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.

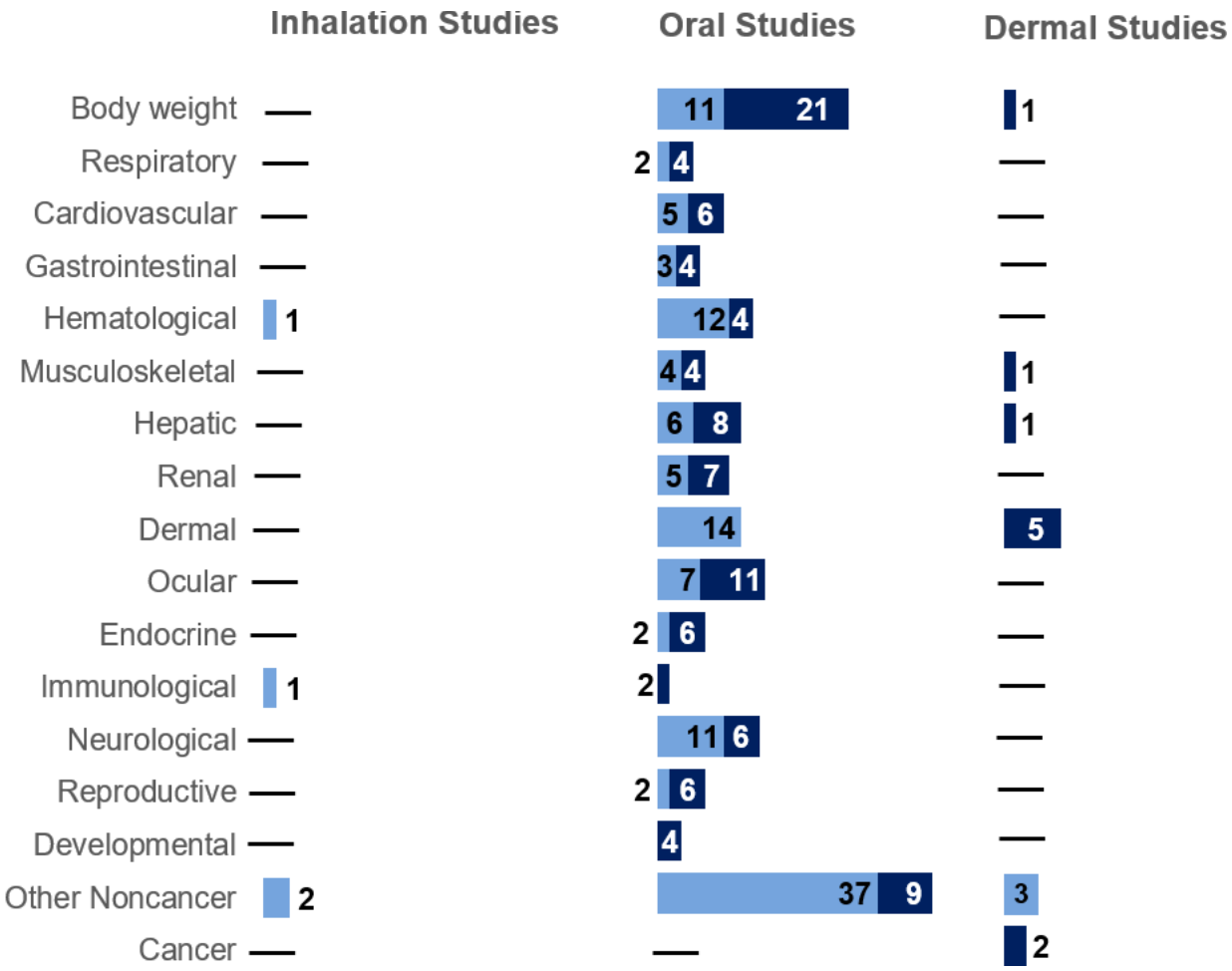
No studies were located regarding health effects in humans or animals after inhalation, oral, or dermal exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNP. The only study regarding health effects of 2,6-DNP in humans or animals after these routes of exposure was an acute-duration oral study of cataract formation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