; the study authors reported that her weight ranged between 120 and 109 kg (average of 114.5 kg). Agranulocytosis was diagnosed.	Goldman and Haber 1936
Fatalities lacking dose or duration Information				
Male, "young adult"	ND	Once	Ingestion of DNP capsules thought to be ecstasy. Postmorten blood level was 60 µg/mL.	Duflou 2019
Female, 27 years old	ND	ND	DNP intake for weight loss. The patient reportedly took twice the dose recommended by the website from which she purchased the chemical. No other information on exposure was provided, nor were blood levels.	Tewari et al. 2009
Male, 30 years old	ND	ND	DNP intake for body building. DNP was considered to be a contributing factor in the death, along with citalopram. Postmortem blood level was 48.4 mg/L.	Politi et al. 2007
Female, 17 years; male, 28 years old	ND	ND	DNP intake for weight loss (female) and body building (male). Blood levels were 36.1 and 28 mg/L in the female and male, respectively, on admission to the hospital.	Miranda et al. 2006
Male, 24 years old	ND	ND	DNP intake for weight loss/body building. No information on exposure or blood levels was provided.	Suozzi et al. 2005

Table 2-3. Case Reports of Human Fatalities After Oral Exposure to 2,4-Dinitrophenol

Gender and age	Approximate lethal dose (mg/kg/day)	Exposure duration	Notes	Reference
Female, 29 years old	ND	See notes	DNP intake for weight loss. Patient took 3–5 tablets a day for several months, discontinued its use for 3 months, and then resumed taking 5 tablets/day for 1 week. The dose per tablet was not provided.	Lattimore 1934

BMI = body mass index; DNP =dinitrophenol; ND = no data; TWA = time-weighted average

Autopsies were performed in some, but not all, cases; findings on autopsy are typically secondary effects of 2,4-DNP. For example, an autopsy revealed pulmonary edema and pale spots on the liver indicative of ischemic necrosis in a 17-year-old girl who died 10 hours after ingesting ~31–38 mg/kg 2,4-DNP with suicidal intent (Hsiao et al. 2005). Autopsy and histological examination of a woman who died after ingesting 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days showed hyperemic and hemorrhagic lungs, degeneration of renal tubules and liver cells, segmentation and fragmentation of cardiac muscles, and hemorrhagic spleen, stomach mucosa, spinal cord, pons, and medulla (Poole and Haining 1934).

Intermediate-duration exposures (41–46 days) to oral doses as low as 1–5 mg/kg/day have resulted in human deaths (Dameshek and Gargill 1934; Goldman and Haber 1936; Silver 1934; Zack et al. 2016). In three early studies, deaths were attributed to agranulocytosis (Dameshek and Gargill 1934; Goldman and Haber 1936; Silver 1934). In the case of an obese man who died after taking between 1.7 and 5.4 mg/kg/day of 2,4-DNP for 44 days, autopsy findings consisted of hemorrhagic pulmonary edema, slight coronary sclerosis, ectasia of the thoracic artery, and recent thrombosis of single peripheral pulmonary arteries (Zack et al. 2016). Other human studies from the 1930s of intermediate-duration exposure to doses of 4–5 mg/kg/day 2,4-DNP for weight loss reported no deaths (Cutting et al. 1934; Grant and Schube 1934; Looney and Hoskins 1934; MacBryde and Taussig 1935; Simkins 1937a, 1937b; Tainter et al. 1934a, 1935b).

Deaths have also occurred after inhalation and dermal exposure to 2,4-DNP. Two workers exposed to mists and dust of 2,4-DNP in a U.S. chemical plant for a few months developed signs of toxicity (fever, profuse sweating, restlessness); following treatment, rest, and a return to work, both collapsed, and died (Gisclard and Woodward 1946). The warmer weather during the second period of exposure (duration not specified) was thought to be a contributing factor because of the greater skin exposure and potential for

increased dermal absorption. In addition, warmer temperatures could potentially exacerbate effects of 2,4-DNP. Exposures levels in the workroom were described by study authors as "2,4-DNP dust" that is "normally present" at a concentration of "at least" 40 mg/m^3. However, the workroom air level was determined after the death occurred and it is not known if the measured levels were obtained from the breathing zone. In addition, significant dermal exposure and even oral absorption may have contributed to the total dose.

Limited information on death associated with chronic exposure to 2,4-DNP is available. Fatal cases of 2,4-DNP poisoning were reported among workmen in the munitions industry in France (Perkins 1919). These men were exposed to airborne vapor and dust of 2,4-DNP and had direct dermal contact with the chemical in solid form. There was poor quantitation in this study since neither duration nor level of exposure was reported. The deaths were preceded by sudden onset of extreme fatigue, elevation of the body temperature to ≥40°C, and other clinical signs of 2,4-DNP poisoning, such as profuse sweating, thirst, and labored respiration. No characteristic lesions were found at autopsy. Following the institution of better ventilation, use of masks, and other industrial hygiene measures to minimize exposure in the French munitions industry, the numbers of deaths per 10,000 tons 2,4-DNP manufactured per year decreased from 16.3 to 1.2 (Perkins 1919).

Animal studies of mortalities after acute gavage (stomach tube) exposure to 2,4-DNP had deficiencies in experimental protocol (group sizes were small; statistical analysis was not performed) and reporting (doses, strain, sex, and numbers of animals were often not reported). The available data are summarized in Table 2-4. As the table shows, LD_{50} values for animals treated once by gavage ranged between 30 and 320 mg/kg for rats; an LD_{50} of 72 mg/kg was reported for weanling CF1 mice. Two studies in dogs using group sizes between one and three animals per dose reported no mortality at 20 mg/kg (Tainter and Cutting 1933b) and death at doses ≥25 mg/kg (Kaiser 1964). No mortality was observed in pregnant mice given 38.3 mg/kg/day 2,4-DNP on gestation days 10–12 (Gibson 1973).

Table 2-4. Mortality in Laboratory Animals Given a Single Gavage Dose of 2,4-Dinitrophenol

Species	Strain	Sex	Age	Number per dose	Dose (mg/kg)	Effect	Reference
Rat	White	NR	NR	NR	20	No mortality	Dow Chemical Co. 1940
					60	100% mortality	
Rat	White	NR	NR	NR	30	LD_{50}	Dow Chemical Co. 1950

Table 2-4. Mortality in Laboratory Animals Given a Single Gavage Dose of 2,4-Dinitrophenol

Rat	NR	Male	Weanling	NR	71	LD_{50}	Kaiser 1964
Rat	NR	Male and female	"Mature"	9–40	27	No mortality	Spencer et al. 1948
					30	37% mortality	
					100	100% mortality	
Rat	Harlan Fischer	Female	NR	4	140	No mortality	Eli Lilly and Co. 1992
					320	LD_{50}	
					500	100% mortality	
Mouse	CF1	Male	Weanling	NR	72	LD_{50}	Kaiser 1964
Dog	NR	NR	NR	1–3	25	33% mortality	Kaiser 1964
					125	100% mortality	
Dog	NR	NR	NR	1–3	20	No mortality	Tainter and Cutting 1933b
					30	100% mortality	

DNP = dinitrophenol; NR = not reported

While the acute lethality data are limited, the available information suggests that species differences in the lethality of 2,4-DNP are relatively small; apart from the study by Eli Lilly and Co. (1992), most of the data suggest that single bolus doses in the range of 30 mg/kg can be fatal to rats and dogs (and possibly mice). In addition, the estimated doses (~31–75 mg/kg) in cases of human fatalities after single oral exposures (see Table 2-3) are similar in magnitude to lethal doses in animals. Animals that survived near-lethal doses often had few other effects. In one study, the authors indicated that rats treated once by gavage either died within 1–2 hours or recovered completely (Spencer et al. 1948). In an LD_{50} test in rats, survivors of a single gavage dose had a temporary increase in respiration rate but gained weight at the same rate as controls during the 7-day observation period (Kaiser 1964).

Studies of lethality after acute-duration exposure to 2,4-DNP via the diet are of little utility, as food intake was not reported in the mouse studies (rendering doses uncertain), guinea pigs were exposed while receiving a vitamin C-deficient diet, and other studies are in birds. Adult yellow adipose and albino mice (sex not reported) exposed to 108 mg/kg/day 2,4-DNP in the diet all died approximately 8 hours later, while 2/8 young albino mice (initial age 5–6 weeks; sex not reported) died following dietary exposure to 325 mg/kg/day for nearly 1 week (Bettman 1946). In a study examining whether vitamin C would prevent cataracts, one of four guinea pigs exposed to 40 mg/kg/day of 2,4-DNP in a vitamin C-deficient diet without vitamin supplementation died in 11 days (Ogino and Yasukura 1957). When bobwhite quail were exposed to 2,4-DNP in the diet, one of six hens in a group consuming 56.1 mg/kg/day died on the eighth and final day of exposure (Dominguez et al. 1993). Necropsy revealed a marked scarcity of

subcutaneous fat, reduced visceral fat, and possibly some shrinkage of leg and breast muscles. No deaths were recorded in a study of 20 young broiler chickens exposed to 2,4-DNP in feed between days 7 and 20 of life at estimated doses of 16.5, 36.3, and 77.9 mg/kg/day (Toyomizu et al. 1992).

Studies regarding death in animals after gavage exposure to 2,4-DNP for intermediate durations were limited, but studies conducted under modern protocols showed that daily gavage doses of 30 mg/kg/day for 18 days were fatal to most (6/10) newborn rats (postnatal day [PND] 4 at exposure initiation), but older rats (5–6 weeks old at exposure initiation) survived for 6 weeks at this dose, succumbing (8/24 died) only at doses of 80 mg/kg/day for up to 28 days (Koizumi et al. 2001, 2002; Takahashi et al. 2009). Earlier studies by Dow Chemical Co. provided contradictory information for deaths following intermediate-duration exposure, compared to acute-duration exposure. A 4-week gavage study in rats of unspecified age survived gavage doses of 30 mg/kg/day 2,4-DNP, 5 days/week for 4 weeks (Dow Chemical Co. 1940). In contrast, Dow Chemical Co. (1950) reported an LD_{50} in rats administered a single dose of 2,4-DNP of 30 mg/kg 2,4-DNP; no explanation was given for this apparent contradiction.

A number of intermediate-duration studies of lethality used dietary administration of 2,4-DNP. Kaiser (1964) fed male and female weanling rats diets containing 2,4-DNP for 4 weeks, and no mortality was observed at author-estimated doses ≤59 mg/kg/day. Other dietary studies of intermediate duration lacked a concurrent control group (Pugsley 1935), used very small numbers of animals (Ogino and Yasukura 1957; Tainter et al. 1934b), or neglected to report doses or feed intake by animals exposed to 2,4-DNP *ad libitum* in the diet (Spencer et al. 1948; Tainter 1938). The lack of dose or feed intake information, particularly given the well-demonstrated effect of 2,4-DNP exposure on body weight (see Section 2.3), renders the dose-response information from these studies uncertain. Sixteen rats of unspecified age exposed to an author-estimated dose of 110 mg/kg/day 2,4-DNP in the diet for 26 days consumed a "normal" amount of food; the authors did not report mortality rate, but the presentation of the data implied 100% survival (Pugsley 1935). Rats exposed to 0.20–0.24% (≥350 mg/kg/day based on default body weight and food intake values from EPA [1988a]) 2,4-DNP failed to eat or grow and died within 5–94 days (Spencer et al. 1948; Tainter 1938). No mortality was observed after a 6-month dietary exposure to ≤0.10% 2,4-DNP (Spencer et al. 1948). One of two guinea pigs exposed to 40 mg/kg/day of 2,4-DNP in a vitamin C-deficient diet but with vitamin C injections of 2 mg/day died in 28 days (Ogino and Yasukura 1957). No mortality was observed in three male dogs fed 10 mg/kg/day in capsules for 6 months (Tainter et al. 1934b).

Only three chronic-duration experiments with 2,4-DNP exposure are available: two used dietary administration in rats (both reported by Tainter [1938]) and the third was a drinking water study in mice (Caldeira da Silva et al. 2008). In rats, dietary doses ≥60 mg/kg/day (estimated based on food factor of 0.05 kg diet/kg body weight/day reported by the authors) reduced lifespan by 50% compared to controls, but lifespan was not affected at 40 mg/kg/day (Tainter 1938). In a well-conducted and reported study of drinking water exposure in mice (Caldeira da Silva et al. 2008), a dose between 0.03 and 0.105 mg/kg/day from 18 weeks of age through natural death was associated with a statistically significant increase in lifespan compared with controls.

One study examined lethality in guinea pigs exposed dermally to 2,4-DNP for 4 hours. No mortality was observed at 100 or 200 mg/kg, while 300 mg/kg resulted in 20% mortality (1/5) and 700 mg/kg resulted in 100% mortality (Spencer et al. 1948).

The mechanism of 2,4-DNP lethality is pyrexia induced by the uncoupling of oxidative phosphorylation (see also Section 2.18.1). During uncoupling, the energy produced by electron transport from NADH to oxygen, which is normally stored as the chemical energy of ATP, is instead released as heat. Physiological responses aimed at dissipating the heat ensue, but fatal hyperpyrexia can occur if the body temperature becomes severely elevated. Death from 2,4-DNP exposure results from the action of the parent compound rather than metabolites. Available data on the lethality of 2,4-DNP metabolites shows rat oral LD_{50} values of 2,400 and >4,000 mg/kg for 2-amino-4-nitrophenol (Lloyd et al. 1977) and 2-amino-5-nitrophenol (Burnett et al. 1977), respectively; these values are far higher than rat oral LD_{50} values (30–320 mg/kg) reported for 2,4-DNP (Dow Chemical Co. 1950; Eli Lilly and Co. 1992; Kaiser 1964).

The acute lethality of other DNP isomers was evaluated in rats and mice exposed by intraperitoneal injection (Harvey 1959). In rats tested at moderate temperature (64–70°F), LD_{50} values were 190, 35, 150, 38, 98, and 45 mg/kg for 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNP, respectively. In other experiments by the same author, mice were exposed at different ambient temperatures to evaluate the effect of temperature on the toxicity of DNPs (Harvey 1959). Table 2-5 shows the results. Taken together with the rat data, the acute lethal potencies reflected by these data suggest that 2,4- and 2,6-DNP are of comparable lethality, followed by 3,5- and 3,4-DNP-, while 2,3-DNP and 2,5-DNP are the least potent. The data suggest increasing lethality of 2,4-DNP and 2,6-DNP with higher ambient temperature, which is consistent with the hyperthermic properties of 2,4-DNP. The potencies of the other isomers show less change with temperature, indicating that these may induce death via other mechanisms.

Table 2-5. Temperature Dependence of Intraperitoneal LD_{50} Values in Mice

Ambient temperature		LD_{50} (mg/kg)					
(°F)	(°C)	2,4-DNP	2,6-DNP	3,5-DNP	3,4-DNP	2,3-DNP	2,5-DNP
64–70[a]	18–21	36	45	50	112	200	273
95–99	35–37	35	37	47	115	160–175	250
102–106	39–41	<5 (all died)	<10 (all died)	50	100–110	160–175	200

[a]Experiments in rats at this temperature yielded LD_{50} values very similar to the LD_{50} values in mice. Rats were not tested at other temperatures.

Source: Harvey (1959)

2.3 BODY WEIGHT

The effects of 2,4-DNP on body weight have been known since the 1930s, when the compound was widely prescribed for weight loss. While no longer used in medicine due to its risk of cataracts (see Section 2.12), death (see Section 2.2), and other adverse effects, the compound is still marketed for weight loss by unregulated internet sources. Body weight losses result from the uncoupling of oxidative phosphorylation (see Section 2.18.1), which increases the basal metabolic rate.

Body weight losses have been described in a number of clinical studies from the 1930s examining acute- and intermediate-duration exposure to 2,4-DNP for weight loss. These studies indicate that body weights are reduced at doses between 1 and 4 mg/kg/day for as little as 7 days. When four volunteers were placed on various diets (balanced, high carbohydrate, high fat, or high protein) and given an average dose of 4 mg/kg/day 2,4-DNP for 7–16 days, the average weight loss was ~2 pounds (0.92 kg) during the 2,4-DNP treatment period (Cutting and Tainter 1933). The type of diet did not appear to influence the degree of weight loss. Thirty-seven obese patients who took 1 mg/kg/day 2,4-DNP as the sodium salt for an average of 14 days had an average weight loss of 0.43 kg/week (Tainter et al. 1935). They had not been losing weight at the time treatment began and had been given instructions to continue the same food intake as before treatment.

Several clinical studies reported body weight losses averaging ~0.5–1 kg/week following administration of 2,4-DNP in obese or psychiatric patients for intermediate durations (Bayer and Gray 1935; Looney and Hoskins 1934; MacBryde and Taussig 1935; Masserman and Goldsmith 1934; Simkins 1937a, 1937b;

Tainter et al. 1935; Whalman 1936). In these studies, doses of 2–4 mg/kg/day were administered for durations ranging between 1 week and 18 months. A number of individual case reports have also described substantial weight losses in patients taking 2,4-DNP doses between 1 and 4 mg/kg/day for durations between 21 days and 18 months (Beinhauer 1934; Epstein and Rosenblum 1935; Goldman and Haber 1936; Horner et al. 1935; Nadler 1935; Zack et al. 2016). Two of the cases died (Goldman and Haber 1936; Zack et al. 2016; see Section 2.2).

Workmen exposed to 2,4-DNP (via inhalation and dermal contact) in the French munitions industry reportedly experienced weight loss to the point of excessive thinness after several months of exposure (Perkins 1919); however, the amount of weight loss, exposures, and durations were not characterized.

Although most of the available acute-duration oral exposure studies in rodents described body weight losses, reporting deficiencies in some of the studies preclude accurate dose estimates. Body weight was not affected in Jcl:SD rats after five daily gavage doses of 30 mg/kg/day 2,4-DNP (Takahashi et al. 2004) or in Harlan Fischer rats after a single dose of up to 140 mg/kg/day (Eli Lilly and Co. 1992). In mice exposed to DNP in drinking water for 7 days, body weight losses (magnitude not reported) were seen at a dose of 89 mg/kg/day; food and water intake were not affected at this dose (Goldgof et al. 2014). Marked decreases in body weight of 12–36% were observed in rats fed 0.2% 2,4-DNP (~350 mg/kg/day) in the diet *ad libitum* for 9–14 days (England et al. 1973; Maayan 1968; Wilkins et al. 1974). Food intake was not reported in these studies, and other studies demonstrated markedly reduced food intake at this dietary concentration (Spencer et al. 1948; Tainter 1938), rendering the dose estimates uncertain and raising the possibility that starvation may have contributed to the body weight effects.

In intermediate-duration oral studies of 2,4-DNP, significant decreases in body weight parameters without concurrent decreases in food consumption were observed in rats exposed by gavage to doses of 20 mg/kg/day from PND 4 to 21 (Koizumi et al. 2001, 2002) or 30 mg/kg/day for 45 days (Takahashi et al. 2009), in rats given dietary doses of 110 mg/kg/day for ~25 days (Pugsley 1935) or 50 mg/kg/day for 6 months (Spencer et al. 1948), in C57Bl/6J mice given 89 mg/kg/day 2,4-DNP in drinking water for 8 weeks (Goldgof et al. 2014), and in mice exposed to 0.03–0.105 mg/kg/day 2,4-DNP in drinking water for 20–50 weeks (Caldeira da Silva et al. 2008). A small (5.2%), statistically significant decrease in body weight was observed in rats after 28 days of exposure to 2,4-DNP in drinking water at a dose of 30 mg/kg/day (Schlagowski et al. 2014). However, body weight was not significantly altered in rats after 4 weeks of dietary exposure to 59 mg/kg/day 2,4-DNP (Kaiser 1964) or in three male dogs fed 10 mg/kg/day in capsules 6 days/week for 6 months (Tainter et al. 1934b). At higher dietary doses,

marked body weight loss and death have been reported after intermediate-duration exposure; taste aversion and starvation may have contributed to these effects. An 18% decrease in body weight gain was observed in rats exposed to 0.2% 2,4-DNP (~350 mg/kg/day) for 30 days (Bakke and Lawrence 1965). Weanling rats fed 0.24% 2,4-DNP (~420 mg/kg/day) in the diet ate very little, gained weight at 1/15 the rate of controls, and died within 5–94 days (Tainter 1938). Rapid weight loss and mortality (within 21 days) were observed in weanling rats exposed to 0.2% 2,4-DNP (~350 mg/kg/day) in the diet (Spencer et al. 1948). Two dogs fed capsules containing 5, 10, 15, 17.5, or 20 mg/kg/day 2,4-DNP intermittently (between 1 and 19 days between doses) for 45–77 days did not exhibit changes in body weight (Tainter and Cutting 1933b).

Decreased body weight was also reported in the single available chronic animal study. Tainter (1938) reported a 25% decrease in body weight gain, without a significant change in food intake, among male white rats exposed to ≥30 mg/kg/day 2,4-DNP in feed for their lifespan.

Studies in birds also showed body weight changes with administration of 2,4-DNP. In female chickens fed 2,4-DNP-containing feed from age 7 to 20 days, body weight gain was significantly different from controls (~12% lower) at a dose of 77.9 mg/kg/day, but not at 16.5 or 36.3 mg/kg/day (Toyomizu et al. 1992). No decrease in feed consumption was observed. Total body fat as a percentage of body weight was slightly decreased at doses ≥36.3 mg/kg/day (10.2% at 36.3 mg/kg/day and 9.4% at 77.9 mg/kg/day versus 12.7% in controls). The body weights of female bobwhite quail aged 22–26 weeks fed 2,4-DNP-containing feed for 8 days fell approximately 13% in quail that consumed 56.1 mg/kg/day, but were unaffected at a dose of 33.6 mg/kg/day (Dominguez et al. 1993). These authors also reported no effect of 2,4-DNP on feed consumption except for a reduction on the first 2 days at the 56.1 mg/kg/day dose. Necropsy of birds that consumed 56.1 mg/kg/day revealed a marked scarcity of subcutaneous fat, reduced visceral fat, and possibly some shrinkage of leg and breast muscles; these changes were not seen at 33.6 mg/kg/day.

While the predominant mechanism for body weight losses from exposure to 2,4-DNP is likely to be uncoupling of oxidative phosphorylation (see Section 2.18.1), metabolites may also play a role, albeit a somewhat small role. No effect on body weight was seen in a 15-day study in rats and mice exposed to gavage doses up to 5,000 mg/kg 2-amino-4-nitrophenol 5 days/week (NTP 1988a). Decreased body weight was observed in male rats (but not female rats or male or female mice) exposed to the 2,4-DNP metabolite, 2-amino-4-nitrophenol, at 500 mg/kg on 5 days/week for 13 weeks or 250 mg/kg for 2 years (NTP 1988a). Exposure to 4-amino-2-nitrophenol in the diet did not affect body weights in rats or mice

exposed for 6 weeks, or in mice or male rats exposed for 2 years (NCI 1978). However, in female rats receiving dietary doses ≥253 mg/kg/day for 2 years, body weights were ~20% lower than controls (NCI 1978).

2.4 RESPIRATORY

Literature reports of respiratory effects of 2,4-DNP consist largely of dyspnea, tachypnea, pulmonary edema, and hemorrhage in cases of fatal human exposures. Some of these effects occur as physiological responses to hyperthermia/hyperpyrexia caused by uncoupling of oxidative phosphorylation (see Section 2.18.1); however, other effects, such as pulmonary edema and hemorrhage are pathological, not adaptive, effects.

A clinical study of 2,4-DNP reported increased respiratory rates (15–30 breaths/minute) in eight subjects exposed once to doses >10 mg/kg (exact doses not specified) (Cutting et al. 1933). Tachypnea was observed in a woman who took ≥10 mg/kg 2,4-DNP once for weight loss and survived severe poisoning (van Veenendall et al. 2011). A clinical study in which obese patients took capsules of the sodium salt of 2,4-DNP at 4 mg/kg/day 2,4-DNP for 4–12 days, showed no change in vital capacity (Stockton and Cutting 1934). A patient who took 2 mg/kg/day 2,4-DNP for 14 days and had severe dermatological reactions did not exhibit dyspnea (Anderson et al. 1933).

Other case reports have also reported tachypnea and/or dyspnea in patients who died after taking 2,4-DNP for acute durations (Bartlett et al. 2010; Geiger 1933; Holborow et al. 2016; Hsiao et al. 2005; Lee et al. 2014; Miranda et al. 2006; Mustonen et al. 2004; Purvine 1936; Siegmueller and Narasimhaiah 2010; Suozzi et al. 2005; Tainter and Wood 1934; Tewari et al. 2009). In a man who died after ingesting 46 mg/kg 2,4-DNP as the sodium salt in two doses taken 1 week apart, autopsy showed pulmonary edema (Tainter and Wood 1934). Dyspnea, a respiratory rate as high as 48 respirations/minute, and coarse rales were noted in a woman who subsequently died after taking 3 mg/kg/day for 2 weeks (Masserman and Goldsmith 1934). In another fatal case, the respiratory rate was 56 respirations/minute when a patient who took 7 mg/kg/day for 5 days was admitted to the hospital (Poole and Haining 1934). Autopsy revealed hyperemic and hemorrhagic lungs, congestion of alveolar walls, and edema in alveoli.

Elevated respiratory rates and dyspnea have also been reported in patients who took 1–4 mg/kg/day 2,4-DNP for intermediate durations (Epstein and Rosenblum 1935; Goldman and Haber 1936; Imerman and Imerman 1936; Simkins 1937a, 1937b; Zack et al. 2016). Two of these patients died (Goldman and

Haber 1936; Zack et al. 2016); vascular congestion was found in the lungs of the one patient for whom autopsy was performed (Goldman and Haber 1936)

A study from the early 1900s reported that workmen exposed to 2,4-DNP in the French munitions industry exhibited short and labored respiration, but the lungs were clear on physical examination (Perkins 1919). Autopsies of fatal cases did not reveal any characteristic lesions other than edema of the lungs, which was thought to be secondary to "intoxication of the vasomotor system." Exposure levels, durations, and incidences were not characterized.

Increased respiratory rates (quantitative data not reported) were observed in dogs exposed to 25 mg/kg/day for 1–14 days and to 125 mg/kg/day for 1 day (Kaiser 1964). Temporary increases in respiration (quantitative data not reported) were observed in survivors of a single-dose lethality test (doses not reported) in rats and mice (Kaiser 1964).

No gross or histological evidence of treatment-related pulmonary damage was reported following 2,4-DNP treatment of rats exposed in the diet to 5–50 mg/kg/day for 6 months (Spencer et al. 1948), rats exposed in the diet to 60 mg/kg/day for life (Tainter 1938), dogs (one to two per group) given 20 mg/kg/day via capsule 7–12 times in 45–77 days (Tainter and Cutting 1933b), or dogs (three per dose group) exposed via capsules to 5 or 10 mg/kg/day for 6 months (Tainter et al. 1934b). More recently-conducted animal studies did not evaluate respiratory effects or gross or microscopic pathology of the lungs (Caldeira da Silva et al. 2008; Goldgof et al. 2014; Haasio et al. 2002a, 2002b; Koizumi et al. 2001, 2002; Perry et al. 2015a,b; Schlagowski et al. 2014; Takahashi et al. 2004, 2009).

2.5 CARDIOVASCULAR

Cardiovascular effects observed in humans and animals exposed to 2,4-DNP stem from its uncoupling of oxidative phosphorylation and consequent increases in basal metabolic rate and body temperature (see Section 2.18). Increases in pulse rate, heart rate, and blood pressure, common findings in people who took 2,4-DNP for weight reduction (Cutting et al. 1933; Dunlop 1934; Tainter and Wood 1934), are physiological responses to higher body temperature and metabolic rate.

An average increase in venous blood pressure (measured directly in the median cubital vein) as high as 37% was seen in normal subjects who ingested the sodium salt of 2,4-DNP at 4 mg/kg/day 2,4-DNP for 4–12 days (Stockton and Cutting 1934). Systolic and diastolic blood pressures were not affected; pulse

rate increased as much as 12%. The changes in venous pressure and pulse rate tended to occur during episodes of peripheral vasodilatation and appeared, therefore, to be compensatory mechanisms for the maintenance of normal blood pressure. An intermediate-duration clinical study of six patients treated for 1–8 weeks with 2,4-DNP at 4 mg/kg/day showed changes in the electrocardiograms (increased size or inversion of T wave, depression of ST interval, notching of QRS complex) of three patients (MacBryde and Taussig 1935). The changes began to appear at the end of the second week of dosing and became more marked toward the end of the 8 weeks, persisting in two patients at 2 weeks after cessation of treatment. In an extensive clinical study of 159 people, pulse, blood pressure, and electrocardiograms were monitored in 16 of the individuals taking about 3 mg/kg/day (Simkins 1937a, 1937b). No abnormal electrocardiographic tracings were found, but bradycardia was observed in two cases. Blood pressure was reduced in 10 formerly hypertensive patients, but no blood pressure changes were found in normotensive individuals. The reasons for the fall in blood pressure in the hypertensive patients and for the bradycardia are not apparent. In 13 psychiatric patients given 2,4-DNP to determine whether the drug had a beneficial effect on depression, no changes in blood pressure were found, but pulse rates increased from 4 to 22 beats per minute above predosing rates (Masserman and Goldsmith 1934). In two clinical studies in obese patients given 4 mg/kg/day 2,4-DNP for 88 days (Tainter et al. 1935) and schizophrenic patients given 3 mg/kg/day 2,4-DNP for 7 weeks (Looney and Hoskins 1934), no appreciable changes in pulse or blood pressure were found.

Case reports of individuals hospitalized after 2,4-DNP exposure showed increased pulse rates (106–136 beats per minute) in patients taking ~3–4 mg/kg/day for 1–6 months (Epstein and Rosenblum 1935; Imerman and Imerman 1936). A woman taking 4 mg/kg/day 2,4-DNP for 2 months complained of heart palpitations while taking the drug (Rank and Waldeck 1936). A case report of a patient who took 2 mg 2,4-DNP for 14 days and developed severe dermatological symptoms reported no changes in blood pressure or heart rate during the dosing period (Anderson et al. 1933).

At lethal doses (see Section 2.2), very rapid pulse and heart rates have been reported prior to death (Goldman and Haber 1936; Masserman and Goldsmith 1934; Poole and Haining 1934). In a woman who took 7 mg/kg/day for 5 days, a pulse of 140 was reported prior to her death (Poole and Haining 1934). Autopsy findings included marked segmentation and fragmentation of the cardiac muscles (Poole and Haining 1934). Prior to her death, a psychiatric patient given 3 mg/kg/day 2,4-DNP as the sodium salt for 14 days had an increased pulse of 148 beats per minute (Masserman and Goldsmith 1934). In addition, her respirations increased to 48 per minute, and her temperature increased to 102°F; she became comatose, and her blood pressure fell from 144/68 to 36/0 (Masserman and Goldsmith 1934). Autopsy,

conducted 4 days after death, revealed slight scarring of the tricuspid and mitral valves, hypertrophy of the right ventricle, and small scattered fatty deposits in the aorta (Masserman and Goldsmith 1934). A young girl took 1 mg/kg/day for 46 days and after being admitted to the hospital had a pulse of 136 beats per minute and irregular heart sounds before she died (Goldman and Haber 1936). In another fatal case in which the dose was not reported, pulse rate and temperature were reported to be normal, despite other symptoms of 2,4-DNP poisoning (diaphoresis, nausea, and vomiting); at autopsy, her death was attributed to myocarditis (Lattimore 1934).

Cardiovascular effects were observed in an acute oral exposure study in dogs, but most intermediate- and chronic-duration studies did not report cardiovascular effects. Increased heart rates (quantitative data not shown) and highly abnormal electrocardiogram tracings were observed in dogs fed capsules containing 25 mg/kg/day for 1–14 days or 125 mg/kg for 1 day (Kaiser 1964). Relative heart weight was significantly increased (7%, without change in body weight) in female rats exposed by gavage to 30 mg/kg/day 2,4-DNP for 40–47 days; hearts were not examined for histopathology (Takahashi et al. 2009). No gross or histological evidence of treatment-related cardiac damage was reported following 2,4-DNP treatment of rats exposed in the diet to 5–50 mg/kg/day for 6 months (Spencer et al. 1948), rats exposed in the diet to 60 mg/kg/day for life (Tainter 1938), dogs (one to two per group) given 20 mg/kg/day via capsule 7–12 times in 45–77 days (Tainter and Cutting 1933b), or dogs (three per dose group) exposed via capsules to 5 or 10 mg/kg/day for 6 months (Tainter et al. 1934b).

2.6 GASTROINTESTINAL

Gastrointestinal effects, including nausea, vomiting, diarrhea, and hemorrhagic lesions of the gastrointestinal tract, are classified as secondary effects in the presence of hyperthermia or primary effects in the absence of hyperthermia. Nausea, vomiting, and diarrhea, were common findings in people who took 2,4-DNP for acute durations in an effort to lose weight. Gastrointestinal disturbances and vomiting occurred in 5 of 15 patients who ingested 2,4-DNP at 4.3 mg/kg/day for 1–8 weeks; the duration of treatment for the affected patients was not specified (MacBryde and Taussig 1935). Body temperature was not reported in this study; therefore, it is possible that the gastrointestinal effects are primary effects, rather than secondary effects, of 2,4-DNP. In case reports of human fatalities after ingesting 2,4-DNP for 1–13 days (see Section 2.2), nausea, vomiting, and diarrhea were frequently reported (Bartlett et al. 2010; Hsiao et al. 2005; Lattimore 1934; Poole and Haining 1934; Purvine 1936; Siegmueller and Narasimhaiah 2010); in these cases, hyperthermia was present. At autopsy in one of the cases, the stomach mucosa was edematous and hemorrhagic, and the glandular epithelium was disintegrated (Poole and Haining 1934).

Reports of other fatal cases in which dose and exposure duration were not known also noted nausea and vomiting (Miranda et al. 2006; Suozzi et al. 2005; Tewari et al. 2009). A woman who took 4.4 mg/kg/day 2,4-DNP for 4 days experienced a burning sensation in her throat immediately after the first dose (Dintenfass 1934). Her pharyngitis became progressively worse, leading to inflammation of the eustachian tubes and hearing impairment. Nausea and diarrhea did not occur in a patient who developed severe dermal reactions after taking 2.3 mg/kg/day 2,4-DNP for 14 days (Anderson et al. 1933).

Gastrointestinal effects have also been reported in patients taking 2,4-DNP for intermediate durations. In two case reports of fatalities at doses between 1 and 3 mg/kg/day for 44–46 days, nausea and vomiting occurred (Goldman and Haber 1936; Zack et al. 2016). Autopsy of one case showed no pathological changes in the stomach, but the small intestine contained numerous focal hemorrhagic necroses (Goldman and Haber 1936). Nausea was among the side effects in 23 patients taking an average of 1.94 mg/kg/day 2,4-DNP as the sodium salt for 51–62 days (Bayer and Gray 1935). In an extensive clinical study of 159 people taking about 3 mg/kg/day for 22–89 days, an unspecified number of individuals experienced temporary diarrhea, vomiting, and heartburn (Simkins 1937a, 1937b). In a group of psychiatric patients given 2,4-DNP for 3–4 months to determine whether the drug would have a beneficial effect on depression, none of the patients experienced gastrointestinal disturbances (Masserman and Goldsmith 1934). Information was insufficient to calculate a dose.

Nausea and vomiting were also reported in workmen in the French munitions industry exposed to 2,4-DNP via inhalation and dermal contact (Perkins 1919). Exposure levels, durations, and incidences were not reported.

Gastrointestinal effects have also been observed in animal studies. Increased salivation was reported in 1/12 female and 2/12 male rats given 30 mg/kg/day 2,4-DNP by gavage for 28 days (Koizumi et al. 2001, 2002). No gross or microscopic lesions of the gastrointestinal tract were noted. Other intermediate-duration studies in rats (Haasio et al. 2002a, 2002b; Perry et al. 2015a,b; Schlagowski et al. 2014; Takahashi et al. 2004, 2009) and mice (Goldgof et al. 2014) at similar doses did not report any clinical signs of gastrointestinal distress. Adult beagles fed capsules containing 2,4-DNP daily at 12.5 mg/kg/day (one female for 14 days), 25 mg/kg/day (two females for 1 day, one male for 14 days), or 125 mg/kg/day (one female for 1 day) displayed emesis; no emesis was observed at 5 mg/kg/day (one male for 14 days) (Kaiser 1964). No gross or histological evidence of treatment-related gastrointestinal damage was reported following 2,4-DNP treatment of rats exposed in the diet to 5–50 mg/kg/day for 6 months (Spencer et al. 1948), dogs (one to two per group) given 20 mg/kg/day via capsule 7–12 times in 45–

77 days (Tainter and Cutting 1933b), or dogs (three per dose group) exposed via capsules to 5 or 10 mg/kg/day for 6 months (Tainter et al. 1934b).

Persistent diarrhea was reported in female bobwhite quail consuming 33.6 or 56.1 mg/kg/day of 2,4-DNP over an 8-day period (Dominguez et al. 1993).

Gastrointestinal effects have also been reported in rats and mice exposed to metabolites of DNPs for intermediate and chronic durations. Diarrhea was observed in rats exposed to 2-amino-4-nitrophenol at doses (administered by gavage 5 days/week) ≥625 mg/kg/day for 15 days, ≥ 500 mg/kg/day for 13 weeks, or ≥125 mg/kg/day for 2 years (NTP 1988a). In the chronic study, exposed male rats (doses of 125 or 250 mg/kg/day) had a higher incidence of digestive ulcers and erosive lesions of the gastrointestinal tract. In similar studies of 2-amino-5-nitrophenol administered by gavage 5 days/week, rats receiving ≥625 mg/kg/day for 13 weeks and male mice receiving 5,000 mg/kg/day had loose stools. Doses ≥400 mg/kg/day in rats and 1,600 mg/kg/day in mice for 13 weeks resulted in acute and chronic perivasculitis of the vessels of the caecum and the colon (NTP 1988b). In 2-year studies, acute and chronic inflammation of the caecum and colon (and in some cases the rectum), often accompanied by focal ulceration in the mucosa, was observed at ≥ 100 mg/kg/day in rats and ≥ 400 mg/kg/day in mice (NTP 1988b).

2.7 HEMATOLOGICAL

There are several reported cases of agranulocytosis following acute-, intermediate-, and chronic-duration oral treatments with 2,4-DNP or its sodium salt for weight reduction. Agranulocytosis is a syndrome characterized by marked decrease in the number of granulocytes, lesions of the throat and other mucous membranes, and fever; it is also referred to as granulocytopenia, malignant neutropenia, or agranulocytic angina. Cases of agranulocytosis were reported for women who ingested 6 mg/kg/day of 2,4-DNP for 2 weeks (Hoffman et al. 1934), an obese woman who took the sodium salt of 2,4-DNP at 3.5 mg/kg/day 2,4-DNP for 20 days (Davidson and Shapiro 1934), an obese woman who took 4 mg/kg/day 2,4-DNP for 35 days (Imerman and Imerman 1936), and an obese young girl who took 1.0 mg/kg/day 2,4-DNP for 46 days (Goldman and Haber 1936). In other cases, dose regimens were complicated and were not clearly delineated in these reports. For example, an obese woman took 2,4-DNP at 2 mg/kg/day, increasing to 3 mg/kg/day and then to 5 mg/kg/day for a total duration of 9 weeks, at which time she developed signs of illness (Dameshek and Gargill 1934). Another obese woman took the sodium salt of 2,4-DNP at 0.6 mg 2,4-DNP/kg/day, increasing to 3 mg/kg/day 2,4-DNP over 5 weeks and then to

4 mg/kg/day 2,4-DNP for 6 days, at which time signs of illness began to appear (Silver 1934). A fatal case of agranulocytosis occurred following ingestion of 2,4-DNP at 3 mg/kg/day increasing to 5 mg/kg/day for a total duration of ~6 weeks (Dameshek and Gargill 1934). The signs of illness developed rapidly while the patients were taking 2,4-DNP, at which time the drug was usually discontinued, and the patients were admitted to the hospital, where the diagnosis was made. The fatal case had no indications of abnormality in a blood smear performed ~2 weeks before hospitalization. Agranulocytosis and mild anemia were also diagnosed in a woman who had been taking 100–200 mg of 2,4-DNP intermittently for 1 year before she became ill (Imerman and Imerman 1936).

Clinical studies and additional case reports found other or no hematological effects following acute- and intermediate-duration exposures to 2,4-DNP. A case report of a "young adult" male who ingested a single, fatal dose of 2,4-DNP found elevated methemoglobin on post-mortem analysis (Duflou 2019). From the time of admission to the hospital (approximately 6 hours after ingestion) to postmortem evaluation, methemoglobin increased from 1 to 45%. Slight anemia was found upon hematological analysis of a woman who had taken 1.86 mg/kg/day 2,4-DNP for 2 weeks (Hitch and Schwartz 1936). Other case reports and clinical studies reported no hematological effects in people taking 2,4-DNP. No hematological effects were found in women who had taken 2 mg/kg/day for 1 week (Anderson et al. 1933), 2 mg/kg/day for 37 days (Beinhauer 1934), or 3 mg/kg/day for 182 days (Epstein and Rosenblum 1935). In a group of psychiatric patients given 2,4-DNP for 3–4 months to determine whether the drug would have a beneficial effect on depression, none of the patients had abnormal blood cytology (Masserman and Goldsmith 1934). Information was insufficient to calculate a dose. In an extensive clinical study of 159 people taking about 3 mg/kg/day for 22–89 days, no clinical cases of agranulocytosis were found, and hematological examination of 11 individuals revealed no abnormalities (Simkins 1937a, 1937b). Similarly, in a clinical study of 2,4-DNP for treatment of obesity, hematological examination of 62 people taking 4 mg/kg/day for an average of 88 days revealed no abnormalities (Tainter et al. 1935).

There are few data on the hematological effects of 2,4-DNP in animals, but available data do not suggest hematologic changes at nonlethal doses. Neonatal rats exposed to 20 mg/kg/day 2,4-DNP by gavage for 18 days, and young (5–6 weeks old) rats exposed by gavage to doses up to 30 mg/kg/day for 28 days exhibited no changes in hematology parameters including clotting parameters; young rats receiving 80 mg/kg/day for 28 days experienced significant decreases in erythrocyte count, hemoglobin concentration, and hematocrit, but this dose was also lethal to 8/24 animals (Koizumi et al. 2001, 2002). Rats exposed to 5–50 mg/kg/day 2,4-DNP in the diet for 6 months had no abnormalities with respect to blood erythrocyte count, hemoglobin concentration, total or differential leukocyte count, or total or

nucleated cell counts in the bone marrow (Spencer et al. 1948). Dogs (three per dose group) exposed to 5 or 10 mg/kg/day 2,4-DNP via capsules for 6 months had no hematological abnormalities (including hemoglobin concentration, erythrocyte count, oxygen capacity and fragility of red blood cells, and total or differential leukocyte counts); histological examination of bone marrow revealed no abnormalities (Tainter et al. 1934b).

2.8 MUSCULOSKELETAL

Data pertaining to musculoskeletal effects of 2,4-DNP in humans are very limited; available information suggests that the uncoupling of oxidative phosphorylation may account for most of the observed effects (see Section 2.18.1).

A number of case studies have documented muscle weakness or pain following oral exposure to 2,4-DNP, as well as rhabdomyolysis resulting from hyperpyrexia. Exercise tests revealed considerable loss of strength and reduced endurance in a limited number of obese patients who ingested 4 mg/kg/day of 2,4-DNP for 1–8 weeks (MacBryde and Taussig 1935). Details of testing methods and results were not reported. Weakness in the legs and arthritic or rheumatoid-like pains in the arms and fingers were experienced by four women who had been taking 2,4-DNP at doses of 0.91 or 1.45 mg/kg/day for 8 days, 4 mg/kg/day for 21 days, or 3.53 mg/kg/day for 105 days (Nadler 1935). These pains may be related to the development of peripheral neuritis. Another woman with a history of chronic hypertrophic arthritis of the cervical spine and knees developed pain in her fingers and all large joints after taking 2 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Anderson et al. 1933). The pains subsided within 4–5 days after she stopped taking 2,4-DNP, but swelling and tenderness persisted in the left wrist and fingers. The authors suggested that 2,4-DNP exacerbated her arthritis. In patients who ultimately succumbed to 2,4-DNP poisoning, muscle pain or weakness, rigidity, and elevated creatine kinase or rhabdomyolysis have been reported prior to death (Bartlett et al. 2010; Holborow et al. 2016; Poole and Haining 1934; Miranda et al. 2006; Tewari et al. 2009). These effects are consistent with those seen with fatal hyperthermia (Bunai et al. 2012).

Female rats exposed to a single dose of ≥10 mg/kg exhibited temporary leg weakness (Eli Lily and Co. 1992). Similarly, animal studies have also observed musculoskeletal effects following intermediate-duration oral exposure to 2,4-DNP. Haasio et al. (2002a, 2002b) observed mitochondrial swelling, deformed or broken mitochondrial cristae, and reduced matrix density in the mitochondria in the skeletal muscle of male rats given 20 mg/kg/day 2,4-DNP by gavage for 15 days; no changes to skeletal muscle

were seen by light microscopy. After 28 days of exposure to 30 mg/kg/day 2,4-DNP in drinking water, significantly decreased maximal running speed and running economy (VO_2/running speed) were reported in male rats (Schlagowski et al. 2014). Histochemical staining showed slightly increased glycogen content, without a change in triglyceride level, in the skeletal muscle; the increase was considered to be an adaptive response to the reduced energy supply associated with uncoupling of oxidative phosphorylation (Schlagowski et al. 2014). No gross or histological evidence of treatment-related damage to muscle and/or skeletal tissue was reported following 2,4-DNP treatment of rats exposed by gavage for 15 days in the diet to 5–50 mg/kg/day (Spencer et al. 1948) or dogs exposed via capsules to 5 or 10 mg/kg/day, each for 6 months (Tainter et al. 1934b).

As noted above, uncoupling of oxidative phosphorylation and consequent hyperthermia by 2,4-DNP likely accounts for most of the musculoskeletal symptoms seen in animals and humans exposed to 2,4-DNP (see also Section 2.18.1). Mitochondrial changes seen in skeletal muscle (Haasio et al. 2002a, 2002b) provide support for this relationship. The depletion of ATP associated with mitochondrial uncoupling may also produce hyperkalemia (reviewed by Grundlingh et al. 2011). 2,4-DNP has been reported to induce potassium accumulation in rabbit kidney slices (Mudge 1951), and hyperkalemia has been observed in humans poisoned with 2,4-DNP (Bartlett et al. 2010; Jiang et al. 2011). Hyperkalemia, in turn, may produce the muscle pain and weakness often reported by humans after exposure to 2,4-DNP. Ribeiro et al. (2005) observed that 2,4-DNP inhibited isometric force and enhanced the stability of weakly bound cross-bridges in isolated mammalian muscle fibers (without skin), thereby inhibiting actin-myosin interactions and thus muscle contraction. These effects may also play a role in the muscle weakness and rigidity observed in 2,4-DNP poisonings.

2.9 HEPATIC

Information on the potential hepatic effects of 2,4-DNP in humans is limited to case reports of poisonings that lack information on pre-existing conditions and clinical studies from the 1930s, which used relatively insensitive methods to assess liver function. These limited data in humans do not suggest that 2,4-DNP exerts strong effects, if any, on the liver. In human cases of hyperpyrexia associated with ingestion of 2,4-DNP, however, acute liver injury can occur (see Section 2.2).

Increased liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) were reported in 14 survivors aged >8 years after an incident at a Chinese factory in which workers and their families were exposed to 2,4-DNP (doses unknown) by inhalation and dermal contact (Lu et al. 2011). A

palpable and tender liver was observed in a patient who took 2 mg/kg/day 2,4-DNP for 37 days, but no tests of liver function were performed (Beinhauer 1934). Impaired liver function as measured by a bromsulphalein (BSP) test[1] was observed in an obese woman who took 4 mg/kg/day 2,4-DNP as the sodium salt for 20 days (Davidson and Shapiro 1934). Liver function, as assessed by the icteric index[2] (Lichtman 1953) or Van den Bergh test[3], was not affected in two women who took 2–3 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Anderson et al. 1933; Masserman and Goldsmith 1934); one of the women subsequently died (Masserman and Goldsmith 1934). In other fatal cases, autopsy findings included slight detachment of the liver cells in a man who died after taking two doses of 46 mg/kg 2,4-DNP as the sodium salt 1 week apart (Tainter and Wood 1934); disintegration of hepatocytes in the periphery of the lobules and granular cytoplasm and pyknotic nuclei in periportal cells in the liver of a woman who took 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days (Poole and Haining 1934); necrosis of hepatocytes and hemorrhage in the liver of a woman who took an indeterminate dose of 2,4-DNP for 1 week (Lattimore 1934); and severe fatty changes in the liver of a young girl who took 1.0 mg/kg/day of 2,4-DNP for 46 days (Goldman and Haber 1936). In the absence of information on health conditions prior to poisoning, it is uncertain whether the few hepatic effects seen in case reports are attributable to 2,4-DNP exposure. In addition, some of the autopsy findings (e.g., hepatocyte necrosis and hemorrhage) are consistent with fatal hyperthermia (Bunai et al. 2012).

In clinical studies from the early 1900s, liver function tests were rarely affected by exposure to 2,4-DNP at doses recommended for weight loss; however, the sensitivity of the metrics used to assess liver function is uncertain, and most are no longer used clinically. The icteric index of 17 patients who ingested 4 mg/kg/day 2,4-DNP as the sodium salt for 2–50 weeks did not differ from the icteric index of an unspecified number of "nonmedicated patients" (Tainter et al. 1934a). Icteric indices were also normal in a group of psychiatric patients given 2,4-DNP (information insufficient to estimate doses) for 3–4 months to determine whether the drug would have a beneficial effect on depression (Masserman and Goldsmith 1934). In obese patients given 2,4-DNP at 4.3 mg/kg/day for 1–8 weeks, increased phenoltetraiodophthalein dye retention (above pretreatment values and above the normal range) was seen in three of five patients tested at 1–2 weeks of treatment and in three of three patients tested at 3–8 weeks

[1]The BSP test measured the ability of the liver to remove bromsulphalein dye from the blood after intravenous injection. Retention of BSP in the blood indicated decreased blood flow, biliary obstruction, or hepatic cell damage.
[2]The icteric index was a calorimetric estimation of bilirubin in the serum by comparison with the absorbance of a standard solution of potassium dichromate.
[3]The Van den Bergh test is a measure of serum bilirubin, in which color changes in diazotized serum indicate defects in bilirubin production, hepatic uptake, or conjugation that cause increases in the serum level of free (unconjugated) bilirubin.

of treatment (MacBryde and Taussig 1935). However, the clinical tests relevant to liver function (icteric index, serum bilirubin, galactose tolerance test, urinary urobilinogen) were normal (MacBryde and Taussig 1935). In an extensive clinical study of 159 people taking about 3 mg/kg/day for 22–89 days, results of icteric index determinations, Van den Bergh tests, and BSP retention tests revealed no evidence of liver damage in the 14 or 15 patients to whom the tests were given (Simkins 1937a, 1937b). In 45 patients given 4 mg/kg/day 2,4-DNP for 2–50 weeks, the bilirubin content of blood serum was elevated in only two patients, neither of whom had other clinical evidence of liver disturbance (Tainter et al. 1934a). No consistent microscopic changes of the liver were revealed during autopsies of workers who died from exposure to 2,4-DNP (primarily via inhalation and dermal contact) in the French munitions industry (Perkins 1919); exposure levels, durations, and incidences were not characterized.

Acute-duration animal studies have not adequately evaluated the hepatic effects of 2,4-DNP. No histological abnormalities were observed in rats treated by gavage with 1 mg/kg/day, 5 days/week for 4 weeks; higher doses (not reported) produced "typical indications of general passive congestion and anoxemia" (Dow Chemical Co. 1940). Two dogs intermittently fed capsules containing 2,4-DNP at dose levels ≤20 mg/kg (with "recovery periods" of 1–19 days between doses), followed by a "fatal dose" (dose not reported) had normal liver function tests and gross and microscopic histology of the liver (Tainter and Cutting 1933b). Overall, these studies do not provide a clear picture of the potential for heptatotoxicity after acute-duration 2,4-DNP exposure.

Well-conducted intermediate-duration studies using modern methods have provided some evidence of 2,4-DNP effects on the liver, while older studies (before 1950) have not. Increased relative liver weight (15% higher than controls, in the absence of body weight changes), along with centrilobular hypertrophy, minor necrotic foci, and mitochondrial changes in the liver were reported in rats exposed to 20 mg/kg/day 2,4-DNP by gavage for 15 days (Haasio et al. 2002a, 2002b). Increased relative liver weight (magnitude not reported) in the absence of histopathology changes was also observed in rats exposed to 80 mg/kg/day, but not 30 mg/kg/day, by gavage for 28 days; deaths were also seen at 80 mg/kg/day (Koizumi et al. 2001, 2002). In male and female parental rats exposed to 30 mg/kg/day for 46 days in a reproduction and developmental toxicity screening study, relative liver weights were increased by 12–14% (Takahashi et al. 2009). While a significant decrease in body weight in male parental rats receiving this dose may explain the change in relative liver weight in males, females did not exhibit changes in body weight. In a study comparing the effects of 2,4-DNP with those of a controlled-release formulation of the compound, statistically significant, but modest (<2-fold) increases in liver enzymes (AST and ALT) were observed in rats exposed to 10 mg/kg/day as a daily bolus dose in peanut butter for 6 weeks

(Perry et al. 2015a,b). This small increase in liver enzymes is not considered to be toxicologically significant. Gross and microscopic pathology were not assessed. No gross or histological evidence of treatment-related liver damage was reported following 2,4-DNP treatment of rats exposed in the diet to 5–50 mg/kg/day for 6 months (Spencer et al. 1948), rats exposed in the diet to 60 mg/kg/day for life (Tainter 1938), or dogs (three per dose group) exposed via capsules to 5 or 10 mg/kg/day for 6 months (Tainter et al. 1934b); in addition, normal results were observed in the dogs with respect to the icteric index of liver function (a measure of serum bilirubin) (Tainter et al. 1934b).

2.10 RENAL

Renal effects have been reported in many case reports of human 2,4-DNP poisoning, as discussed below. In most cases, the effects appear to be part of the multiorgan dysfunction that accompanies severe and/or fatal hyperthermia (see Sections 2.18 and 2.18.1). Acute renal failure occurs commonly with exertional heatstroke (Bunai et al. 2012). Tubular necrosis results from hypovolemia (as blood is moved to peripheral blood vessels) and diaphoresis, and there can also be direct thermal injury to the kidneys (Bunai et al. 2012). Hemorrhagic conditions precipitated by severe hyperthermia, including disseminated intravascular coagulation (in which the clotting cascade is activated throughout the small blood vessels of the body), also contribute to renal failure, as does myoglobinuria that results from rhabdomyolysis (Bunai et al. 2012).

Renal failure was noted in a case report of a fatality after DNP intake (~40 mg/kg) with suicidal intent (Bartlett et al. 2010), and in another fatality presumed to be associated with DNP exposure but without dose or duration information (Suozzi et al. 2005). Mild nephrotic changes were seen during histopathological examination of tissues from a man who died after ingesting two doses of 46 mg/kg 2,4-DNP as the sodium salt of 2,4-DNP 1 week apart (Tainter and Wood 1934). In other fatal cases, cloudy swelling, pyknosis, and necrosis in the renal tubules, edema in interstitial tissue, distention of capillary and arterial loops in the glomerulus, and hemorrhage were seen in the kidneys of a woman who took 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days (Poole and Haining 1934); marked destruction of the epithelium lining the renal tubules with hemorrhage into the glomeruli was found in the kidneys of a woman who took an indeterminate dose of 2,4-DNP for 1 week (Lattimore 1934); and hemorrhagic nephritis was found in the kidneys of a young girl who took 1.0 mg/kg/day 2,4-DNP for 46 days (Goldman and Haber 1936). The blood nonprotein nitrogen level was normal in a psychiatric patient who subsequently died after being given 3 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Masserman and Goldsmith 1934). Upon autopsy, no gross evidence of kidney damage was found, but microscopic

examination was inconclusive due to autolysis, because autopsy was delayed by 4 days. Autopsies of workers who died from exposure to 2,4-DNP (via inhalation and dermal contact) in the French munitions industry did not reveal any consistent changes of the kidney; no information on exposure levels or durations was provided (Perkins 1919).

Nonfatal poisonings with 2,4-DNP also resulted in renal effects. A woman who ingested 2,4-DNP at a single dose of ≥10 mg/kg for weight loss developed transient renal failure but recovered within 4 days (van Veenendaal et al. 2011). No changes in blood tests for renal function were seen in healthy male bodybuilders who took ~1 mg/kg/day 2,4-DNP daily for 10 days (Lee et al. 2014) or ~4 mg/kg/day for 6 days (Le et al. 2015). Similarly, the blood nonprotein nitrogen level was normal in a woman who took 2 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Anderson et al. 1933). Moderate and marked albuminuria was found in 2 women who took 2 mg/kg/day (Beinhauer 1934) or 4 mg/kg/day 2,4-DNP (Imerman and Imerman 1936) for 37 or 35 days, respectively. In the woman who took 2 mg/kg/day, kidney function as determined by phenolsulfonphthalein retention was normal (Beinhauer 1934). Tests of renal function (examination of urine for albumin, red and white cells and casts; concentration-diuresis tests with measurement of specific gravity; phenolsulfonphthalein excretion; blood nonprotein nitrogen determinations) performed repeatedly on three patients over a period of 8 weeks while they underwent treatment with 4 mg/kg/day 2,4-DNP showed no changes; the data were not provided (MacBryde and Taussig 1935).

A few clinical studies found transient or no renal effects following 2,4-DNP exposure. In an extensive clinical study of 159 patients taking an average of 3 mg/kg/day 2,4-DNP as the sodium salt for 22–89 days, kidney function, as assessed by phenolsulfonphthalein retention, was normal in the 15 patients to whom the test was given (Simkins 1937a, 1937b). However, 4 of 15 had transient albuminuria and 2 of 15 had persistent albuminuria. In a group of psychiatric patients given 2,4-DNP at various doses for 34 months to determine whether the drug would have a beneficial effect on depression, no changes in urinary constituents were found (Masserman and Goldsmith 1934).

Two acute-duration animal studies reported mild or no renal effects following 2,4-DNP exposure, but lacked statistical analysis or dose data. Eight rats treated once by gavage with 20 mg/kg 2,4-DNP displayed very mild tubular necrosis in 5 of 16 kidneys examined 12 hours after dosing (Arnold et al. 1976). No statistical analysis of the data was reported. Two dogs repeatedly fed capsules of 2,4-DNP at dose levels of ≤20 mg/kg, with “recovery periods” of about 5 days between doses, followed by a “fatal

dose" (dose level not reported) had no abnormalities with respect to gross and microscopic histology of the kidney (Tainter and Cutting 1933b).

Intermediate-duration animal studies generally reported mild or no renal effects following nonfatal exposures, but some had reporting limitations. A 28-day study in rats exposed to 2,4-DNP by gavage revealed increased kidney weight and histopathology changes (mineralization in the corticomedullary junction) at 80 mg/kg/day, a dose that also resulted in the death of 2/12 males and 6/12 females (Koizumi et al. 2001, 2002). A NOAEL of 1 mg/kg/day for kidney histology was reported for rats exposed for 5 days/week for 4 weeks; higher doses (not reported) produced chronic tubular necrosis characterized by degeneration of the tubular epithelium (Dow Chemical Co. 1940). The degeneration varied from slight cloudy swelling of the epithelium to complete necrosis with extensive desquamation and sloughing into the tubular lumina. Marked pyknosis and degeneration were observed in the nuclei of the epithelial cells, but the glomeruli were essentially normal. In rats exposed to 2,4-DNP by daily gavage for 40–47 days, relative kidney weights were increased by 11–14% in both males and females at a dose of 30 mg/kg/day but not at 10 mg/kg/day; histopathology was not evaluated (Takahashi et al. 2009). Rats exposed to 5–50 mg/kg/day 2,4-DNP in the diet for 6 months had no gross or histological evidence of damage to the kidney (Spencer et al. 1948). Blood urea nitrogen (BUN) was greatly elevated in 2 /14 and 2/9 rats exposed to 25 and 50 mg/kg/day, respectively, but the mean values in each group were similar to those of the controls (Spencer et al. 1948). Dogs (three per dose group) exposed to 5 or 10 mg/kg/day 2,4-DNP via capsules for 6 months had normal levels of blood urea and urinary sugar; urinary albumin was increased at 12 weeks at both exposure levels but was otherwise normal throughout the experiment (Tainter et al. 1934b). In addition, no gross or histological evidence of kidney damage was observed. The authors concluded that the treatment did not produce progressive damage to the kidney (Tainter et al. 1934b). In rats exposed to dietary 2,4-DNP (60 mg/kg/day) in a lifetime study, no treatment-related gross or histopathological findings in the kidney were observed relative to the control group (Tainter 1938).

In rats treated with a metabolite of 2,4-DNP (2-amino-4-nitrophenol) by gavage for 5 days/week, mineralization of the renal cortex and degeneration of the renal tubular epithelium were observed after 13 weeks at ≥ 500 mg/kg, and increased incidences of nephropathy and renal tubular cell hyperplasia were seen in males after 2 years of exposure to 250 mg/kg (NTP 1988a).

2.11 DERMAL

Yellow discoloration of the skin and pruritic skin rashes were common findings in people taking 2,4-DNP for weight loss. Some early studies (e.g., Bayer and Gray 1935) attributed the yellow discoloration to jaundice, but this finding more likely results from 2,4-DNP excretion in sweat (e.g., Holborow et al. 2016). A woman who took 4 mg/kg/day 2,4-DNP for 4 days developed a rash on her chest (Dintenfass 1934). Two women who took 0.91 or 1.45 mg/kg/day 2,4-DNP for 8 days developed marked pruritic rashes that disappeared within 2–5 days after dosing was discontinued but reappeared upon resumption of treatment (Nadler 1935). Generalized maculopapular rashes covering much of the body were observed in two young, healthy bodybuilders who ingested 2,4-DNP; one patient took 72 mg 2,4-DNP per tablet once per day for 10 days (~1 mg/kg/day Lee et al. 2014), while the other took 200 or 400 mg/day for 6 days (~4 mg/kg/day; Le et al. 2015). For the latter individual, the rash resolved within 5 days (Le et al. 2015). Severe skin lesions developed in two women who took 2 mg/kg/day 2,4-DNP for 14 days (Anderson et al. 1933; Hitch and Schwartz 1936). In one case, the lesions were characterized by severe exfoliating dermatitis with redness, edema, oozing of serum, scaling, and crusting over 100% of the body surface (Hitch and Schwartz 1936). In the other case, severe pruritus, edema, maculopapular eruptions covered the entire body, with the exception of the face and scalp (Anderson et al. 1933). No dermal effects were seen in 37 obese patients taking 1 mg/kg/day 2,4-DNP as the sodium salt of 2,4-DNP for an average of 14 days (Tainter et al. 1935). Serious skin reactions (not otherwise specified) were observed in 3 of 15 obese patients taking 4 mg/kg/day 2,4-DNP for 1–8 weeks; the duration of 2,4-DNP treatment for the affected patients was not specified (MacBryde and Taussig 1935).

Case reports of people taking 2,4-DNP for longer durations reported pruritis and urticaria. A woman who took 4 mg/kg/day for 21 days developed pruritis (Nadler 1935). Urticaria developed in one or all of two women and one man who took 3 mg/kg/day for 41–49 days (Hunt 1934), in addition to one patient (sex not specified) taking 2 mg/kg/day for 110 days (Simkins 1937a, 1937b). Transient pruritic spots occurred in a woman who had been taking 100–200 mg of 2,4-DNP intermittently for 1 year (Imerman and Imerman 1936). Beinhauer (1934) reported a severe case of pruritus involving the entire body of a woman who took 2 mg/kg/day 2,4-DNP for 37 days The pruritus was characterized by swelling of both eyelids, lips, and neck; giant wheals covering the entire body, which were tense to the touch and marked by numerous deep excoriating and intense urticaria; distended and swollen hands and feet; and numerous herpetic lesions in the mouth.

Intermediate-duration clinical studies have also reported dermal effects following 2,4-DNP exposure. In an extensive clinical study of 159 patients taking an average of 3 mg/kg/day 2,4-DNP as the sodium salt for 22–89 days, 32 developed skin lesions, including 4 cases of pruritus, 3 of macular rashes, 12 of maculopapular rashes, 4 of swelling and redness of hands, and 10 of urticaria (Simkins 1937a, 1937b). Skin reactions were observed in 23 of 170 obese patients who ingested an average of 4 mg/kg/day 2,4-DNP from sodium 2,4-DNP for an average of 88 days (Tainter et al. 1935). The treatment regimen involved an initial dose of 1 mg/kg/day 2,4-DNP, usually for 1 week, increasing to 2 mg/kg/day for several weeks, and then to 4 mg/kg/day with continued small incremental increases until symptoms or loss of body weight contraindicated further increases. The dermal effects occurred only among the 100 patients who took ≥4 mg/kg/day for ≥6 weeks. One-third of the 23 affected patients experienced transient itching without a rash; the remaining two-thirds experienced itching and visible urticarial or maculopapular skin lesions. In one case, the reaction was severe, with massive urticarial wheals covering the body and extensive localized edema. Patients sometimes recovered while remaining on treatment, but usually treatment was discontinued, and recovery ensued. In an experimental study involving 13 men of average weight given an average dose of 5 mg/kg/day 2,4-DNP for 20 days, no skin lesions were observed (Grant and Schube 1934).

Studies of dermal irritation in animals following acute dermal exposure to 2,4-DNP had deficiencies in experimental protocol (statistical analysis was not performed) and reporting (strain, sex, and numbers of animals, duration of each application, and number of applications per day were not reported). Twenty applications of a 3% 2,4-DNP solution in 95% ethanol to the ears of rabbits produced no significant signs of dermal irritation (Spencer et al. 1948). When similar treatment was applied to a bandage on the shaved abdomen, the result was very slight irritation, including mild hyperemia, edema, and exfoliation. No evidence of toxic absorption was apparent, but the criteria used to assess toxicity were not reported (Spencer et al. 1948). Twenty applications of a 4% 2,4-DNP solution in propylene glycol to the ears of rabbits produced no significant signs of dermal irritation (Dow Chemical Co. 1940). In the same study, six applications of a similar solution onto the shaved abdomen resulted in a “moderate simple irritation,” as indicated by hyperemia, edema, and denaturation.

2.12 OCULAR

Cataracts developed in some patients who took 2,4-DNP or sodium 2,4-DNP as a weight reduction aid for acute, intermediate, and chronic durations. The cataracts developed rapidly, sometimes while the patient was still ingesting the drug and sometimes after cessation of treatment, and were bilateral and irreversible,

progressing to total blindness. The case report literature in regard to this effect is voluminous, with at least 164 cases in the published literature (Hitch and Schwartz 1936; Horner 1942; Horner et al. 1935; Rank and Waldeck 1936; Rodin 1936; Simkins 1937a, 1937b; Whalman 1936). Cataract formation appears to be the primary reason that 2,4-DNP was withdrawn from medical use. The doses resulting in cataracts ranged from 2 to 4 mg/kg/day 2,4-DNP. In some cases, marked swelling of the lens occurred and occasionally caused acute secondary glaucoma. The cataracts developed in patients who were at an age when senile cataracts do not occur. One patient who took 2 mg/kg/day 2,4-DNP for 2 weeks developed a generalized skin eruption that worsened to the point where she was admitted to the hospital 8 months later (Hitch and Schwartz 1936). While her eyes were normal on admission, after 40 days in the hospital, she developed blurred vision, which was attributed to bilateral cataracts. The incidence of cataracts among patients treated with an average of 4 mg/kg/day 2,4-DNP from sodium 2,4-DNP for an average of 88 days was 1 of 170 (Tainter et al. 1935); among patients treated with an unspecified dosage/duration of 2,4-DNP, the incidence was 1 of 68 (Hill 1936). A report of 19 cases of cataracts mentions that among these cases were a mother and her daughter, possibly indicating familial susceptibility (Hessing 1937). A genetic role in susceptibility was also suggested by cases of cataract development in identical twins who had taken 2,4-DNP (Buschke 1947).

Attempts to find a suitable animal model for cataract development in humans exposed to 2,4-DNP have generally been unsuccessful. As discussed below, normal mammalian animals have not developed cataracts after oral exposure to 2,4-DNP, although cataracts could be induced in a special strain of mouse (yellow adipose), in vitamin C-deficient guinea pigs, in ducks, and in chickens. No evidence of corneal opacity or cataract formation was observed in rats exposed to 0.2% 2,4-DNP (350 mg/kg/day) in the diet (Spencer et al. 1948). Food consumption was not reported; however, the authors indicated that the rats ate very little, lost weight rapidly, and were all dead after 24 days of treatment. No evidence of corneal opacity or cataract formation was observed in rats fed 2,4-DNP for 6 months at dietary concentrations ≤0.10% (50 mg/kg/day) (Spencer et al. 1948). Rats exposed for their lifetime to dietary levels ≤60 mg/kg/day 2,4-DNP (Tainter 1938) and rabbits exposed to 0.25% 2,4-DNP in the diet for 8 hours (total dose 41 mg/kg) (Bettman 1946) did not develop cataracts. However, cataracts were induced in rabbits injected intraperitoneally with 2,4-DNP (Gehring and Buerge 1969a). Immature rabbits (10 days old) were more susceptible than 62-day-old rabbits, while no cataracts were induced in 90-day-old rabbits. This age-related susceptibility to the cataract formation was attributed to a decreased ability to metabolize substances and an increased permeability of the blood-ocular fluid barrier in the very young rabbits.

No cataracts developed in rats on a vitamin A- or vitamin B2-deficient diet to which 2,4-DNP was added at a dose of 50 mg/kg/day for 58–173 days (Tainter and Borley 1938). Likewise, no cataracts developed in guinea pigs on a vitamin C-deficient diet to which 2,4-DNP was added at a dose of 80 mg/kg/day for 21–37 days (Tainter and Borley 1938).

Cataracts developed in 3 of 40 congenitally obese mice (yellow adipose mice) exposed to 0.1% 2,4-DNP (130 mg/kg/day) in the diet for 6 months (Bettman 1946). Cataracts developed within 4–8 weeks of treatment and were initially "immature" with fine posterior subcapsular opacities. Later, the nucleus developed definite cortical spikes, producing a milky appearance. The cataracts did not progress beyond this stage, in spite of continued treatment. No cataracts developed in 40 yellow adipose mice exposed to a control diet for 6 months. Adult albino and black mice (sex not reported) were exposed to 130 mg/kg/day 2,4-DNP in the diet for ≥11 months; a group of albino mice received a control diet (Bettman 1946). The incidences of cataract formation were 1 of 20, 0 of 20, and 0 of 20 for treated albino and black mice and untreated albino mice, respectively. The author indicated that formation of the cataract occurred in the mouse just prior to death after 11 months of exposure to 2,4-DNP and was therefore not comparable to the cataracts developing in 4–8 weeks in yellow adipose mice. Exposure of adult yellow adipose and adult albino mice to a diet containing 2,4-DNP at a concentration that would be equivalent to 325 mg/kg/day did not result in cataracts, but 100% of the adult mice died within 8 hours (Bettman 1946). Since these mice were exposed to the diet for only 8 hours, their estimated total dose was only 108 mg/kg. Exposure of young albino mice to 325 mg/kg/day 2,4-DNP in the diet for 1 week produced 25% mortality, but no cataracts (Bettman 1946).

Cataracts developed within hours to days after chicks were exposed to dietary concentrations of 0.10–0.25% 2,4-DNP and in ducks exposed to 0.25% 2,4-DNP in feed, both for 31 days (Robbins 1944). Dose levels and food intake were not reported in this study, and no standard reference values of daily intake were available for these species (EPA 1986a); therefore, doses were not calculated. The percentage of birds developing cataracts and the persistence of the cataracts in chicks were positively correlated with the concentration of 2,4-DNP in the feed; at 0.15%, 57% of the chicks developed cataracts within 24 hours, but the cataracts had regressed after 31 days of treatment. In chicks and ducks exposed to 0.25% 2,4-DNP in the feed, gross opacities were observed in lenses of 84–100% of the birds after 1 day of treatment. The author provided a detailed description of development and subsequent regression of cataracts in birds exposed to 0.25% 2,4-DNP, based on observations in living birds and histological examination of lenses in birds sacrificed throughout the study. The author indicated that the progression of cataracts in birds fed 2,4-DNP was "remarkably similar" to that reported in humans exposed to

2,4-DNP (Horner 1942; Robbins 1944). In humans, however, the cataracts did not regress (Horner 1942). Chicks exposed to 0.5% 2,6-DNP in the diet developed slight lens opacity on days 2 and 3 of a 6-day exposure; no cataracts were present in chicks when the exposure was terminated. The author indicated that the effects of dietary 2,6-DNP on cataract formation in chicks were much less pronounced than those of 2,4-DNP (Robbins 1944). Gavage administration of 2,6-DNP at a dose of 79 mg/kg to chickens produced equivocal evidence of cataract formation in 5 days (Buschke 1947). The activity of 2,6-DNP was far less than that of 2,4-DNP (11 mg/kg in this study).

Administration of 2,4-DNP by gavage in peanut oil or by intramuscular injection produced cataracts in baby chicks and adult chickens (Buschke 1947). This treatment produced cataracts within 1–1.5 hours "in any number of different strains," at a threshold dose of 20 mg/kg. In addition, a gavage dose of 11 mg/kg produced cataracts in 3.5 hours in chicks. No cataracts were produced at 6 mg/kg. Dietary concentrations of 2,4-DNP caused cataracts in baby chicks, but not in adult chickens. The authors indicated that the threshold concentration of 2,4-DNP in the diet was 0.1% (Buschke 1947). These cataracts disappeared after a few days, in spite of continued exposure to 2,4-DNP in the feed; however, food consumption was not reported, so the possible role of a decreasing daily dose over time due to reduced food consumption in the resolution of the cataracts is unclear. This study is limited by incomplete reporting of doses and numbers of chickens used.

Giant White Pekin ducks (initial age 16–30 days, initial body weight 400–800 g) were treated once by gavage with 2,4-DNP (Gehring and Buerge 1969a). The percentages of ducks developing cataracts (bilateral opacities in lenses) were 0, 0, 38, 75, 100, and 100% at dose levels of 12, 15, 20, 25, 28, and 30 mg/kg, respectively. The ED_{50} (effective dose in 50% of animals) with 0.95% confidence limits was 21.5 (17.9–25.8) mg/kg. Cataracts were generally observed for the first time between 1 and 3 hours after dosing and usually disappeared completely within 12 hours after the first observation. The authors suggested that the rapid development of cataracts indicated that the parent compound, not the metabolite, was causing the effect. The validity of this suggestion is supported by an experiment in which 2,4-DNP (0.10–10.0 μg) was injected directly into the posterior chamber of the eyes of ducks (Gehring and Buerge 1969b). Cataracts developed within 30 minutes of injection regardless of dose and within 10 minutes of injection at doses ≥1.0 μg. Ducks exposed to 0.25% sodium salt of 2,4-DNP in the diet all developed bilateral cataracts after 1 day of treatment (Spencer et al. 1948).

A study in rabbits, with supporting *in vitro* experiments, suggested that 2,4-DNP binds to serum proteins; concentrations of 2,4-DNP in the eye are related to the unbound 2,4-DNP, but there appears to be a blood-

aqueous humor barrier preventing free diffusion (Gehring and Buerge 1969b). This barrier was more effective in mature rabbits than in immature rabbits. In addition, the mature rabbit eliminated 2,4-DNP more rapidly from serum and the eye. Differences in sensitivity of animals to the cataractogenic properties of 2,4-DNP may be related to the levels of 2,4-DNP attained and maintained in the eye (see Sections 3.1.2 and 3.1.4).

The uncoupling of mitochondrial electron transport from oxidative phosphorylation (see Section 2.18.1) with resultant decreased production of ATP by 2,4-DNP appears to be related to the cataractogenesis of 2,4-DNP. The lens epithelium is the chief source of available energy for the lens (Kuck 1970). In most animals, the energy needs are met principally by anaerobic glycolysis, and <30% by oxidative phosphorylation. In incubated bovine lenses, oxygen was not necessary for maintaining sodium levels in the presence of glucose, suggesting anaerobic respiration in the lens (Trayhurn and van Heyningen 1971). Energy evolved from the breakdown of ATP by Na+/K+-activated ATPase is required for the transport of these cations across the lens epithelium to maintain proper ionic balance. Sodium is actively transported from the lens to the aqueous humor, while potassium is actively transported in the reverse direction. Interference with this active transport mechanism across the lens epithelium can result in increased sodium in the lens, disruption of the ionic balance between the lens and aqueous humor, and subsequent cataract formation. An *in vitro* study with rabbit lenses also demonstrated that 2,4-DNP does not cause calcium-induced cataracts by interfering with active transport of calcium from the lens, because the energy for calcium transport is derived from anaerobic glycolysis and not oxidative phosphorylation (Hightower and Reddy 1981).

Effects of 2,4-DNP on oxidative phosphorylation may be more important in the lens epithelial cells of humans, rabbits, and domestic birds (e.g., chicks and ducklings), as these species appear to be more susceptible to cataract formation after 2,4-DNP exposure (Kuck 1970). In domestic birds, cataracts occur almost immediately after exposure to 2,4-DNP and are reversible (Buschke 1947). However, in humans, cataracts can occur sometime after treatment is terminated and may not be reversible. This phenomenon has not been fully explained.

2.13 ENDOCRINE

A case report and two clinical studies reported thyroid and/or glucose tolerance effects following acute- and intermediate-duration exposures to 2,4-DNP. Autopsy of a woman who died after taking 1.03 mg 2,4-DNP for 46 days revealed extensive vascularization of the spleen and pituitary accompanied by goiter

in the thyroid (Goldman and Haber 1936). Decreased glucose tolerance was seen in one clinical study in five of eight patients after 1–2 weeks of treatment and in four of four patients after 3–4 weeks of treatment with 4.3 mg/kg/day 2,4-DNP (MacBryde and Taussig 1935). An additional finding in humans given 2,4-DNP for short durations was a 21% decrease in serum protein-bound iodine in 11 non-obese subjects who ingested 3 mg/kg/day 2,4-DNP for 2 days (Castor and Beierwaltes 1956). Thyroidal uptake and fecal and urinary excretion of ^{131}I, tested in two of these subjects, did not appear to be affected. Hence, the toxicological significance of this finding is unclear.

Two intermediate-duration exposure rodent studies observed 2,4-DNP effects on glucose regulation. Rats exposed to 2,4-DNP (20 mg/kg/day) by daily gavage for 15 days exhibited a 43% increase in blood glucose concentrations compared with controls (Haasio et al. 2002a, 2002b). In contrast, mice given 2,4-DNP in drinking water at a concentration (1 mg/L) yielding doses between 0.03 and 0.105 mg/kg/day exhibited decreased levels of serum glucose and insulin after 14 weeks of exposure (Caldeira da Silva et al. 2008).

Four studies were located that addressed potentially toxic effects of 2,4-DNP on the hypothalamic-pituitary-thyroid axis in rats (Bakke and Lawrence 1965; England et al. 1973; Maayan 1968; Wilkins et al. 1974). In these studies, rats were exposed for 7–30 days to dietary 2,4-DNP at a concentration of 0.2%. These studies all reported extremely rapid body weight loss (as much as 1% of body weight per day), implying that the animals were starving and/or wasting away, and diet-matched control groups were not used. Investigation of subtle endpoints of toxicity (e.g., pituitary levels of thyroid-stimulating hormone, daily fractional turnover rates of thyroxin, serum protein-bound iodine, and pituitary cyclic adenosine monophosphate [cAMP] concentrations) are inappropriate in circumstances in which animals are starving and dying. Thus, these four studies (Bakke and Lawrence 1965; England et al. 1973; Maayan 1968; Wilkins et al. 1974) were considered inadequate to estimate the endpoints addressed.

In a yeast two-hybrid assay for estrogenic activity, 2,4-DNP was not active (Jung et al. 2004). No other information on potential estrogenic effects of 2,4-DNP, and no information on potential androgenic effects, was located.

2.14 IMMUNOLOGICAL

Agranulocytosis has been reported in several case reports of people who took 2,4-DNP as a diet pill. These reports are discussed in the hematological effects section (Section 2.7). A cohort study of

9 patients with acute occupational 2,4-DNP "poisoning" through combined inhalation and dermal exposure and 30 healthy controls evaluated the relationship between exposure and peripheral blood lymphocyte subpopulations (Jiang et al. 2016). Patients were exposed for approximately 5–6 hours; however, the time from exposure to assessment was not reported, although symptoms of poisoning (fever, fatigue, and erythema) appeared within 2–30 hours. The mean 2,4-DNP plasma concentration upon admission was 19.27 mg/L (range 2.01–41.99 mg/L). On day 1 of admission, lymphocyte subpopulations in blood of exposed workers were significantly (≤0.001) different from the recruited control group as follows: total lymphocytes count decreased by 76%; $CD4^+/CD8^+$ ratio increased by 47%; $CD3^+$ cell count decreased by 77%; $CD3^+CD4^+$ cell count decreased by 79%; $CD3^+CD8^+$ cell count decreased by 85%; B cell count decreased by 70%; and natural killer cell counts decreased by 62%.

Whether the dermal effects (urticarial and macropapular rashes discussed in Section 2.11) of 2,4-DNP are related to sensitization is unclear. As described in Section 2.11, a woman who had been taking 2 mg/kg/day 2,4-DNP for 37 days developed severe skin reactions over her entire body (Beinhauer 1934). When she was given contact skin tests with 2,4-DNP, a mildly positive reaction occurred with a 1:2 dilution, and a negative reaction was obtained with 1:10 dilution; the starting 2,4-DNP material used for the dilution was not reported. The authors, however, did not comment on whether they considered her condition to be due to sensitization. Three methods of direct skin testing for sensitization (patch, scratch, and intradermal injection) were performed on 157 people, 117 of whom were patients with hay fever, asthma, or urticaria (Matzger 1934). In addition, an indirect or passive transfer test was performed, in which blood serum from a patient with a violent clinical reaction to DNP was introduced intradermally in nonallergic subjects, followed by challenge 24 hours later with intradermal injection of 2,4-DNP. Both the direct and the passive transfer test were negative. Twelve of the subjects whose tests were negative were given "therapeutic doses" (not further specified) of sodium 2,4-DNP by mouth for an unspecified duration (Matzger 1934). A definite urticarial reaction developed in three of the subjects, at which time they discontinued using the drug. Following disappearance of the dermal lesions, the subjects resumed taking the drug in the same or even larger (unspecified) doses, without any recurrence of the dermal effects. Other studies have noted that some patients who experienced dermal effects were able to resume treatment with no further difficulties or even experienced a disappearance of the rash while still on treatment (Bortz 1934; Tainter et al. 1935). This evidence argues against sensitization.

No gross or histological evidence of treatment-related damage to the spleen was reported following 2,4-DNP treatment of rats exposed in the diet to 5–50 mg/kg/day for 6 months (Spencer et al. 1948) or of dogs (three per dose group) exposed via capsules to 5 or 10 mg/kg/day for 6 months (Tainter et al.

1934b). In addition, no gross or histological evidence of effects was seen in the bone marrow or lymph nodes of the dogs.

2.15 NEUROLOGICAL

Often, the earliest clinical sign of poisoning with 2,4-DNP is agitation or restlessness, progressing rapidly to hyperpyrexia and/or cardiovascular collapse. Case reports of poisoning with 2,4-DNP included other neurological symptoms of headache, weakness or fatigue, malaise, dizziness, delirium, and confusion (Bartlett et al. 2010; Dintenfass 1934; Eichert 1936; Geiger 1933; Goldman and Haber 1936; Holborow et al. 2016; Hsiao et al. 2005; Imerman and Imerman 1936; Masserman and Goldsmith 1934; McFee et al. 2004; Poole and Haining 1934; Tainter and Wood 1934); in fatal cases, patients often lost consciousness and/or convulsed prior to death (Goldman and Haber 1936; Masserman and Goldsmith 1934; Purvine 1936). A patient taking an undetermined dose of 2,4-DNP intermittently for 1 year was semiconscious and occasionally irrational (Imerman and Imerman 1936). These signs are consistent with hyperthermia (Bunai et al. 2012) induced by uncoupling of oxidative phosphorylation by 2,4-DNP (see Sections 2.18 and 2.18.1). In a woman who died after taking 2,4-DNP at a dose of 7 mg/kg/day for 5 days (Poole and Haining 1934), autopsy findings were consistent with death from hyperthermia (Bunai et al. 2012): hyperemia of the spinal cord, pons, and medulla, slight degeneration of ganglion cells in the pons, and capillaries distended with blood. In another case, involving a young woman who died after taking ~1 mg/kg/day 2,4-DNP for 46 days, an autopsy revealed no pathological changes in the cortex, medulla, cerebellum, pons, or proximal portion of the spinal cord, although hemorrhages in several other tissues were seen (Goldman and Haber 1936). Neurological signs in workmen exposed to 2,4-DNP in the French munitions industry included rapid onset of fatigue and agitation in mild and severe cases, and convulsions preceding death in fatal cases; exposure levels, durations, and incidences were not characterized (Perkins 1919).

In an experimental study to determine if 2,4-DNP would be beneficial in the treatment of depression, various responses were observed among 18 psychiatric patients with pre-existing symptoms of listlessness, indifference, mild depression, or lethargy (Masserman and Goldsmith 1934). No psychological change was found for eight patients, lethargy and depression increased in severity in four patients, while alertness increased and depression decreased in six patients. Discontinuation of the drug resulted in marked regression in three of the six patients that benefitted from the treatment.

Clinical studies reported peripheral nervous system effects following acute- and intermediate-duration exposures to 2,4-DNP. No symptoms of peripheral neuritis were reported by 37 patients who took the sodium salt of 2,4-DNP at an estimated dose of 1 mg/kg/day 2,4-DNP for an average of 14 days (Tainter et al. 1935). However, symptoms of peripheral neuritis occurred in 18 of 170 obese patients who ingested an average of 4 mg/kg/day 2,4-DNP from sodium 2,4-DNP for an average of 88 days (Tainter et al. 1935). The treatment regimen involved an initial dose of 1.2 mg/kg/day 2,4-DNP with small increases over time. The neurological effects occurred only among the 100 patients who took ≥4 mg/kg/day for ≥6 weeks and were characterized by abnormal sensations of numbness, "pins and needles," heat and cold, and heightened sensation of pain in the extremities, or loss of taste and numbness and tingling of the tongue. In a clinical study of 15 obese women given 4 mg/kg/day 2,4-DNP for 1–8 weeks, 1 woman experienced a virtual loss of taste that persisted for several weeks after discontinuation of dosing (MacBryde and Taussig 1935). In an extensive clinical study of 159 patients taking 3 mg/kg/day 2,4-DNP as the sodium salt for 22–89 days, 4 frank cases of peripheral neuritis occurred after dosing for 4–10 weeks, persisted for weeks, and gradually abated when dosing was discontinued (Simkins 1937a, 1937b). Five patients lost the sense of taste and developed numbness and tingling of the tongue, usually within the 5^{th}–7^{th} week of dosing. These symptoms generally persisted for 2 days to several weeks but disappeared spontaneously during the continuation of dosing. Several individual case reports described symptoms consistent with peripheral neuritis in patients taking 2,4-DNP for weight reduction. In these reports, doses ranged from 2 to 15.7 mg/kg/day and durations ranged from 10 days to >18 months (Anderson et al. 1933; Bortz 1934; Epstein and Rosenblum 1935; Hitch and Schwartz 1936; Hunt 1934; Nadler 1935; Phillips and Singer 2013).

A few animal studies reported neurological and neurodevelopmental effects following acute and intermediate exposures to 2,4-DNP. In a developmental toxicity study, mouse dams treated by gavage with 38.3 mg/kg/day 2,4-DNP on gestational days 10–12 displayed hyperexcitability (Gibson 1973). In contrast, a decrease in locomotor activity was observed in rats exposed to 30 mg/kg/day (a dose also associated with deaths) from PND 4 to 21 or for 28 days starting at 5–6 weeks of age (Koizumi et al. 2001, 2002). The lower dose (25.5 mg/kg/day) apparently did not produce maternal toxicity. Dogs exposed to 20 mg/kg doses of 2,4-DNP periodically over 45–77 days (7–12 times) or to 5 or 10 mg/kg/day 2,4-DNP for 6 months had no gross or histological evidence of brain damage or spinal cord lesions (Tainter and Cutting 1933b; Tainter et al. 1934b).

2.16 REPRODUCTIVE

Three case reports and a clinical study described reproductive effects in women taking 2,4-DNP for weight reduction. A young girl who subsequently died after taking 1.03 mg/kg/day 2,4-DNP was found to have a small and infantile uterus and numerous follicular cysts in the ovary (Goldman and Haber 1936). Physical examination of a woman who took 2 mg/kg/day 2,4-DNP revealed fibroid degeneration of the uterus and a cystic left ovary (Beinhauer 1934). Whether or not these were preexisting conditions is not known. A woman who had been taking 3 mg/kg/day 2,4-DNP as the sodium salt for 98 days was found to be pregnant (Epstein and Rosenblum 1935). After taking the drug for an additional 45 days (at approximately 14 weeks of pregnancy), she was hospitalized for profuse vaginal bleeding, and no evidence of a fetus was found (Epstein and Rosenblum 1935). The authors suggested that 2,4-DNP caused a premature separation of the placenta, resulting in miscarriage; however, the precise cause of the miscarriage is uncertain. In a clinical study of 159 patients taking 3 mg/kg/day 2,4-DNP as the sodium salt for 22–89 days, 15 women developed altered menstrual cycles or amenorrhea and 18 women experienced excessive menstrual edema (Simkins 1937a, 1937b). Because the menstrual changes in many of the women were marked and occurred so soon after 2,4-DNP dosing, the study author concluded that the altered menstrual cycles were likely attributable to 2,4-DNP intake (Simkins 1937a, 1937b).

2,4-DNP administered by gavage at 30 mg/kg/day for 5 days resulted in a slight, but not statistically significant, increase in the incidence of tailless sperm (Takahashi et al. 2004); however, a longer study at the same dose did not show any effects on spermatogenesis or histopathologic changes to male reproductive organs (Takahashi et al. 2009). In a Hershberger assay validation study (Yamasaki et al. 2006), 2,4-DNP did not alter reproductive organ weights in castrated 6- or 7-week-old male rats exposed to 2,4-DNP by gavage for 10 consecutive days. When administered with testosterone propionate (testing for androgen receptor antagonism), 2,4-DNP resulted in an increase in the weight of the levator ani and bulbocavernosus muscle complex (LABC), but did not affect the weights of other organs; the authors concluded that 2,4-DNP did not exhibit androgen receptor antagonism (Yamasaki et al. 2006). In a reproductive toxicity screening study conducted according to OECD Guideline 421, Takahashi et al. (2009) reported no effects of 2,4-DNP on spermatogenesis at gavage doses up to 30 mg/kg/day for 46 days. In that study, no effects were seen on estrous cyclicity, length of gestation, copulation, fertility and nursing indices, or weights or histology of testes, epididymides, or ovaries (Takahashi et al. 2009). Longer-duration studies also showed no gross or histological evidence of treatment-related testicular damage in rats exposed in the diet to 5–50 mg/kg/day for 6 months (Spencer et al. 1948), rats exposed in the diet to 60 mg/kg/day for life (Tainter 1938), or dogs exposed via capsules to 5 or 10 mg/kg/day for

6 months (Tainter et al. 1934b). Rats exposed at 350 mg/kg/day did show signs of testicular atrophy (Spencer et al. 1948), but this may have been the result of starvation.

2.17 DEVELOPMENTAL

Data on developmental effects following gestational exposure to 2,4-DNP are limited to animal studies involving maternal oral exposure and one study of neonatal exposure. Data from animal studies indicate that oral exposure to 2,4-DNP can be embryotoxic, resulting in stillbirths, increased resorptions, and decreased fetal body weight. The potential teratogenicity of 2,4-DNP has not been adequately studied. The most reliable information comes from a reproduction/ developmental toxicity screening study (Takahashi et al. 2009) conducted according to OECD Guideline 421. In this study, rats were exposed to 2,4-DNP by daily gavage for 2 weeks prior to mating and through mating, gestation, and through PND 3. The numbers of live pups on PNDs 0 and 4 were significantly reduced, as were the body weights of live male and female pups on PNDs 0 and 1 at a dose of 30 mg/kg/day (Takahashi et al. 2009). The pups were observed for internal and external malformations, but not for skeletal malformations. The effects on pup viability are supported by an early study of white rats that were dosed for 8 days prior to mating, during pregnancy, and during lactation (Wulff et al. 1935). This study did not indicate clearly whether the dams received 10 or 20 mg/kg twice daily; thus, it is not known whether the dose was 20 or 40 mg/kg/day. The study did not evaluate potential teratogenicity. While maternal body weight gain was not affected by the treatment, dams dosed with 2,4-DNP had a 25% stillborn rate compared with 7% in controls; in addition, the mortality rate for pups during lactation was 30.9% for the treated rats and 13.4% for the control groups (Wulff et al. 1935).

Two studies in mice (Gibson 1973; Kavlock et al. 1987) showed no evidence of developmental toxicity; however, in both studies, the mice were exposed during only a portion of organogenesis, only Gibson (1973) evaluated teratogenicity, and reporting of 2,4-DNP effects in both studies was limited. In a developmental toxicity screening study of 46 chemicals, CD-1 mice were treated by gavage with 125 mg/kg/day 2,4-DNP on gestation days 8–12 and sacrificed following parturition (Kavlock et al. 1987). Two dams each died in the control (40 mice) and treatment (30 mice) groups; the causes of death were not reported. Maternal body weight gain in treated mice was not significantly ($p>0.05$) different from controls. The percent of live litters and resorptions and the numbers and weights of live offspring on PNDs 1 and 3 were not significantly different ($p>0.05$) from controls. Teratogenicity was not evaluated. When Swiss-Webster mice were treated by gavage with 0, 25.5, or 38.3 mg/kg/day 2,4-DNP on gestation days 10–12 and then sacrificed on gestation day 19, the percentage resorptions was

increased, but the effect was not statistically significant or dose-related (Gibson 1973). No treatment-related changes in the incidences of external, visceral, or skeletal anomalies were observed (Gibson 1973). The authors indicated that higher doses produced overt toxicity (hyperexcitability and hyperthermia) in dams, but no deaths.

In a neonatal exposure study, a dose of 30 mg/kg/day administered by gavage to rats from PND 4 to 21 (18 days) resulted in the death of 6/10 animals and decreased locomotor activity in 1/4 survivors (Koizumi et al. 2001, 2002). In contrast, older (5–6 weeks of age) rats survived the same dose administered for 28 days (Koizumi et al. 2001, 2002). Neonatal exposure to 20 mg/kg/day for 18 days resulted in a 14% decrease in terminal body weight (Koizumi et al. 2001, 2002). These data demonstrate greater susceptibility of young rats to the lethal effects of 2,4-DNP.

Parenteral studies examining limited endpoints (Gibson 1973; Goldman and Yakovac 1964) provide support for the developmental toxicity findings seen after oral exposure to 2,4-DNP. In mice, intraperitoneal injection of 2,4-DNP at a dose of 13.6 mg/kg/day on gestational days 10–12 resulted in a nonsignificant increase in the percentage of resorptions (mean of 14.1% per litter versus 4.4% in untreated controls) and significant decreases in fetal body weight and crown-rump length (Gibson 1973). The numbers of implantations and fetuses were not affected, and no other endpoints were evaluated. Decreased fetal weight and length and more early resorptions were also reported in rats injected subcutaneously with 1,000 mg of 2,4-DNP as the sodium salt (Goldman and Yakovac 1964).

A study in zebrafish showed developmental effects of 2,4-DNP. When zebrafish embryos were cultured with 2,4-DNP (≥3 mg/L) for 4 days, developmental effects included lack of somite formation, incomplete head and eye formation, tail curvature, weak pigmentation, kyphosis, scoliosis, and nonpigmentation (Ceylan et al. 2016).

2.18 OTHER NONCANCER

2.18.1 Metabolic Effects from Uncoupling of Oxidative Phosphorylation

The characteristic effects of 2,4-DNP exposure are consequences of the uncoupling of oxidative phosphorylation (see Section 2.18.2): elevation of the basal metabolic rate and elevation of body temperature, with increased perspiration. The body compensates for these effects by increasing the

respiratory rate to deliver more oxygen to the tissues. As body temperature rises, peripheral vasodilation occurs as a cooling mechanism and the pulse rate rises to maintain the circulation.

In the studies described in this section, metabolic rate was measured indirectly by oxygen consumption. True metabolic rates (heat generated per unit time) are measured by calorimetry, which is very expensive and technically difficult. Oxygen consumption was found to correlate reasonably well with true metabolic rates when expressed as liters of oxygen consumed per unit of time per square meter of body surface area. Advances in measuring basal metabolic rate will provide increased accuracy and precision in measurements (Lam and Ravussin 2016).

Case reports, experimental studies, and clinical studies of exposure to 2,4-DNP, taken together, suggest dose-related changes in basal metabolic rate, with little change seen across acute, intermediate, and chronic exposure durations. In general, basal metabolic rate increases on the order of ~10% were reported for each 100 mg (~1 mg/kg) increase in dose. In studies with one obese patient per dose regimen, exposure to 1 mg/kg/day 2,4-DNP for ~6 days resulted in an increase in basal metabolic rate of 12%, single or repeated doses of 2 mg/kg/day resulted in increases of 25–27%, and repeated doses of 3 mg/kg/day in a patient with severe hypothyroidism (myxedema) resulted in increases of 35–42% (Dunlop 1934). In an experimental study, in which four volunteers were placed on various diets (balanced, high carbohydrate, high fat, or high protein) and given an average dose of 4 mg/kg/day 2,4-DNP for 7–16 days, increases in basal metabolic rates of 27–55% were found regardless of diet type (Cutting and Tainter 1933). Clinical studies with larger numbers of patients have reported increases in basal metabolic rates of 26% in non-obese subjects who received 3 mg/kg/day 2,4-DNP for 2 days (Castor and Beierwaltes 1956); 38% in normal or obese subjects with normal pretreatment basal metabolic rates given 4 mg/kg/day 2,4-DNP as the sodium salt for 1–2 weeks (Cutting et al. 1934); 23% in subjects (most of whom had low pretreatment basal metabolic rates and obesity/hypothyroidism) given 4 mg/kg/day 2,4-DNP as the sodium salt for 3–13 weeks (Cutting et al. 1934); 50% in schizophrenic patients with low pretreatment basal metabolic rates given 3–4 mg/kg/day 2,4-DNP for 7 weeks (Looney and Hoskins 1934); and 32.9% in 13 psychiatric patients given increasing doses for 3–4 months (Masserman and Goldsmith 1934). An increase in basal metabolic rates of 30–70% was seen within the first 24 hours in obese patients who ingested 4 mg/kg/day 2,4-DNP for 1–8 weeks, and was maintained throughout the treatment period; pretreatment values were not reported (MacBryde and Taussig 1935). In clinical studies of patients treated for 22–89 days, the average increase in basal metabolic rate rose ~11% for each 100 mg (1 mg/kg) increase in dose (Simkins 1937a, 1937b; Tainter et al. 1935). In general, basal metabolic rate increases of 10–29% result in increased body temperature that may be adverse and

increases of ≥30% may result in severe pyrexia and potentially death. The basal metabolic rate increased by 38% in a psychiatric patient who subsequently died after being given 3 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Masserman and Goldsmith 1934). However, Epstein and Rosenblum (1935) reported an 82% increase in basal metabolic rate in the case of a woman who survived exposure to 3 mg/kg/day 2,4-DNP over a period of 182 days.

Symptoms and signs related to the increased metabolic rate, such as a sensation of warmth, increased perspiration, and increased body temperature, have been noted in the above studies at doses as low as 1 mg/kg/day 2,4-DNP; these symptoms and signs became much more severe at higher doses. Normal body temperature in humans is 36.5–37.5°C (97.7–99.5°F); hyperthermia consists of temperatures greater than 37.5–39.3°C (99.5–100.9°F), while temperatures above 40°C (104°F) are generally characterized as severe hyperthermia or hyperpyrexia, which is life-threatening. In fatal cases of 2,4-DNP poisoning, body temperatures between 100 and 110°F (38–43°C) have been reported (Dameshek and Gargill 1934; Geiger 1933; Goldman and Haber 1936; Masserman and Goldsmith 1934; Poole and Haining 1934; Purvine 1936; Suozzi et al. 2005; Tainter and Wood 1934; Tewari et al. 2009); other reports of fatal cases described extreme or uncontrollable fever (Holborow et al. 2016; Hsiao et al. 2005) or just reported fever (McFee et al. 2004; Miranda et al. 2006; Siegmueller and Narasimhaiah 2010). Some of the variability in temperatures reported in case reports of fatalities may stem from differences in the sensitivity of the thermometer instrument used, physiological site of measurement (oral, axillary, rectal, tympanic membrane), timing of temperature measurement relative to exposure and death, and therapeutic efforts to control hyperthermia. Bartlett et al. (2010) reported only slightly elevated temperature (37.8°C) in a fatal case of 2,4-DNP poisoning; in another fatal case (Lattimore 1934), the body temperature was also reported to be normal. Symptoms of life-threatening hyperthermia/hyperpyrexia include tachycardia and tachypnea; profuse sweating; headache; nausea and vomiting; muscle pain or weakness; and behavioral changes such as confusion, agitation, and delirium. Seizures and coma may occur, and multi-organ failure is common. These symptoms and findings are seen in virtually all fatal cases of 2,4-DNP poisoning (see Section 2.2).

In nonfatal case reports of individuals seeking medical attention, many symptoms of hyperthermia, especially diaphoresis, have been reported (Anderson et al. 1933; Bortz 1934; Davidson and Shapiro 1934; Dintenfass 1934; Eichert 1936; Epstein and Rosenblum 1935; Hunt 1934; Imerman and Imerman 1936; Le et al. 2015; Lee et al. 2014; Rank and Waldeck 1936; van Veenendaal et al. 2011). Actual increases in body temperature were not seen at repeated doses of 3 mg/kg/day (Dunlop 1934) or single doses between 5 and 10 mg/kg, but increases of ≥3°C were seen with single doses >10 mg/kg (exact

doses not specified) (Cutting et al. 1933) and after two doses of 46 mg/kg/dose taken 1 week apart (Tainter and Wood 1934). A woman who took 4.4 mg/kg/day 2,4-DNP for 4 days experienced profuse perspiration within a few hours after the first dose (Dintenfass 1934).

Similar findings were reported in clinical studies. A group of 13 obese patients given 4 mg/kg/day for 4–12 days experienced sensations of warmth and excessive perspiration (Stockton and Cutting 1934). In an experimental study, in which four volunteers were placed on various diets (balanced, high carbohydrate, high fat, or high protein) and given an average dose of 4 mg/kg/day 2,4-DNP for 7–16 days, all subjects experienced a feeling of warmth and excessive perspiration regardless of diet type (Cutting and Tainter 1933). Side effects experienced by 23 obese patients taking ~2 mg/kg/day 2,4-DNP as the sodium salt for 51–62 days included perspiration and elevated temperature (not specified) (Bayer and Gray 1935). High body temperature (102.8°F or 39.3°C) and/or excessive perspiration were also found in patients taking 3–4 mg/kg/day for 35–60 days (Hunt 1934; Imerman and Imerman 1936; Rank and Waldeck 1936). A woman complained of profuse perspiration and had a body temperature as high as 103°F (39.4°C) after taking an indeterminate dose of 2,4-DNP intermittently for 1 year (Imerman and Imerman 1936).

Rats given 20 mg/kg/day 2,4-DNP by gavage for 15 days had significant increases in body temperature (1.3°C higher than controls at the peak; Haasio et al. 2002a, 2002b). Electron microscopy revealed mitochondrial swelling, deformed or broken mitochondrial cristae, and reduced matrix density in the mitochondria in the liver and skeletal muscle of the exposed animals. In dogs, dose-related increases in body temperature have been reported after acute exposure to 2,4-DNP. Single doses of 10 mg/kg/day 2,4-DNP caused slight elevations in body temperatures of dogs; higher elevations in body temperature (1–2.5°C) were associated with exposure levels of 15–20 mg/kg/day (Tainter and Cutting 1933a, 1933b). Increased body temperature (quantitative data not reported) was observed in dogs fed capsules containing 25 mg/kg/day 2,4-DNP for 1 or 14 days (Kaiser 1964) and in rats consuming 350 mg/kg/day (Bakke and Lawrence 1965). Hyperthermia (quantitative data not reported) was observed in pregnant mice treated by gavage with 38.3 mg/kg/day 2,4-DNP on gestation days 10–12 (Gibson 1973).

Resting oxygen uptake was significantly increased in eight adult male Wistar rats given 30 mg/kg/day 2,4-DNP in drinking water for 28 days (Schlagowski et al. 2014). A 30–85% increase in oxygen consumption was observed in mice exposed to 110 mg/kg/day in the diet for 26 days (Pugsley 1935). Fecal excretion of calcium and urinary excretion of creatine and creatinine were increased to 200, 45, and 400%, respectively, of preexposure levels. The toxicological significance of these changes in excretion

was not clear, but high calcium excretion would be expected to result in neuromuscular toxicity and high excretion of creatine and creatinine are often a result of muscle toxicity.

In bobwhite quail hens exposed to doses of 2,4-DNP of 33.6 and 56.1 mg/kg/day (as calculated from feed consumption) over an 8-day period, metabolic rates (as measured by oxygen and carbon dioxide exchange) and energy consumption were increased in a dose-related manner during exposure, and returned to normal after exposure was discontinued (Dominguez et al. 1993). Birds receiving 33.6 mg/kg/day had metabolic rates 31–41% higher than controls during the dark period (nighttime) and 23–32% higher during the light period (daytime). Birds receiving 56.1 mg/kg/day had dark period metabolic rates 48–77% higher than controls and light period rates 41–67% higher than controls. At 33.6 mg/kg/day, the birds expended 32% more energy than controls during exposure and at 56.1 mg/kg/day, the birds expended 60% more energy.

2.18.2 Mechanism of Action—Oxidative Phosphorylation Uncoupling

The ability of 2,4-DNP to uncouple oxidative phosphorylation underpins many of the clinical observations and physiological effects of its toxicity in both humans and animals. Such effects include elevated basal metabolic rate or oxygen consumption, elevated respiration and pulse rates, increased perspiration, and increased body temperature, and are related to the uncoupling of oxidative phosphorylation. *In vitro* demonstrations of 2,4-DNP's ability to uncouple oxidative phosphorylation began as early as 1948 (Loomis and Lipmann 1948; see also Ilivicky and Casida 1969; Muscatello et al. 1975; Pinchot 1967; Weinbach and Garbus 1969). During the Krebs cycle, 2,4-DNP and other lipophilic weak acids uncouple oxidative phosphorylation from electron transport by picking up protons, diffusing across the inner mitochondrial membrane, deprotonating, and returning to pick up more protons, thereby dissipating the pH gradient and membrane electrochemical potential needed for the formation of ATP (Lou et al. 2007; Stryer 1988). During this uncoupling, electron transport from NADH to oxygen can increase several-fold, but the energy produced, which is normally stored as the chemical potential of ATP, is released as heat. The prevention of ATP formation by 2,4-DNP means that all energy-dependent biochemical processes are likely to be affected. In addition, the depletion of ATP associated with mitochondrial uncoupling may produce hyperkalemia. 2,4-DNP has been reported to induce potassium accumulation in rabbit kidney slices (Mudge 1951), and hyperkalemia has been observed in humans poisoned with 2,4-DNP (Jiang et al. 2011).

Studies conducted in mice have shown that the severity of toxicity produced by 2,4-DNP can be modified by ambient temperature, with increased toxicity observed at higher ambient temperatures (Harvey 1959). Facultative thermogenesis (e.g., brown fat thermogenin pathway) is a contributing factor in the temperature modulation of 2-4-DNP toxicity. In mice maintained at low temperatures, facultative thermogenesis is activated. This physiological response at low temperature is suppressed by thermogenesis induced by 2,4-DNP (Goldgof et al. 2014).

The body attempts to dissipate the increased heat by inducing dilation of blood vessels. When heat production exceeds the organism's capacity to dissipate heat, fatal hyperthermia may result (Murphy 1986). Symptoms reported in humans who were poisoned by 2,4-DNP exposure are similar to those seen in heat stroke, including headache; nausea and vomiting; muscle pain or weakness; and behavioral changes such as restlessness, confusion, agitation, and delirium. Hyperkalemia in turn may produce muscle pain and weakness often reported by humans after exposure to 2,4-DNP. With both 2,4-DNP exposure and hyperthermia, these symptoms may progress to seizures and coma, and may be accompanied by rhabdomyolysis, acute renal failure, and other signs of multiorgan failure; the cause of death is typically cardiac arrest (see Section 2.2 for effects of 2,4-DNP, and Power et al. [2014] and Trujillo and Fragachán [2011] for hyperthermia). Autopsy findings after fatal 2,4-DNP poisoning often show widespread tissue hyperemia, congestion, and hemorrhage resulting from vasodilation (see Section 2.2).

Both endogenous and exogenous chemicals that uncouple oxidative phosphorylation, including 2,4-DNP, have recently been explored in animal models as potential therapies for prevention or mitigation of obesity, Type II diabetes, and aging (reviewed by Divakaruni and Brand 2011). Uncoupling of oxidative phosphorylation has been shown to induce weight loss and improve glucose homeostasis, effects also seen with exposure to 2,4-DNP (Caldeira da Silva et al. 2008; Goldgof et al. 2014; see also Sections 2.3 and 2.13). Furthermore, because superoxide anion is a byproduct of oxidative phosphorylation as well as a potent cellular oxidant, uncoupling has been shown to reduce oxidative stress, thought to be an important mechanism of aging (reviewed by Divakaruni and Brand 2011). Indeed, several measures of oxidative stress were decreased, and survival was prolonged, in mice exposed to low levels of 2,4-DNP for their natural lifespan (Caldeira da Silva et al. 2008). However, the narrow margin of safety between a 2,4-DNP dose that is beneficial and that which is toxic, even lethal, limits the potential therapeutic uses of 2,4-DNP (Divakaruni and Brand 2011; Lou et al. 2007).

Little information is available on the uncoupling potency of other DNP isomers. In experiments designed to facilitate development of a quantitative structure-activity relationship (QSAR) for uncoupling activity of substituted phenols, Escher et al. (1999) showed that 3,4-DNP uncoupled oxidative phosphorylation in Rhodobacter membrane vesicles, with higher rates of uncoupling observed at lower pH (tested from pH 5.3 to 8.25). In isolated rat liver mitochondria, the effective concentrations for uncoupling by the DNPs were 20, 30, 40, 40, 100, and 100 µM for 3,5-, 2,4-, 2,6-, 3,4-, 2,3-, and 2,5-DNP, respectively (Burke and Whitehouse 1967). This order is not entirely congruent with the acute lethality of the isomers in animals exposed via intraperitoneal injection (LD_{50} values were 45, 35, 38, 98, 190, and 150 mg/kg in rats for the same order of DNPs; Harvey 1959), but does provide support for the lower relative potency of 2,3- and 2,5-DNP compared with the others. The lower potency of 2,3- and 2,5-DNP is also supported by the observation that mouse intraperitoneal LD_{50} values for these isomers did not change with increasing temperature (Harvey 1959), suggesting lower potential for inducing chemical hyperthermia.

Metabolic effects of 2,4-DNP also appear to influence intracellular calcium levels. Hudman et al. (2002) investigated the basis for DNP-induced increase in cytoplasmic calcium in rat cardiac myocytes. Their results indicated that the increase in cytoplasmic calcium occurs in two phases. The first phase appears to result from the release of mitochondrial calcium due to mitochondrial depolarization. The second phase appears to be the result of a progressive release of calcium from the sarcoplasmic reticulum following depletion of intracellular ATP.

In addition, 2,4-DNP-induced toxicity may involve activation of ATP-sensitive K+ channels (Ravesloot and Rombouts 2000). Wu et al. (2000) reported increased ATP-sensitive K+ channel activity in pituitary GH3 cells treated with 2,4-DNP.

The DNP metabolites for which toxicity information is available appear to have much lower systemic toxicity than 2,4-DNP. This is most likely due to the fact that they are much less potent in uncoupling oxidative phosphorylation than 2,4-DNP. However, while 2,4-DNP is metabolized to compounds with lower systemic toxicity, the aminonitrophenols produced are mutagenic in test systems and show some evidence of carcinogenicity in chronic-duration tests in rats and mice. These data are difficult to reconcile with the generally negative results obtained with 2,4-DNP with S9 activation since the S9 fraction contains both microsomes and the soluble enzymes that metabolize 2,4-DNP to the aminonitrophenols (Eiseman et al. 1972). The generally negative results for genotoxicity of 2,4-DNP in test systems where metabolic activation was present may be related to the dependence of 2,4-DNP

reduction on ATP. 2,4-DNP metabolism requires ATP; unless the S9 fraction contains an ATP-regenerating system, 2,4-DNP may not be metabolized.

2.18.3 Effects on Hearing

Two case reports described secondary effects on the ear and hearing. In one report, a woman who developed severe dermal lesions over 100% of the body surface about 10 months after taking 1.86 mg/kg/day 2,4-DNP for 2 weeks also developed hearing difficulty (Hitch and Schwartz 1936). The hearing impairment was attributed to a reactive exudation in the middle ear rather than to nerve impairment. In the second report, a woman who experienced pharyngitis after taking one dose of 4.4 mg/kg/day 2,4-DNP complained of pain and fullness in the ears, which became more severe and led to hearing impairment after the fourth dose (Dintenfass 1934). This condition persisted for another 2 months, at which time an examination revealed bulged, reddened eardrums, with obliterated landmarks and a 30% decrement in hearing. The condition was secondary to congestion and inflammation of the pharynx and persisted for an additional 7 months.

2.19 CANCER

Data on cancer in humans or animals exposed to DNPs are limited to skin-painting studies in mice. In mouse skin-painting initiation-promotion protocol studies, 2,4-DNP was not effective as a promotor of skin tumors initiated by exposure to 7,12-dimethylbenz(a)anthracene (DMBA), with or without co-treatment with the known promoter croton oil. Female Sutter mice (initial age 2–3 months) received a single initiating dose of 0.3% DMBA in acetone applied to a shaved area of the back (Boutwell and Bosch 1959). 2,4-DNP was then applied to the same area twice weekly for 12 weeks at a time-weighted average (TWA) dose of 80 mg/kg/day. The survival rate was 100%; no evidence of skin papillomas or carcinomas was observed. In female Swiss mice, papillomas occurred in 6 of 30 mice (20%) receiving DMBA followed by 2,4-DNP plus croton oil (twice weekly for 50 weeks); in 8 of 30 mice (27%) receiving DMBA followed by acetone plus croton oil; in 4 of 30 mice (13%) receiving DMBA followed by acetone alone; in 2 of 30 mice (7%) receiving DMBA followed by 2,4-DNP plus acetone; and in 30 of 50 mice (60%) receiving DMBA followed by croton oil alone (Stenback and Garcia 1975). In another experiment, 2,4-DNP was applied to the back 2 days before initiation with DMBA, during initiation, and 2 days after initiation, followed by promotion with croton oil. The incidence of papillomas was 32 of 50 in this group compared with 30 of 50 in the group receiving DMBA alone followed by croton oil

(Stenback and Garcia 1975). Thus, 2,4-DNP had no significant influence on DMBA initiation of tumors promoted by croton oil.

Metabolites of 2,4- and 2,5-DNP administered orally have increased tumor incidences in male rats, but not in female rats or in mice. Male rats exposed to 4-amino-2-nitrophenol exhibited renal cortical adenomas in one study (NTP 1988a) and a significant increase in the incidence of transitional cell carcinomas of the urinary bladder in an earlier study (NCI 1978). Male rats exposed to 2-amino-5-nitrophenol showed an increased incidence of pancreatic cell adenomas (NTP 1988b).

2.20 GENOTOXICITY

No studies were located regarding genotoxic effects in humans after exposure to DNP.

2,4-DNP has been tested for genotoxicity in several *in vivo* and numerous *in vitro* test systems; 2,3-, 2,5-, 2,6-, 3,4-, and 3,5-DNP were tested *in vitro* for mutagenicity (see Tables 2-6 and 2-7). *In vivo* studies have evaluated effects on DNA synthesis and clastogenic effects. Regarding effects on DNA synthesis, some chemical mutagens and carcinogens bind covalently to deoxyribonucleic acid (DNA) and inhibit DNA synthesis. Two studies assessed the effects of 2,4-DNP administered once by gavage on DNA synthesis in testicular cells (Friedman and Staub 1976; Seiler 1981). In one study, the rate of DNA synthesis in mice treated with 20 mg/kg 2,4-DNP was essentially the same as that of untreated mice. The authors concluded that 2,4-DNP was not genotoxic under these experimental conditions (Friedman and Staub 1976). In another study, DNA synthesis (as determined by the ratio of the rate of uptake of tritiated thymidine injected 3 hours after drug administration to the rate of uptake of 14C-thymidine injected 16 hours before drug administration) in testicular cells of mice treated with 30 mg/kg 2,4-DNP was 55% less than that of untreated mice (Seiler 1981). Based on further *in vitro* experiments, the study author suggested that the inhibition of DNA synthesis by 2,4-DNP was due to some other mechanism than genotoxicity, probably produced by 2,4-DNP-induced suppression of cellular metabolism and, therefore, DNA synthesis. One study evaluated the clastogenic effects of 2,4 DNP. Mice were injected intraperitoneally with 0.25, 0.50, and 1 mL of a saturated solution of 2,4-DNP and then sacrificed 24 hours posttreatment for analysis of bone marrow cells for chromosomal aberrations (Mitra and Manna 1971). A dose-related increase in percentage of these aberrations was observed. The authors concluded that 2,4-DNP was clastogenic under the assay conditions and attributed the effect to the compound's electrophilic properties. No studies were located regarding *in vivo* testing for genotoxicity after exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

Table 2-6. Genotoxicity of 2,4-Dinitrophenol *In Vivo*

Species (test system)	Endpoint	Results	Reference	Isomer
Mammalian cells:				
Mouse (intraperitoneal)	Chromosomal aberrations (bone marrow cells)	+	Mitra and Manna 1971	2,4-DNP
Mouse (gavage)	Reduced DNA synthesis (testicular cells)	+	Seiler 1981	2,4-DNP
Mouse (gavage)	Reduced DNA synthesis (testicular cells)	–	Friedman and Staub 1976	2,4-DNP

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

Table 2-7. Genotoxicity of Dinitrophenols *In Vitro*

		Results			
		Activation			
Species (test system)	Endpoint	With	Without	Reference	Isomer
Prokaryotic organisms:					
Salmonella typhimurium					
TA98	Reverse mutation	–	–	Kubo et al. 2002	2,4-DNP
TA100		–	–		
TA98	Reverse mutation	No data	–	Chiu et al. 1978	2,4-DNP
TA100		No data	–		
TA1538	Reverse mutation	–	–	Garner and Nutman 1977	2,4-DNP
TA98	Reverse mutation	–	No data	Anderson and Styles 1978	2,4-DNP
TA100		–	No data		
TA1535		–	No data		
TA1538		–	No data		
TA1530	Reverse mutation	No data	–	Kleinhofs and Smith 1976	2,4-DNP
TA98	Reverse mutation	–	–	Probst et al. 1981	2,4-DNP
TA100		–	–		
TA1535		–	–		
TA1538		–	–		
G46		–	–		
C7036		–	–		
D3052		–	–		

Table 2-7. Genotoxicity of Dinitrophenols *In Vitro*

Species (test system)	Endpoint	Results		Reference	Isomer
		Activation			
		With	Without		
TA98	Reverse mutation	–	–	De Flora 1981	2,4-DNP
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
TA1538		–	–		
TA98	Reverse mutation	–	(+)	Kawai et al. 1987	2,4-DNP
TA100		–	(+)		
TA98	Reverse mutation	+	+	Kawai et al. 1987	2,3-DNP
TA100		+	+		
TA98	Reverse mutation	+	+	Kawai et al. 1987	2,5-DNP
TA100		+	+		
TA98	Reverse mutation	–	–	Kawai et al. 1987	2,6-DNP
TA100		–	–		
TA98	Reverse mutation	–	–	Kawai et al. 1987	3,4-DNP
TA100		+	+		
TA98	Reverse mutation	+	+	Neuwoehner et al. 2007	3,5-DNP
TA100		+	+		
TA1535/Psk1002	DNA damage (induction of sister chromatid exchange response)	–	–	Nakamura et al. 1987	2,4-DNP
Salmonella choleraesius subsp. chol.					
TA1535/pSK1002	DNA damage	–	+	Neuwoehner et al. 2007	3,5-DNP
TA1535/pSK1002/ pNM12		+	+		
Escherichia coli					
WP2	Reverse mutation	–	–	Probst et al. 1981	2,4-DNP
WP2(uvrA-)		–	–		
B/Sd-4/1,3,4,5	Reverse mutation	No data	+	Demerec et al. 1951	2,4-DNP
B/Sd-4/3,4		No data	+		
K-12(lambda)	Phage induction	No data	–	Heinemann and Howard 1964	2,4-DNP
PQ37	DNA damage	+	–	Neuwoehner et al. 2007	3,5-DNP

Table 2-7. Genotoxicity of Dinitrophenols *In Vitro*

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference	Isomer
Eukaryotic organisms: Mammalian cells					
Human lymphoblasts (TK6)	Chromosomal aberrations	No data	(+)	Hilliard et al. 1998	2,4-DNP
Human blood peripheral lymphocytes	Chromosomal aberrations	No data	+	Huang et al. 1995, 1996	2,4-DNP
CHO cells	Chromosomal aberrations	No data	+	Hilliard et al. 1998	2,4-DNP
CHO cells V79	DNA damage (alkali elution)	–	–	Swenberg et al. 1976	2,4-DNP
Rat hepatocytes	Unscheduled DNA synthesis	No data	–	Probst et al. 1981	2,4-DNP
Mouse leukemia L1210	DNA damage (alkali elution)	No data	+[a]	Hilton and Walker 1977	2,4-DNP
Human HeLa cells	DNA damage (alkali elution)	No data	+[a]	Hilton and Walker 1977	2,4-DNP
Chinese hamster V79 cells	Inhibition of replicative DNA synthesis	No data	+	Richard et al. 1991	2,4-DNP

[a]Removal of 2,4-DNP allowed for repletion of ATP pools and repair of DNA damage; therefore, positive finding is related to depletion of DNA pools.

– = negative result; + = positive result; (+) = weakly positive result; ATP = adenosine triphosphate; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; DNP = dinitrophenol

In *in vitro* studies of prokaryotic organisms, 2,4-DNP was negative for reverse mutations using one or more standard stains of *Salmonella typhimurium* (TA98, TA100, TA1530, TA1535, TA1537, TA1538, G46, C7036, D3052) with and/or without metabolic activation by rat liver S9 microsomes (Anderson and Styles 1978; Chiu et al. 1978; De Flora 1981; Garner and Nutman 1977; Kleinhofs and Smith 1976; Probst et al. 1981). For reverse mutation, a weakly positive response was observed in *Salmonella* strains TA98 and TA100 without metabolic activation; with metabolic activation, 2,4-DNP was negative (Kawai et al. 1987). The negative results for mutagenicity of 2,4-DNP with S9 are surprising in light of the fact that the two major metabolites of 2,4-DNP (2-amino-4-nitrophenol and 4-amino-2-nitrophenol) are genotoxic in several test systems. The S9 fraction contains both microsomal and soluble enzymes that metabolize 2,4-DNP to amino nitrophenols (Eiseman et al. 1972). However, 2,4-DNP metabolism

requires ATP; unless the S9 fraction contains an ATP regenerating system, 2,4-DNP may not be metabolized.

Among the other DNP isomers, 2,3-, 2,5-, and 3,5-DNP were positive for reverse mutations in the TA98 and TAl00 strains of *S. typhimurium* with or without metabolic activation; 2,6-DNP was negative in both strains with or without metabolic activation; and 3,4-DNP was negative in TA98 and positive in TAl00 both with and without metabolic activation (Kawai et al. 1987; Neuwoehner et al. 2007).

Using *Escherichia coli* as the test organism, 2,4-DNP was negative for reverse mutation in the Wp2 and Wp2(uvrA-) strains with and without metabolic activation (Probst et al. 1981). Positive results for mutagenicity were reported for reverse mutation in the B/Sd-4/1,3,4,5 and B/Sd-4/3,4 strains of *E. coli* without metabolic activation (Demerec et al. 1951). The authors concluded that 2,4-DNP was clearly positive for mutagenicity; however, the data appeared unreliable, based on extreme variation in survival and mutation rates within exposure groups.

In *in vitro* studies, 2,4-DNP generally did not produce DNA damage in prokaryotic or eukaryotic organisms, but 3,5-DNP did. 2,4-DNP was negative for DNA damage in the TA1535/pSK1002 strain of *S. typhimurium* (as determined by induction of the SOS response) with and without metabolic activation (Nakamura et al. 1987); in the K12(λ) strain of *E. coli* (as determined by phage induction) without metabolic activation (Heinemann and Howard 1964); in rat hepatocytes (as determined by unscheduled DNA synthesis) (Probst et al. 1981); and in Chinese hamster ovary cells (as determined by alkali elution) with or without metabolic activation (Swenberg et al. 1976). One study reported increases in DNA damage (as determined by alkali elution) in mouse leukemia L1210 cells and human HeLa cells (Hilton and Walker 1977); however, the observed effects were related to depletion of ATP pools, and the removal of the 2,4-DNP allowed for repletion of the pools and repair of DNA damage. Based on the weight of evidence presented, 2,4-DNP was negative for DNA damage, either with or without metabolic activation. In contrast, 3,5-DNP induced DNA damage in *Salmonella choleraesius* subsp. chol. (Neuwoehner et al. 2007).

Numerous *in vitro* studies reported decreased DNA synthesis and/or changes in the mitotic index in mammalian cells exposed to 2,4-DNP (Garrett and Lewtas 1983; Gautschi et al. 1973; Ghosh et al. 1989; Miyagawa 1977; Richard et al. 1991; Tsuda 1974). Typically, large decreases in ATP and/or protein synthesis were also observed. Because a primary effect of 2,4-DNP in cells is to uncouple oxidative phosphorylation, cellular processes dependent on production of ATP by oxidative phosphorylation likely

will be adversely affected by the actions of 2,4-DNP. DNA synthesis depends, to some extent, on ATP. Thus, assessing this endpoint as an indicator of genotoxicity may lead to "false positives" for genotoxicity. In these studies, the effects of 2,4-DNP on mitosis and/or DNA synthesis were related to lower ATP levels in cells exposed to 2,4-DNP, resulting in decreases in energy-dependent processes, including mitosis and DNA synthesis (Garrett and Lewtas 1983; Gautschi et al. 1973; Ghosh et al. 1989; Miyagawa 1977; Richard et al. 1991; Tsuda 1974). Thus, these changes probably do not indicate a positive response for genotoxicity.

No studies were located regarding genotoxicity in humans after inhalation, oral, or dermal exposure or in animals after inhalation or dermal exposure to 2,4-DNP. 2,4-DNP was negative for genotoxicity in one *in vivo* gavage assay in mice assessing DNA synthesis in testicular cells (Friedman and Staub 1976) and positive in another (Seiler 1981); 2,4-DNP was negative for mutagenicity in assays on prokaryotic organisms; and DNP was negative for DNA damage *in vitro* using prokaryotic and mammalian cells (Anderson and Styles 1978; Chiu et al. 1978; De Flora 1981; Garner and Nutman 1977; Heinemann and Howard 1964; Kleinhofs and Smith 1976; Nakamura et al. 1987; Probst et al. 1981; Swenberg et al. 1976). In mice injected intraperitoneally with 2,4-DNP, the incidence of chromosomal aberrations was increased (Mitra and Manna 1971). Other studies producing positive results for genotoxicity were either equivocal for mutagenicity in prokaryotic organisms or were "false positives" for genotoxicity in assays measuring DNA synthesis or mitotic indices that could be explained by a 2,4-DNP induced decrease in cellular metabolic rate (Demerec et al. 1951; Garrett and Lewtas 1983; Gautschi et al. 1973; Ghosh et al. 1989; Kawai et al. 1987; Miyagawa 1977; Seiler 1981; Tsuda 1974). Thus, the weight of evidence indicates that 2,4-DNP is not genotoxic. However, one study demonstrated an increase in chromosomal aberrations *in vivo*, indicating that it might be useful to further test 2,4-DNP for clastogenicity (Mitra and Manna 1971). Furthermore, considerable data indicate that the metabolites of 2,4-DNP (2-amino-nitrophenol, 4-amino-2-nitrophenol, and 2,4-diaminophenol) are mutagenic in *S. typhimurium* (Garner and Nutman 1977). Since 2,4-DNP was negative with metabolic activation with rat liver S9, which contains the enzymes that reduce 2,4-DNP to these metabolites, the positive results with the metabolites are difficult to reconcile. A study that specifically addresses the metabolism of 2,4-DNP in the presence of the S9 activating system and an appropriate ATP-regenerating system would resolve this apparent contradiction.

In a study screening 102 chemicals for reverse mutations of *S. typhimurium*, 2,3- and 2,5-DNP were positive for mutagenicity in the TA98 and TA100 strains, 2,6-DNP was negative for mutagenicity in the TA98 and TA100 strains, and 3,4-DNP was positive and negative for mutagenicity in the TA100 and

TA98 strains, respectively (Kawai et al. 1987). Thus, data indicate a potential for mutagenicity in 2,3-, 2,5-, and 3,4-DNP. Further studies in bacterial and mammalian culture assays of these isomers would be useful to better determine their potential genotoxicity.

Hilliard et al. (1998) reported that 2,4-DNP was an oxidative phosphorylation uncoupler that induced marked increases in chromosome aberrations with 26 and 38% cell aberrations. These were associated with considerable reductions in cell counts in Chinese hamster ovary cells.

Genotoxicity of DNP Metabolites. 2-Amino-4-nitrophenol was mutagenic in *S. typhimurium* strain TA98 both with and without metabolic activation (Shahin et al. 1982; Zeiger et al. 1987), and in strain TA1538 with and without activation (Ames et al. 1975; Garner and Nutman 1977; Shahin et al. 1982). 2-Amino-4-nitrophenol was not mutagenic in strains TAl00, TA1535, and TA1537, both with and without activation (Shahin et al. 1982; Zeiger et al. 1987). Results for 2-amino-4-nitrophenol were equivocal in a test of phage induction in *E. coli* without activation (Kvelland 1985). 2-Amino-4-nitrophenol was mutagenic in the neurospora *Sorduriu brevicollis* (Yu-Sun et al. 1981) without activation. In eukaryotic cells, 2-amino-4-nitrophenol was mutagenic without activation in mouse lymphoma L5178Y cells (NTP 1988a), and caused chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells both with and without activation (Anderson et al. 1990; NTP 1988a). 2-Amino-4-nitrophenol was negative in a dominant lethal mutation test after intraperitoneal administration to rats (Burnett et al. 1977). Genotoxicity information is summarized in Tables 2-8 and 2-9.

Table 2-8. Genotoxicity of Dinitrophenol Metabolites *In Vivo*

Species (test system)	Endpoint	Results	Reference	Isomer
Mammalian cells:				
Rat (intraperitoneal)	Dominant lethal mutation	–	Burnett et al. 1977	2-a-4np
Rat (intraperitoneal)	Dominant lethal mutation	–	Burnett et al. 1977	2-a-5np
Rat (intraperitoneal)	Dominant lethal mutation	–	Burnett et al. 1977	4-a-2np

– = negative result; + = positive result; 2-a-4np = 2-amino-4-nitrophenol; 4-a-2np = 4-amino-2-nitrophenol; 2-a-5np = 2-amino-5-dinitrophenol

Table 2-9. Genotoxicity of Dinitrophenol Metabolites *In Vitro*

Species (test system)	Endpoint	Results		Reference	Isomer
		Activation			
		With	Without		
Prokaryotic organisms:					
Escherichia coli					
B, CR63, K12(λh)	Phage induction	No data	(+)	Kvelland 1985	2-a-4np
Salmonella typhimurium					
TA98	Reverse mutation	(+)	+	Shahin et al. 1982	2-a-4np
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
TA1538		(+)	+		
TA1538	Reverse mutation	+	No data	Ames et al. 1975	2-a-4np
TA1538	Reverse mutation	+	+	Garner and Nutman 1977	2-a-4np
TA98	Reverse mutation	+	(+)	Zeiger et al. 1987	2-a-4np
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
Sordaria brevicollis	Reverse mutation	No data	+	Yu-Sun et al. 1981	2-a-2np
E. coli					
B, CR63, K12(λh)	Reverse mutation	–	–	Kvelland 1985	2-a-5np
S. typhimurium					
TA98	Reverse mutation	+	+	Shahin et al. 1982	2-a-5np
TA100		–	(+)		
TA1535		–	+		
TA1537		+	+		
TA1538		+	+		
TA1538	Reverse mutation	No data	+	Ames et al. 1975	2-a-5np
TA98	Reverse mutation	No data	+	Chiu et al. 1978	2-a-5np
TA100		No data	–		
TA98	Reverse mutation	+	+	Zeiger et al. 1987	2-a-5np
TA1000		(+)	(+)		
TA1535		–	–		
TA1537		(+)	(+)		
TA98	Reverse mutation	+	+	Garner and Nutman 1977	4-a-2np
TA1538		+	+		
TA97	Reverse mutation	+	+	Zeiger et al. 1987	4-a-2np
TA98		+	+		

Table 2-9. Genotoxicity of Dinitrophenol Metabolites *In Vitro*

Species (test system)	Endpoint	Results		Reference	Isomer
		Activation			
		With	Without		
TA98	Reverse mutation	–	–	Shahin et al. 1982	4-a-2np
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
TA1538		–	–		
TA1538	Reverse mutation	+	–	Dybing and Thorgeirsson 1977	2,4-dap
Eukaryotic organisms: Mammalian cells					
Mouse lymphoma L518Y cells	Gene mutation	No data	+	NTP 1988a	2-a-4np
CHO cells	Sister chromatid exchange	+	+	NTP 1988a	2-a-4np
CHO cells	Sister chromatid aberrations	+	+	NTP 1988a	2-a-4np
CHO cells	Sister chromatid aberrations	+	+	Anderson et al. 1990	2-a-4np
Mouse lymphoma L518Y cells	Gene mutation	No data	+	NTP 1988a	2-a-5np
CHO cells	Sister chromatid exchange	+	+	NTP 1988a	2-a-5np
CHO cells	Chromosomal aberrations	+	+	NTP 1988a	2-a-5np
Rat 344 hepatocyte primary culture	Unscheduled DNA synthesis	No data	–	Williams et al. 1982	4-a-2np
Mouse lymphoma L518Y cells	Gene mutation	+	+	Mitchell et al. 1988	4-a-2np

– = negative result; + = positive result; (+) = weakly positive or equivocal result; 2-a-4np = 2-amino-4-nitrophenol; 2-a-5np = 2-amino-5-nitrophenol; 4-a-2np = 4-amino-2-nitrophenol; 2,4-dap = 2,4-diaminophenol; CHO = Chinese hamster ovary; DNA =deoxyribonucleic acid

Commercial-grade 4-amino-2-nitrophenol was reported to be mutagenic in *S. typhimurium* strains TA98 and TA1538 with and without activation (Garner and Nutman 1977). Highly purified 4-amino-2-nitrophenol was not mutagenic in strains TA98, TAl00, TA1535, TA1537, or TA1538 (Shahin et al. 1982), leading the authors to conclude that the mutagenic activity of the commercial grade was due to a contaminant. However, in other studies, highly purified 4-amino-2-nitrophenol was mutagenic with and without activation in strains TA97, TA98, and TA1538 (Shahin 1985; Zeiger et al. 1987). 4-Amino-

2-nitrophenol also caused forward mutations at the TK locus in mouse lymphoma L5178Y cells with and without activation (Mitchell et al. 1988). 4-Amino-2-nitrophenol was negative when administered intraperitoneally in a dominant lethal mutation study (Burnett et al. 1977) and did not induce unscheduled DNA synthesis in Fischer 344 rat primary hepatocyte cultures (Williams et al. 1982).

In a phage induction test for mutagenicity in *E. coli*, 2-amino-5-nitrophenol was mutagenic without activation (Kvelland 1985). In *S. typhimurium*, 2-amino-5-nitrophenol was mutagenic in strain TA98 with and without activation (Chiu et al. 1978; Shahin et al. 1982; Zeiger et al. 1987); negative or equivocal in strain TA100 without activation, and negative or equivocal with activation (Chiu et al. 1982; Shahin et al. 1982; Zeiger et al. 1987); positive or negative in strain TA1535 without activation (Shahin et al. 1982; Zeiger et al. 1987) and negative with activation (Shahin et al. 1982; Zeiger et al. 1987); positive or weakly positive in strain TA1537 with and without activation (Shahin et al. 1982; Zeiger et al. 1987); and positive in strain TA1538 with and without activation (Ames et al. 1975; Shahin et al. 1982). 2-Amino-5-nitrophenol was also mutagenic in the mouse lymphoma L5178Y cell mutation test without activation (NTP 1988b) and caused sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells with and without activation. 2-Amino-5-nitrophenol was negative in a dominant lethal mutation test in CD rats given the test chemical intraperitoneally (Burnett et al. 1977).

2,4-Diaminophenol was reported to be mutagenic only with activation in *S. typhimurium* strain TA1538 (Dybing and Thorgeirsson 1977). Another report (Kawai et al. 1987) stated that 2,4-diaminophenol was mutagenic, but did not provide further information.