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).

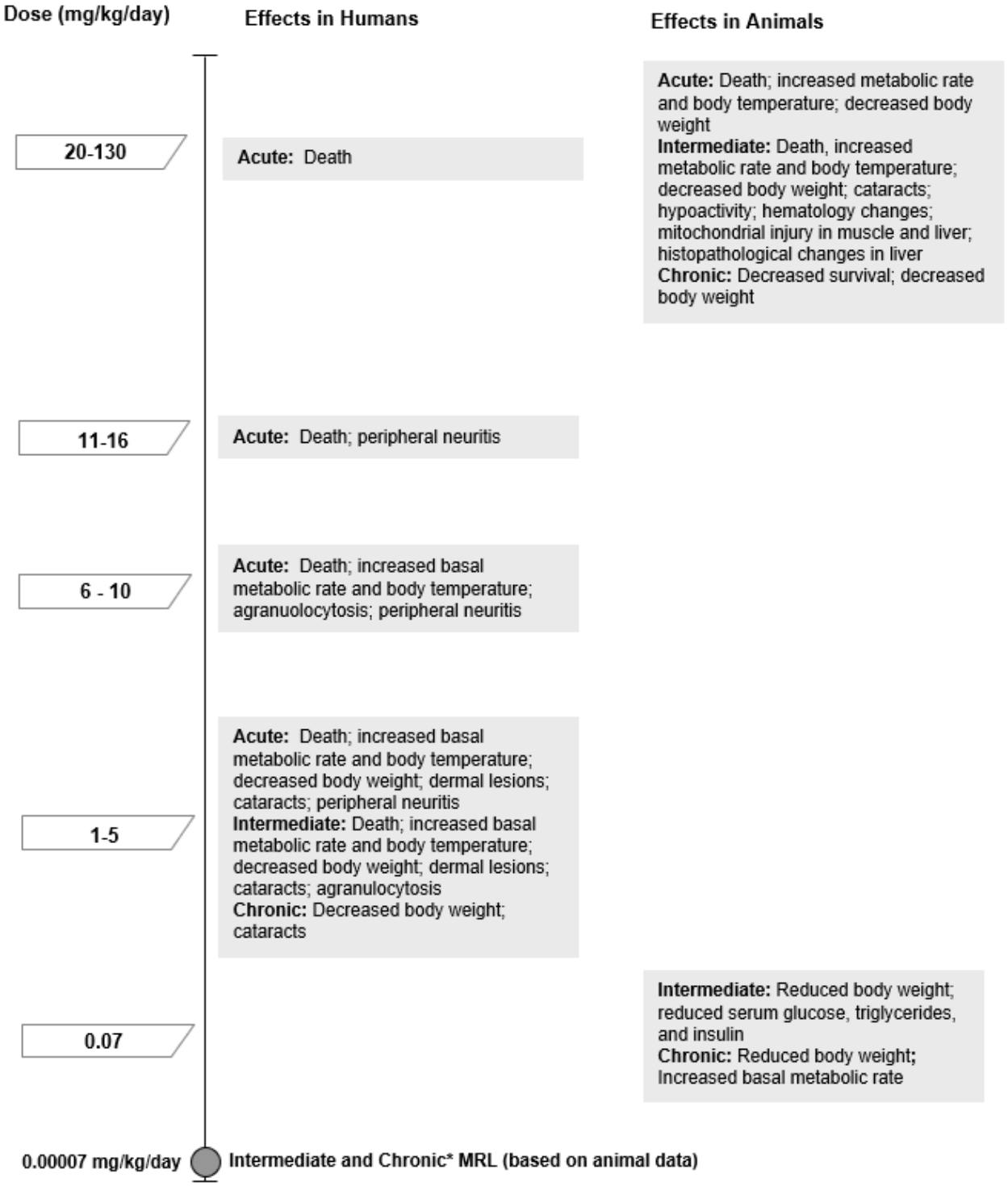
1.2 SUMMARY OF HEALTH EFFECTS

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

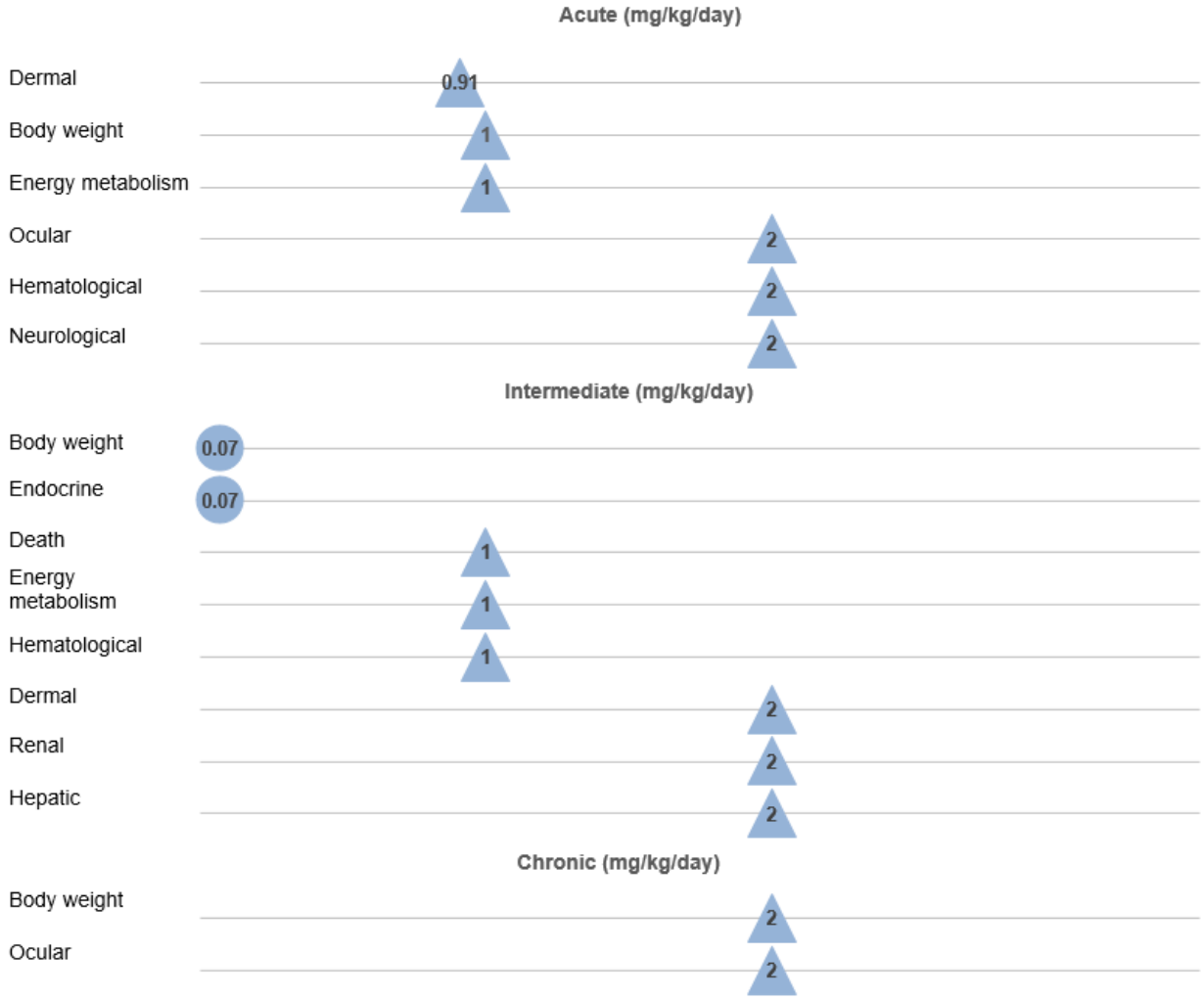


Table 1-1. Minimal Risk Levels (MRLs) for 2,4-Dinitrophenol[a,b]

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