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).

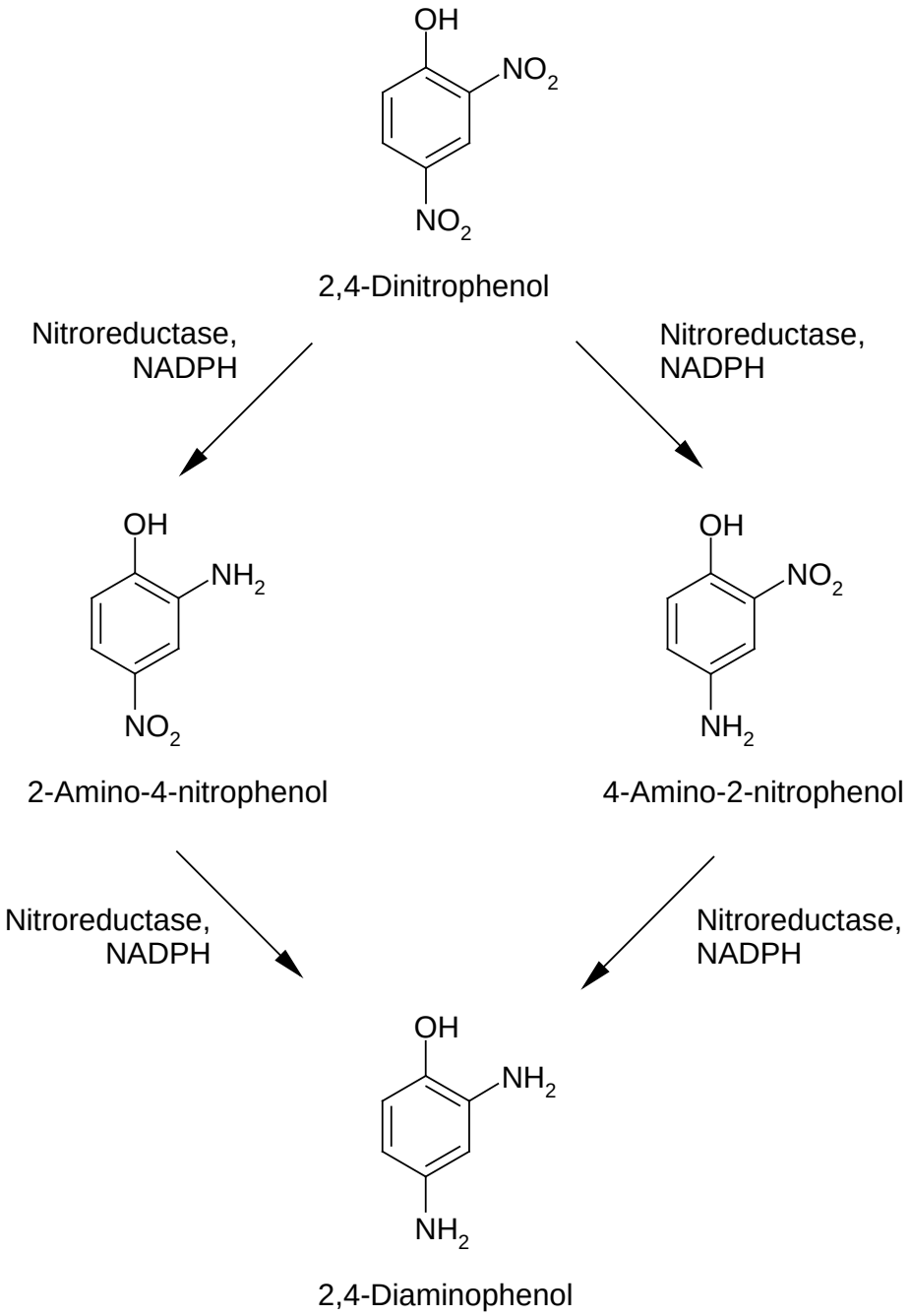
In vitro data are consistent with the available *in vivo* data on metabolism. An extensive investigation of the *in vitro* metabolism of 2,4-DNP by rat liver homogenates found that, under optimal pH and cofactor levels, 81% of the 2,4-DNP was metabolized. 2-Amino-4-nitrophenol accounted for 75%, 4-amino-2-nitrophenol 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.