Toxicological Profile for Dinitrophenols

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U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

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DISCLAIMER

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FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

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

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance due to associated acute, intermediate, and chronic exposures;

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

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

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

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

VERSION HISTORY

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August 1995	Final toxicological profile released

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ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

Dinitrophenols (DNPs) are a class of synthetic organic chemicals that exist in six isomeric forms: 2,3-DNP, 2,4-DNP, 2,5-DNP, 2,6-DNP, 3,4-DNP, and 3,5 DNP. They do not occur naturally in the environment. DNPs are yellow solids that dissolve slightly in water and can be explosive when dry and when heated or subjected to flame, shock, or friction (WHO 2015). DNPs have no known odor.

DNPs are used in the manufacture of dyes, wood preservatives, photographic developers, and explosives, and as a pH indicator. In addition to current industrial uses, 2,4-DNP was previously used as an insecticide, although no products containing 2,4-DNP are currently registered for use in the United States (NLM 2020). In the 1930s, 2,4-DNP was prescribed by physicians as a weight-reducing agent; however, the U.S. Food and Drug Administration (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" (FDA 2020a), and use of 2,4-DNP was discontinued due to serious adverse health effects, including fatality (Bartlett et al. 2010; FDA 2020a; NLM 2020). In recent years, however, 2,4-DNP in tablet and powder form has been illegally marketed for weight loss and body building by unregulated internet sources, leading to a number of human fatalities (Cairns et al. 2020; Sousa et al. 2020). As a result of the growth in availability of 2,4-DNP to the general public, there is increased potential for exposure. Results of a study in laboratory animals indicate that the toxicity of 2,4-DNP is greater at high ambient temperatures (Harvey 1959). Although the specific mechanism for this effect has not been established, susceptibility to the toxic effects may increase for workers at high workroom temperatures or in the general population at high environmental temperatures.

2,4-DNP and other DNPs are released to the environment primarily during their manufacture and use, and from waste disposal sites. The most likely routes of exposure near hazardous waste sites would be breathing contaminated air, drinking contaminated water, eating contaminated food, or skin contact with contaminated soil. No recent monitoring data were identified for DNP in air or drinking water. Recent monitoring data (~2010–2020) did not detect DNPs in surface water, soil, or sediment (NWQMC 2020). DNPs and their metabolites have not been measured in the tissues or body fluids of humans in the general population who did not deliberately ingest the compound (as a diet pill or in a suicide attempt).

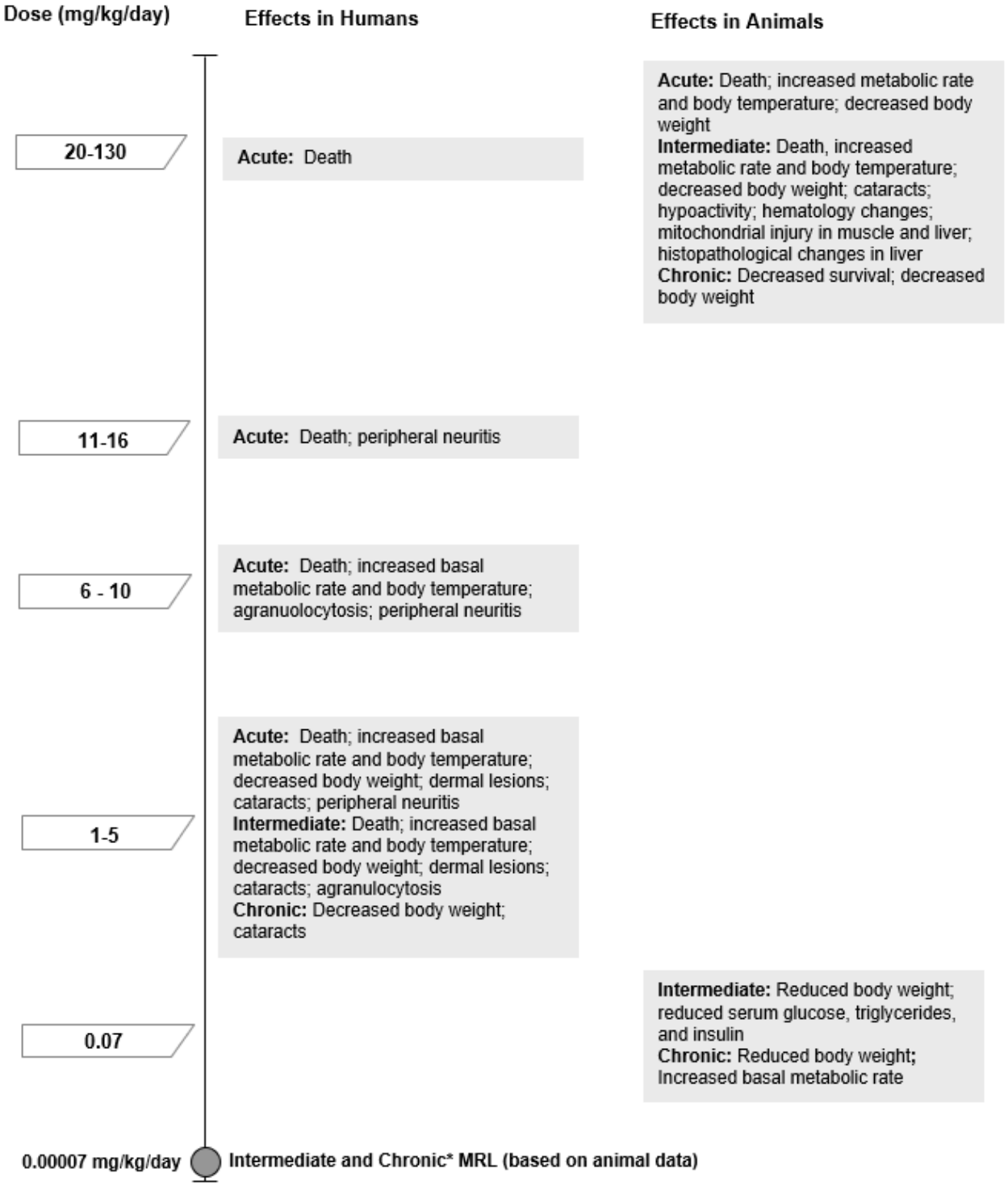
1.2 SUMMARY OF HEALTH EFFECTS

The health effect literature on DNPs is largely limited to information on 2,4-DNP. Much of the scientific literature on effects of 2,4-DNP consists of case reports of human poisonings, clinical studies of its use as a weight-loss agent in the 1930s, and animal studies from the early 1900s. Recent years have seen additional case reports of human poisonings or fatalities, because 2,4-DNP continues to be marketed for weight loss by unregulated internet sources (Cairns et al. 2020; Sousa et al. 2020). A handful of animal studies examining focused endpoints have been conducted in the past 2 decades. Information on the remaining isomers is restricted to an animal lethality study using intraperitoneal administration (Harvey 1959), an oral study in chickens (Robbins 1944), and *in vitro* genotoxicity or mechanistic data. Data from the acute intraperitoneal LD_{50} study indicate 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 (Harvey 1959); however, no data are available for comparison of DNP isomers for other routes or durations of exposure.

Abundant data in humans document 2,4-DNP-induced dangerous increases in body temperature (hyperpyrexia) and basal metabolic rate (generally measured as oxygen consumption) that elicit secondary effects (Anderson et al. 1933; Bayer and Gray 1935; Bortz 1934; Castor and Beierwaltes 1956; Cutting and Tainter 1933; Cutting et al. 1934; Dameshek and Gargill 1934; Davidson and Shapiro 1934; Dintenfass 1934; Dunlop 1934; Eichert 1936; Epstein and Rosenblum 1935; Geiger 1933; Goldman and Haber 1936; Holborow et al. 2016; Hsiao et al. 2005; Hunt 1934; Imerman and Imerman 1936; Le et al. 2015; Lee et al. 2014; Looney and Hoskins 1934; MacBryde and Taussig 1935; Masserman and Goldsmith 1934; McFee et al. 2004; Miranda et al. 2006; Poole and Haining 1934; Purvine 1936; Rank and Waldeck 1936; Siegmueller and Narasimhaiah 2010; Simkins 1937a, 1937b; Stockton and Cutting 1934; Suozzi et al. 2005; Tainter and Wood 1934; Tainter et al. 1935; Tewari et al. 2009; van Veenendaal et al. 2011). In case reports of fatal exposures, autopsy findings consist of edema, hyperemia, congestion, and/or hemorrhage in the lungs, liver, stomach, and small intestine; these effects are consistent with those seen in fatal hyperthermia. Findings on autopsy are generally secondary effects. Studies in animals (Bakke and Lawrence 1965; Caldeira da Silva et al. 2008; Dominguez et al. 1993; Gibson 1973; Haasio et al. 2002a, 2002b; Kaiser 1964; Pugsley 1935; Schlagowski et al. 2014; Tainter and Cutting 1933a, 1933b) confirm the dose-related effects of 2,4-DNP on body temperature and basal metabolic rate. Figure 1-1 shows health effects found in humans and animals following oral exposure to 2,4-DNP.

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.

Figure 1-1. Health Effects Found in Humans and Animals Following Oral Exposure to 2,4-Dinitrophenol



*A chronic-duration oral MRL was not derived. The intermediate-duration oral MRL is expected to be protective for chronic-duration exposures.

Effects are considered primary if they occur in the absence of increased body temperature. Secondary effects have been identified based on underlying pathophysiological changes that are associated with hyperthermia (Bunai et al. 2012); these include:

- decreased body weight or 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.

Mechanistic data indicate that DNP effects are related to the uncoupling of mitochondrial electron transport from oxidative phosphorylation, which results in the release of energy as heat, rather than storage in the chemical potential of adenosine triphosphate (ATP) (see Section 2.18.1). The uncoupling of oxidative phosphorylation has the potential to affect all tissues and organs. Exposure of humans to 2,4-DNP results in increased basal metabolic rate, increased perspiration, weight loss, and, at higher doses, increased heart and respiratory rates and hyperthermia. These effects occur rapidly (over several hours) and may present a significant risk of death. Stopping exposure to 2,4-DNP often leads to a complete recovery. Very limited data on the other DNP isomers indicate that 2,6-, 3,4-, and 3,5-DNP may have equivalent potential for increasing basal metabolic rate as 2,4-DNP, while 2,3- and 2,5-DNP appear to have lower potential.

Primary effects include skin discoloration and rashes, cataract formation, and developmental effects. However, the underlying mechanisms of these effects have not been well investigated. Possible primary effects of 2,4-DNP are discussed below.

Hepatic Effects. Limited available data from humans do not suggest hepatic effects of 2,4-DNP apart from those related to its pyrexic effects; these data consist of case reports of poisonings, which lack

information on pre-existing conditions, as well as clinical studies from the 1930s. Early human studies attributed yellow discoloration of the conjunctiva, sclera, and skin in exposed persons to jaundice, but these effects appear to result from direct discoloration by the compound itself. There are insufficient data to assess the hepatic effects of acute- or chronic-duration exposure to 2,4-DNP in animals, but well-conducted intermediate-duration studies in rats have reported increased liver weights along with histopathologic changes (centrilobular hypertrophy, necrotic foci, and mitochondrial changes).

Dermal Effects. Human case reports of poisoning with 2,4-DNP after acute and intermediate oral exposures document yellow discoloration of skin, erythema, and pruritis, as well as maculopapular eruptions of the skin, sometimes covering the entire body.

Ocular Effects. Use of 2,4-DNP as a weight-loss agent in the 1930s was discontinued primarily because a small percentage of patients developed cataracts. Cataracts have also been observed in the yellow adipose strain of mouse, in vitamin C-deficient guinea pigs, and in ducks and chickens exposed orally, as well as in rabbits exposed intraperitoneally to 2,4-DNP. Rats and other mouse strains appear to be resistant. Although the mechanism for cataract formation is uncertain, uncoupling of oxidative phosphorylation may play an important role in this effect as well.

Developmental Effects. No information was located on developmental effects of 2,4-DNP in humans. Exposure to 2,4-DNP has resulted in developmental effects after gestational exposure of rats exposed orally and rats and mice exposed parenterally. Increases in the numbers of stillborn pups and neonatal pup deaths, as well as decreases in pup body weight in the early postnatal period were reported in rats exposed orally to 2,4-DNP in an Organisation for Economic Co-operation and Development (OECD) guideline reproduction/developmental toxicity screening study (Takahashi et al. 2009) and similar effects were reported in an earlier study (Wulff et al. 1935). Decreased fetal weight and length and increased resorptions were also reported in rats and mice exposed to 2,4-DNP via parenteral routes (Gibson 1973; Goldman and Yakovac 1964).

Cancer Effects. There are no epidemiological studies of cancer in humans exposed to any DNPs. 2,4-DNP has not been adequately tested for carcinogenicity in animals, and no studies were located regarding carcinogenicity in animals exposed to the other DNP isomers. The U.S. Environmental Protection Agency (EPA) (IRIS 2005), the Department of Health and Human Services (NTP 2016), and the International Agency for Research on Cancer (IARC 2017) have not evaluated the potential carcinogenicity of any of the DNPs. 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. Metabolites, 2-amino-4-nitrophenol and 2-amino-5-nitrophenol, have been designated as "not classifiable as to their carcinogenicity to humans" (IARC 1993a, 1993b).

No data show unequivocally that 2,4-DNP is genotoxic. The positive results of some of the DNA tests may reflect its cytotoxicity (decreased cellular metabolic rate).

1.3 MINIMAL RISK LEVELS (MRLs)

No studies were located regarding health effects in humans or animals (other than chickens) after inhalation or oral exposure to any isomer of DNP other than 2,4-DNP. Accordingly, the following discussion will focus on 2,4-DNP. The available information is considered insufficient to derive inhalation MRLs for 2,4-DNP. Although health effects have occurred in humans exposed to 2,4-DNP occupationally (Gisclard and Woodward 1946; Jiang et al. 2011; Perkins 1919), exposure appeared to involve both the inhalation and dermal routes, and exposure concentrations were not known or inadequately characterized. No studies were located regarding health effects in animals after inhalation exposure to 2,4-DNP. As shown in Figure 1-2, available oral data from humans identify death, body weight, effects on energy metabolism, and dermal, ocular, hematological, and neurological endpoints as the sensitive targets of 2,4-DNP toxicity; laboratory animal data support the body weight and energy metabolism findings in humans (and also support the intermediate-duration oral MRL for 2,4-DNP). The MRL value for intermediate-duration oral exposure to 2,4-DNP is summarized in Table 1-1 and discussed in greater detail in Appendix A. Data are insufficient to determine if the intermediate-duration oral MRL for 2,4-DNP would be protective for other DNP isomers.

Figure 1-2. Summary of Sensitive Targets of 2,4-Dinitrophenol – Oral

Body weight and energy metabolism are the most sensitive targets of 2,4-dinitrophenol oral exposure.

Numbers in triangles and circles are the lowest LOAELs among health effects in humans and animals, respectively

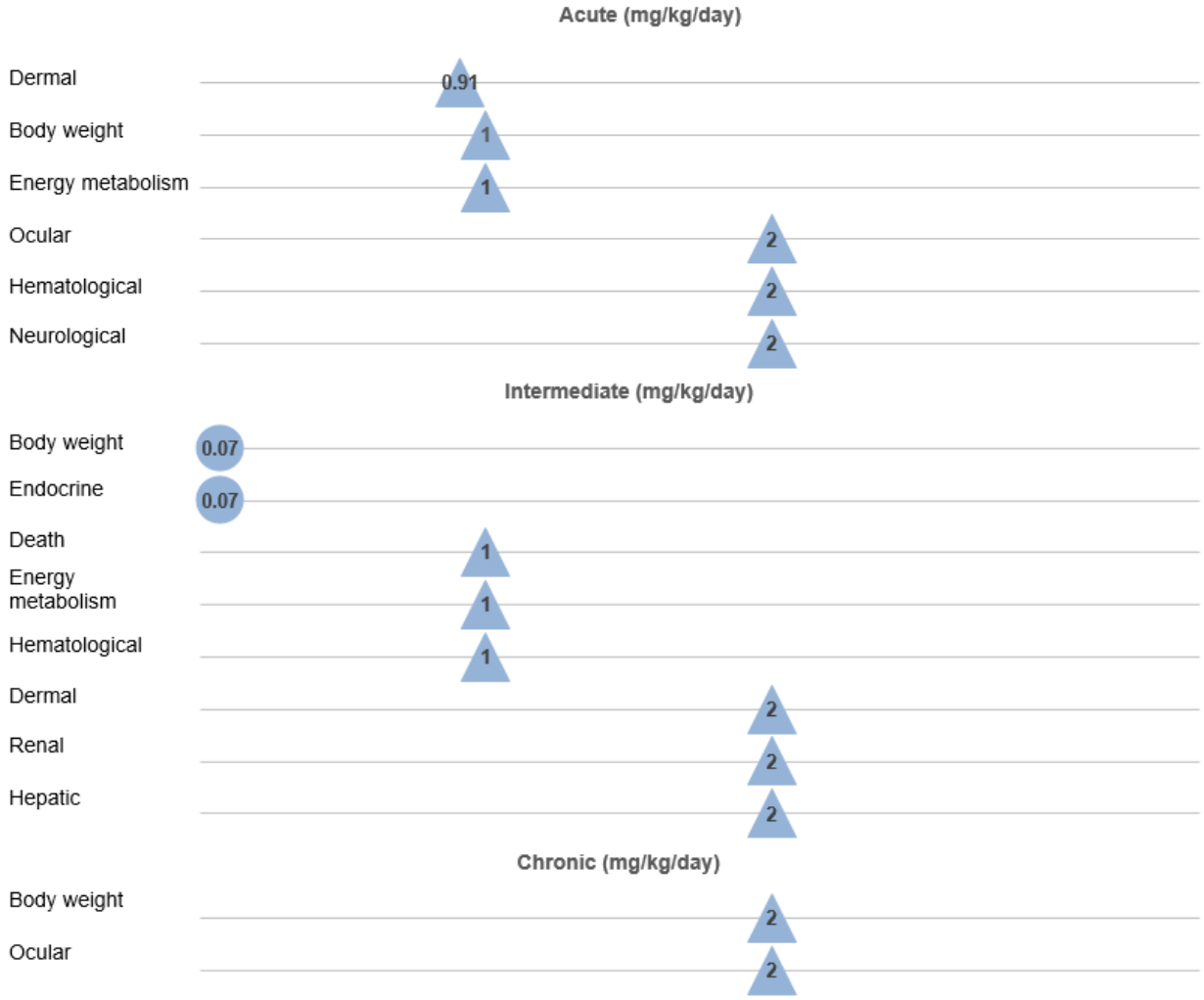


Table 1-1. Minimal Risk Levels (MRLs) for 2,4-Dinitrophenol[a,b]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure (ppm)					
Acute	Insufficient data for MRL derivation				
Intermediate	Insufficient data for MRL derivation				
Chronic	Insufficient data for MRL derivation				
Oral exposure (mg/kg/day)					
Acute	Insufficient data for MRL derivation				
Intermediate	0.00007	Decreased body weight	0.07 (LOAEL)	UF: 1,000	Caldeira da Silva et al. 2008
Chronic	Insufficient data for MRL derivation; however, the intermediate MRL is believed to be protective for chronic exposures				

[a]See Appendix A for additional information.
[b]Data are insufficient to derive MRLs for other dinitrophenol isomers.

LOAEL = lowest-observed-adverse-effect level; UF = uncertainty factor

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.

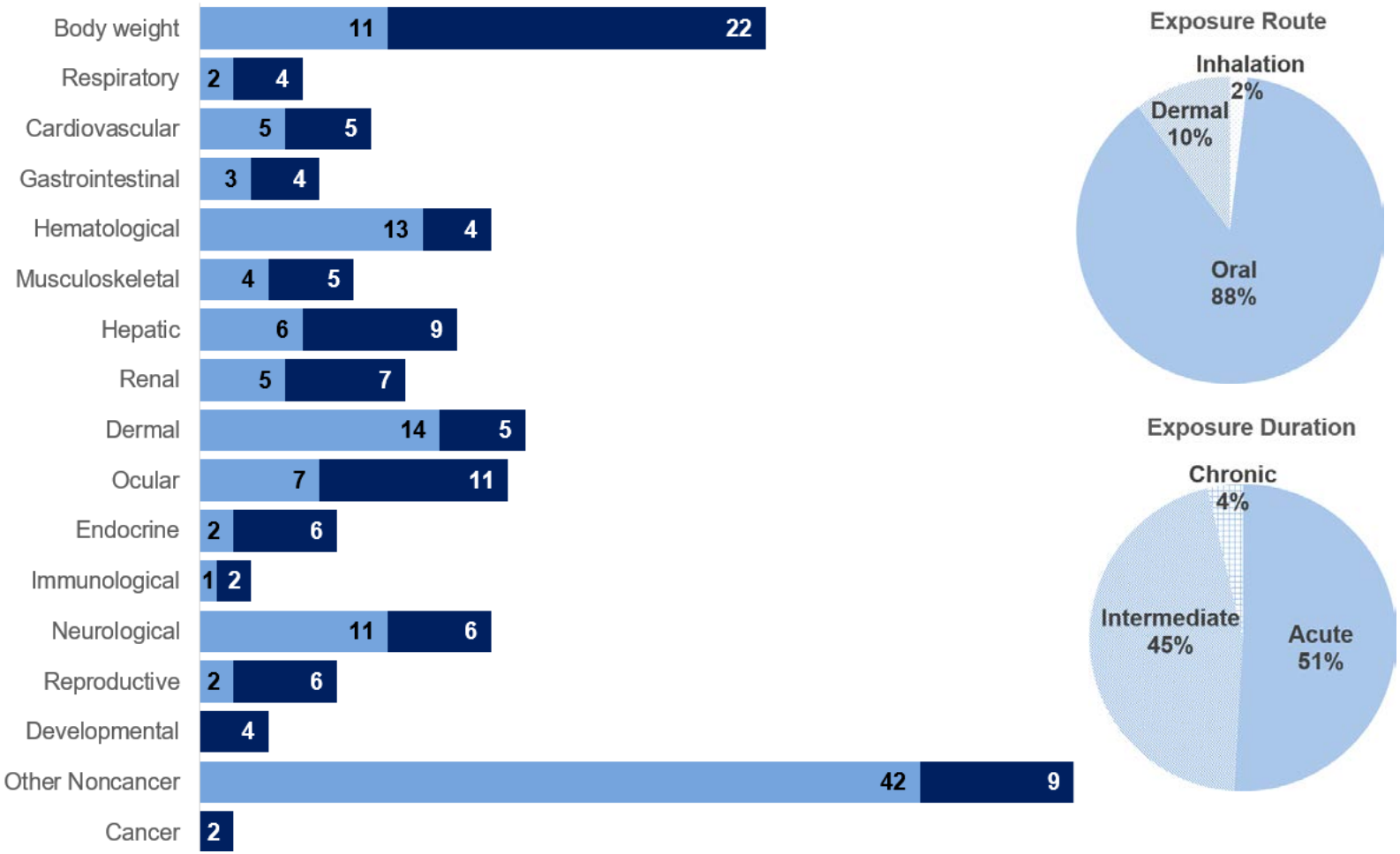
- **Hematological Endpoint:** Agranulocytosis in humans.

- **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									

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
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									

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
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									

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
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									

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
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									

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
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									

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
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									

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
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									

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
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									

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
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)

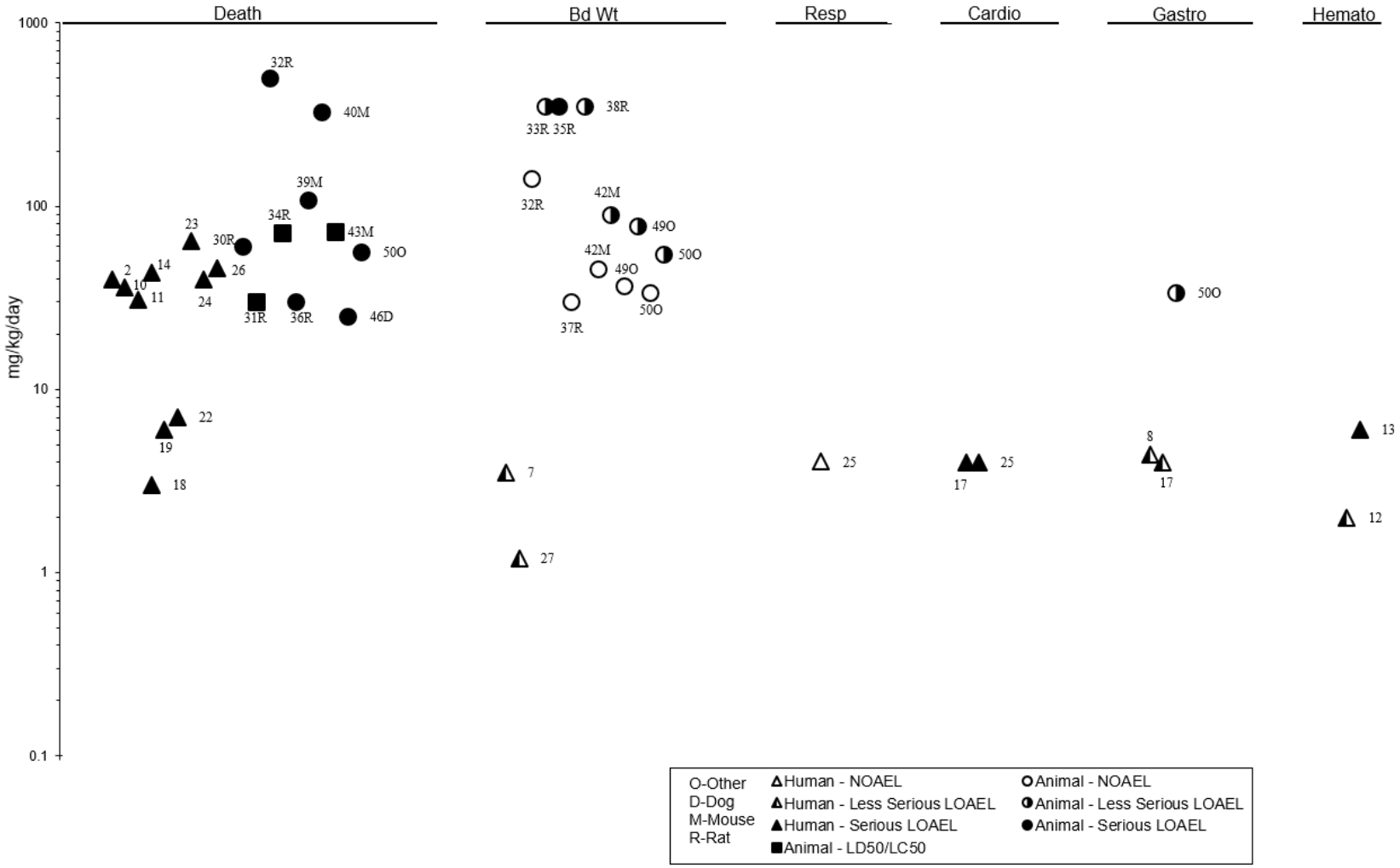


Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Acute (≤14 days)

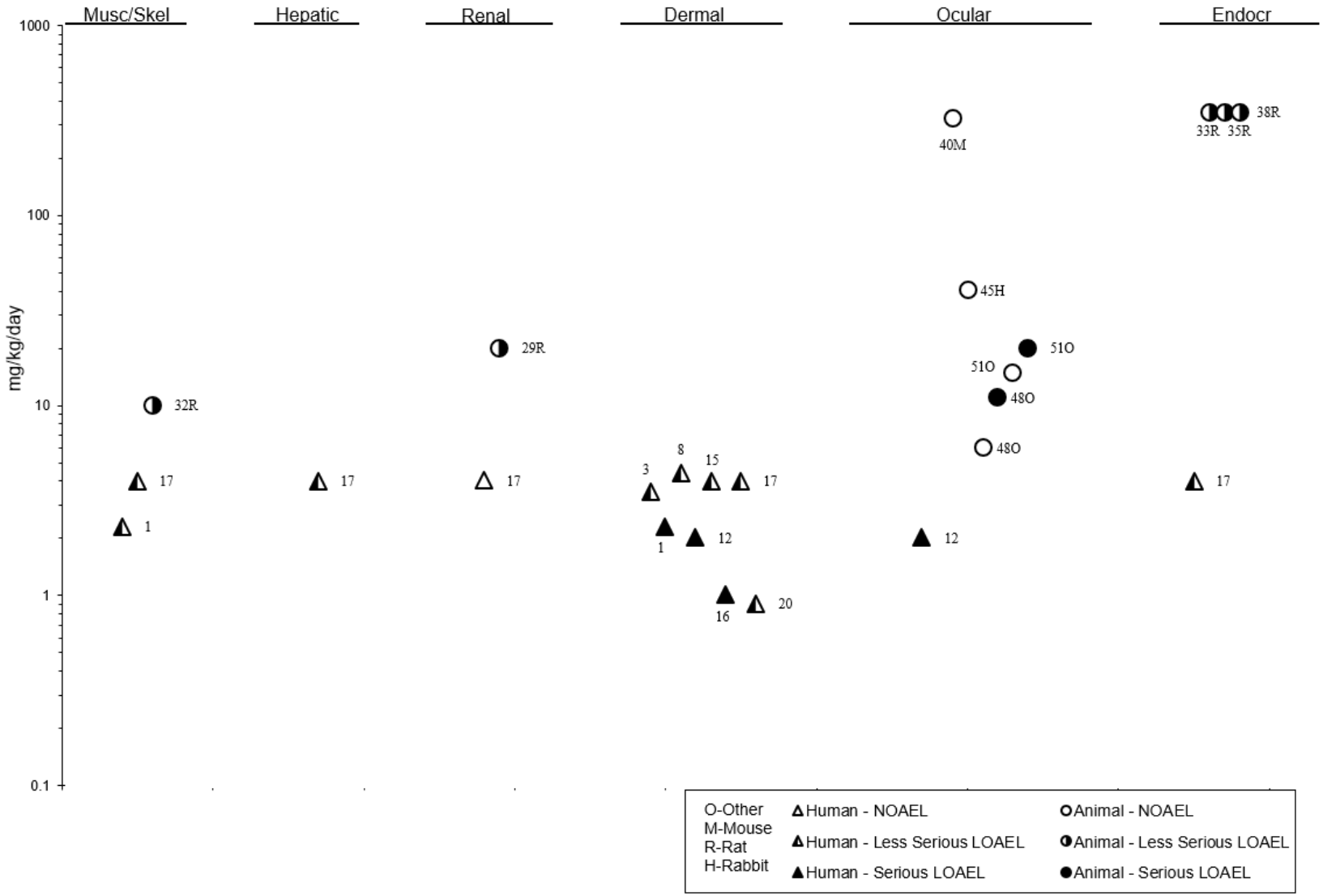


Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Acute (≤14 days)

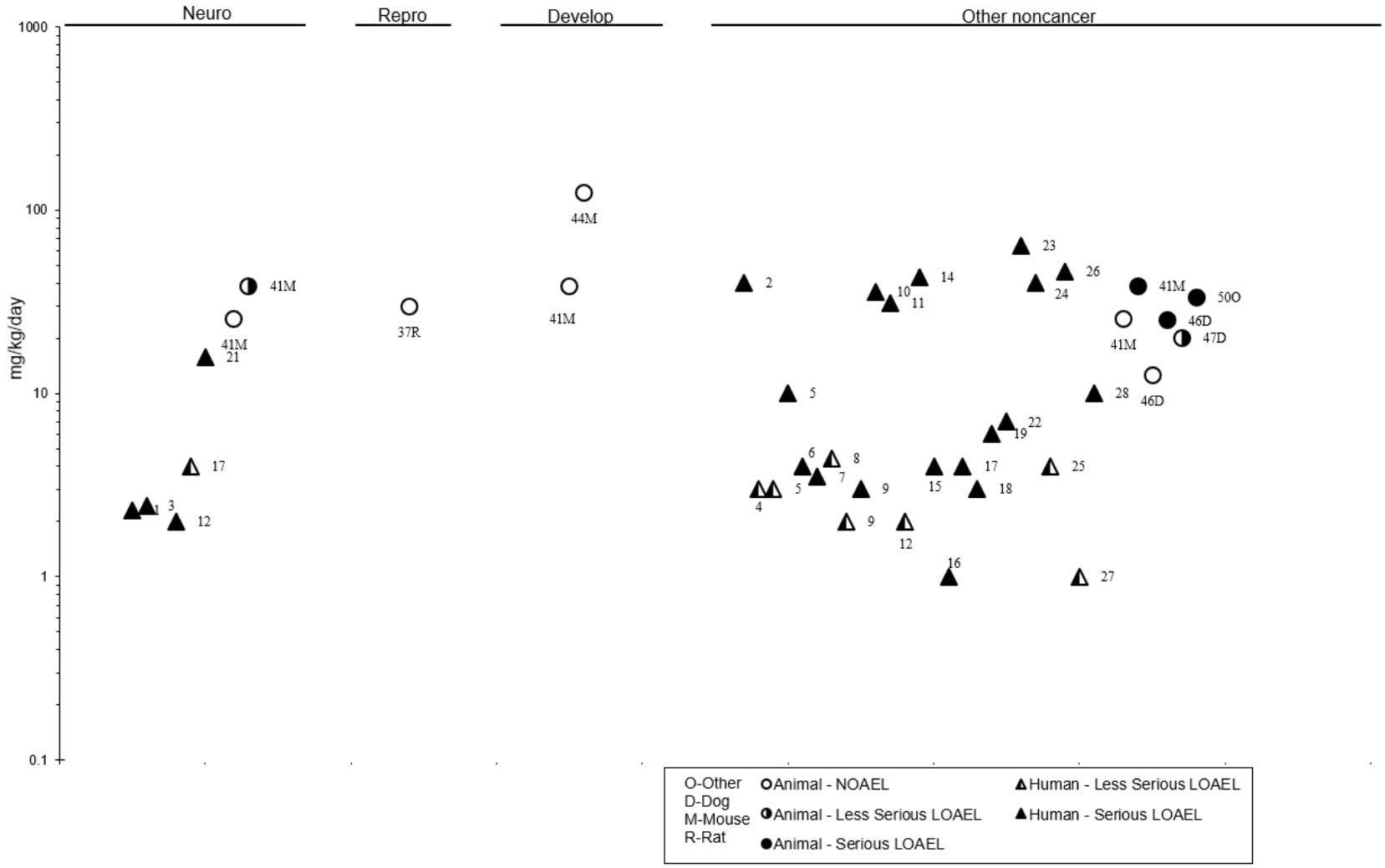


Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral
Intermediate (15-364 days)

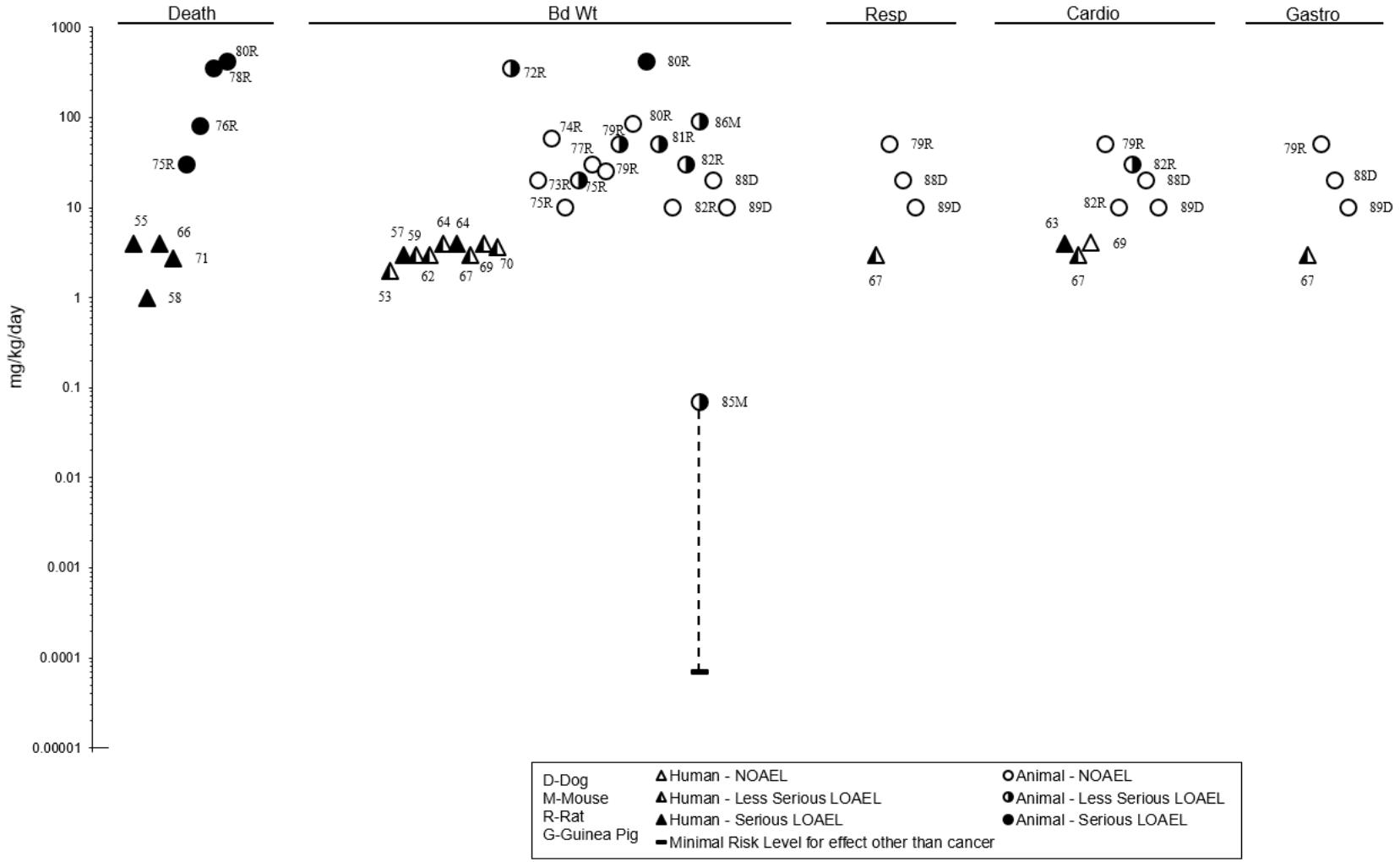


Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Intermediate (15-364 days)

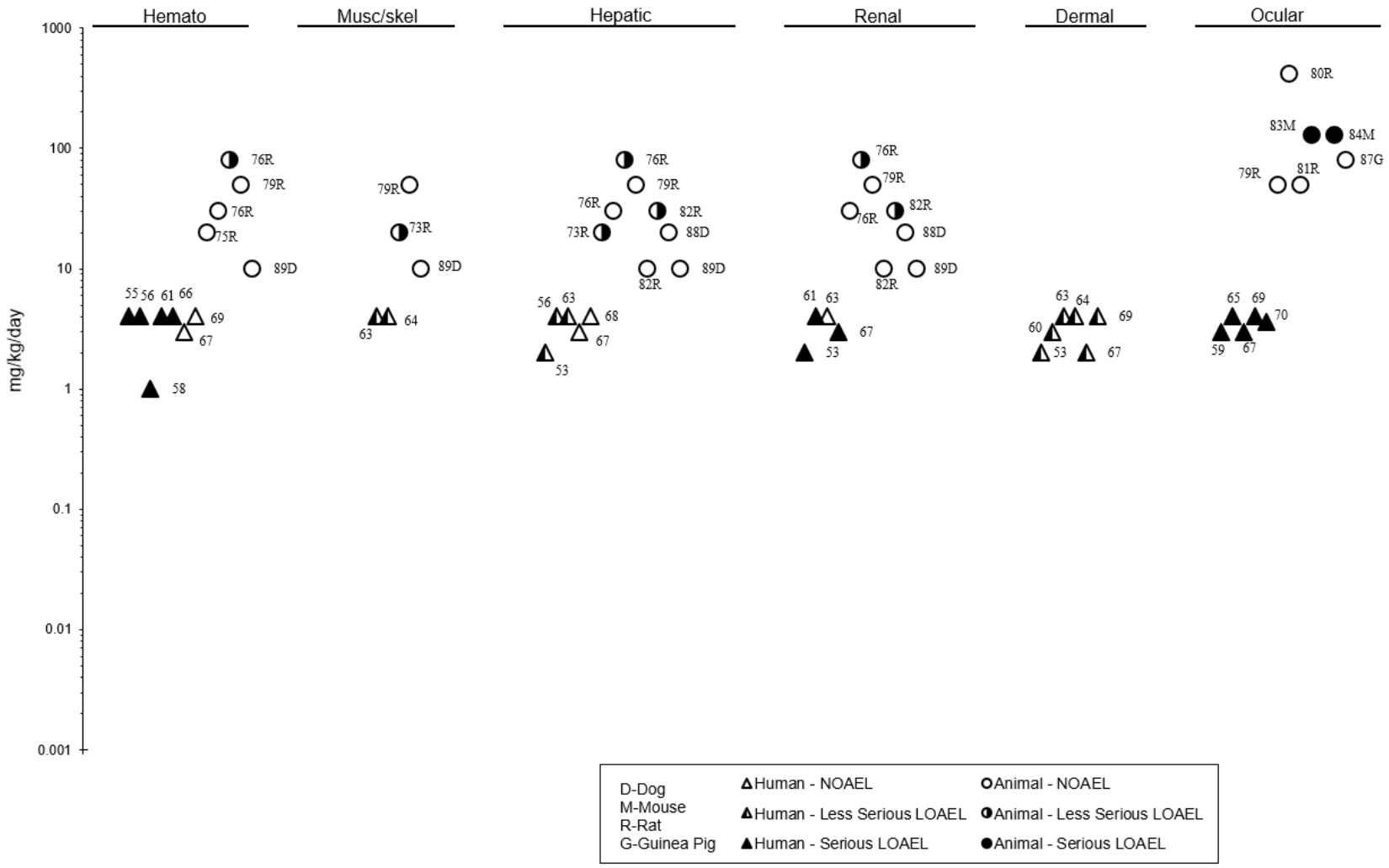


Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Intermediate (15-364 days)

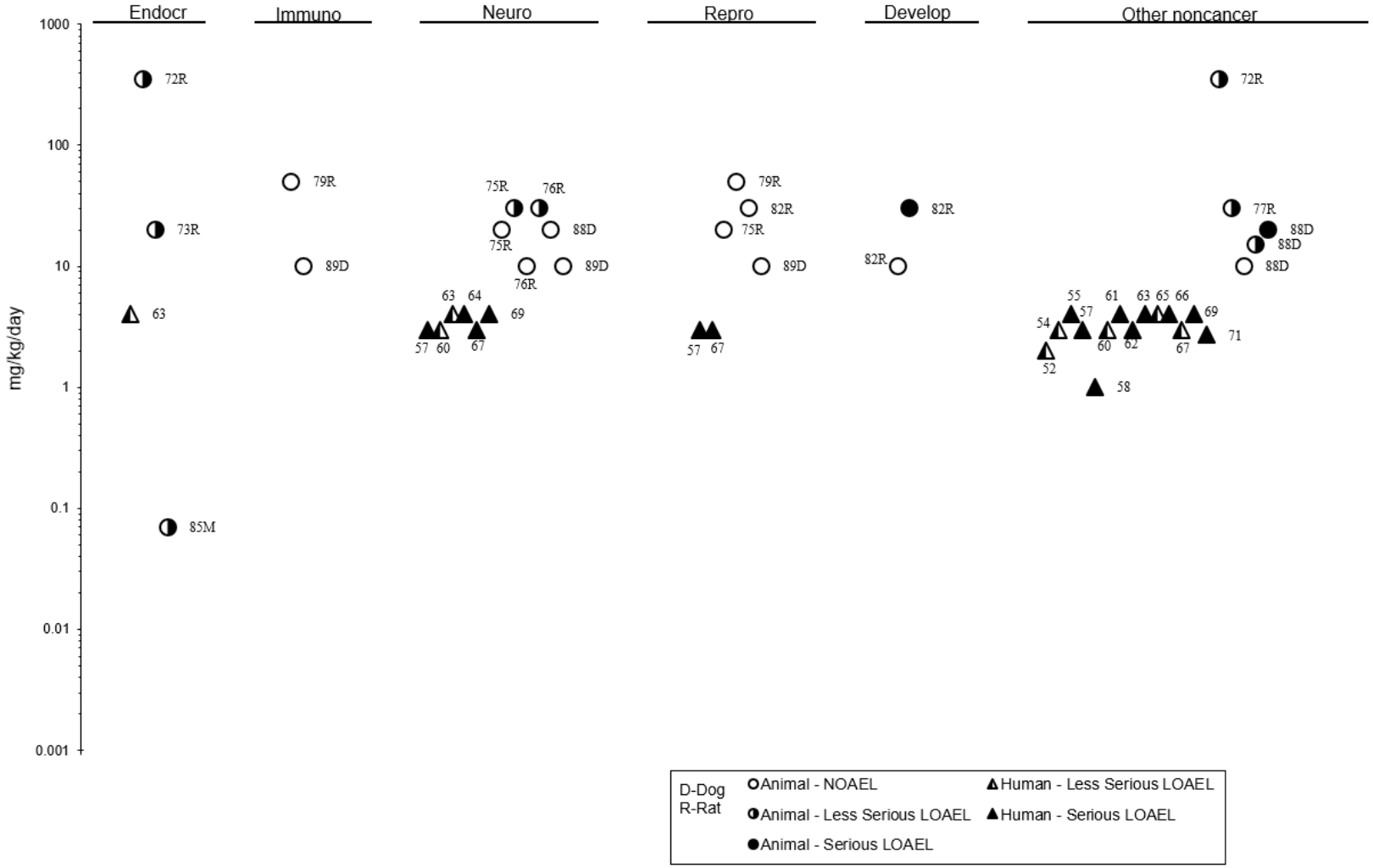


Figure 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Oral

Chronic (≥365 days)

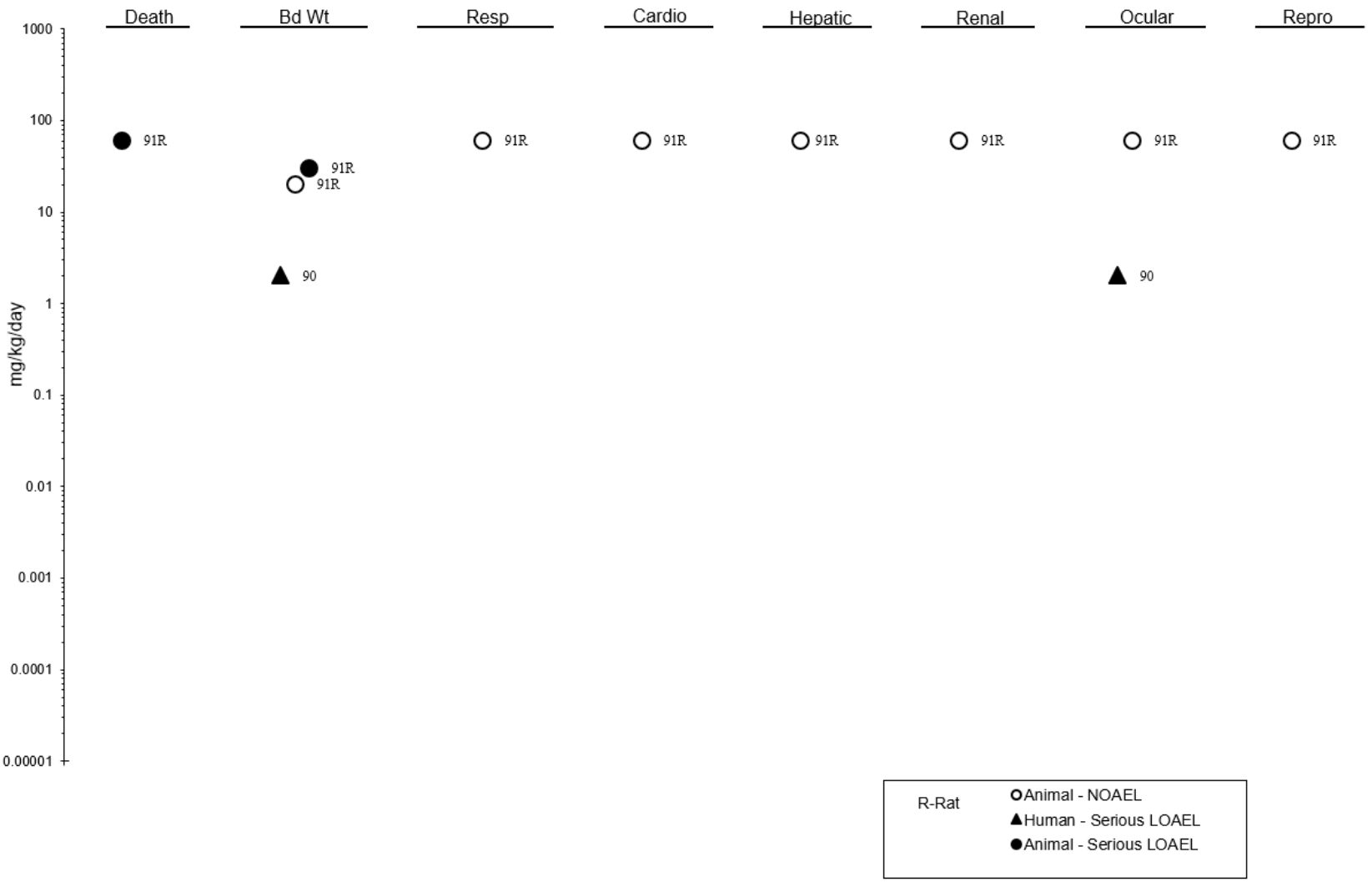


Table 2-2. Levels of Significant Exposure to 2,4-Dinitrophenol – Dermal

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.

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

- Information on absorption, distribution, and metabolism of DNPs is limited to the 2,4-isomer.
- 2,4-DNP is rapidly absorbed by the oral and inhalation routes, and possibly by the dermal route.
- A portion of 2,4-DNP in the blood is bound to serum proteins, and the unbound fraction enters organs such as the eye.
- 2,4-DNP is metabolized via sequential nitro group reduction to 2-amino-4-nitrophenol and 4-amino-2-nitrophenol and 2,4-diaminophenol.
- 2,4-DNP and its metabolites are excreted in the urine.
- With the exception of 2,6-DNP, other isomers are eliminated much more rapidly than is 2,4-DNP, but this is based on limited information.

3.1.1 Absorption

Qualitative evidence for absorption of 2,4-DNP after inhalation and/or dermal exposure is provided by reports of health effects in workers; however, the exposures may have included uptake by the oral route. A metabolite of 2,4-DNP, 2-amino-4-nitrophenol, was commonly detected in the urine of workers (predominantly male) exposed to 2,4-DNP in the munitions industry in France (Perkins 1919). Results of autopsies performed on workers who died indicated the presence of 2,4-DNP and its metabolites in blood, unspecified organs, and urine, but quantitative data were not provided (Perkins 1919). In a case of fatal occupational poisoning from exposure to mists and airborne dust of 2,4-DNP in the U.S. chemical industry, the urine contained 2.08 g/L of 2,4-DNP and 50 mg/L of 2-amino-4-nitrophenol (Gisclard and Woodward 1946). Workroom air levels of 2,4-DNP, determined subsequent to the death, were "normally" ≥40 mg/m^3. More recent reports (Jiang et al. 2011; Lu et al. 2011) of occupational poisoning with 2,4-DNP provide additional support for absorption via dermal and inhalation routes. Two workers were exposed by recycling nylon bags that had contained 2,4-DNP, while wearing only facial masks and no protective covering on the skin (Jiang et al. 2011).

The data regarding absorption in humans after oral exposure are limited. Case reports of poisoning have documented symptoms of toxicity as early as 4–9 hours, and death as soon as 10–15 hours, after a single oral exposure (Holborow et al. 2016; Hsiao et al. 2005; Siegmueller and Narasimhaiah 2010), suggesting

rapid oral absorption. Evidence of substantial 2,4-DNP absorption was obtained from the case of an 80-kg man who ingested two 4.5-g doses of the sodium salt of 2,4-DNP (each equivalent to 46 mg 2,4-DNP/kg) 1 week apart and died 11 hours after the second dose (Tainter and Wood 1934). Analysis of a blood sample for 2,4-DNP and estimation of the total body burden, assuming the drug was evenly distributed between blood and tissues, gave a body burden of ≈2.72 g 2,4-DNP, which corresponds to 3.31 g of the sodium salt of 2,4-DNP at the time of death. Since some of the drug would have been metabolized, and some excretion of parent compound and metabolites probably would have occurred during the interval between ingestion and death, this value is not inconsistent with complete absorption of the second dose. 2,4-DNP and 2-amino-4-nitrophenol were detected in the urine of a woman who had taken sodium 2,4-DNP at 3.5 mg/kg/day 2,4-DNP for 20 days (Davidson and Shapiro 1934), indicating that absorption had occurred. Quantitative data were not reported. Indirect evidence of rapid absorption is provided by the maximal increases in basal metabolic rate that occurred within 1 hour of ingestion of 2–5 mg/kg 2,4-DNP from 2,4-DNP (Cutting et al. 1933) or sodium 2,4-DNP (Dunlop 1934) by patients in clinical studies.

Limited information provided by animal studies suggests rapid absorption after oral exposure, but the extent of absorption and its relationship to dose have not been adequately assessed. The half-time for absorption of 2,4-DNP following gavage administration of a single 22.5 mg/kg dose to mice was 0.5 hours based on serum concentrations of 2,4-DNP measured 1, 3, 6, 12, and 24 hours after dosing (Robert and Hagardom 1983). Similarly, peak plasma concentrations occurred within the first 0.5–2 hours of gavage doses up to 22.5 mg/kg in mice (Robert and Hagardom 1985) or 25 mg/kg/day in rats (Perry et al. 2015a,b), and within the first 0.5–4 hours of oral doses up to 125 mg/kg in dogs (Kaiser 1964).

No studies were located regarding the rate or extent of absorption in animals after dermal exposure to 2,4-DNP. However, the death of one of five guinea pigs dermally exposed to 300 mg/kg (Spencer et al. 1948) suggests that dermal absorption occurred. No information quantifying dermal absorption or dermal permeability of 2,4-DNP was identified.

3.1.2 Distribution

No reliable information on the distribution of 2,4-DNP in humans after inhalation or dermal exposure was identified in the literature. 2,4-DNP and its metabolites were reportedly detected in the blood and organs of workmen who died from exposure to 2,4-DNP in the munitions industry in France (Perkins 1919);

however, the organs, concentrations, and details of extraction and analytical methods were not reported. Analysis of unspecified organs from two workmen who died following exposure to 2,4-DNP in the United States did not demonstrate the presence of the chemical or its metabolites, despite the fact that 2,4-DNP and its metabolite were detected in the urine of one worker (Gisclard and Woodward 1946).

Limited information is available regarding distribution in animals after oral exposure to 2,4-DNP. In mice given a gavage dose of 22.5 mg/kg of 2,4-DNP, concentrations of 2,4-DNP were much lower in liver and kidney than in serum (Robert and Hagardom 1983), despite similar half-times for absorption ($t_{1/2}$=0.50–0.62 hours) in all three tissues. Elimination of 2,4-DNP from kidney was very slow compared with liver and serum (see Section 3.1.4). The authors suggested that the apparent persistence of 2,4-DNP in the kidney could be related to tissue binding of the compound.

The time course of plasma concentrations of 2,4-DNP following oral administration to dogs (one per dose) at 5, 12.5, or 25 mg/kg gave no evidence of a trend towards higher plasma levels with continued daily dosing (Kaiser 1964). Hence, 2,4-DNP did not appear to accumulate.

In coordination with toxicity studies (Gehring and Buerge 1969a) (see Section 2.12, Ocular Effects), a study was performed to determine whether susceptibility to 2,4-DNP cataractogenesis could be related to the concentrations of 2,4-DNP in the compartments of the eye (aqueous humor, vitreous humor, lens) after intraperitoneal injection (Gehring and Buerge 1969b). The concentration of 2,4-DNP in the ocular compartments appeared to be more important than the elimination rates (see Section 3.1.4) in determining susceptibility to developing cataracts. Although initial concentrations of 2,4-DNP in the serum of all three animal models were similar, initial concentrations of 2,4-DNP in the compartments of the eye were higher in the more susceptible immature rabbits (~10 µg/g in all compartments) and ducklings (~3, 10, and 10 µg/g in lens, aqueous humor, and vitreous humor, respectively) than in the less susceptible mature rabbits (~1, 4, and 3 µg/g, respectively).

Additional experiments, including *in vitro* investigations and pharmacokinetic analysis, indicated that some of the 2,4-DNP in serum was bound to protein and some was free; the fraction of free DNP was similar among the animals tested (mature and immature rabbits, ducklings) (Gehring and Buerge 1969b). The concentration of DNP in the aqueous humor was related to, but lower than, the concentration of free 2,4-DNP in the serum; hence, there appeared to be a blood-aqueous humor barrier preventing free diffusion. This barrier appeared to be most effective in the mature rabbit and least effective in the duckling.

3.1.3 Metabolism

In both humans and animals, available data indicate that 2,4-DNP is metabolized by gut microflora by sequential nitro group reduction via the enzyme nitroreductase to form 2-amino-4-nitrophenol and 4-amino-2-nitrophenol and 2,4-diaminophenol. These metabolites may be conjugated with glucuronic acid or sulfate prior to excretion in the urine.

Limited information on 2,4-DNP metabolites in humans is available from an occupational health study and a few case reports. Examination of the blood and organs of workmen who died from exposure to 2,4-DNP in the French munitions industry revealed the presence of 2,4-DNP and its reduced metabolites (not further specified) (Perkins 1919). The author reported that urinary metabolites in workers included 2,4-DNP, 2-amino-4-nitrophenol, 4-amino-2-nitrophenol, 2,4-diaminophenol, and other unidentified nitrogen compounds (Perkins 1919), which may have been glucuronide conjugation products (NRC 1982). In cases of serious 2,4-DNP poisoning, large quantities of 2-amino-4-nitrophenol were detected in the urine; this finding formed the basis of the Derrien test, a colorimetric test used as an indicator of exposure to 2,4-DNP. The color reaction depended on the presence of a NO_2 group; thus, the test was not very specific, and it detected only aminonitrophenol metabolites, and not diaminophenols. However, the intensity of the Derrien test apparently showed some correlation with the degree of intoxication (Perkins 1919). A woman who ingested sodium 2,4-DNP at 3.5 mg/kg/day 2,4-DNP for 20 days tested positive for the presence of 2-amino-4-nitrophenol (Derrien test) and 2,4-DNP ("indicator test" not further described) in the urine (Davidson and Shapiro 1934). In a case of fatal occupational 2,4-DNP poisoning from exposure to mists and airborne dust of 2,4-DNP in the U.S. chemical industry, the urine contained 2.08 g/L of 2,4-DNP and 50 mg/L of 2-amino-4-nitrophenol (Gisclard and Woodward 1946).

Limited studies in rats, mice, and rabbits also indicate that 2,4-DNP is metabolized to aminonitrophenols (predominantly 2-amino-4-nitrophenol) and, to a lesser extent, diaminophenols. 2,4-DNP, 2-amino-4-nitrophenol, and 4-amino-2-nitrophenol, were measured by high-performance liquid chromatography (HPLC) in plasma collected from mice for up to 96 hours following a single gavage dose of 22.5 mg/kg (Robert and Hagardom 1985). Plasma concentrations of these two metabolites reached their highest levels within the first half hour after dosing, indicating rapid metabolism. The authors indicated that the amount of 2-amino-4-nitrophenol was 7.9 times the amount of 4-amino-2-nitrophenol, and that 50% of 2,4-DNP elimination involved direct conversion to these two compounds. In a study from the older literature, 2,4-diaminophenol was identified in the urine of rabbits treated orally with 2,4-DNP, and was

concluded to be a metabolite of 2,4-DNP (Ogino and Yasukura 1957). However, the study lacked adequate reporting of dose, route, and number of animals; in addition, the relative lack of specificity in the identification methods available at the time, and the lack of experiments to quantify losses of metabolite during the extraction and purification processes limit the conclusions that can be drawn regarding the metabolite identity and percentage of the administered dose. Analysis of 24-hour urine samples for 2,4-DNP and its aminonitrophenol metabolites following a single subcutaneous injection of 20 mg/kg 2,4-DNP into rats revealed only parent compound and 2-amino-4-nitrophenol; 4-amino-2-nitrophenol was not detected (Parker 1952).

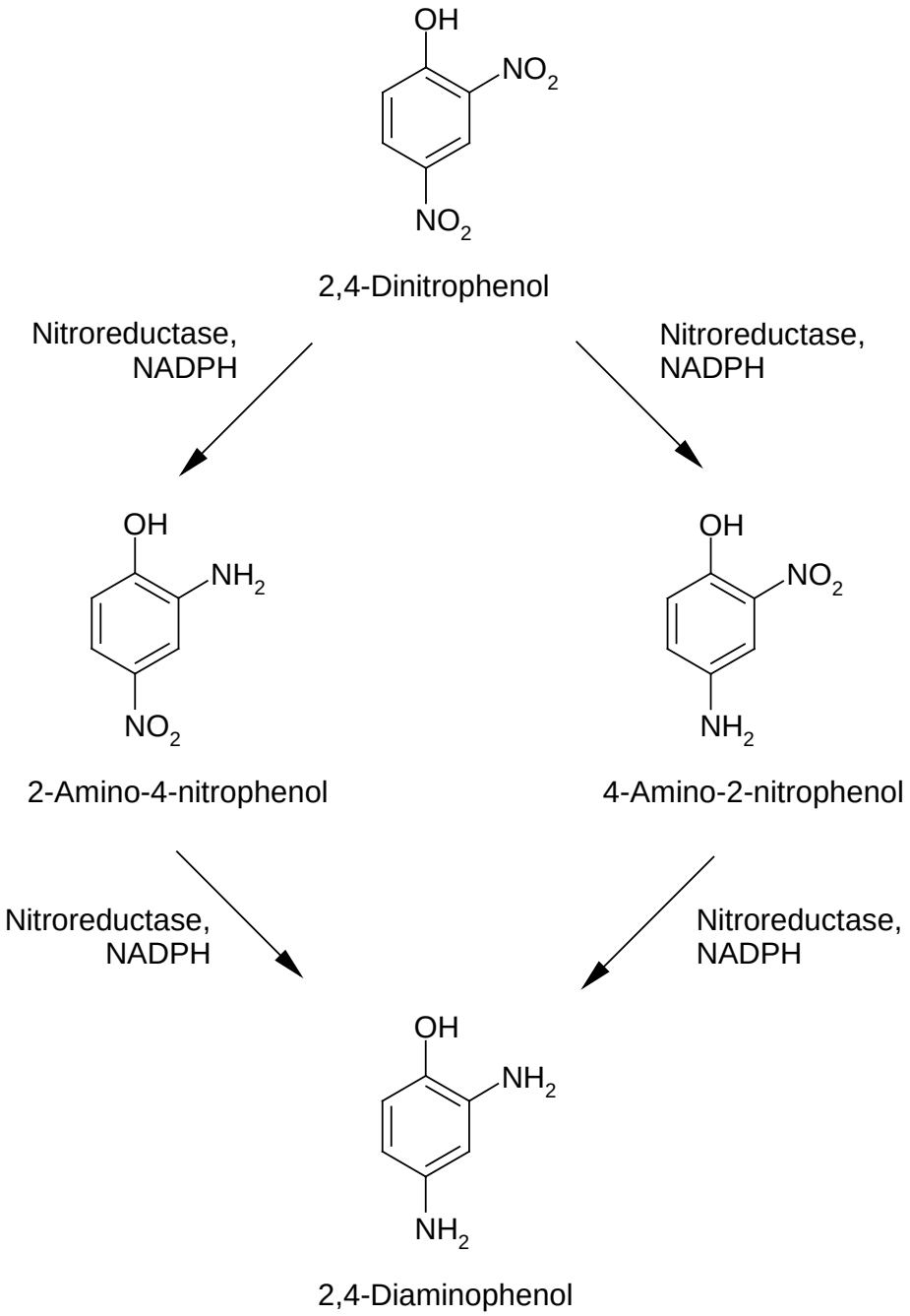
In vitro data are consistent with the available *in vivo* data on metabolism. An extensive investigation of the *in vitro* metabolism of 2,4-DNP by rat liver homogenates found that, under optimal pH and cofactor levels, 81% of the 2,4-DNP was metabolized. 2-Amino-4-nitrophenol accounted for 75%, 4-amino-2-nitrophenol accounted for 23%, and 2,4-diaminophenol accounted for ≈1% of the total amine metabolites produced (Eiseman et al. 1972). Even under suboptimal conditions, 2-amino-4-nitrophenol was the predominant metabolite. An earlier *in vitro* study of 2,4-DNP metabolism in rat liver homogenates identified 2-amino-4-nitrophenol and 4-amino-2-nitrophenol as metabolic products, with 4-amino-2-nitrophenol present in greater abundance (Parker 1952). In that study, an additional ether-insoluble metabolite was tentatively identified as 2,4-diaminophenol. When 2-amino-4-nitrophenol or 4-amino-2-nitrophenol was incubated with rat liver homogenates, the 2-amino-4-nitrophenol was slowly metabolized to the ether-insoluble compound, while 4-amino-2-nitrophenol rapidly disappeared but with very little accumulation of the ether-insoluble compound. The reduction of the aminonitrophenols to the ether-insoluble compound appeared to be catalyzed by the same nitroreductase that reduces 2,4-DNP to the aminonitrophenols.

A comparison of the activity of homogenates of various tissues in the rat and rabbit revealed higher metabolic rates in rat tissue homogenates than in rabbit and showed that liver homogenate metabolized 2,4-DNP at a higher rate than did other tissue homogenates (Parker 1952). The rates of 2,4-DNP metabolism in the rat liver, kidney, and heart were 2–20-fold higher than in the rabbit; in addition, there was substantial metabolic activity in the rat fat, muscle, and spleen, while no detectable activity occurred in these tissues in the rabbit. In the rat, enzyme activities (normalized to wet tissue weight) relative to that of the liver homogenate (100%) were 60% in kidney, 59% in spleen, 47% in intrascapular fat, 29% in heart, 16% in muscle, and 3% in brain homogenates. In the rabbit, kidney homogenate activity was 41%, and heart homogenate activity was 3%, relative to liver homogenate activity. The rabbit spleen

homogenate had no activity. The other rabbit tissues (intrascapular fat, muscle, and brain) were not analyzed. No metabolic activity was found in the blood of rats or rabbits (Parker 1952).

Nitro reduction of 2,4-DNP is depicted in Figure 3-1.

Figure 3-1. Nitro Reduction of 2,4-Dinitrophenol



Sources: Eiseman et al. 1972; Parker 1952; Robert and Hagardom 1985

The distribution of enzyme activity was analyzed in subcellular fractions: nucleic, mitochondrial, microsomal, and cytosol (Eiseman et al. 1972). The maximum activity was found in the cytosol, which is the site of other nitroreductases, although nitroreductases can also be located in microsomes (Fouts and Brodie 1957; Juchau et al. 1970; Kamm and Gillette 1963; Kato et al. 1969; Parker 1952). The properties of nitroreductases have been extensively studied for the reduction of p-nitrobenzoic acid (Kato et al. 1969). Two separate enzyme systems are involved: one located in the cytosol and the other in the microsomes. Both forms require the presence of reduced nicotinamide adenine dinucleotides (NADH or NADPH) (Kato et al. 1969). The cytosolic reducing activity for 2,4-DNP required NADPH since the activity in both the whole homogenate and the cytosol was enhanced by adding glucose-6-phosphatase and NADP (Eiseman et al. 1972). The fact that the washed microsomal fraction contained no appreciable activity with 2,4-DNP could be due to the absence of soluble NADPH-generating enzymes, such as a glucose-6-phosphate dehydrogenase. Oxygen partially inhibited the formation of the aminonitrophenols. This inhibition is consistent with a reoxidation of cofactors FADH2 or NADPH in the presence of oxygen (Kamm and Gillette 1963). Reduction of [14C]2,4-DNP to 2-amino-4-nitrophenol and 4-amino-2-nitrophenol by rat liver homogenates was not affected by the addition of p-nitrobenzoic acid, suggesting that different nitroreductases are involved (Eiseman et al. 1974). However, p-nitrophenol, o-nitrophenol, and 2,4-dinitro-6-sec-butylphenol inhibited the reduction of 2,4-DNP. The reduction was competitively inhibited by o-nitrophenol and noncompetitively inhibited by p-nitrophenol and 2,4-dinitro-6-sec-butylphenol. These results indicate separate metabolic pathways for 2,4-DNP and p-nitrobenzoic acid. The competitive inhibition by o-nitrophenol, however, suggests that 2,4-DNP and o-nitrophenol compete for the same active site on the nitroreductase, while the noncompetitive inhibition by the other two nitro compounds suggests binding at different sites on the enzyme.

Limited information indicates that 2,4-DNP may also be conjugated to glucuronic acid or sulfate in the liver and then excreted in the urine (NRC 1982).

No studies were located regarding possible fecal metabolites of 2,4-DNP.

Politi et al. (2007) used liquid chromatography-mass spectrometry to analyze biological fluids from a fatal poisoning case. They tentatively identified three possible conjugated DNP metabolites: 2-amino-4-nitrophenol glucuronide, 2,4-dinitrophenol glucuronide, and 2,4-dinitrophenol sulfate.

3.1.4 Excretion

In humans exposed to 2,4-DNP by any exposure route, both the parent compound and metabolites appear to be excreted in the urine. 2,4-DNP and its metabolites were detected in the urine of workmen who died from exposure to 2,4-DNP in the munitions industry in France; the metabolite, 2-amino-4-nitrophenol, was commonly detected in the urine of workers who survived as well (Perkins 1919). Quantitative exposure or urinary data were not provided. In a case of fatal occupational 2,4-DNP poisoning in the United States, the urine contained 2.08 g/L of 2,4-DNP and 50 mg/L of 2-amino-4-nitrophenol (Gisclard and Woodward 1946). In both occupational studies, exposure may have occurred by the dermal as well as inhalation routes. Both 2,4-DNP and its metabolite, 2-amino-4-nitrophenol, were detected in the urine of a woman who had taken the sodium salt of 2,4-DNP at 3.5 mg/kg/day 2,4-DNP for 20 days and was admitted to the hospital 5 days after cessation of DNP treatment because of severe illness (agranulocytosis) (Davidson and Shapiro 1934). Detection of parent compound (method not described) and 2-amino-4-nitrophenol (Derrien test) occurred on the second day of hospitalization and detection of parent compound occurred on the third. The bromsulphalein test for liver function showed evidence of impaired function, which may have accounted for the persistence of 2,4-DNP and 2-amino-4-nitrophenol in the body 7–8 days after cessation of intake. More recently, 2,4-DNP was measured at a concentration of 53 mg/L in the urine of a 28-year-old man who died (Miranda et al. 2006).

Yellow staining of the skin was observed in French munitions workers exposed to 2,4-DNP who perspired profusely (Perkins 1919), indicating that 2,4-DNP was also excreted in the sweat.

In dogs (one per dose) that received 1, 12.5, or 25 mg/kg/day 2,4-DNP orally, 24-hour excretion of parent compound in the urine at 1, 3, and 6 days of treatment was erratic (Kaiser 1964), raising the suspicion that collection may have been incomplete in some instances. Analysis of 24-hour urine samples following a single subcutaneous injection of 20 mg/kg 2,4-DNP into rats revealed parent compound and 2-amino-4-nitrophenol (Parker 1952).

Pharmacokinetic analysis indicated that a two-compartment open model best characterized the disposition of 2,4-DNP in the serum, liver, and kidney of mice given a gavage dose of 22.5 mg/kg of 2,4-DNP (Robert and Hagardom 1983). Serum and tissue levels of the parent compound were quantified by a highly specific capillary gas chromatography-mass spectrometry (GC-MS) method at 1–24 hours postdosing. Half-times for the slow terminal elimination phases were 7.7 hours for serum, 8.7 hours for liver, and 76.2 hours for kidney. The authors suggested that the apparent persistence of 2,4-DNP in the

kidney could be related to tissue binding of the compound. In a related study employing the same analytical methods, 2,4-DNP and its metabolites, 2-amino-4-nitrophenol and 4-amino-2-nitrophenol, were monitored in plasma for 0.5–96 hours following a single gavage dose of 22.5 mg/kg in mice (Robert and Hagardom 1985). Pharmacokinetic analysis indicated that two-compartment open models best characterized the disposition of 2,4-DNP and 2-amino-4-nitrophenol from plasma, whereas a three-compartment open model best characterized the disposition of 4-amino-2-nitrophenol from plasma. The elimination half-lives for the terminal phase were estimated at 10.3 hours for 2,4-DNP, 46.2 hours for 2-amino-4-nitrophenol, and 25.7 hours for 4-amino-2-nitrophenol.

Elimination of 2,4-DNP from various compartments of the eye may partially explain age-related differences in susceptibility to 2,4-DNP-induced cataract formation. Elimination rates were compared in animal models of higher (ducklings) and intermediate (immature rabbits) susceptibility and those of low susceptibility (mature rabbits) after intraperitoneal exposures (Gehring and Buerge 1969a). In mature rabbits, the apparent first-order rate constants for elimination of 2,4-DNP from the media studied were 0.82 $hours^{-1}$ for the first phase of elimination from serum, 0.89 $hours^{-1}$ for aqueous humor, and 0.41 $hours^{-1}$ for vitreous humor. These values were substantially higher than those of immature rabbits (0.15 $hours^{-1}$ for the first phase of elimination from serum, 0.13 $hours^{-1}$ for aqueous humor, and 0.16 $hours^{-1}$ for vitreous humor) and the serum, but not the aqueous and vitreous humor values for ducklings (0.21 $hours^{-1}$ for the first phase of elimination from serum, 0.84 $hours^{-1}$ for aqueous humor, and 1.10 $hours^{-1}$ for vitreous humor). The apparent first-order rate constant for elimination from lens of the mature rabbit was 0.27 $hours^{-1}$, but no values for lens could be calculated for the other two animal groups. The ducklings eliminated 2,4-DNP from eye compartments more rapidly than did immature rabbits, which may account for the faster disappearance of cataracts in ducklings than in immature rabbits.

Species differences in elimination of 2,4-DNP do not appear to be large (<2-fold) on the basis of a single limited study. The elimination rate constants for 2,4-DNP from blood of rats, rabbits, guinea pigs, and mice following unspecified single oral doses of 2,4-DNP were 0.062, 0.10, 0.12, and 0.098 $hours^{-1}$, respectively (Lawford et al. 1954). In a companion experiment, rate constants for 2,4-DNP elimination from blood following a single unspecified intraperitoneal dose of 2,4-DNP were 0.122, 0.22, 0.135, and 0.21 $hours^{-1}$, in rats, rabbits, guinea pigs, and mice, respectively, (Lawford et al. 1954).

Data from a very limited study suggest that, in rats and mice, 2,4- and 2,6-DNP may be eliminated more slowly than the other four DNP isomers (Harvey 1959); however, the small number of sampling times, short duration of sampling, and rough estimation of half-lives severely limit any conclusions that could be

drawn from this study. After a single large intraperitoneal dose, half-times for elimination of 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNP were roughly estimated as 12.5, 225.0, 13.0, 210.0, 11.5, and 2.1 minutes, respectively, in rats; and 2.7, 54.0, 3.3, 238.0, 3.5, and 2.7 minutes, respectively, in mice (Harvey 1959).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

No PBPK models were located for any DNPs.

3.1.6 Animal-to-Human Extrapolations

Available data do not provide a clear picture of the relative sensitivity of humans and animals to the toxicity of 2,4-DNP; lethality data suggest little interspecies variability, but species differences in metabolism may impact susceptibility. While the acute lethality data are limited, the available information suggests that species differences in the lethality of 2,4-DNP are 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 fatal doses in humans (~31–75 mg/kg single oral exposures) (see Table 2-2) are similar in magnitude to fatal doses in animals. Similarly, species differences in elimination of 2,4-DNP by laboratory mammals do not appear to be large (<2-fold) on the basis of a single limited study. The elimination rate constants for 2,4-DNP from blood of rats, rabbits, guinea pigs, and mice following unspecified single oral doses of 2,4-DNP were 0.062, 0.10, 0.12, and 0.098 $hours^{-1}$, respectively (Lawford et al. 1954). In a companion experiment, rate constants for 2,4-DNP elimination from blood following a single unspecified intraperitoneal dose of 2,4-DNP were 0.122, 0.22, 0.135, and 0.21 $hours^{-1}$, in rats, rabbits, guinea pigs, and mice, respectively (Lawford et al. 1954). *In vitro* data suggest species differences in the rate of 2,4-DNP metabolism that could indicate species differences in susceptibility. As the parent compound, and not its metabolites, is responsible for the cellular change (uncoupling oxidative phosphorylation) that leads to most of its noncancer health effects, metabolism of

2,4-DNP is expected to have a detoxifying effect. In rat tissue homogenates, the rates of 2,4-DNP destruction in the liver, kidney, and heart were 2–20-fold higher than in the rabbit; in addition, there was substantial metabolic activity in the rat fat, muscle, and spleen, while no detectable activity occurred in these tissues in the rabbit (Parker 1952). No information on rates of 2,4-DNP destruction in human tissues was located.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to DNPs are discussed in Section 5.7, Populations with Potentially High Exposures.

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

3.2.1 Increased Susceptibility due to Age

Koizumi et al. (2001) reported that newborn rats were more susceptible to the toxic effects of 2,4-DNP than were older rats. In the newborn rat study of 2,4-DNP, animals died at 30 mg/kg in the dose-finding study. In the main study, significant lowering of body and organ weights was observed at 20 mg/kg. In the 28-day young (5–6 weeks old) rat study, clear toxic signs followed by death occurred at 80 mg/kg, but no definitive toxicity was observed at 20 mg/kg. The authors concluded that the toxic response in newborn rats is at most 4 times higher than that in young rats. In addition, immature rabbits were more susceptible than older rabbits to ocular effects (cataracts) of 2,4-DNP (Gehring and Buerge 1969a). Results of these studies suggest that infants and children could be more susceptible to the toxicity of 2,4-DNP than adolescents or adults.

3.2.2 Pre-existing Conditions that Increase Susceptibility

2,4-Dinitrophenol is metabolized by the liver and metabolites are excreted in urine; therefore, it is possible that pre-existing hepatic or renal disease may increase susceptibility to 2,4-DNP. Increased numbers of clinical cases of poisoning were seen during the warmer months of the year (Gisclard and Woodward 1946; Perkins 1919), but it is uncertain whether this finding was related to greater exposure and absorption through the skin, or to a lessened capacity to dissipate body heat when environmental temperatures were high. Studies in animals indicate that high environmental temperature increases the toxicity of 2,4-DNP (Harvey 1959). Some human subpopulations that are predisposed to a syndrome known as malignant hyperthermia may be more likely to develop fatal hyperthermia following exposure to 2,4-DNP. Malignant hyperthermia is an inherited disease of skeletal muscle characterized by a drug-induced hyperpyrexia (Schroeder and McPhee 1990). Humans with this inherited disease are predisposed to acute hyperthermic reactions triggered by stress or drugs (such as inhalation anesthetic agents, skeletal muscle relaxants, and amide local anesthetics) (Britt 1979). Although no data were located linking 2,4-DNP with malignant hyperthermia, persons with the genetic predisposition may be more susceptible to the hyperthermic effects of 2,4-DNP.

Salicylate at very high doses can increase respiratory rates and produce hyperthermia in humans (Brody 1956) and possibly exaggerate these signs in persons acutely exposed to 2,4-DNP. Therefore, people who take high doses of aspirin regularly may be at an increased risk of 2,4-DNP-induced toxicity.

A single case report suggests that impaired liver function may be a factor in susceptibility to the hematological effects of ingested 2,4-DNP (Davidson and Shapiro 1934).

3.2.3 Factors Increasing Susceptibility to Cataracts

Cataracts have been seen at a relatively low incidence among humans ingesting 2,4-DNP or sodium 2,4-DNP for weight loss. Based on cases of cataracts in a mother and daughter (Hessing 1937) and in identical twins who had taken the drug, Buschke (1947) suggested that a genetic predisposition may play a role in susceptibility to 2,4-DNP cataractogenesis.

Rats fed diets deficient in vitamin A or B2 to which 2,4-DNP was or was not added did not develop cataracts (Tainter and Borley 1938). Similarly, guinea pigs fed a diet deficient in vitamin C to which 2,4-DNP or no 2,4-DNP was added also did not develop cataracts. These results indicated that cataracts could not be induced in rats or guinea pigs by 2,4-DNP, even if deficient in vitamins. However, in a later study, guinea pigs fed a vitamin C-deficient diet and treated orally with 2,4-DNP developed cataracts, while guinea pigs on a vitamin C-deficient diet but given ascorbic acid and 2,4-DNP did not (Ogino and Yasukura 1957), indicating that vitamin C deficiency made the guinea pigs susceptible to 2,4-DNP cataractogenesis. Human subpopulations with diets deficient in vitamins C, E, (Robertson et al. 1989), or B2 (Prchal et al. 1978) may be more susceptible to cataract formation in general. The concentration of ascorbic acid in the aqueous humor of adult animals is generally higher than that in young animals (Kinsey et al. 1945). Ascorbic acid concentration in the eyes of rabbits younger than 8 days of age did not differ significantly from the concentration in the blood. Beyond 8 days of age, the concentration in the aqueous humor increased. This suggests that low levels of ascorbic acid may be associated with DNP-induced cataracts in young animals. However, no studies were located to indicate that low levels of ascorbic acid in the eyes of young animals may predispose them to 2,4-DNP-induced cataracts or that high concentrations of ascorbic acid in adults prevents 2,4-DNP-induced cataracts.

An increased risk of cataracts secondary to lactose and galactose ingestion is present in subpopulations with a deficiency in galactokinase activity (Couet et al. 1991). In addition, people with hyperparathyroidism, hypocalcemia, or hypoglycemia are predisposed to cataracts (Lloyd et al. 1992). People with diabetes mellitus also develop cataracts (Muller-Breitenkamp and Hockwin 1991). Evidently, defects in the metabolism of hexose sugars, such as in diabetes and galactosemia, can lead to osmotically induced cataracts (Lloyd et al. 1992). Therefore, people with these metabolic disorders and/or vitamin deficiencies may be more susceptible to 2,4-DNP cataractogenesis. Other physical and chemical agents that are cataractogenic in humans include ultraviolet, x-ray, or microwave radiation,

cigarette smoke, trinitrotoluene, and polyvinyl chloride (Muller-Breitenkamp and Hockwin 1991). Thus, people exposed to these agents or who smoke may be at increased risk to 2,4-DNP cataractogenesis.

Immature rabbits and ducklings were more susceptible to 2,4-DNP cataractogenesis than mature rabbits (Gehring and Buerge 1969a). However, incubation of lenses from mature rabbits with 2,4-DNP resulted in cataract formation. It therefore appears that age-related difference in susceptibility is related to a difference in the rate of clearance of 2,4-DNP from the blood and/or to the presence of a blood-aqueous humor barrier. Higher concentrations of 2,4-DNP were found in the aqueous humor, vitreous humor, and lenses of immature rabbits and ducklings than in similar tissues of older animals (Gehring and Buerge 1969b). These investigators proposed that the presence of a physiological blood-aqueous humor barrier in older rabbits and ducks maintains a lower concentration of 2,4-DNP in the aqueous humor than in the serum. This study also confirmed that 2,4-DNP was cleared faster from the serum of mature rabbits than from young rabbits. Mature animals are therefore less susceptible to cataract formation because the lens is protected physically and metabolically from cataractogenic 2,4-DNP concentrations. This suggests that human infants may be more susceptible than adults, but no studies were located that address this issue.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to 2,4-DNP are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for DNPs from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health

impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by DNPs are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Yellow staining of the skin or sclera has occurred in humans exposed to 2,4-DNP, and may be an initial indicator of exposure and/or poisoning.

2,4-DNP and its metabolites have been detected or measured in blood, urine, and tissues of humans and animals (Davidson and Shapiro 1934; Gehring and Buerge 1969b; Gisclard and Woodward 1946; Kaiser 1964; Lawford et al. 1954; Parker 1952; Perkins 1919; Robert and Hagardom 1983, 1985). The predominant compounds in blood and urine appear to be unchanged 2,4-DNP, 2-amino-4-nitrophenol, and a small amount of 4-amino-2-nitrophenol. Blood and urine levels of DNP and its metabolites can be used as biomarkers of exposure; however, systematic attempts to correlate levels of 2,4-DNP or its metabolites in blood or urine with exposure levels have not been made. Observations in the French munitions industry in 1917–1918 suggested that the presence and amount of 2-amino-4-nitrophenol in the urine, as indicated by a color test (Derrien test) (Perkins 1919), could be used as a rough indicator of intensity of exposure (Tainter et al. 1934a), but the test lacked specificity. m-Dinitrobenzene is metabolized to 2-amino-4-nitrophenol (Parke 1961) and would also give a positive Derrien test. The total mass or concentration of 2,4-DNP and its principal metabolite(s) in blood and urine would probably be a better indicator of exposure than either alone. However, because 2,4-dinitroanisole is metabolized to 2,4-DNP in the body, the possibility of 2,4-dinitroanisole exposure should also be considered when 2,4-DNP is found in blood or urine (Hayes 1982).

The other DNP isomers have also been monitored in blood in animal studies (Harvey 1959), so blood concentrations could potentially be used to quantify exposure to those isomers.

3.3.2 Biomarkers of Effect

It is well established from human studies that 2,4-DNP exposure increases the basal metabolic rate; causes increased perspiration, a sensation of warmth, weight loss; and, at higher levels, increases the pulse, respiratory rate, and body temperature (Castor and Beierwaltes 1956; Cutting et al. 1934; Gisclard and Woodward 1946; Looney and Hoskins 1934; MacBryde and Taussig 1935; Perkins 1919; Tainter et al. 1935). The increase in basal metabolic rate and the weight loss may be fairly sensitive indices of the profound metabolic disturbances caused by 2,4-DNP. Other chemicals that uncouple oxidative phosphorylation (e.g., 4,6-dinitro-o-cresol) also increase the basal metabolic rate and cause weight loss in humans; amphetamines and heat stress can also mimic the effects of 2,4-DNP.

As cataracts develop in some humans exposed to 2,4-DNP and can lead to blindness (Horner 1942), the appearance of lens opacities can serve as an early warning that more serious cataracts could eventually develop.

3.4 INTERACTIONS WITH OTHER CHEMICALS

No human data on potential interactions between 2,4-DNP and other chemicals were located. 2,4-DNP appears to markedly increase the rate of ethanol metabolism in rat liver slices by 100–160% (Videla and Israel 1970) and in rats *in vivo* by 20–30% (Israel et al. 1970). Because 2,4-DNP uncouples mitochondrial electron transport from oxidative phosphorylation, the oxidation of NADH to NAD+ is accelerated in the mitochondria. Reoxidation of NADH rather than the activity of alcohol dehydrogenase is the rate-limiting step in the metabolism of ethanol; thus, the metabolic effect of 2,4-DNP enhances the clearance of ethanol (Eriksson et al. 1974).

In an attempt to determine the best treatment regimen for mice given intraperitoneal doses of 4,6-dinitro-o-cresol (DNOC), which also uncouples oxidative phosphorylation and is hyperthermic, the effect of 2,3-, 2,4-, 2,5-DNP, and 3,4-DNP on the lethality of DNOC was studied (Harvey 1959). At a dose of 10 mg/kg, DNOC itself resulted in 100% mortality. When the other DNPs were given immediately after DNOC, mortality was 60% after 2,3-DNP and 50% after 3,4-DNP. 2,4-DNP and 2,5-DNP afforded no protection of the mice. Harvey (1959) suggested that the protection resulted from hypothermic effects of

the other isomers; however, no other information suggesting hypothermic properties of these isomers was located.

2,4-DNP administration by intracerebroventricular injection enhanced the induction of convulsions by potassium cyanide administration in mice (Yamamoto 1995). The authors suggested that ATP depletion may be involved in the mode of action for cyanide-induced convulsions.

In isolated perfused rat livers, 2,4-DNP caused a depletion of the mitochondrial calcium pool, without altering the extramitochondrial calcium pool (Kleineke and Söling 1985). Because 2,4-DNP uncouples oxidative phosphorylation from electron transport by dissipating the electrochemical potential, which provides the energy for the accumulation of calcium in the mitochondrial matrix, only the calcium pool in the mitochondria was affected. 2,4-DNP also caused a rapid increase in NAD, along with a decrease in NADH, a rapid decrease in protein thiol content, but only a slow decrease in nonprotein thiol (e.g., reduced glutathione [GSH]), and an increase in cytoplasmic calcium concentration in isolated rat intestinal cells (Nishihata et al. 1988a). This DNP-induced protein thiol loss and/or increase of cytoplasmic calcium concentration induced cell rounding and decreased cell viability. Incubation of salicylate and 2,4-DNP with intestinal cells caused a reduction in the 2,4-DNP-induced increase in cytosolic free calcium concentration by complexation, which facilitated the release of calcium from cells. Salicylate also inhibited DNP-induced cell rounding and increased cell viability in the small intestine.

Salicylate (aspirin), which also uncouples oxidative phosphorylation in mitochondrial preparations (although at much higher concentrations than 2,4-DNP) (Brody 1956), partially inhibited a protein thiol loss induced by 2,4-DNP, but not nonprotein thiol loss by 2,4-DNP in the small intestine of rats (Nishihata et al. 1988b). Although DNP inhibits the absorption of the hydrophilic drug, cefmetazole, incubation of DNP and salicylate removed this inhibitory effect in the small intestine. By preventing the DNP-induced protein thiol loss in the small intestine, salicylate appears to enhance diffusivity of cefmetazole in the small intestines. However, salicylate at very high doses can also increase respiratory rates and produce hyperthermia in humans (Brody 1956), and possibly exaggerate these signs in persons acutely exposed to 2,4-DNP.

Spontaneous release of acetylcholine, as measured electrophysiologically as increased miniature endplate potential (MEPP) frequency, was determined in myofibers from rat hemidiaphragms exposed to 2,4-DNP and/or methylmercury (Levesque and Atchison 1987). Tissues exposed to 2,4-DNP caused the same increase in MEPP frequency as when methylmercury was administered. However, pretreatment with

2,4-DNP did not block methylmercury-induced stimulation of MEPP frequency. Although 2,4-DNP and methylmercury were capable of individually increasing cytoplasmic calcium and stimulating spontaneous release of acetylcholine, there was no interaction between DNP and methylmercury. The authors proposed that methylmercury and DNP do not share a common mechanism for increasing cytoplasmic calcium.

Additional information regarding interactions of any isomer of DNP with chemicals other than drugs (haloperidol, salicylates, anticholinergics) was not located. Pretreatment of rats with haloperidol significantly diminished the hyperpyrexia and lethality of 2,4-DNP by interfering with the uncoupling of oxidative phosphorylation by 2,4-DNP (Gatz and Jones 1972). The protection by haloperidol may have occurred by an indirect action on the mitochondrial membrane. Although no studies were located regarding interactions between 2,4-DNP and anticholinergics, anticholinergics may also cause hyperpyrexia, and aspirin and other salicylates also uncouple oxidative phosphorylation (Brody 1956; Ellenhorn and Barceloux 1988; Flower et al. 1985; Haddad and Winchester 1990). In addition, 4,6-DNOC uncouples oxidative phosphorylation (Ilivicky and Casida 1969). Therefore, these agents may exacerbate the effects of 2,4-DNP. Animal studies have established environmental temperature as a factor in the toxicity of 2,4-DNP, in that high temperatures increase the toxicity and low temperatures have a protective effect (Harvey 1959). Data from occupational exposure studies suggest that this phenomenon may hold for humans as well (Gisclard and Woodward 1946; Perkins 1919).

2,4-Dinitro-6-sec-butylphenol (the pesticide Dinoseb), p-nitrophenol, and o-nitrophenol inhibit the metabolism of 2,4-DNP (Eiseman et al. 1974). o-Nitrophenol is a competitive inhibitor and is metabolized by the same enzyme as 2,4-DNP. 2,4-Dinitro-6-sec-butylphenol and p-nitrophenol are non-competitive inhibitors of 2,4-DNP metabolism. If an individual is exposed simultaneously to 2,4-DNP and any of these inhibitors, it is possible that the toxic effects of 2,4-DNP would be enhanced.

DNOC was also used as a diet pill in the 1930s and also was associated with cataracts. DNOC also uncouples oxidative phosphorylation and may have an additive or synergistic effect with 2,4-DNP if a person were simultaneously exposed.

As discussed in Section 2.21, 2,4-DNP uncouples oxidative phosphorylation, thereby preventing the generation of ATP. Since many biochemical processes depend on the energy released during the breakdown of ATP, the limited supply of ATP may affect detoxification or the activation of other xenobiotic chemicals.

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

DNPs are a class of synthetic organic chemicals that exist in six isomeric forms: 2,3-DNP, 2,4-DNP, 2,5-DNP, 2,6-DNP, 3,4-DNP, and 3,5 DNP. Information regarding the chemical identity of DNPs is located in Table 4-1.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of DNPs is located in Table 4-2.

Table 4-1. Chemical Identity of Isomers of Dinitrophenols[a]

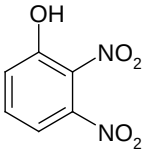
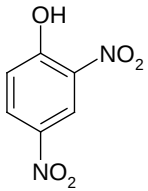
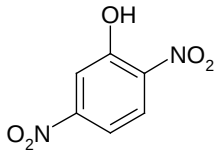
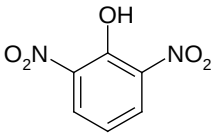
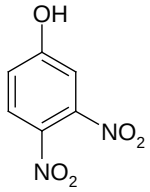
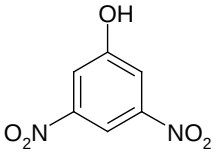
Characteristic	Information			
Chemical name	2,3-Dinitrophenol	2,4-Dinitrophenol	2,5-Dinitrophenol	2,6-Dinitrophenol
Synonym(s)	No data	1-Hydroxy-2,4-dinitrobenzene; α-Dinitrophenol; 2,4-DNP	γ-Dinitrophenol; 2,5-DNP[b]	β-Dinitrophenol[b]; 2,6-DNP
Registered trade name(s)	No data	Caswell No. 392; Sulfo Black B; Sulfo Black 2b Supra; Nitro Kleenup; others	No data	No data
Chemical formula	$C_6H_4N_2O_5$	$C_6H_4N_2O_5$	$C_6H_4N_2O_5$	$C_6H_4N_2O_5$
Chemical structure				
CAS Registry Number[c]	66-56-8	51-28-5	329-71-5	573-56-8

Table 4-1. Chemical Identity of Isomers of Dinitrophenols[a]

Characteristic	Information		
Chemical name	3,4-Dinitrophenol	3,5-Dinitrophenol	Dinitrophenol mixture
Synonym(s)	Δ-Dinitrophenol[c]; 4,5-Dinitrophenol[c]	θ-Dinitrophenol[c]	Dinitrophenol solution[c]
Registered trade name(s)	No data	No data	No data
Chemical formula	$C_6H_4N_2O_5$	$C_6H_4N_2O_5$	$(C_6H_4N_2O_5)$
Chemical structure			Usually a mixture of 2,3-, 2,4-, and 2,6- isomers
CAS Registry Number[c]	577-71-9	586-11-8	25550-58-7

[a]All information obtained from CHEMID (1992) and HSDB (1994), except where noted.
[b]RTECS 1992.
[c]SANSS 1992.

CAS = Chemical Abstracts Services

Table 4-2. Physical and Chemical Properties of Dinitrophenols

Property	2,3-Dinitrophenol	2,4-Dinitrophenol	2,5-Dinitrophenol	2,6-Dinitrophenol
Molecular weight	184.1	184.1[a]	184.1[a]	184.1[a]
Color	Yellow[b]	Yellow[a]	Yellow[a]	Yellow[a]
Physical state	Solid[b]	Solid[a]	Solid[a]	Solid[a]
Melting point	No data	Sublimes[a]	No data	No data
Boiling point	144°C[c]	112–114°C[a]	108°C[a]	63–64°C[a]
Density (g/cm^3)	1.681[c]	1.683[a]	No data	No data
Odor	No data	No data	No data	No data

Table 4-2. Physical and Chemical Properties of Dinitrophenols

Odor threshold:				
Water	No data	No data	No data	No data
Air	No data	No data	No data	No data
Solubility:				
Water (at 18°C)	No data	5,600 mg/L[d]	385 mg/L at 20°C[e]	No data
Water (at 35–36°C)	2,200 mg/L[b]	790 mg/L[b]	680 mg/L[b]	420 mg/L[b]
Organic solvents	No data	Solubility at 15°C (g/100 g solution): 15.55 in ethyl acetate; 35.90 in acetone; 5.39 in chloroform; 20.05 in pyridine; 0.423 in carbon tetrachloride; 6.36 in toluene; soluble in alcohol and benzene[a]	Slightly soluble in cold alcohol; soluble in hot alcohol, ether, and fixed alkali hydroxides[a]	Slightly soluble in cold alcohol; freely soluble in chloroform, ether, boiling alcohol, and fixed alkali hydroxides[a]
Partition coefficients:				
Log K_{ow}	No data	1.54[f]	1.75[f]	1.37[f]
K_{oc}	No data	1.69[g,e]	No data	No data
pKa	4.89[b]	4.09[h]	5.22[h]	3.71[h]
Vapor pressure	No data	1.49×10^{-5} mmHg[d] at 18°C	1.05×10^{-3} mmHg[e] at 20°C	No data
Henry's law constant	No data	2.82×10^{-7} atm-m^3/mol[e,g]	6.61×10^{-7} atm-m^3/mol[e,g]	No data
Autoignition temperature	No data	No data	No data	No data
Flashpoint	No data	No data	No data	No data
Flammability limits	No data	No data	No data	No data
Conversion factors	1 ppm=7.65 mg/m^3 [i] 1 mg/m3=0.13 ppm	1 ppm=7.65 mg/m^3 [i] 1 mg/m3=0.13 ppm	1 ppm=7.65 mg/m^3 [i] 1 mg/m3=0.13 ppm	1 ppm=7.65 mg/m^3 [i] 1 mg/m^3=0.13 ppm
Explosive limits	No data	An explosive solid	No data	Moderate explosion hazard exposed to heat[i]

Property	3,4-Dinitrophenol	3,5-Dinitrophenol	Dinitrophenol mixture
Molecular weight	184.1	184.1	184.1
Color	Pale brown[b]	Colorless[b]	Yellow[c]
Physical state	Solid[b]	Solid[b]	Solid[c]
Melting point	134°C[b]	122–123°C[b]	No data
Boiling point	No data	No data	No data
Density (g/cm^3)	1.672[b]	1.702[b]	No data

Table 4-2. Physical and Chemical Properties of Dinitrophenols

Odor	No data	No data	No data
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water (at 18°C)	No data	No data	No data
Water (at 35–36°C)	230 mg/L[b]	160 mg/L[b]	No data
Organic solvents	No data	No data	Soluble in alcohol, ether, benzene, and chloroform[c]
Partition coefficients:			
Log K_{ow}	No data	2.36[f]	No data
K_{oc}	No data	No data	No data
pKa	5.42[h]	3.68[b]	No data
Vapor pressure	No data	No data	No data
Henry's law constant at 25°C	No data	No data	No data
Autoignition temperature	No data	No data	No data
Flashpoint	No data	No data	No data
Flammability limits	No data	No data	No data
Conversion factors (at 25ºC, 1 atmosphere)	1 ppm=7.65 mg/m^3 [i] 1 mg/m^3=0.13 ppm	1 ppm=7.65 mg/m^3 [i] 1 mg/m3=0.13 ppm	1 ppm=7.65 mg/m^3 [i] 1 mg/m3=0.13 ppm
Explosive limits	No data	No data	Severe explosion hazard when dry[c, j]

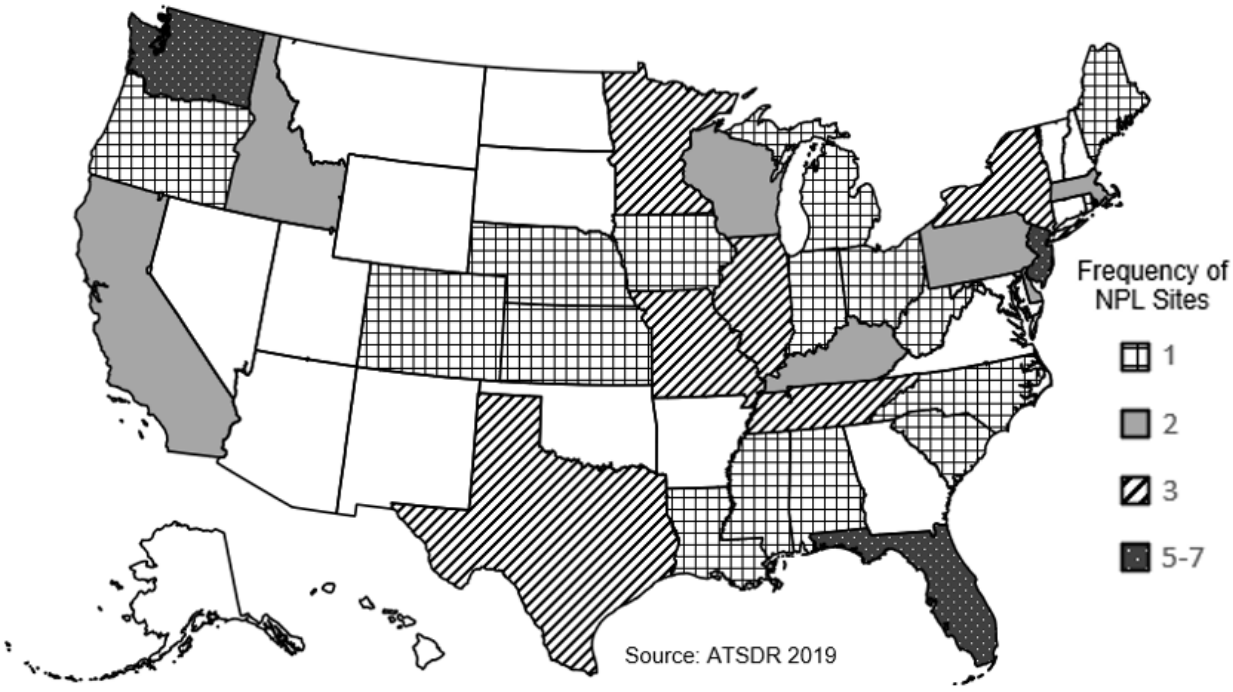
[a]Budavari et al. 1989.
[b]Harvey 1959.
[c]Sax and Lewis 1987.
[d]Mabey et al. 1981.
[e]Schwarzenbach et al. 1988.
[f]Hansch and Leo 1985.
[g]Estimated value.
[h]Pearce and Simkins 1987.
[i]HSDB 1994.
[j]WHO 2015.

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

2,4-DNP has been identified in at least 65 of the 1,867 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2019). 2,6-DNP has been identified in at least one of these sites. However, the number of sites in which DNPs have been evaluated is not known. The number of sites in each state is shown in Figure 5-1.

Figure 5-1. Number of NPL Sites with Dinitrophenol Contamination



- The general population could be exposed to DNPs by inhaling contaminated air and ingesting contaminated food and drinking water.
- A daily intake of DNP from air, food, or drinking water has not been estimated, as there are no pertinent data.
- DNPs are used as pH indicators and in the synthesis of dyes, picric acid, picramic acid, wood preservatives, photographic developers, and explosives.
- DNPs are not likely to volatilize appreciably from water or soil. Leaching from soil to groundwater will vary by soil type and degree of DNP adsorption. 2,4-DNP may biodegrade slowly.

DNPs do not occur naturally in the environment; they are released to the environment primarily during their manufacture and use, and from waste disposal sites that contain DNPs (Games and Hites 1977; McLuckey et al. 1985; Patil and Shinde 1988). DNPs also form in the atmosphere from the reaction of benzene with NOx in ambient air (Nojima et al. 1983). Significant removal of DNPs from the atmosphere due to photochemical or other chemical reactions is not likely. Dry and wet deposition of particulate DNPs are the two significant removal processes in the air (Alber et al. 1989; Capel et al. 1991; Levsen et al. 1990). Neither photochemical nor other chemical processes have been identified that are significant for the transformation/degradation of DNPs in natural waters (EPA 1979a; Lipczynska-Kochany 1992; Tratnyek and Hoigne 1991; Tratnyek et al. 1991). The loss of DNPs from water due to volatilization is negligible (EPA 1979a). Moderate amounts of DNPs are removed from water to sediment due to adsorption (EPA 1979a). Biodegradation may be the most important loss process for DNPs in natural waters (EPA 1979b; Chambers et al. 1963; Games and Hites 1977; Tabak et al. 1981a, 1981b). As in water, no chemical process has been identified that would be significant for transformation/degradation of DNPs in soil. Biodegradation may be the most significant process for destroying DNPs in soil (Kincannon and Lin 1985; EPA 1989; O'Connor et al. 1990). The loss of DNPs from soil due to volatilization is not significant (Wild and Jones 1992). The mobility of DNPs in soils decreases with increase in acidity, clay, and organic matter content, but the mobility in soil will increase as the basicity of soil-water increases because the ionized form is more water soluble and moves faster through soil (Kaufman 1976). 2,4-DNP has been measured in groundwater from waste disposal sites (ATSDR 1988; Plumb 1991), indicating the possibility that these compounds leach from soil. Depending on the nature of soil and climatic conditions, the residence time of 2,4-DNP in soil has been determined to be <8–120 days (Kincannon and Lin 1985; EPA 1989; O'Connor et al. 1990).

Other than in workplace air, information on DNPs in ambient air in the United States was not located; however, DNPs have been detected in air in other countries (see Section 5.5.1). DNPs were detected in effluent from a dye-manufacturing plant at a maximum concentration of 3.2 mg/L (Games and Hites 1977), but no monitoring data for drinking water in the United States are available. The maximum detected 2,4-DNP concentration in groundwater from a Superfund site was 30.6 mg/L in Minnesota (ATSDR 1988). No data are available on the levels of DNPs in food or total diet samples in the United States. Within the overall population, workers at facilities producing or using DNPs are likely to be exposed to higher concentrations of DNPs than the rest of the general population. It is possible that people who live near hazardous waste sites that contain these pollutants may also have higher exposures; however, the extent of such exposures for residents around waste sites has not been documented.

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

The production volume of 2,4-DNP is Confidential Business Information (CBI) (EPA 1991). Therefore, information on the annual production volumes of 2,4-DNP in the United States in recent years has not been published, although the production volumes are substantial (EPA 1991). The most recent information on production volumes based on non-confidential information lists the production range of 10,000–500,000 pounds for 2002 (NLM 2020). Older data, providing historical data on production, indicate that production volume has substantially decreased. For example, NLM (2020) lists the range for production volume in 1986 as >1–10 million pounds. In 1977, the production volume for 2,4-DNP was <1,000 pounds by Alpine Laboratories, Inc., in Bay Minette, Alabama. Production volumes in 1977 for two alternate production sites (Mobay Chemical Corp. in Charleston, South Carolina, and Martin Marietta Corp. in Charlotte, North Carolina) and one plant, whose name remains confidential, were not reported (TSCAPP 1993). According to data from the U.S. International Trade Commission publication on U.S. production and sales of synthetic organic chemicals (USITC 1970), 863,000 pounds of 2,4-DNP were produced in 1968. One chemical company, Sandoz Chemicals Corp., in Charlotte, North Carolina, was listed as a domestic manufacturer of 2,4-DNP in 1992 (SRI 1994) and its yearly production volume was in the range 0.1–1.0 million pounds.

Table 5-1 reports data from 2018 on the number of facilities in each state that manufacture and process 2,4-DNP and the range of maximum amounts of 2,4-DNP stored on-site. The data reported in Table 5-1 are derived from the Toxics Release Inventory (TRI) of EPA (TRI18 2020). The TRI data should be used with caution since only certain types of facilities were required to report. Thus, this is not an exhaustive list. Neither production sites nor volumes for any of the other DNP isomers were located in the available literature.

Table 5-1. Facilities that Produce, Process, or Use 2,4-Dinitrophenol

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
KY	1	1,000	9,999	12
LA	2	100	9,999	1, 5, 13

Table 5-1. Facilities that Produce, Process, or Use 2,4-Dinitrophenol

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
MS	1	1,000	9,999	1, 5, 13
TX	2	0	9,999	1, 5, 12

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

Source: TRI18 2020 (Data are from 2018)

The commercial DNP mixture is produced by heating phenol with dilute sulfuric acid, cooling the product, and then nitrating while keeping the temperature below 50°C, or by nitrating with a mixed acid under careful temperature control (Sax and Lewis 1987). 2,3-, 2,5-, and 3,4-DNP are prepared by nitration of m-nitrophenol. 3,5-DNP is prepared by the replacement of one nitro group by methoxyl in 1,3,5-trinitrobenzene and demethylation of the dinitroanisole by anhydrous aluminum chloride. 2,6-DNP is prepared by sulfonation and nitration of o-nitrophenol (Harvey 1959). 2,6-DNP is also produced as a byproduct in the synthesis of 2,4-DNP by way of 2,4-dinitrochlorobenzene. Heating with 6% aqueous sodium hydroxide at 95–100°C for 4 hours hydrolyzes 2,4-dinitrochlorobenzene. 2,4-DNP in the hydrolyzed product is precipitated by adding acid; the precipitate is removed by filtration. The residue is washed to remove added acid and the more soluble 2,6-DNP (Booth 1991).

5.2.2 Import/Export

During 1985, 102,000 pounds of 2,4-DNP were imported into the United States (NLM 2020); no information on export of 2,4-DNP from the United States was located, nor were more recent import data. Neither import nor export data for any of the other DNP isomers were located in the available literature.

5.2.3 Use

DNP (commercial mixture of the 2,3- and 2,6- isomers, but mostly the 2,4- isomer) is used in the synthesis of dyes, picric acid, picramic acid, wood preservatives, diaminophenol dihydrochloride (a photographic developer), and explosives, and as a pH indicator. 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). In recent years, however, DNP has been sold illegally for this purpose by unregulated sources via the internet. 2,5-DNP is used in the manufacture of dyes, organic chemicals, and as a pH indicator. DNPs are also used in the manufacture of styrene as inhibitors in the purification stills to reduce polymerization (Coulter et al. 1969; Budavari et al. 1989; Sax and Lewis 1987).

5.2.4 Disposal

2,4-DNP has been identified as a hazardous waste by EPA, and disposal of this waste is regulated under the Federal Resource Conservation and Recovery Act (RCRA). Specific information regarding federal regulations on 2,4-DNP disposal on land, in municipal solid waste landfills, and in incinerators is available in the Code of Federal Regulations (EPA 1990, 1992, 1993).

A method that utilizes the classical Fenton reaction (reaction with ferrous chloride and hydrogen peroxide) has been suggested for the treatment of phenolics in waste waters (Lipczynska-Kochany 1991; Vella and Munder 1993). At a nitrophenol to ferrous chloride concentration ratio of 1:3 and in the presence of excess hydrogen peroxide (nitrophenol/hydrogen peroxide ratio=1:18), >90% of nitrophenol was destroyed in <7 hours and no aromatic degradation products were detected (Lipczynska-Kochany 1991). However, the Fenton's system is very susceptible to inhibitors and hydroxyl radical scavengers (e.g., phosphate and carbonate) (Vella and Munder 1993). Alternative treatment methods for the reduction of nitrophenol concentrations in waste water include activated carbon treatment and biological treatment methods (Vella and Munder 1993). Ultimate disposal of DNP isomers can be accomplished in an incinerator equipped with an acid scrubber for removing oxides of nitrogen (NOx). EPA suggests rotary kiln and fluidized bed incineration as methods for disposal of DNP. Because DNP has been used as a pesticide, the disposal of containers that contained DNP is required by EPA. Combustible containers from organic or many metalloorganic pesticides may be disposed of in pesticide incinerators or in specified landfill sites.

5.3 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing

facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005).

5.3.1 Air

Estimated releases of 114 pounds (~0.05 metric tons) of DNPs to the atmosphere, from five domestic manufacturing and processing facilities in 2018, accounted for <1% of the estimated total environmental releases from facilities required to report to the TRI (TRI18 2020). These releases are summarized in Table 5-2.

Releases of 2,4- and 2,6-DNPs in air may occur through automobile exhaust (Nojima et al. 1983). Photochemical reactions of benzene with nitrogen oxides in air also produce DNP in the atmosphere (Nojima et al. 1983). DNPs have been detected in emissions from hazardous waste combustion (James et al. 1984). DNPs may be present in the aerosol or vapor phase near hazardous waste disposal sites.

Table 5-2. Releases to the Environment from Facilities that Produce, Process, or Use 2,4-Dinitrophenol[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
KY	1	97	0	0	0	0	97	0	97
LA	2	0	0	0	0	0	0	0	0
MS	1	0	0	0	0	0	0	0	0

Table 5-2. Releases to the Environment from Facilities that Produce, Process, or Use 2,4-Dinitrophenol[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
TX	2	17	0	709,267	0	0	709,267	0	709,284
Total	5	114	0	709,267	0	0	188,429	0	709,381

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown.
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI18 2020 (Data are from 2018)

5.3.2 Water

There were no releases of DNPs to surface water from five domestic manufacturing and processing facilities in 2018 required to report to the TRI (TRI18 2020).

DNPs may be released into surface water from production facilities and by industries that use them. 2,4-DNP has been detected in the waste water from nitrobenzene-manufacturing plants (Patil and Shinde 1988). Other industries or plants that can release DNPs in surface water are explosives producers (McLuckey et al. 1985), dye-manufacturing plants (Games and Hites 1977), and sewage treatment plants where the influent waters already contain DNPs (DeWalle et al. 1982). Since 2,4-DNP is also used to produce picric acid, picramide, and photographic developer (diaminophenol hydrochloride), and in preserving wood (Hawley 1981), waste waters or land runoffs from these industries may release 2,4-DNP to surface water. Small amounts will also enter surface water by wet and dry deposition of atmospheric DNPs (Alber et al. 1989; Bohm et al. 1989; Levsen et al. 1990).

5.3.3 Soil

DNPs may be released in soils in the vicinity of the sites where they are manufactured and used. These releases are summarized in Table 5-2. There were 709,267 pounds (~321 metric tons), which accounted for >99% of the total environmental emissions, were released via underground injection (TRI18 2020). DNPs were also found in the soil of a decommissioned wood preserving facility (EPA 1988b). However, in 2018, there were no releases of DNPs to soil from five domestic manufacturing and processing facilities that are required to report to the TRI (TRI18 2020). Since vehicular exhaust contains DNPs (Nojima et al. 1983), roadside soil might contain these compounds. Small amounts of DNPs will enter soil due to dry and wet deposition of these compounds present in the atmosphere. 2,4-DNP was previously used as an insecticide, acaricide, and fungicide; however, no pesticides containing 2,4-DNP are actively registered in the United States (NLM 2020).

5.4 ENVIRONMENTAL FATE

Very little data regarding the fate and transport of 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP in the environment were located in the literature; however, more data are available for 2,4-DNP. While there are differences in physico-chemical properties (pKa, water solubility, vapor pressure, and other relevant properties) among the isomers, the environmental fate and transport of other DNPs are likely to follow the same general pattern of environmental behavior as that of 2,4-DNP.

5.4.1 Transport and Partitioning

Air. The vapor pressure of 2,4-DNP is 1.49×10^{-5} mm Hg at 18°C (EPA 1981). Organics with vapor pressures of 10^{-4}–10^{-8} mm Hg at ambient temperature should exist partly in the vapor and partly in the particulate phase in the atmosphere (Eisenreich et al. 1981). Nitrophenols were detected experimentally in the particulate phase in air (Nojima et al. 1983), although the method used to collect atmospheric particulate matter was not suitable for collecting vapor-phase DNPs. The distance of atmospheric transport of DNPs will depend on atmospheric residence times. The residence time of DNPs, based on the estimated rates of various reactions, is long enough to allow atmospheric transport (see Section 5.3.1). The removal and transport of atmospheric DNPs to land and water by physical processes, such as wet and dry deposition, will depend on the physical states of these compounds in the atmosphere. Since DNPs have been detected in rain, snow, and fog (Alber et al. 1989; Capel et al. 1991; Levsen et al. 1990), at least partial removal of these compounds occurs by physical processes. Because of faster rates of travel, unreacted vapor-phase DNPs in the atmosphere have a greater chance to be transported longer distances

than DNPs present as aerosols. No monitoring data, however, are available to substantiate long-distance atmospheric transport of DNPs.

Based on its vapor pressure, water solubility, and presence predominantly in ionic forms in most natural waters (pKa=4.09 for 2,4-DNP), EPA (1979a) concluded that the volatilization of these compounds from water to air is not significant.

Water. The partitioning of DNPs from water to suspended solids and sediment due to adsorption would transport these compounds from water to sediment. The sorption of DNPs by soil or sediment would depend on the organic carbon content, clay content, and pH of soil and sediment (EPA 1979a; Kaufman 1976). An increase in clay and organic carbon content and a decrease in pH (thereby increasing the concentration of the un-ionized form) would increase the amount sorbed. Therefore, the sorption and subsequent transport of DNPs from water to suspended solids and sediment would be significant in natural waters that are acidic and/or have high organic matter and clay content.

No experimental data on the bioconcentration potential for DNPs or their metabolites in aquatic organisms were located. Based on bioconcentration factors (BCFs) in the range of 8–24 estimated from octanol/water partition coefficients (EPA 1986b), bioconcentration is not expected to be significant for DNPs in aquatic organisms. In addition, the fact that DNPs exist predominantly in ionic forms in most natural waters (pH 5–9) further indicates that bioconcentration is not significant (bioconcentration is usually significant for hydrophobic and non-ionic compounds). Using the equation log BCF = 1.02xlog K_{ow}-1.82 to correct the BCF due to ionization in water (McCarty et al. 1993) and a value of 1.54 for log K_{ow} (see Table 4-2), the BCF of 2,4-DNP in fish can be estimated to be 0.56. Therefore, the concentration of 2,4-DNP in fish may be even lower than its concentration in water.

Takahashi et al. (1994) provided information on the environmental fate of DNPs. Five nitrophenols and two synthetic dyes were ozonated to evaluate: (1) the biodegradability of reaction products from water quality parameters, and (2) the relationship between biodegradability and the behavior of the reaction products and the nitrogen forms. From the comparison of the biodegradability of the target compound, Takahashi et al. (1994) confirmed that the variation of biodegradability was deeply associated with the behavior of the nitrogen forms.

Asman et al. (2005) monitored wet deposition of nitrophenols (including 2,4-DNP) at two sites in Denmark and determined the contributions from regional sources. The authors measured concentrations

of selected pesticides and of nitrophenols in rain. Several of the compounds, including 2,4-DNP, could be detected but not quantified. The deposition of 2,4-DNP was up to a factor of 40 higher than that of most of the pesticides tested. Asman et al. (2005) concluded that although these pesticides were not allowed in Denmark, they came in from other places and were transported at least 60–80 km.

Sediment and Soil. The possibility of transport of DNPs from soil to air via volatilization needs examination. Based on its low volatility and reasonably high water solubility, Wild and Jones (1992) concluded that 2,4-DNP would not volatilize from most soils. Although the possibility of volatilization of phenolic compounds in soil via co-distillation with water has been suggested (Kaufman 1976) and release to air via aerosol formation is possible, no volatilization of 2,4-DNP was observed in 21 days from soil columns (Kincannon and Lin 1985). The transport of DNPs from soil to groundwater also may occur via leaching. The amount of DNP leached depends on the DNP adsorption capability of soils. Adsorption of phenols in soil increases with a decrease in pH and an increase in organic carbon, goethite (one of the most common iron oxides in soil), and clay content (Hudson-Baruth and Seitz 1986; Kaufman 1976; O'Connor et al. 1990; Shea et al. 1983; Stone et al. 1993). In soils containing goethite, 2,4-DNP adsorption is due to surface complex formation at protonated mineral surfaces (Stone et al. 1993). The adsorption of 2,4-DNP on goethite was shown to be maximum near pH 4.5 and negligible at neutral and alkaline pHs (Stone et al. 1993). The adsorption of 2,4-DNP in calcareous soils low in organic carbon, goethite, and clay content was low at a pH >7 (Hudson-Baruth and Seitz 1986; O'Connor et al. 1990). The leaching of DNPs is high in such soils. Conversely, the leaching of DNPs from soils will decrease when the organic carbon, clay, or goethite content of soil increases and the pH of soil-water attains a pH of <6. The transport of DNP from soil to adjacent land or surface water may occur as a result of lateral movement via runoff and/or hydrogeological movement of contaminated groundwater to surface water. DNPs have been detected in agricultural watersheds (Wegman and Wammes 1983). This indicates that transport of DNPs to adjacent surface water or land occurs via runoff or as a result of hydrogeological movement of groundwater to surface water from fields where DNPs have been used as pesticides, or other pesticides have been applied (DNPs may also originate as impurities in certain pesticides such as Dinoseb). Such movement of DNPs from soils and groundwater in waste sites is also possible.

Some loss of DNPs from soil could also occur by plant uptake. High concentrations of these compounds are toxic to the growth and development of plants, especially at lower pH (WRSIC 1982). At concentrations likely to cause maximum bioaccumulation (10 mg/kg), the bioaccumulation factor (concentration in plant over concentration in soil) in lettuce, carrot (tops, peels, and root), hot pepper foliage, and fruits was <0.01 at a soil pH of 6.7–7.2 (O'Connor et al. 1990). Since DNPs undergo

metabolism in plants, plant accumulation of DNPs due to uptake would not be significant (O'Connor et al. 1990). The uptake and translocation could be significant in soil with low pH where the concentration of non-ionized DNPs (more readily adsorbed than the ionized form) are higher (Shea et al. 1983). Since the concentration of the non-ionized form is only <0.25% of total DNP at pH 6.7 (O'Connor et al. 1990), the soil pH has to be considerably lower for uptake to be significant in plants.

5.4.2 Transformation and Degradation

Air. The reactions of DNPs with hydroxyl and nitrate radicals may be important in determining the residence times of DNPs in the atmosphere (Atkinson et al. 1992). However, the products of these reactions have not been identified. No kinetic data are available for the atmospheric reactions of DNPs with these species. Using the method of Atkinson (1988), the estimated rate constant for the reaction of vapor-phase 2,4-DNP with hydroxyl radicals is 5.76×10^{-13} cm^3/molecule-second. Based on an average ambient atmospheric concentration of hydroxyl radicals of 5×10^5 radicals/cm^3 (Atkinson 1988), the estimated half-life for the reaction is 28 days. Since DNPs are expected to be present partly in the particulate phase in the air, the reaction rate is expected to be even slower than the estimated value for the gas phase reaction. Grosjean (1985) has concluded that the atmospheric reaction of chemically related 4-methyl-2-nitrophenol with nitrate radicals was not of major significance. By structural analogy, the reaction of nitrate radicals with 2,4-DNP is not likely to be significant, and the loss of DNPs in the air due to chemical reactions should not be a significant environmental fate process. The overall atmospheric half-life (due to all chemical and physical processes) of 2,4-DNP remains unknown.

Water. The estimated rate constants for the reaction of 2,4-DNP with singlet oxygen ($^1O_2$) and peroxy radicals (RO_2) are 3×10^4/molar-hour and 5×10^5/molar-hour, respectively (EPA 1981). The reaction of hydroperoxy radicals (HO_2) with 2,4-DNP produces a ring hydroxylated product (Lipczynska-Kochany 1992). Based on an assumption that the concentrations of singlet oxygen and peroxy radicals in typical eutrophic waters are 10^{-12} and 10^{-9} molar, respectively (Mill and Mabey 1985), these reactions would not be significant. The experimental value for the rate constant for 2,4-DNP's reaction with singlet oxygen is 4.05×10^5/molar-second (Tratnyek and Hoigne 1991; Tratnyek et al. 1991). Based on an estimated average singlet oxygen concentration of 4×10^{-14} molar in typical eutrophic fresh water (Tratnyek and Hoigne 1991) and the experimental reaction rate constant, this reaction still would not be important in water (half-life of ≈500 days). Although it has been speculated that 2,4-DNP may hydrolyze while adsorbed to clay in water in a manner analogous to mono-nitrophenols (EPA 1979a), no experimental evidence exists of such a reaction in natural water.

The direct photolysis of 2,4-DNP in water is too slow to be an important environmental fate process (Lipczynska-Kochany 1992). 2,4-DNP may be photoreduced to 2-amino-4-nitrophenol in the presence of ascorbic acid or ferrous ions, and the reaction is sensitized by chlorophyll (Massini and Voorn 1967). The possibility of such photoreduction exists in natural water in which the suspended reducing matter may act as a reducing agent and humic substances or algae may serve as a sensitizer.

2,4-DNP is biodegraded by several pure cultures of microorganisms, such as *Pseudomonas* sp. (Bruhn et al. 1987; Sudhakar-Barik et al. 1976), *Scenedesmus obliquus* (Klekner and Kosaric 1992), *Haloanaerobium praevalens, Sporohalobacter marismortui* (Oren et al. 1991), *Fusarium oxysporum* (Madhosingh 1961), two strains of *Rhodococcus* sp. (Lenke et al. 1992; Schmidt et al. 1992), *Janthinobacterium* sp. (Gier et al. 1989; Hess et al. 1990; Schmidt et al. 1992), *Corynebacterium simplex* (Gundersen and Jensen 1956; Jensen and Gundersen 1955), a gram-positive bacterium (Suwa et al. 1992), and a filamentous bacterium (Schmidt et al. 1992) isolated from soil, water, and sediment. DNP is also anaerobically biodegraded by *Veillonella alkalescens* in the presence of hydrogen (McCormick et al. 1976). A sulfate-reducing bacterium, *Desulfovibrio* sp., isolated from a continuous anaerobic digester, used 2,4-DNP as sole source of nitrogen but not of carbon and energy for growth (Boopathy and Kulpa 1993). Usually, the pure cultures were able to biodegrade 2,4-DNP after a certain adaptation period and as long as the concentration of 2,4-DNP was below a certain toxic level. The degradation pathway depended on the microorganisms and the conditions of aeration. Typically, with aerobic organisms and aerobic conditions, the biodegradation proceeded by replacement of nitro groups by hydroxyl groups and liberation of nitrite, or by hydroxylation of the aromatic ring positions 3, 5, or 6 (Raymond and Alexander 1971). The biodegradation by anaerobic organisms and under anaerobic conditions proceeded by reduction of nitro groups to amino groups (Madhosingh 1961; McCormick et al. 1976). The bacterium, *Desulfovibrio* sp., reduced the nitro groups in 2,4-DNP to amines and reductively deaminated the amino groups, leaving the aromatic ring intact with the formation of phenol (Boopathy and Kulpa 1993). Although these pure culture studies are important for establishing degradative pathways, they do not reflect real environmental situations where mixed microorganisms and different nutritional conditions are present.

Complete or partial biodegradation of 2,4-DNP was observed under aerobic conditions with mixed microorganisms from activated sludge (Kincannon et al. 1983a, 1983b; Patil and Shinde 1989; Pitter 1976), enriched sewage (Brown et al. 1990; Wiggins and Alexander 19SS), adapted sediment from rivers or waste lagoons (EPA 1979b; Chambers et al. 1963; Tabak et al. 1964), settled domestic waste water

(Tabak et al. 1981a, 1981b), and aeration lagoons and settling ponds (Games and Hites 1977). Typically, aerobic biodegradation occurred if the concentration of 2,4-DNP was below toxic levels (≈10–20 mg/L) and adapted microorganisms were used. The adaptation period is usually short (Tabak et al. 1981b). Ozonation prior to aerobic biodegradation increases the biodegradation rate (Medley and Stover 1983). The biodegradation of 2,4-DNP under methanogenic conditions with anaerobic digester sludge was observed with little or no inhibitory effect at concentrations ≤20 mg/L (O'Connor and Young 1989). As the concentration of 2,4-DNP increased to >100 mg/L, however, the microorganisms needed an adaptation period of 40–90 days before the commencement of biodegradation (Battersby and Wilson 1989; O'Connor and Young 1989). Controlled chemical oxidation of 2,4-DNP with Fenton's reagent or ozone significantly enhances anaerobic biodegradability by reducing the toxicity of 2,4-DNP to methanogens (Wang 1992). No kinetic data were located that estimated 2,4-DNP's biodegradation rate in natural water. It can be concluded from the above discussions, however, that in natural waters in which the degrader populations are small, biodegradation of 2,4-DNP will be slow until microorganisms multiply to give a large enough degrader population to cause a detectable degradation.

Sediment and Soil. No study was located that reported the abiotic degradation/transformation of DNPs in soil. It has been speculated that 2,4-DNP in soil may be reduced to 2-amino-4-nitrophenol by sunlight in the presence of a reductant, such as ferrous ions, and a sensitizer, such as chlorophyll (Kaufman 1976; WRSIC 1982; Shea et al. 1983). Considering, however, that sunlight would not penetrate below the surface layer of soil, photolysis would not be significant at subsurface levels.

Biodegradation is the most important process for the loss of DNPs in soils. Both aerobic and anaerobic degradation of DNPs by pure cultures of microorganisms isolated from soil have been reported (Schmidt and Gier 1989, 1990; Simpson and Evans 1953; Sudhakar-Barik and Sethunathan 1978). Shea et al. (1983) concluded from isolated culture studies that biodegradation in soil may proceed either by reduction of the nitro group or displacement of a nitro group by a hydroxyl group with the release of nitrite ions. The optimum pH for microbial degradation is near neutrality because of the low rate of sorption of DNP to soils and sediment (increasing bioavailability to microorganisms) and low toxicity of DNP towards microorganisms at this pH (O'Connor et al. 1990). A certain concentration of the degrader population must be reached before the onset of detectable DNP mineralization. The biodegradation will occur as long as the initial concentrations of DNP in soils and sediments do not exceed the level of ≈100 mg/kg. Above this level, DNP may be toxic to the degrader microorganisms (Bartha et al. 1967; Namkoong et al. 1988; Schmidt and Gier 1989). Multiphasic (involving several types of reaction kinetics) mineralization kinetics have been observed for the degradation of DNPs in soils. The presence of a consortium of

bacteria in soil is responsible for these multiphasic degradation kinetics (Schmidt and Gier 1990). Depending on the soil (pH, organic matter content), the length of acclimation phase, as well as the initial concentration, the residence time of DNPs for the aerobic biodegradation of soil may vary from 8 to 120 days (Kincannon and Lin 1985; EPA 1989; O'Connor et al. 1990). 2,4-DNP also undergoes biotransformation in anaerobic soil and sediments. 2,4-DNP disappeared within hours due to reduction of the nitro groups to yield diaminophenol as a result of anaerobic activity (Kohping and Wiegel 1987). In contrast to the fast initial reductive biotransformation, the rate of conversion of 2,4-DNP to methane and carbon dioxide was found to be slow. Slow and partial mineralization of 2,4-DNP at low concentrations (<20 mg/kg) to methane was observed under anaerobic conditions after a suitable adaptation period (DOE 1986).

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to DNPs depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of DNPs in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on DNP levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-3 shows the lowest limits of detection that are achieved by analytical analysis in environmental media. No monitoring data were identified for DNPs in air (see Section 5.5.1). 2,4-DNP was not detected in groundwater or surface water (see Section 5.5.2). Data on other DNP isomers were not available.

Table 5-3. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Isomer	Reference
Air	~0.05 mg/kg in particulate matter	2,4- and 2,6-DNP	Nojima et al. 1983
Drinking water	0.005 µg/L	2,4-DNP	Schultz 1983
Surface water and groundwater	13 µg/L	2,4-DNP	EPA 1986c
Soil	0.05 mg/kg	2,3-, 2,5-, 2,6-DNP	Wegman and Wammes 1983
	0.01 mg/kg	2,4- and 3,5-DNP	

Table 5-3. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Isomer	Reference
Sediment	0.05 mg/kg	2,3-, 2,5-, 2,6-DNP	Wegman and Wammes 1983
	0.01 mg/kg	2,4- and 3,5-DNP	
Serum and plasma	<0.05 mg/L	2,4-DNP	Robert and Hagardom 1983

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

Detections of 2,4-DNP in water and soil at NPL sites are summarized in Table 5-4; there were no detections of other isomers at NPL sites.

Table 5-4. 2,4-Dinitrophenol Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
Water (ppb)	400	260	33	5	5
Soil (ppb)	460,000	190,000	20	8	7
Air (ppbv)			No data		

[a]Concentrations found in ATSDR site documents from 1981 to 2019 for 1,867 NPL sites (ATSDR 2019). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

5.5.1 Air

No monitoring data reporting ambient, indoor, or occupational air levels of DNPs in the United States were located. Air monitoring data from foreign countries report air concentrations ranging from 0.01 to 53.5 ng/m^3 (Delhomme et al. 2010; Nojima et al. 1983). A review of vapor intrusion data from ATSDR public health assessments completed between 1994 and 2009 did not identify any sites with detected levels of DNPs in groundwater, soil gas, or air (Burk and Zarus 2013). Since DNP is solid at typical ambient conditions, vapor intrusion is not expected to be of concern.

5.5.2 Water

No monitoring data regarding the concentrations of DNPs in U.S. drinking water were located. 2,4-DNP was not detected in over 1,300 surface water samples collected in 2016–2020 from all across the United

States (NWQMC 2020). In a study completed in 2013 by the Oregon Department of Environmental Quality, 2,4-DNP was not detected at or above the lower reporting limit of 209–250 ppt (NWQMC 2020). Recent groundwater testing reports indicate that 2,4-DNP has not been detected at or above reporting limits of 0.5–50 ppb in over 1,000 samples collected across the United States (NWQMC 2020). Foreign monitoring data for rain water reported 2,4-DNP concentrations ranging from <1.0 to 0.367 µg/L (Quaghebeur et al. 2004; Schussler and Nitschke 2001). The ranges of DNP (unspecified isomers) concentrations in raw waste waters and final effluents from a dye-manufacturing plant were 400–3,200 and <1–2,700 µg/L, respectively (Games and Hites 1977). 2,4-DNP was not detected in storm water or wastewater treatment effluent samples collected by the Oregon Department of Environmental Quality in 2010 and 2015, respectively (NWQMC 2020).

5.5.3 Sediment and Soil

As with other media, monitoring results were located only for the 2,4-DNP isomer. 2,4-DNP was not detected at or above quantification (2.1–7,000 µg/kg) or lower reporting limits (26–990 µg/kg) in approximately 3,000 sediment samples collected across the United States from 2010 to 2020 (NWQMC 2020). In surface and subsurface soil samples collected from Bainbridge Island, Seattle, Washington, 2,4-DNP was not detected (NWQMC 2020). 2,4-DNP was also not detected in approximately 350 soil samples collected from other sites in the United States (NWQMC 2020). At superfund sites in Alaska, Colorado, and Utah, 2,4-DNP was not detected in soil samples (NWQMC 2020). Soil samples collected post-Katrina in 2005 and 2006 also did not contain 2,4-DNP (NWQMC 2020).

5.5.4 Other Media

Despite the finding that aqueous solutions of 2,4-DNP quickly permeate plant cuticles and probably metabolize fast in plants, this chemical has been detected in conifer needles. Concentrations of 2,4-DNP in the needles collected from different areas and of different ages ranged from <3.7 to 33.1 µg/kg (Hinkel et al. 1989). The concentrations of 2,4-DNP were higher in needles obtained from roadside and urban areas than rural areas. The higher concentrations of 2,4-DNP in air and in soil, caused primarily by automobile exhaust near the roadside and by automobile exhaust and other anthropogenic sources in urban areas, may be responsible for the higher concentrations of 2,4-DNP in pine needles collected from these two areas. 2,4-DNP content was also higher in needles from older trees. Hinkel et al. (1989) explained that this was due to continuous input and accumulation rates exceeding degradation rates.

5.6 GENERAL POPULATION EXPOSURE

The general population could be exposed to DNPs by inhaling contaminated air and ingesting contaminated food and drinking water. The concentration of DNPs in urban air is expected to be higher than in rural air due to smog and heavy vehicular traffic. The concentration values for 2,4-DNP in ambient air, drinking water, or food from any location in the United States, let alone typical values, were not located. Although the use of 2,4-DNP in diet pills is no longer legal, the availability of this product from unregulated internet sources may lead to exposure of the general population. The general population may be dermally exposed to 2,4-DNP from using wood treated with 2,4-DNP. The occurrence of this exposure, however, has not been verified. ATSDR's three-compartment Shower and Household Water-Use Exposure (SHOWER) model predicts air concentrations in the shower stall, bathroom, and main house throughout the day for households with up to eight members; the model is a desktop application that is available by sending a request to showermodel@cdc.gov. Using concentrations in water and human activity patterns, the model estimates a daily TWA exposure concentration from breathing indoor air. Inhalation exposure from bathing in 2,4-DNP contaminated water cannot be estimated using the model because volatilization factors are not available for 2,4-DNP. The model can also estimate a dermal dose from skin contact while bathing and washing hands if the 2,4-DNP concentration in water is known.

Occupational exposure to 2,4-DNP may occur in the chemical manufacturing industry and munitions manufacturing industry. The level of 2,4-DNP in the air of one workroom area (where the chemical was handled) of an industry in the 1940s that manufactured picric acid was typically ≥40 mg/m^3 (Gisclard and Woodward 1946). Occupational exposures to DNPs would also occur in picramide manufacturing, dye manufacturing, the photographic industry, and wood treatment facilities. Studies establishing occupational exposure to DNPs from these industries were not located, however. The National Occupational Exposure Survey (NOES) of 1983 estimated that a total of 130 and 28 workers are potentially exposed to 2,4- and 2,6-DNP, respectively (RTECS 1992). The NOES database does not contain data on the frequency, duration, concentration, or route of exposure of workers to DNPs or any other chemical.

2,4-DNP and its metabolites have not been measured in the tissues and body fluids of humans in the general population who did not deliberately ingest the compound (as a diet pill or in a suicide attempt). 2-Amino-4-nitrophenol, a metabolite of 2,4-DNP, was qualitatively detected (Derrien test) in the urine of workmen in a munitions factory in France following inhalation exposure to 2,4-DNP-contaminated dust and vapor and dermal exposure to this compound (Perkins 1919). In a case of fatal occupational

poisoning from inhaling dust and vapor of 2,4-DNP in a U.S. chemical industry, the urine of the worker contained 2.08 g/L of 2,4-DNP and 50 mg/L of the metabolite, 2-amino-4-nitrophenol (Gisclard and Woodward 1946). 2,4-DNP and its metabolite, 2-amino-4-nitrophenol, were detected in the urine of a woman who had taken sodium salt of 2,4-DNP as a diet pill at a dose of 4 mg/kg body weight/day (Davidson and Shapiro 1934).

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Within the general population, workers in facilities using DNPs have potentially high exposures to DNPs. Persons who reside with, or otherwise come into regular contact with persons who work with DNPs or obtain 2,4-DNP illegally may have potentially high exposures.

People who live in urban areas where vehicular traffic is high and atmospheric inversion is known to occur may be exposed to higher levels of DNP formed by photochemical reactions than populations of less polluted areas. It is possible that people who live near hazardous waste sites that contain these pollutants may also have higher exposures from inhaling contaminated air or ingesting contaminated groundwater; however, the extent of such exposures for residents around waste sites has not been documented.

CHAPTER 6. ADEQUACY OF THE DATABASE

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

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.1 Information on Health Effects

Nearly all of the available health effect data on DNPs pertain to 2,4-DNP. Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to 2,4-DNP that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of 2,4-DNP. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

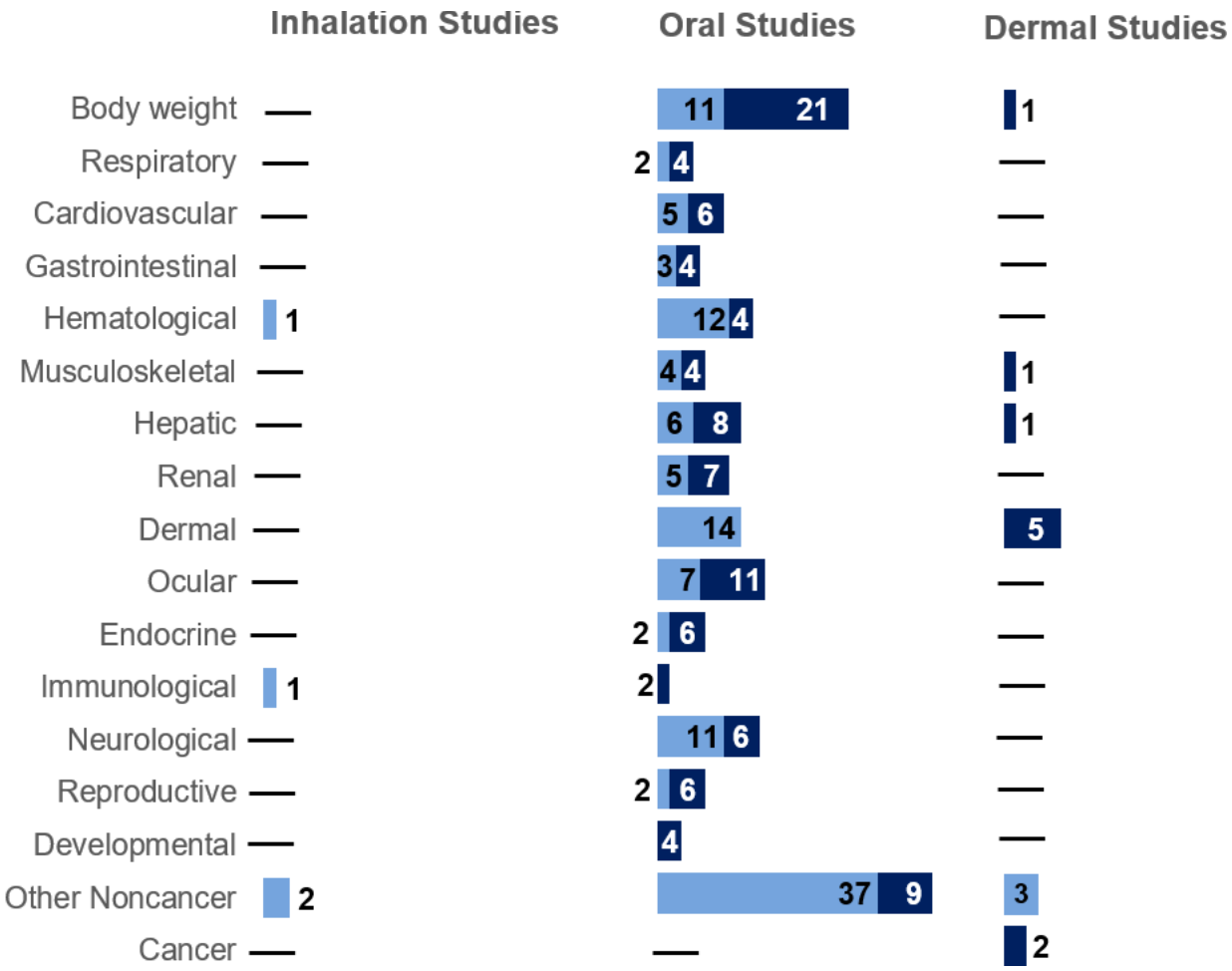
No studies were located regarding health effects in humans or animals after inhalation, oral, or dermal exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNP. The only study regarding health effects of 2,6-DNP in humans or animals after these routes of exposure was an acute-duration oral study of cataract formation in chickens (Robbins 1944).

6.2 Identification of Data Needs

Missing information in Figure 6-1 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 6-1. Summary of Existing Health Effects Studies on 2,4-Dinitrophenol By Route and Endpoint*

Potential body weight, energy metabolism, and ocular effects were the most studied endpoints
The majority of the studies examined oral exposure in **humans** (versus **animals**)



*Includes studies discussed in Chapter 2; a total of 108 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints.

Virtually all of the available quantitative data on the toxicity of DNPs are from studies of 2,4-DNP. Data from animals or humans exposed to other isomers by oral, dermal, and inhalation routes are needed to identify exposure-response information for 2,3-, 2,5-, 2,6-, 3,4-, and 3,5-DNP.

Inhalation MRLs. No inhalation MRLs have been derived for DNPs because inhalation data are limited to two human studies without reliable exposure route or level information. Acute-, intermediate-, and chronic-duration studies are needed to determine sensitive effects after inhalation exposure.

Oral MRLs. Data were adequate to derive an intermediate-duration oral MRL of 0.00007 mg/kg/day based on a LOAEL of 0.07 mg/kg/day for reduced body weight in mice exposed for 50 weeks (Caldeira da Silva et al. 2008). Although data were not adequate to derive a chronic-duration oral MRL, the intermediate oral MRL is believed to be protective for chronic exposures (see Appendix A). Accordingly, additional chronic studies of 2,4-DNP do not appear to be necessary.

Available data on 2,4-DNP were not adequate to derive an acute-duration oral MRL; thus, additional animal studies may fill this data gap. No acute-duration studies of 2,4-DNP in animals used dose levels below those associated with lethality in humans, and studies in humans identified LOAELs at doses only slightly below those resulting in fatalities. Nonfatal LOAELs in humans were 0.9–2 mg/kg/day (Anderson et al. 1933; Hitch and Schwartz 1936; Lee et al. 2014; Nadler 1935; Tainter et al. 1935) compared with fatal doses of 3–7 mg/kg/day (Masserman and Goldsmith 1934; McFee et al. 2004; Poole and Haining 1934). Oral studies in animals exposed for 14 days to doses between 0.07 mg/kg/day (the LOAEL for intermediate-duration exposure) and 0.9 mg/kg/day 2,4-DNP (lowest available acute-duration LOAEL in humans or animals) that include sensitive endpoints such as basal metabolic rate, body weight, and neurological effects might provide information to enable the derivation of an acute-duration MRL. Studies in mice or rabbits would be more useful than studies in rats, which appear to be somewhat less sensitive to the effects of 2,4-DNP. Additional information on the toxicity of other DNP isomers may provide information to determine if the intermediate-duration oral for 2,4-DNP would be protective for other isomers.

Health Effects. Available information from data in humans and animals exposed orally show effects in multiple physiological systems and organs as a result of uncoupling of mitochondrial electron transport from oxidative phosphorylation. Acute-, intermediate-, and chronic-duration studies are needed to determine sensitive effects after inhalation exposure. Other specific data needs are as follows:

Neurological. There are no neurotoxicity or neurobehavioral studies of 2,4-DNP in animals. Human case reports (Anderson et al. 1933; Bortz 1934; Epstein and Rosenblum 1935; Hitch and Schwartz 1936; Nadler 1935; Phillips and Singer 2013) and clinical studies (Simkins 1937a, 1937b; Tainter et al. 1935) have documented peripheral neuritis after oral exposure to 2,4-DNP at low doses (2–16 mg/kg/day) for acute and intermediate durations. Comprehensive neurotoxicity and neurobehavioral studies are needed to evaluate the potential for subtle adverse neurological effects of exposure.

Immunological. No studies examining sensitive immunological effects in humans or animals after oral, inhalation, or dermal exposure to 2,4-DNP were located. A battery of immune function tests may be useful in determining whether the immune system is affected by exposure to 2,4-DNP.

Developmental. The potential teratogenicity of 2,4-DNP has not been adequately studied. 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. Reliable developmental toxicity data are available only from a combination reproduction/developmental toxicity screening study in rats exposed orally (Takahashi et al. 2009); however, this study did not evaluate skeletal malformations in pups. The only study that evaluated external, visceral, and skeletal malformations in pups dosed mice only during part of organogenesis (gestation days 10–12; Gibson 1973) and did not report the data for these evaluations.

Cancer. Further evaluation of the potential carcinogenicity of 2,4-DNP is needed. Two skin painting studies in female mice using DMBA as an initiator reported that 2,4-DNP was clearly not effective as a tumor promotor (Boutwell and Bosch 1959; Stenback and Garcia 1975), and 2,4-DNP was negative for genotoxicity in most *in vivo* and *in vitro* studies (see Section 2.19, Cancer, and Section 2.20, Genotoxicity). However, metabolites of 2,4-DNP (2-amino-4-nitrophenol, 4-amino-2-nitrophenol, and 2,4-diaminophenol) appear to be mutagenic, and both 2-amino-4-nitrophenol and 4-amino-2-nitrophenol showed some evidence of carcinogenicity in male rats (NCI 1978; NTP 1988a, 1988b). For this reason, carcinogenicity studies with 2,4-DNP may be justified.

Epidemiology and Human Dosimetry Studies. Studies of workers currently exposed to 2,4-DNP and people who live or work near waste sites contaminated with 2,4-DNP could help define relationships

among exposure, blood and urine levels of parent compounds and metabolites, and the sensitive effects. No epidemiology studies of workers or other populations exposed to 2,4-DNP were located; aside from individual case reports, human studies are limited to an occupational health study from 1919 (Perkins 1919) and clinical studies of 2,4-DNP use as a diet pill in the 1930s (Bortz 1934; Castor and Beierwaltes 1956; Cutting et al. 1933, 1934; Looney and Hoskins 1934; MacBryde and Taussig 1935; Simkins 1937a, 1937b; Tainter et al. 1934a, 1935b). The limitations of these studies were common to studies of that time, and include the lack of a control worker population or placebo-treated control group, anecdotal style of reporting results, and lack of statistical analysis. The available studies show that endpoints related to the uncoupling of oxidative phosphorylation are the most sensitive. These endpoints include body weight loss, increased basal metabolic rate, and characteristic signs and symptoms including increased perspiration and a sensation of warmth. Other effects reported in people taking 2,4-DNP orally for weight reduction purposes included agranulocytosis, peripheral neuritis, and cataract development. No consistent correlations between effects and dose or duration were discerned, indicating that individual sensitivity varies widely.

Biomarkers of Exposure and Effect. Biomarkers of effect specific to 2,4-DNP would be useful. 2,4-DNP and its metabolites have been monitored in body fluids and tissues of humans and animals. Systematic attempts to correlate levels of 2,4-DNP or its metabolites in blood or urine with exposure levels or durations have not been made, but would facilitate medical surveillance and epidemiological studies. The increase in basal metabolic rate and weight loss, along with the characteristic clinical signs and symptoms (increased perspiration, sensation of warmth) seen with oral and occupational exposure of humans to 2,4-DNP, appear to be fairly sensitive indices of the profound metabolic disturbances caused by 2,4-DNP. However, these effects are not specific to 2,4-DNP; thus, research to develop specific biomarkers of effect would be beneficial.

Absorption, Distribution, Metabolism, and Excretion. Available data on the absorption, distribution, metabolism, and excretion of 2,4-DNP in humans and animals exposed orally are very limited, and no reliable studies in humans or animals exposed by inhalation or dermal routes were located. Pharmacokinetic studies in animals exposed to 2,4-DNP by the inhalation, oral, and dermal routes would help to identify differences among these routes and may help to identify a suitable model to assess potential differences in pharmacokinetics via these routes in humans.

Comparative Toxicokinetics. Comparative data on *in vitro* metabolic rates in human, rat, and mouse tissues may inform species extrapolation of toxicity data in animals. Because the fundamental effect of

2,4-DNP (uncoupling of oxidative phosphorylation) occurs in all species and tissues, effects would be expected to occur nonspecifically in the organs and tissues and to be similar across species. Nevertheless, some differences are apparent with regard to ocular and hematological effects and *in vitro* metabolic rates.

Children's Susceptibility. A study in rats (Koizumi et al. 2001) indicated that neonatal animals are more susceptible to 2,4-DNP toxicity than young (5–6 weeks old) animals; thus, additional data to confirm age-related changes in vulnerability may be useful.

Physical and Chemical Properties. It would be helpful to develop more reliable data on certain physical properties important in predicting the environmental fate of DNPs. More experimental and estimated data on the physical and chemical properties for 2,4-DNP are available than for other DNPs (see Table 4-2). Even in the case of 2,4-DNP, reliable experimental or estimated values are not available for vapor pressure, Henry's law constant, and log K_{oc}. This is not surprising since DNPs exist predominantly in the ionic forms at pH >6 with very low vapor pressure. If available, the physical constants are important in predicting the environmental transport of DNPs.

Production, Import/Export, Use, Release, and Disposal. Since each individual isomer of the DNPs was manufactured by one or two U.S. companies (SRI 1994), the production volumes of DNPs in recent years are considered confidential business information and are unavailable in open literature. No data were located that would project future production volume or permit comparison in the trend of DNP production rates in recent years. Additional information about the efficiency of the different methods of disposal and destruction would be helpful. Other than treated woods, very few consumer products are known to contain DNPs. Considering the industrial uses of DNPs, both water and soil are likely to be contaminated with significant quantities (ATSDR 1988; EPA 1988c; Games and Hites 1977; Plumb 1991; Wegman and Wammes 1983). It would be useful if information on the amounts of DNPs disposed by the two principal methods (land disposal and incineration) were available. Specific information regarding federal regulations on disposal of 2,4-DNP in land, in water, and by incineration is available (EPA 1990, 1992, 1993).

Environmental Fate. From the data in the literature, it is difficult to conclude whether DNPs will partition in a particular environmental medium; therefore, it would be useful to study the concentration distribution of these compounds between the water and sediment in a natural body of water. The environmental transport of DNPs from water by volatilization would not be significant (EPA 1979a), but transport of these compounds from soil to groundwater was observed (ATSDR 1988; Plumb 1991).

Although it is known that these compounds degrade slowly via biodegradation in water (Games and Hites 1977) and at a faster rate in soil (Kincannon and Lin 1985; EPA 1989; O'Connor et al. 1990), it would be helpful to develop more quantitative data on the rate of biodegradation, particularly in natural water. The importance of abiotic processes, particularly photolysis and oxidation (by radicals, such as OH, HO_2, NO_3), in the transformation/degradation of these compounds in the environment needs further evaluation. Whether vapor-phase DNPs undergo long-distance transport in the atmosphere needs further study.

Bioavailability from Environmental Media. Information on the bioavailability of DNPs in water, soil, and air would improve assessments of hazard and risk from exposure to these compounds in environmental media. Available absorption kinetics of DNPs following ingestion and dermal contact are discussed in Section 3.1.1; however, no information about the bioavailability of DNPs from natural air, water, or soil was located. The observation that DNPs are found at least partly in the particulate-sorbed state in the air (Nojima et al. 1983) indicates that their bioavailability from air is less than 100%. The adsorption of DNPs to soil and sediment depends on the nature of soil and sediment (e.g., organic matter and clay content) and the pH of the medium (EPA 1979a; Kaufman 1976). Therefore, the bioavailability of particle-sorbed DNPs due to desorption from soil and sediment containing a high percent of organic matter and clay may be less than that of the free form (unabsorbed) of DNP.

Food Chain Bioaccumulation. The bioconcentration of DNPs from water to aquatic organisms and from soil to plants is not expected to be important (EPA 1986b; O'Connor et al. 1990). Data on the biomagnification potential for DNPs in predators that consume contaminated prey would be useful.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of DNP in contaminated media at hazardous waste sites are needed; the information obtained on levels of DNPs in the environment could be used in combination with the known body burden of DNPs to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites. No monitoring data are available for DNP levels in ambient air, drinking water, and total diet samples (typical food consumed by a person in the United States) of the general population. Consequently, daily human intake of these compounds from inhalation and ingestion routes remains unknown. Although the intake of DNPs by the general population is expected to be low, studies that evaluate the daily intake would be useful. Since vehicular exhaust contains DNPs (Nojima et al. 1983), it would be helpful to analyze roadside soil to determine whether such soils contain elevated levels of DNPs.

Exposure Levels in Humans. Studies that determine the levels of 2,4-DNP and its major metabolite (2-amino-4-nitrophenol) in the blood and urine of the general population and in people living near hazardous waste sites containing these pollutants would be useful. This information is necessary for assessing the need to conduct health studies on these populations. Only limited data on the levels of 2,4-DNP in human tissue and body fluids are available. Most of these data are quite dated, come from autopsies of fatalities, and were obtained using outdated analytical methods.

Exposures of Children. Studies evaluating potential sources of exposure to children would inform the need for additional assessment of childhood susceptibility. Underlying conditions, such as hepatic and renal diseases, may increase susceptibility to DNPs. A single study (Koizumi et al. 2001, 2002) suggested that neonatal rats may be more susceptible to 2,4-DNP effects than young (5–6 weeks old) rats.

6.3 Ongoing Studies

No relevant ongoing studies of DNPs were identified in the National Institute of Health (NIH) RePORTER (2020) database.

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding DNPs in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for 2,4-DNP.

Table 7-1. Regulations and Guidelines Applicable to Dinitrophenols

Agency	Description	Information	Reference
	Air		
EPA	RfC		IRIS 2005
	2,4-Dinitrophenol	Information reviewed but value not estimated	
WHO	Air quality guidelines	Not listed	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories	Not listed	EPA 2018a
	National primary drinking water regulations	Not listed	EPA 2009
	RfD		IRIS 2005
	2,4-Dinitrophenol	$2x10^{-3}$ mg/kg/day	
	Provisional Peer Reviewed Toxicity Value		EPA 2007
	2,4-Dinitrophenol		
	Provisional subchronic RfD	$2x10^{-2}$ mg/kg/day	
WHO	Drinking water quality guidelines	Not listed	WHO 2017
FDA	Substances Added to Food[a]	Not listed	FDA 2020b
	Cancer		
HHS	Carcinogenicity classification	No data	NTP 2016
EPA	Carcinogenicity classification	No data	IRIS 2005
IARC	Carcinogenicity classification	No data	IARC 2020
	Occupational		
OSHA	PEL (8-hour TWA) for general industry, shipyards and construction	No data	OSHA 2020a, 2020b, 2020c
NIOSH	REL (up to 10-hour TWA)	No data	NIOSH 2018

Table 7-1. Regulations and Guidelines Applicable to Dinitrophenols

Agency	Description	Information	Reference
	Emergency Criteria		
EPA	AEGLs-air	Not listed	EPA 2018b
DOE	PACs-air		DOE 2018a
	Dinitrophenol		
	PAC-1[b]	0.25 mg/m^3	
	PAC-2[b]	2.7 mg/m^3	
	PAC-3[b]	16 mg/m^3	
	2,3-Dinitrophenol		
	PAC-1[b]	1.1 mg/m^3	
	PAC-2[b]	13 mg/m^3	
	PAC-3[b]	75 mg/m^3	
	2,4-Dinitrophenol		
	PAC-1[b]	0.61 mg/m^3	
	PAC-2[b]	6.8 mg/m^3	
	PAC-3[b]	16 mg/m^3	
	2,6-Dinitrophenol		
	PAC-1[b]	0.23 mg/m^3	
	PAC-2[b]	2.5 mg/m^3	
	PAC-3[b]	15 mg/m^3	

[a]The Substances Added to Food inventory replaces EAFUS and contains the following types of ingredients: food and color additives listed in FDA regulations, flavoring substances evaluated by FEMA or JECFA, GRAS substances listed in FDA regulations, substances approved for specific uses in food prior to September 6, 1958, substances that are listed in FDA regulations as prohibited in food, delisted color additives, and some substances "no longer FEMA GRAS".
[b]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2018b).

AEGL = acute exposure guideline levels; DOE = Department of Energy; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; FEMA = Flavor and Extract Manufacturers Association of the United States; GRAS = generally recognized as safe; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; JECFA = Joint FAO/WHO Expert Committee on Food Additives; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = protective action criteria; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TWA = time-weighted average; WHO = World Health Organization

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

APPENDIX A

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Office of Innovation and Analytics, Toxicology Section, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Office of Innovation and Analytics, Toxicology Section, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Dinitrophenols
CAS Numbers: 66-56-8; 51-28-5; 329-71-5; 573-56-8; 577-71-9; 586-11-8; 2550-58-7
Date: August 2021
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL.

Rationale for Not Deriving an MRL: Although health effects have occurred in humans exposed to 2,4-DNP occupationally (Gisclard and Woodward 1946; Perkins 1919), exposure appeared to involve both the inhalation and dermal routes, and exposure concentrations were not known or inadequately characterized. No studies of health effects in animals exposed to DNP via inhalation were identified in the available literature. Therefore, data are insufficient to derive MRLs for acute-duration inhalation exposure to DNP.

Agency Contacts (Chemical Managers): Jennifer Przybyla

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Dinitrophenols
CAS Numbers: 66-56-8; 51-28-5; 329-71-5; 573-56-8; 577-71-9; 586-11-8; 2550-58-7
Date: August 2021
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL.

Rationale for Not Deriving an MRL: Although health effects have occurred in humans exposed to 2,4-DNP occupationally (Gisclard and Woodward 1946; Perkins 1919), exposure appeared to involve both the inhalation and dermal routes, and exposure concentrations were not known or inadequately characterized. No studies of health effects in animals exposed to DNP via inhalation were identified in the available literature. Therefore, data are insufficient to derive MRLs for intermediate-duration inhalation exposure to DNP.

Agency Contacts (Chemical Managers): Jennifer Przybyla

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Dinitrophenols
CAS Numbers: 66-56-8; 51-28-5; 329-71-5; 573-56-8; 577-71-9; 586-11-8; 2550-58-7
Date: August 2021
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL.

Rationale for Not Deriving an MRL: Although health effects have occurred in humans exposed to 2,4-DNP occupationally (Gisclard and Woodward 1946; Perkins 1919), exposure appeared to involve both the inhalation and dermal routes, and exposure concentrations were not known or inadequately characterized. No studies of health effects in animals exposed to DNP via inhalation were identified in the available literature. Therefore, data are insufficient to derive MRLs for chronic-duration inhalation exposure to DNP.

Agency Contacts (Chemical Managers): Jennifer Przybyla

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 2,4-Dinitrophenol
CAS Numbers: 51-28-5
Date: August 2021
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for 2,4-DNP.

Rationale for Not Deriving an MRL: An MRL derived based on the lowest LOAELs for effects of acute oral exposure to 2,4-DNP would not provide adequate protection, because the POD would fall within an order of magnitude of levels known to cause death in humans. Fatalities have occurred in humans exposed to 2,4-DNP at doses between ~3 and 7 mg/kg/day for 3–14 days (Masserman and Goldsmith 1934; McFee et al. 2004; Poole and Haining 1934). The only effect levels identified at lower doses after acute-duration exposure were LOAELs between 0.9 and 2 mg/kg/day for dermal lesions (Anderson et al. 1933; Hitch and Schwartz 1936; Lee et al. 2014; Nadler 1935), decreased body weight (Tainter et al. 1935), cataracts (Hitch and Schwartz 1936), and polyneuritis (Hitch and Schwartz 1936) in human case reports or clinical studies from the 1930s. Use of any of these LOAELs as the POD for acute-duration oral MRL derivation may not provide adequate protection against the lethal effects of 2,4-DNP, because the dose estimates for human case reports are in some cases uncertain (when body weight information is not reported) and the difference between death and less severe effects is extremely small. Furthermore, variability in susceptibility to pyrexia with changes in ambient temperature have been reported in animals (Harvey 1959), raising the possibility that lower doses may be fatal in humans exposed to 2,4-DNP at higher ambient temperatures. No acute-duration studies of 2,4-DNP in animals used dose levels below those associated with lethality in humans, so animal data cannot be used to derive an acute MRL. Therefore, data are insufficient to derive an acute MRL for exposure to 2,4-DNP.

Agency Contacts (Chemical Managers): Jennifer Przybyla

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 2,4-Dinitrophenol
CAS Numbers: 51-28-5
Date: August 2021
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL 0.00007 mg/kg/day
Critical Effect: Decreased body weight
Reference: Caldeira da Silva et al. 2008
Point of Departure: LOAEL of 0.07 mg/kg/day
Uncertainty Factor: 1,000
LSE Graph Key: 85
Species: Mouse

MRL Summary: An intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL).

Selection of the Critical Effect: Human fatalities have been reported after acute- and intermediate-duration oral exposures to 2,4-DNP doses as low as 1–5 mg/kg/day (Dameshek and Gargill 1934; Goldman and Haber 1936; Masserman and Goldsmith 1934; Silver 1934; Zack et al. 2016). The only effects reported at lower doses in humans or animals were decreased body weight (at least 10% lower than controls after 20 weeks of exposure) and decreased serum glucose, triglycerides, and insulin (after 12 weeks of exposure) in a chronic mouse study in which 2,4-DNP was administered at an average dose of 0.07 mg/kg/day in drinking water (Caldeira da Silva et al. 2008). The mice were maintained at a lower ambient temperature (22°C) than normal to enable heat to dissipate; thus, it is not possible to assess whether hyperthermia might also occur at this exposure level. A clinical study in humans provides limited support for the effects of 2,4-DNP on serum glucose and insulin, as decreased glucose tolerance was seen after acute and intermediate exposures to 4 mg/kg/day 2,4-DNP (MacBryde and Taussig 1935).

In contrast, there are abundant data indicating that oral 2,4-DNP exposure results in decreased body weight or decreased body weight gain in humans and animals. Decreases in body weight have been reported in humans exposed to acute- (Anderson et al. 1933; Bortz 1934; Cutting and Tainter 1933; Tainter et al. 1935), intermediate- (Beinhauer 1934; Boardman 1935; Cutting et al. 1933, 1934; Horner et al. 1935; Looney and Hoskins 1934; Masserman and Goldsmith 1934; Nadler 1935; Simkins 1937a, 1937b; Tainter et al. 1935; Whalman 1936), and chronic-duration (Horner et al. 1935) doses of 1–4 mg/kg/day. Likewise, body weight decrements have been reported in intermediate- (Bakke and Lawrence 1965; Goldgof et al. 2014; Koizumi et al. 2001, 2002; Pugsley 1935; Spencer et al. 1948; Tainter and Borley 1938; Takahashi et al. 2009) and chronic-duration (Tainter 1938) studies in rats and mice, albeit at much higher doses (≥20 mg/kg/day). Furthermore, the mechanism for 2,4-DNP-induced decreases in body weight is well established: 2,4-DNP uncouples oxidative phosphorylation, leading to an increase in basal metabolic rate and an increase in carbohydrate consumption as the body endeavors to produce ATP needed for cell functions. In summary, decreased body weight occurs in both humans and animals exposed to 2,4-DNP by a well-studied mode of action, and was selected as the critical effect for MRL derivation.

Selection of the Principal Study: The Caldeira da Silva et al. (2008) study provided the lowest adverse effect level.

Summary of the Principal Study:

Caldeira da Silva CC, Cerqueira FM, Barbosa LF, et al. 2008. Mild mitochondrial uncoupling in mice affects energy metabolism, redox balance and longevity. Aging Cell 7(4):552-560.

Six groups (three pairs of control and exposed groups) of 30 female Swiss mice were exposed to DNP in drinking water at a concentration of 0 or 1 mg/L beginning at 18 weeks of age (Caldeira da Silva et al. 2008). The animals were housed at 22°C to allow heat due to uncoupling to dissipate and to prevent hyperthermia. Food and water intake and body weight were recorded weekly. The authors reported that the animals received doses between 0.03 and 0.105 mg/kg/day (midpoint of the dose range is 0.07 mg/kg/day) based on body weight and water intake. At 22 and 32 weeks of age (after 4 and 14 weeks of exposure), one exposed and one control group each were sacrificed for evaluation of oxygen consumption and oxidative stress in the heart, brain, and liver (oxidative stress was measured as peroxide release, protein carbonyl signal, and oxidative DNA adducts). In the groups sacrificed at 32 weeks of age (14 weeks of exposure), blood was collected for analysis of triglycerides, glucose, and insulin. The third sets of exposed and control groups were observed until natural death; the only endpoints assessed in these animals were food and water intake, body weight, body temperature, and survival.

Serum levels of glucose, triglycerides, and insulin were significantly lower than controls after 14 weeks of DNP treatment. Oxygen consumption was significantly increased in the brain and liver of exposed mice, but not in the heart. In all three organs, oxidative stress was reduced by exposure to DNP. In the groups exposed for their natural lifespans, food and water intake, and body temperature at 75 weeks of age did not differ from controls. Exposed mice exhibited significantly reduced body weight (~8–13% less than controls based on digitization of data shown graphically; see Table A-1) after the first 20 weeks of exposure; the decrease persisted throughout the study. The last body weight measurement reported in the study was ~week 68, reflecting at least 50 weeks of exposure. Mean lifespan was significantly higher in exposed mice than in controls.

Table A-1. Body Weight Changes Over Time in Mice Exposed to 2,4-Dinitrophenol in Drinking Water from 18 Weeks of Age until Natural Death[a]

Mouse age (weeks)	Exposure duration (weeks)	Approximate body weight (g)		Approximate percent change from control
		Control	Exposed	
18	Pretreatment	40	39	Not applicable
28	10	48	45	6%
38	20	51	47[b]	8%
48	30	55	50[b]	9%
58	40	56	51[b]	9%
68	50	55	48[b]	13%[c]

[a]Mice were exposed for ~140 weeks, but the only body weight data presented were measurements through the first 50 weeks of exposure.
[b]Significantly different from control mean, $p<0.05$.
[c]Exceeds ATSDR benchmark for adverse change in body weight (10% change from control mean).

Source: Caldeira da Silva et al. (2008). Data digitized from Figure 1 using Grab It!™.

Selection of the Point of Departure for the MRL: The LOAEL of 0.07 mg/kg/day (midpoint of the dose range reported by the authors) for decreased body weight was selected as the POD for deriving an MRL for intermediate-duration oral exposure to DNP. Benchmark dose modeling was not considered for this dataset, as only one drinking water concentration was used in the critical study.

Calculations: None.

Intermittent Exposure: Not applicable.

Uncertainty Factor: The LOAEL of 0.07 mg/kg/day was divided by a total uncertainty factor of 1,000:

- 10 for interspecies extrapolation
- 10 for use of a LOAEL
- 10 for human variability

Other Additional Studies or Pertinent Information that Lend Support to this MRL: See *Selection of the Critical Effect* above.

Agency Contacts (Chemical Managers): Jennifer Przybyla

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 2,4-Dinitrophenol
CAS Numbers: 51-28-5
Date: August 2021
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for 2,4-DNP.

Rationale for Not Deriving an MRL: An MRL derived based on the lowest LOAELs for effects of chronic oral exposure to 2,4-DNP would not provide adequate protection, because the POD would be at or above dose levels known to cause death in humans. However, the intermediate-duration oral MRL is believed to be protective for chronic exposures.

Only two chronic-duration studies of 2,4-DNP (Horner et al. 1935; Tainter 1938) provided enough information to identify effect levels, and the effect levels were at or above doses associated with human fatalities. The NOAELs and LOAELs in the Horner et al. (1935) human study and Tainter (1938) were 2 and 30 mg/kg/day, respectively, and human fatalities have been reported after acute- or intermediate-duration exposures of 1–5 mg/kg/day (Dameshek and Gargill 1934; Goldman and Haber 1936; Masserman and Goldsmith 1934; Silver 1934; Zack et al. 2016).

Caldeira da Silva et al. (2008) included an experiment with chronic-duration exposure (mice were exposed from 18 weeks of age until their natural deaths), but the only endpoints evaluated in the groups exposed until natural death were food and water intake, body weight, body temperature, and survival. Furthermore, the authors reported data on food and water intake and body temperature only at 75 weeks of age (57 weeks of exposure), and on body weights only through 68 weeks of age (50 weeks of exposure). The intermediate-duration MRL is based on the LOAEL for decreased body weight in mice in the study by Caldeira da Silva et al. (2008). In this study, the mice exhibited body weight decrements of 8–13% less than controls between 20 and 50 weeks of exposure. The body weight data, reported graphically, showed a persistent or slightly expanding decrement from control weights over the exposure period.

The intermediate-duration MRL is believed to be protective for chronic exposures, for several reasons. The exposure duration in the critical experiment was just short of 1 year (50 weeks, or 350 days), and thus approximated a chronic duration (≥365 days). In addition, as noted above, the body weight data showed a continued trend in reduced weight throughout the experiment that is likely to have persisted with longer exposure. Furthermore, available chronic-duration studies in humans (Horner et al. 1935) and rodents (Tainter 1938) indicated that weight loss is a common and sensitive effect of chronic exposure to 2,4-DNP. Finally, the mechanism for the weight loss (uncoupling of oxidative phosphorylation and increased basal metabolic rate) is very well characterized, and is expected to be operant at all exposure durations.

Agency Contacts (Chemical Managers): Jennifer Przybyla

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR DINITROPHENOLS

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to DNPs.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen was conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for DNPs. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of DNPs have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of DNPs are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen

Health Effects
Species
Human
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects
Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects
Renal effects
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects
Reproductive effects
Developmental effects

Table B-1. Inclusion Criteria for the Literature Search and Screen

Other noncancer effects
Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

B.1.1 Literature Search

The current literature search was intended to update the draft toxicological profile for DNPs released for public comment in 2019; thus, the literature search was restricted to studies published between April 2016 and June 2020. The following main databases were searched in June 2020:

- PubMed
- National Technical Reports Library (NTRL)
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for DNPs. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to DNPs were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-2. Database Query Strings

Database search date	Query string
PubMed	
06/2020	(((66-56-8[rn] OR "2,3-dinitrophenol"[nm] OR 51-28-5[rn] OR "2,4-Dinitrophenol"[mh] OR 329-71-5[rn] OR "2,5-dinitrophenol"[nm] OR 573-56-8[rn] OR "2,6-dinitrophenol"[nm] OR 577-71-9[rn] OR 586-11-8[rn] OR 25550-58-7[rn] OR "dinitroaminophenol"[nm] OR "3,4-Dinitrophenol"[tw] OR "3,4-DNP"[tw] OR "3,5-Dinitrophenol"[tw] OR "6,5-Dinitrophenol"[tw] OR "Phenol, 3,4-dinitro-"[tw] OR "Phenol, 3,5-dinitro-"[tw]) OR (("1'alpha-2,4-dinitrophenol"[tw] OR "1-hydroxy-2,4-dinitrobenzene"[tw] OR "2,3-dnp"[tw] OR "2,4-dnp"[tw] OR "2,5-dnp"[tw] OR "2,6-dnp"[tw] OR "aldifen"[tw] OR "camello mosquito coils"[tw] OR "chemox pe"[tw] OR "cobra salts"[tw] OR "dinitroaminophenol"[tw] OR "dinitrophenol"[tw] OR "dinitrophenols"[tw] OR "dinofan"[tw] OR "fenoxyl carbon n"[tw] OR "impregna salts"[tw] OR "maroxol-50"[tw] OR "nitro kleenup"[tw] OR "nitrophen"[tw] OR "nitrophene"[tw] OR "osmoplastic-r"[tw] OR "osmotox-plus"[tw] OR "shirakiku brand mosquito coils"[tw] OR "solfo black 2b supra"[tw] OR "solfo black b"[tw] OR "solfo black bb"[tw] OR "solfo black g"[tw] OR "solfo black sb"[tw] OR "tertrosulphur black pb"[tw] OR "tertrosulphur pbr"[tw] OR "2,3-dinitrophenol"[tw] OR "2,4-dinitrophenol"[tw] OR "2,5-dinitrophenol"[tw] OR "2,6-dinitrophenol"[tw] OR "alpha-dinitrophenol"[tw] OR "beta-dinitrophenol"[tw] OR "gamma-dinitrophenol"[tw] OR "o-dinitrophenol"[tw] OR "p-dinitrophenol"[tw]) NOT medline[sb])) AND (2017/04/01:3000[mhda] OR 2017/04/01:3000[crdt] OR 2017/04/01:3000[edat] OR 2016/04/01:3000[dp])) OR ("1,3-dinitro-4-hydroxybenzene"[tw] OR "aldifren"[tw] OR "dinitrophenate"[tw] OR "ek 102"[tw] OR "phenol, 2,3-dinitro-"[tw] OR "phenol, 2,4-dinitro-"[tw] OR "phenol, 2,5-dinitro-"[tw] OR "phenol, 2,6-dinitro-"[tw] OR "phenol, alpha-dinitro-"[tw] OR "phenol, beta-dinitro-"[tw] OR "phenol, dinitro-"[tw] OR "phenol, gamma-dinitro-"[tw] OR "phenol, α-dinitro-"[tw])
NTRL	
06/2020	"3,4-DNP" OR "Phenol, 3,4-dinitro-" OR "Phenol, 3,5-dinitro-" OR "1 alpha-2,4-dinitrophenol" OR "1-hydroxy-2,4-dinitrobenzene" OR "2,3-dnp" OR "2,4-dnp" OR "2,5-dnp" OR "2,6-dnp" OR "aldifen" OR "camello mosquito coils" OR "chemox pe" OR "cobra salts" OR "dinitroaminophenol" OR "dinitrophenol" OR "dinitrophenols" OR "dinofan" OR "fenoxyl carbon n" OR "impregna salts" OR "maroxol-50" OR "nitro kleenup" OR "nitrophen" OR "nitrophene" OR "osmoplastic-r" OR "osmotox-plus" OR "shirakiku brand mosquito coils" OR "solfo black 2b supra" OR "solfo black b" OR "solfo black bb" OR "solfo black g" OR "solfo black sb" OR "tertrosulphur black pb" OR "tertrosulphur pbr" OR "1,3-dinitro-4-hydroxybenzene" OR "aldifren" OR "dinitrophenate" OR "ek 102" OR "phenol, 2,3-dinitro-" OR "phenol, 2,4-dinitro-" OR "phenol, 2,5-dinitro-" OR "phenol, 2,6-dinitro-" OR "phenol, alpha-dinitro-" OR "phenol, beta-dinitro-" OR "phenol, dinitro-" OR "phenol, gamma-dinitro-" OR "phenol, α-dinitro-"

Table B-2. Database Query Strings

Database search date	Query string
	Date Published 2016 to 2020
Toxcenter	
06/2020	FILE 'TOXCENTER' ENTERED AT 11:44:15 ON 05 JUN 2020 CHARGED TO COST=EH038.06.01.LB.02 L1 5846 SEA FILE=TOXCENTER 66-56-8 OR 51-28-5 OR 329-71-5 OR 573-56-8 OR 577-71-9 OR 586-11-8 OR 25550-58-7 L2 5377 SEA FILE=TOXCENTER L1 NOT PATENT/DT L3 337 SEA FILE=TOXCENTER L2 AND ED>=20160101 L4 337 SEA FILE=TOXCENTER L2 AND ED>=2016 ACT TOXQUERY/Q --------- L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L7 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L10 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L11 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L12 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L13 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L14 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L15 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L16 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L17 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L18 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?) L19 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?) L20 QUE (ENDOCRIN? AND DISRUPT?) L21 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L22 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) L23 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L24 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)

Table B-2. Database Query Strings

Database search date	Query string
	L25 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L26 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L27 QUE (NEPHROTOX? OR HEPATOTOX?)
	L28 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L29 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L30 QUE L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29
	L31 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L32 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L33 QUE L30 OR L31 OR L32
	L34 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L35 QUE L33 OR L34

	L36 172 SEA FILE=TOXCENTER L4 AND L35
	L37 47 SEA FILE=TOXCENTER L36 AND MEDLINE/FS
	L38 125 SEA FILE=TOXCENTER L36 NOT L37
	L39 163 DUP REM L37 L38 (9 DUPLICATES REMOVED) ANSWERS '1-163' FROM FILE TOXCENTER
	L*** DEL 47 S L36 AND MEDLINE/FS
	L*** DEL 47 S L36 AND MEDLINE/FS
	L40 47 SEA FILE=TOXCENTER L39
	L*** DEL 125 S L36 NOT L37
	L*** DEL 125 S L36 NOT L37
	L41 116 SEA FILE=TOXCENTER L39
	L42 116 SEA FILE=TOXCENTER (L40 OR L41) NOT MEDLINE/FS D SCAN L42

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS via ChemView	
06/2020	Compounds searched: 66-56-8; 51-28-5; 329-71-5; 573-56-8; 577-71-9; 586-11-8; 25550-58-7
NTP	
06/2020	2016-present: 66-56-8 51-28-5 329-71-5 573-56-8 577-71-9 586-11-8 25550-58-7 "3,4-DNP" "Phenol, 3,4-dinitro-" "Phenol, 3,5-dinitro-" "1 alpha-2,4-dinitrophenol" "1-hydroxy-2,4-dinitrobenzene" "2,3-dnp" "2,4-dnp" "2,5-dnp" "2,6-dnp" "aldifen" "camello mosquito coils" "chemox pe" "cobra salts" "dinitroaminophenol" "dinitrophenol" "dinitrophenols" "dinofan" "fenoxyl carbon n" "impregna salts" "maroxol-50" "nitro kleenup" "nitrophen" "nitrophene" "osmoplastic-r" "osmotox-plus" "shirakiku brand mosquito coils" "solfo black 2b supra" "solfo black b" "solfo black bb" "solfo black g" "solfo black sb" "tertrosulphur black pb" "tertrosulphur pbr" No date limit: "1,3-dinitro-4-hydroxybenzene" "aldifren" "dinitrophenate" "ek 102" "phenol, 2,3-dinitro-" "phenol, 2,4-dinitro-" "phenol, 2,5-dinitro-" "phenol, 2,6-dinitro-" "phenol, alpha-dinitro-" "phenol, beta-dinitro-" "phenol, dinitro-" "phenol, gamma-dinitro-" "phenol, α-dinitro-"
Regulations.gov	
06/2020	Compounds searched: 66-56-8; 51-28-5; 329-71-5; 573-56-8; 577-71-9; 586-11-8; 25550-58-7
NIH RePORTER	
09/2020	Search Criteria: Text Search: "3,4-Dinitrophenol" OR "3,4-DNP" OR "3,5-Dinitrophenol" OR "6,5-Dinitrophenol" OR "Phenol, 3,4-dinitro-" OR "Phenol, 3,5-dinitro-" OR "1'alpha-2,4-dinitrophenol" OR "1-hydroxy-2,4-dinitrobenzene" OR "2,3-dnp" OR "2,4-dnp" OR "2,5-dnp" OR "2,6-dnp" OR "aldifen" OR "camello mosquito coils" OR "chemox pe" OR "cobra salts" OR "dinitroaminophenol" OR "dinitrophenol" OR "dinitrophenols" OR "dinofan" OR "fenoxyl carbon n" OR "impregna salts" OR "maroxol-50" OR "nitro kleenup" OR "nitrophen" OR "nitrophene" OR "osmoplastic-r" OR "osmotox-plus" OR "shirakiku brand mosquito coils" OR "solfo black 2b supra" OR "solfo black b" OR "solfo black bb" OR "solfo black g" OR "solfo black sb" OR "tertrosulphur black pb" OR "tertrosulphur pbr" OR "2,3-dinitrophenol" OR "2,4-dinitrophenol" OR "2,5-dinitrophenol" OR "2,6-dinitrophenol" OR "alpha-dinitrophenol" OR "beta-dinitrophenol" OR "gamma-dinitrophenol" OR "o-dinitrophenol" OR "p-dinitrophenol" OR "1,3-dinitro-4-hydroxybenzene" OR "aldifren" OR "dinitrophenate" OR "ek 102" OR "phenol, 2,3-dinitro-" OR "phenol, 2,4-dinitro-" OR "phenol, 2,5-dinitro-" OR "phenol, 2,6-dinitro-" OR "phenol, alpha-dinitro-" OR "phenol, beta-dinitro-" OR "phenol, dinitro-" OR "phenol, gamma-dinitro-" OR "phenol, α-dinitro-" (Advanced), Search in: Projects AdminIC: All, Fiscal Year: Active Projects

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
Other	Identified throughout the assessment process

The 2020 results were:

- Number of records identified from PubMed, NTRL, and TOXCENTER (after duplicate removal): 205
- Number of records identified from other strategies: 28
- Total number of records to undergo literature screening: 233

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on DNPs:

- Title and abstract screen
- Full text screen

Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

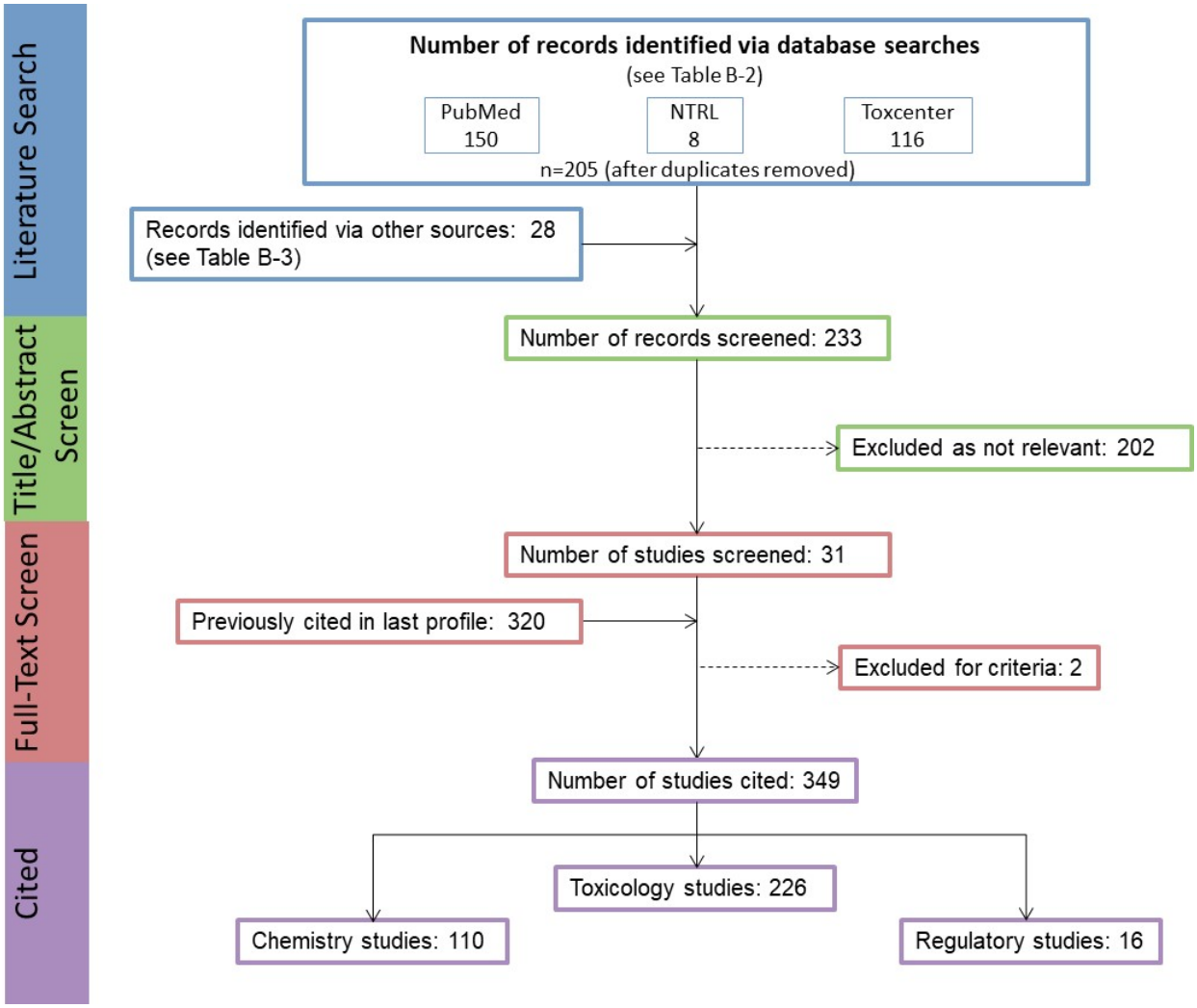
- Number of titles and abstracts screened: 233
- Number of studies considered relevant and moved to the next step: 31

Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 31
- Number of studies cited in the pre-public draft of the toxicological profile: 320
- Total number of studies cited in the profile: 349

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. June 2020 Literature Search Results and Screen for Dinitrophenols



APPENDIX C. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

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

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

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

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

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page C-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), behavioral (BH), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), enzyme activity (EA), food intake (FI), fetal toxicity (FX), gross necropsy (GN), hematology (HE), histopathology (HP), lethality (LE), maternal toxicity (MX), organ function (OF), ophthalmology (OP), organ weight (OW), teratogenicity (TG), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page C-6)

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

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

(13) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

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

(15) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(16) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(17) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral (1)

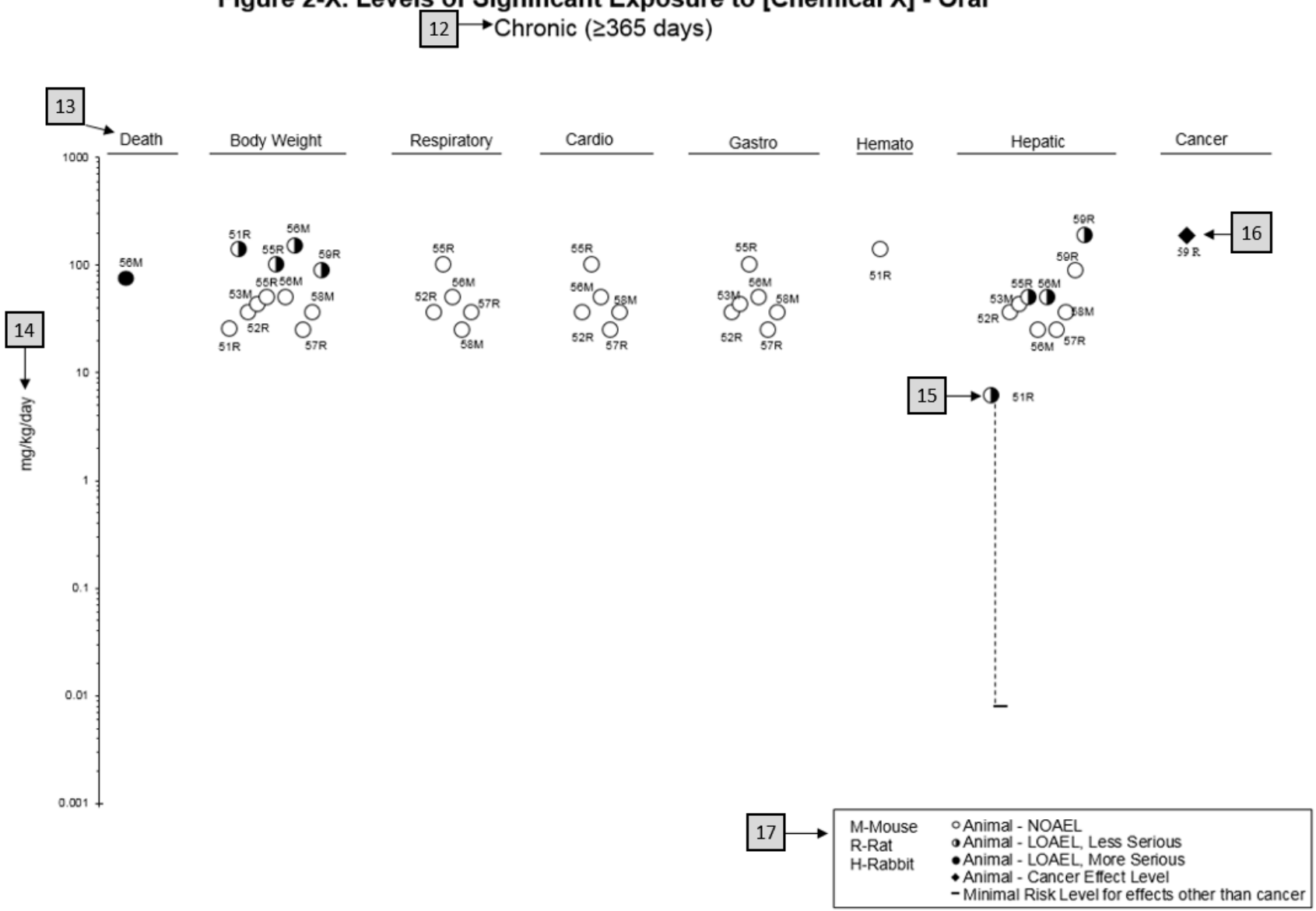
Figure key[a] (4)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
CHRONIC EXPOSURE (2)									
51 (3)	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
Aida et al. 1992 (10)									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

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

(11) [b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral



APPENDIX D. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:

Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional materials are available online:

Case Studies in Environmental Medicine are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

Managing Hazardous Materials Incidents is a set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp).

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX E. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

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

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

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

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

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

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

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

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

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

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

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

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

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

***In Vivo*—**Occurring within the living organism.

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

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

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

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

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

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

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

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

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

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

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

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Point of Departure (POD)—The point on the dose-response curve that defines where low-dose extrapolation commences. The POD may be a NOAEL, LOAEL, or benchmark dose estimated from mathematical modeling of the dose-response relationship.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) $\geq$1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

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

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

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

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

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX F. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act

FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram

NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure level/limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SLOAEL	serious lowest-observed-adverse-effect level
SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor

U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
USNRC	U.S. Nuclear Regulatory Commission
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result