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 of the toxicology of dinitrophenols (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.

There are six isomers of dinitrophenol (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, explosives, and insecticides. 2,5-DNP is also used in the manufacture of dyes and organic chemicals. Information on the use of the other isomers is not available (see Section 4.3). 2,4-DNP was used in the 1930s as a weight-reduction drug, but this was discontinued in 1938 because of the many reports of adverse effects in people who used it. 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. Two oral studies on the ocular effects of 2,6-DNP in chickens were located. An in vitro genotoxicity study of 2,3-, 2,5-, 2,6-, and 3,6-DNP was available (see Section 2.4). The only studies located regarding toxic effects of 2,3-, 2,5-, 3,4-, and 3,5-DNP were conducted by parenteral routes in animals (see Section 2.4). Therefore, the focus of Chapter 2 is on 2,4-DNP.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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 first by route of exposure-inhalation, oral, and dermal; and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of

three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure 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 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 end point 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 end points. 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.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels 1 or MRLs) may be of interest to health professionals and citizens alike.

A User's Guide has been provided at the end of this profile (see APPENDIX A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and MRLs.

2.2.1 Inhalation Exposure

Only two studies were identified that described the effects of inhalation exposure to dinitrophenols (DNPs). Both report cases of illness and death associated with occupational exposure to 2,4-DNP. The first study (Perkins 1919) reports findings in workers exposed to 2,4-DNP in the munitions industry in France during World War I. The second (Gisclard and Woodward 1946) reports two fatalities occurring in the United States during the manufacture of 2,4-DNP as an intermediate in the manufacture of picric acid. Although these studies provide no reliable quantitation of exposure levels, and it is likely that exposure occurred by the dermal and possibly oral routes as well as by inhalation, they do provide useful qualitative information on health effects and are discussed below. No studies were located regarding effects in animals after inhalation exposure to 2,4-DNP.

No studies were located regarding health effects in humans or animals after inhalation exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

2.2.1.1 Death

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. Fatal cases were noted especially among alcoholics or workers with renal or hepatic disease. 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. Incidence data were not available. Following the institution of better ventilation, use of masks, and other industrial hygiene measures to minimize exposure, the numbers of deaths per 10,000 tons 2,4-DNP manufactured per year decreased ≈14-fold. Similarly, 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 and rest, then a return to the job, 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, and may have exacerbated the effects. Workroom air levels, determined after the deaths occurred, were “normally” at least 40 mg/m^3, but this

value may underestimate breathing zone levels. In addition, significant dermal exposure and even oral absorption may have contributed to the total dose.

No studies were located regarding death in animals after inhalation exposure to 2,4-DNP.

2.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, hematological, musculoskeletal, endocrine, dermal, or ocular effects in humans or animals after inhalation exposure to 2,4-DNP. The limited information regarding systemic effects in humans is discussed below.

Respiratory Effects. 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." Details of this study are provided in Section 2.2.1.1; exposure levels, durations, and incidences were not characterized.

No studies were located regarding respiratory effects in animals after inhalation exposure to 2,4-DNP.

Gastrointestinal Effects. Clinical signs in workmen exposed to 2,4-DNP in the French munitions industry included anorexia followed by nausea and vomiting (Perkins 1919). Autopsies of fatal cases revealed no characteristic lesions. Details of this study are provided in Section 2.2.1.1; exposure levels, durations, and incidences were not characterized.

No studies were located regarding gastrointestinal effects in animals after inhalation exposure to 2,4-DNP.

Hepatic Effects. Autopsies of workers who died from exposure to 2,4-DNP in the French munitions industry did not reveal any characteristic lesions; no consistent microscopic changes of the liver were revealed during microscopic examination (Perkins 1919). Details of this study are provided in Section 2.2.1.l; exposure levels, durations, and incidences were not characterized.

No studies were located regarding hepatic effects in animals after inhalation exposure to 2,4-DNP.

Renal Effects. Autopsies of workers who died from exposure to 2,4-DNP in the French munitions industry did not reveal any characteristic lesions; no consistent changes of the kidney were revealed during microscopic examination (Perkins 1919). Details of this study are provided in Section 2.2.1.1; exposure levels, durations, and incidences were not characterized.

No studies were located regarding renal effects in animals after inhalation exposure to 2,4-DNP.

Body Weight Effects. Workmen exposed to 2,4-DNP in the French munitions industry experienced weight loss to the point of excessive thinness after several months of exposure (Perkins 1919). Details of this study are provided in Section 2.2.1.1; amount of weight loss, exposures, and durations were not characterized.

No studies were located regarding body weight effects in animals after inhalation exposure to 2,4-DNP.

Metabolic Effects. Workmen exposed to 2,4-DNP in the French munitions industry had characteristic signs and symptoms of toxicity (Perkins 1919). Details of this study are provided in Section 2.2.1.1; exposure levels and durations were not characterized. Gastrointestinal signs are discussed in the appropriate section above. In mild cases, the workers experienced increased sweating, particularly at night. Moderate cases, which were generally preceded by the signs noted for mild cases, included rapid onset of fatigue, moderately elevated body temperature, profuse sweating, thirst, markedly decreased urinary volume, short and labored respiration (but clear lungs), and agitation. Fatal cases, which occurred particularly among alcoholics and workers with renal or hepatic disease, included rapid onset of extreme fatigue, elevated body temperature (≥40 °C), profuse sweating that stained the skin yellow, intense thirst, dehydration, constriction of the pupils, and convulsions. The temperature continued to rise after death, and rigor mortis set in rapidly. Incidence data were not available. The graphical depiction of data from one factory showed a tendency toward higher percentages of clinical cases of intoxication during the warmer months, and a decrease in total percentages of clinical cases (from as high as 20% to 0-6%) after better industrial hygiene practices were instituted. In a case report of the death of two workers exposed to mists and dusts of 2,4-DNP in a U.S. chemical plant for a few months, signs of metabolic toxicity included fever and profuse sweating (Gisclard and Woodward 1946). Workroom air levels, determined after the deaths, were

"normally" at least 40 mg/m^3, but this value may underestimate breathing zone levels, and significant dermal exposure and even oral exposure may have occurred.

No studies were located regarding metabolic effects in animals after inhalation exposure to 2,4-DNP.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans or animals after inhalation exposure to 2,4-DNP.

2.2.1.4 Neurological Effects

Workmen exposed to 2,4-DNP in the French munitions industry had characteristic signs and symptoms of toxicity (Perkins 1919). Details of this study are provided in Section 2.2.1.1; exposure levels, durations, and incidences were not characterized. Neurological signs included rapid onset of fatigue and agitation in mild and severe cases, and convulsions preceding death in fatal cases. 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, and fatigue) and, following treatment and rest, returned to the job, collapsed, and died (Gisclard and Woodward 1946). Workroom air levels, determined after the deaths occurred, were "normally" at least 40 mg/m^3, but this value may underestimate breathing zone levels, and significant dermal exposure and even oral exposure may have occurred.

No studies were located regarding the following effects in humans or animals after inhalation exposure to 2,4-DNP:

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

2.2.2 Oral Exposure

During the 1930s, 2,4-DNP was used extensively as a diet pill (Hardgrove and Stem 1938; Horner 1942; Parascandola 1974). Both 2,4-DNP and its sodium salt, $C_6H_3(NO_2)_2(ONa)H_2O$, were available in capsules prescribed by physicians and were even available over the counter. The capsules usually contained ≤100 mg of these drugs and were normally taken l-3 times a day with meals, depending on the desired total dose. It was estimated that between 1933 and 1935, 100,000 patients were given 2,4-DNP for weight reduction (Tainter et al. 1935b). The database for human oral exposure consists almost entirely of clinical studies and case reports related to this use from 1932 to about 1938. Most of the patients who took this drug were women. In 1938, the U.S. Food and Drug Administration (FDA) announced that manufacturers of drugs such as DNP that were known to have caused adverse effects even when used under medical supervision would be prosecuted by the FDA whenever the drugs were found in interstate commerce. The use of 2,4-DNP was essentially discontinued at this time (Parascandola 1974). In the early 1980s, a physician in Texas administered 2,4-DNP to patients at his diet clinic for weight reduction, but was stopped by state authorities (Kurt et al. 1986). Much of the database for animal oral exposure also dates from the 1930s. These human and animal studies have limitations common to studies of the time. For example, none of the human clinical studies includes a concurrent, matched, placebo-treated control group, and statistical analyses generally were not mentioned. Frequently, however, each subject was monitored before and sometimes after treatment as a control measure. Dosages were generally reported as mg/day; for this profile, these were converted to mg/kg/day using average body weight values suggested by the EPA (EPA 1986b).

The only oral studies conducted with a DNP isomer other than the 2,4-isomer are two studies regarding the ocular effects of 2,6-DNP in chickens, reviewed in Section 2.2.2.2 below. No studies were located regarding the health effects in humans or animals after oral exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNP.

2.2.2.1 Death

Little information is available regarding death in humans after acute oral exposure to 2,4-DNP. A case report details the death of an 80-kg man who took ≈46 mg 2,4-DNP/kg as the sodium salt, followed by another 46 mg/kg dose 1 week later (Tainter and Wood 1934). The first dose produced a high

fever; the second dose resulted in admission to the hospital 6.5 hours later because of hyperpnea and chest pain. The rectal temperature was 105 °F, and pulse was rapid (as high as 146 beats per minute). Despite the administration of aspirin, the temperature rose to 105.7 °F by 10.5 hours following ingestion of the drug. Death occurred 0.5 hours later, with rigor mortis setting in 10 minutes after death and the temperature rising to ≈115 °F by 20 minutes after death. The clinical signs and the autopsy and histological findings were considered by the authors to be similar to those seen in heat stroke. A woman who took 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days was admitted to the hospital in a comatose condition and subsequently died (Poole and Haining 1934). She had complained of headache, backache, weakness, dizziness, shortness of breath, and excessive perspiration. Her temperature was at least 101.8 °F, pulse 140 beats per minute, and respiratory rate 56 per minute. Upon autopsy and histological examination, 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 were found. Slight ganglion cell degeneration was found in the pons. In another case, a psychiatric patient was given sodium 2,4-DNP in an experimental study to determine whether 2,4-DNP would be beneficial in treating depression (Masserman and Goldsmith 1934). Over the course of 14 days, she had been given 2.66 mg/kg/day 2,4-DNP. She died after her pulse increased to 148 beats per minute and respirations to 48 per minute, her temperature rose to 102 °F, she became comatose, and blood pressure fell to 36/0. Because autopsy was delayed for 4 days, no conclusions regarding histopathological lesions could be made. There were no deaths, however, in a number of clinical and experimental studies in which obese or normal subjects were given 2,4-DNP or its sodium salt at oral dosages of 1.2-4.3 mg/kg/day 2,4-DNP for ≤14 days (Castor and Beierwaltes 1956; Cutting et al. 1934; Cutting and Tainter 1933; MacBryde and Taussig 1935; Stockton and Cutting 1934; Tainter et al. 1935b).

In studies of intermediate-duration oral exposure to 2,4-DNP, cases of death from agranulocytosis (described in the discussion of Hematological Effects) have been attributed to 2,4-DNP. These cases occurred during the usual dosing regimens for weight loss, employing increasing doses in one case from 2.9 to 4.3 mg/kg/day of 2,4-DNP for 6 weeks (Dameshek and Gargill 1934); a dose of 1.03 mg/kg/day 2,4-DNP for 46 days in another case (Goldman and Haber 1936); and in another, from 0.62 to 3.8 mg/kg/day 2,4-DNP as sodium 2,4-DNP for 41 days (Silver 1934). In all cases, the patients were under medical supervision. Several clinical studies regarding the effects of 2,4-DNP or its sodium salt in obese and non-obese humans taking the drug for an intermediate duration at doses of 3.5-5.27 mg/kg/day 2,4-DNP have reported no deaths from this treatment (Cutting et al. 1934; Grant

and Schube 1934; Looney and Hoskins 1934; MacBryde and Taussig 1935; Simkins 1937a, 1937b; Tainter et al. 1934a, 1935b). A woman who took 3-5 tablets a day of 2,4-DNP for several months, discontinued its use for 3 months, and then resumed taking 5 tablets a day for 1 week, became ill only after resumption of dosing and subsequently died (Lattimore 1934). The data reported were insufficient to determine a dose in this case. It is not known why this woman tolerated the treatment for several months without developing any signs of illness, then subsequently became ill and died within 1 week after resumption of the same dose.

Studies regarding death in animals after acute oral 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). LD_{50} values for animals treated once by gavage were 30 mg/kg for white rats (sex and age not reported) (Dow Chemical Co. 1950), 71 mg/kg for weanling male rats, and 72 mg/kg for weanling male CFl mice (Kaiser 1964); the range of doses and the numbers of animals used were not reported. In a fairly reliable study on mature rats of each sex (9-40 per dose group) treated once by gavage, a dose-related increase in mortality was observed, with no mortality at doses of l0-27 mg/kg, 37% mortality at 30 mg/kg, and 100% mortality at 100 mg/kg (Spencer et al. 1948). A "100% survival dose" of 20 mg/kg and a "100% lethal dose" of 60 mg/kg were reported for white rats (strain, sex, and number not reported) treated once by gavage (Dow Chemical Co. 1940). A "100% survival dose" of 20 mg/kg and a "100% lethal dose" of 30 mg/kg were reported in dogs (l-3 per group, strain and sex not reported) treated once by gavage (Tainter and Cutting 1933b). Dogs (l-2 per dose group, strain and sex not specified) had 50% and 100% mortality following single gavage doses of 25 and 125 mg/kg/day, respectively (Kaiser 1964). These studies are limited by the small number of dogs used, by the lack of details, and by the omission of control groups. No mortality was observed in pregnant mice treated by gavage with 38.3 mg/kg/day 2,4-DNP on gestation days 10-12 (Gibson 1973). Adult yellow adipose and albino mice (sex not reported) exposed to 0.25% 2,4-DNP (325 mg/kg/day) in the diet all died approximately 8 hours later (total dose 108 mg/kg) (Bettman 1946). Twenty-five percent mortality was observed in young albino mice (initial age 5-6 weeks; sex not reported) exposed to 0.25% 2,4-DNP (325 mg/kg/day) in the diet for 1 week (Bettman 1946). Food consumption was not reported in this study, and doses were estimated using standard reference values for food intake by mice (EPA 1986b). One of six guinea pigs exposed to 40 mg/kg/day of 2,4-DNP in the diet died in 11 days and another died in 28 days (Ogino and Yasukura 1957). The guinea pig that died in 11 days and three others were on a vitamin C-deficient diet. The guinea pig that died after 28 days and one other were also on

a vitamin C-deficient diet, but received injections of 2 mg/day vitamin C. The study was conducted to determine the affect of vitamin C, an antioxidant known to prevent cataracts in general, on 2,4-DNP-induced cataractogenesis, but whether the antioxidant properties of vitamin C had any effect on survival could not be determined from this study.

The cause of death in these acute studies was generally attributed to the pyretic effect of 2,4-DNP, produced by an increase in metabolic rate. In one study, the authors indicated that rats treated once by gavage either died within l-2 hours or recovered completely (Spencer et al. 1948). In an LD_{50} test, 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). In animal studies, death generally did not occur after single gavage doses ≤10 mg/kg.

In a study where bobwhite quail were exposed to 2,4-DNP in the diet, 1 of 6 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 fed *ad libitum* between days 7 and 20 of life (Toyomizu et al. 1992). Dosages calculated from feed in take data were 16.5, 36.3, and 77.9 mg/kg/day 2,4-DNP.

Studies regarding death in animals after oral exposure to 2,4-DNP for intermediate durations were limited. Neither doses nor measured feed intake by animals exposed to 2,4-DNP *ad libitum* in the diet were reported accurately, and there also were potentially confounding effects of starvation. Rats are known to have a strong taste aversion to 2,4-DNP. Six rats exposed to a dietary concentration of 0.24% 2,4-DNP ate considerably less of the diet than other groups, failed to grow, and all died within 94 days (Tainter 1938). Since rats on the 0.24% 2,4-DNP diet consumed about 50% less of the food than the control group and groups that received lower concentrations (≤0.12%), they gained weight at only l/15 the rate of the other groups. However, because of their greatly reduced body weight, the intake of food on a body weight basis was considerably higher than that of the other groups. Thus, the rats on the 0.24% diet consumed 0.175 kg diet/kg body weight/day compared with 0.07 kg diet/kg body weight/day in the other groups. This higher consumption of food on a body weight basis results in an estimated dose of 420 mg/kg body weight/day 2,4-DNP, rather than 168 mg/kg/day had their food consumption been 0.07 kg diet/kg body weight/day. In other studies, rats exposed to 0.2% 2,4-DNP in the diet for 3 days consumed feed at 20% the rate of rats exposed to feed without

2,4-DNP (Bakke and Lawrence 1965). Thus, it appears that food consumption is also greatly reduced at dietary concentrations of 0.2% 2,4-DNP compared to control feed. Rats exposed to 0.2% 2,4-DNP in their feed (*ad libitum*) lost weight rapidly, with 40% mortality occurring in the first 21 days; the remaining rats were sacrificed on day 24 due to moribund condition (Spencer et al. 1948). Food consumption was not reported in the study; however, the authors indicated that the rats exposed to 0.2% dietary 2,4-DNP ate very little. In the absence of feed consumption data, it would be inappropriate to use the standard reference value for daily intake in a rat (0.05 kg feed/kg body weight/day) (EPA 1986b) to estimate dose from a dietary concentration of 0.2% 2,4-DNP. A more appropriate estimate of dose in animals exposed to 0.2-0.24% would be 350-420 mg/kg/day, based on the intake of 0.175 kg diet/kg body weight/day as determined in rats exposed to 0.24% 2,4-DNP in the feed (Tainter 1938). No mortality was observed after a 6-month exposure to ≤0.10% 2,4-DNP (Spencer et al. 1948). For studies in which rats were exposed to ≤0.12% 2,4-DNP, but food consumption was not reported, evidence indicates that the standard reference value for normal food consumption (EPA 1986b) would be appropriate for calculating dose (Tainter 1938). Thus, the estimated dose for rats exposed to 0.10% 2,4-DNP in feed is 50 mg/kg/day (Spencer et al. 1948).

Rats of unspecified age treated by gavage with 30 mg/kg/day 2,4-DNP, 5 days per week for 4 weeks all survived the treatment (Dow Chemical Co. 1940). The same company reported an LD_{50} of 30 mg/kg 2,4-DNP in rats for a later report (Dow Chemical Co. 1950); no explanation was given for this apparent contradiction. In a reasonably well conducted study, weanling rats were exposed to 2,4-DNP in the feed for 4 weeks; no mortality was observed at ≤59 mg/kg/day (Kaiser 1964). Sixteen rats of unspecified age exposed to 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). No mortality was observed in 3 male dogs fed 10 mg/kg/day in capsules for 6 months (Tainter et al. 1934b). In rats exposed to 2,4-DNP in the feed for their lifetime, 100% mortality was observed at 420 mg/kg/day between days 5 and 94 of treatment, a 50% decrease in life span was observed at 60 mg/kg/day, and no decrease in life span was observed in rats exposed to ≤40 mg/kg/day (Tainter 1938).

Starvation probably contributed to mortality among rats exposed to high levels of dietary 2,4-DNP because rats exposed to 0.20-0.24% (≈350-420 mg/kg/day) 2,4-DNP failed to eat or grow and died quickly (Spencer et al. 1948; Tainter 1938). Rats exposed to 60 mg/kg/day for life had normal food consumption, but growth and life span were decreased; the cause of death in these animals was not

determined, as no histopathological lesions were found in the lungs, heart, liver, kidney, or testis (Tainter 1938). In rat studies involving dietary administration of 2,4-DNP for intermediate and chronic durations, death was not generally observed at dose levels ≤40 mg/kg/day.

Administration of 2,4-DNP by continuous intermittent feeding is less lethal to rats than a single bolus dose by gavage. Rats treated once by gavage had 50% mortality at exposure levels as low as 30 mg/kg, with 100% mortality observed at 60 mg/kg (Dow Chemical Co. 1940, 1950). In dietary studies, rats survived a 30-day treatment with 110 mg/kg/day 2,4-DNP, and the life span was not decreased in rats exposed to 40 mg/kg/day (Pugsley 1935; Tainter 1938). Under most circumstances, dietary studies in animals may be more relevant than single bolus studies to current oral exposure of humans to 2,4-DNP.

The LD_{50} values and the doses associated with death in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-l.

2.2.2.2 Systemic Effects

Studies regarding systemic effects in humans and animals after oral exposure to 2,4-DNP are discussed below. One study of 2,6-DNP is mentioned in the discussion of Ocular Effects. The highest NOAEL values and all LOAEL values from each reliable study for each systemic effect in each species and duration category are recorded in Table 2-l and plotted in Figure 2- 1.

Respiratory Effects. A case report and a clinical study of 2,4-DNP reported increased respiratory rates at very high single doses (>l0 mg/kg [exact doses not specified] and 46 mg/kg followed by another 46 mg/kg 1 week later) (Cutting et al. 1933; Tainter and Wood 1934). This effect is secondary to the elevation of body temperature and basal metabolic rate by 2,4-DNP. Pulmonary edema was seen in a man who died after ingesting the sodium salt of 2,4-DNP in two doses of 46 mg/kg 2,4-DNP 1 week apart (Tainter and Wood 1934). The body temperature was greatly elevated, and the authors concluded that the autopsy findings were similar to those seen in heat stroke. Other case reports have also reported respiratory effects in patients who died after taking 2,4-DNP for acute durations. Dyspnea, a respiratory rate as high as 48 respirations per minute, and course rales were found in a woman who subsequently died after taking 2.66 mg/kg/day for 2 weeks (Masserman and Goldsmith 1934). In another fatal case, the respiratory rate was 56 respirations per minute when

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Human	14 d 1-2x/d (C)				2.66 F (death)	Masserman and Goldsmith 1934
2	Human	5 d (C)				7 F (1/1 died)	Poole and Haining 1934
3	Human	1 wk 2x/wk (C)				46 M (1/1 died)	Tainter and Wood 1934
4	Rat (white)	once (GO)				60 (100% mortality)	Dow Chemical 1940
5	Rat (white)	once (GO)				30 (LD50)	Dow Chemical Co. 1950
6	Rat (Sherman)	once (GW)				71 M (LD50)	Kaiser 1964
7	Rat (white)	once (GO)				30 (11/30 died)	Spencer et al. 1948
8	Mouse (yellow adipose and albino)	8 hr ad lib (F)				108 (12/12 died)	Bettman 1946
9	Mouse (albino)	1 wk ad lib (F)				325 (2/8 died)	Bettman 1946

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key[a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
10	Mouse (CF1)	once (GW)				72 M (LD50)	Kaiser 1964
11	Quail (Bobwhite)	8 d ad libitum				56.1 F (death in 1 of 6)	Dominguez et al. 1993
	Systemic						
12	Human	14 d 3x/d (C)	Resp	2.3 F			Anderson et al. 1933
			Cardio	2.3 F			
			Gastro	2.3 F			
			Hemato	2.3 F			
			Musc/skel		2.3 F (exacerbation of arthritis)		
			Hepatic	2.3 F			
			Renal	2.3 F			
			Derm			2.3 F (severe pruritis, edema, maculopapular eruptions covering most of body)	
13	Human	2 d 3x/d (C)	Metabolic		3.2 (increased basal metabolic rate +29%)		Castor and Beierwaltes 1956
14	Human	7-16 d 1x/d	Bd Wt		3.53 (average weight loss of 0.92 kg)		Cutting and Tainter 1933
		(C)	Metabolic			3.53 (27-55% increase in basal metabolic rate, excessive perspiration)	
15	Human	1-2 wk 7 d/wk 1x/d (C)	Metabolic			3.5 (increased basal metabolic rate +38%)	Cutting et al. 1934

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key[a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
16	Human	3-4 d 1x/d (C)	Gastro		4.4 F (burning in throat, inflammation of pharynx)		Dintenfass 1934
			Derm		4.4 F (rash on chest)		
			Metabolic		4.4 F (profuse perspiration)		
			Other		4.4 F (inflammation of eustachian tube and hearing impairment secondary to pharyngeal inflammation)		
17	Human	2 wk (C)	Hemato		1.86 F (slight secondary anemia)		Hitch and Schwartz 1936
			Derm			1.86 F (severe exfoliating dermatitis over 100% of body surface)	
			Ocular			1.86 F (cataract)	
			Other		1.86 F (temporary hearing impairment due to exudation in middle ear)		
18	Human	2 wk 7 d/wk 4x/d (C)	Hemato			5.7 F (agranulocytosis)	Hoffman et al. 1934

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
19	Human	1-8 wk 7 d/wk 3 x/d (C)	Cardio			4.3 F (abnormalities on ECGs in 3 of 6 tested)	MacBryde and Taussig 1935
			Gastro		4.3 F (gastrointestinal disturbances and vomiting 5/15)		
			Musc/skel		4.3 F (loss of strength and endurance on exercise tests in 4 of 4 tested)		
			Hepatic		4.3 F (increased phenol-tetraiodophthalein retention above pretreatment values and above normal range- in 3 of 5 tested at 1-2 weeks)		
			Renal	4.3 F			
			Derm		4.3 F ("quite severe" skin rashes 3/15)		
			Endocr		4.3 F (decreased glucose tolerance in 5 of 8)		
			Metabolic			4.3 F (basal metabolic rate +30 to +70%, excessive sweating)	
20	Human	14 d 1-2x/d (C)	Resp			2.66 F (dyspnea, tachypnea up to 48/minute, and rales)	Masserman and Goldsmith 1934
			Cardio			2.66 F (increased pulse up to 148 beats/minute)	
			Hepatic	2.66 F			
			Renal	2.66 F			
			Metabolic			2.66 F (basal metabolic rate increase by +38%; 102 degree F body temperature)	
21	Human	8 d 1x/d (C)	Musc/skel		0.91 F (weakness in legs and rheumatoid pain in arms and fingers)		Nadler 1935
			Derm		0.91 F (pruritic rash)		

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
22	Human	5 d (C)	Resp			7 F (respiration rate- 56/min, shortness of breath, hyperemic and hemorrhagic lungs, congestion of alveolar walls, edema in alveoli)	Poole and Haining 1934
			Cardio			7 F (tachycardia, segmentation and fragmentation of cardiac muscles)	
			Gastro			7 F (vomiting, hemorrhage and edema of gastric mucosa and degeneration of glandular epithelium)	
			Hemato		7 F (splenic pulp filled with blood)		
			Musc/skel		7 F (pain in arms and legs)		
			Hepatic			7 F (degeneration of hepatocytes, necrosis)	
			Renal			7 F (cloudy swelling, necrosis of tubular epithelium, edema in tubules, distention of capillary and arterial loops in glomeruli and hemorrhage)	
			Metabolic			7 F (101.8 °F fever; profuse perspiration)	
23	Human	4-12 d 3x/d (C)	Resp	3.5			Stockton and Cutting 1934
			Cardio			3.5 (average increase in venous blood pressure of up to 37% and in pulse of up to 12%)	
			Metabolic		3.5 (sensation of warmth; increased perspiration)		

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
24	Human	1 wk 2x/wk (C)	Resp			46 M (very rapid and deep respiration; pulmonary edema and congestion)	Tainter and Wood 1934
			Cardio			46 M (pulse rate up to 146; 140 systolic and 124 diastolic blood pressure; fragmentation of heart muscle)	
			Hepatic		46M (slight detachment of liver cells)		
			Renal		46M (mild nephrotic changes)		
			Metabolic			46 M (temperature of 105.7 °F rising to 115 °F after death)	
25	Human	14 d 1x/d (C)	Derm	1.2			Tainter et al. 1935b
			Bd Wt		1.2 [b] (0.43 kg/week weight loss)		
			Metabolic		1.2 (sensation of warmth, increased perspiration)		
26	Rat (Wistar)	once (GW)	Renal		20 F (mild tubular necrosis in 5/16 kidneys)		Arnold et al. 1976
27	Rat (NS)	9 d ad lib (F)	Endocr		350M (increased thyroxine secretion)		England et al. 1973
			Bd Wt		350M (12% decrease in body weight)		

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
28	Rat (NS)	7-14 d ad lib (F)	Endocr		350M (21-35% decreased absolute thyroid weight; decreased thyroid function; decreased serum protein bound iodine)		Maayan 1968
			Bd Wt			350 M (24-36% decreased body weight gain)	
29	Rat (Sprague-Dawley)	2 wk ad lib (F)	Endocr		350M (34% decrease in absolute pituitary weight; decreased pituitary function; decreased growth hormone synthesis; decreased thyroid function; decreased serum thyroxin levels)		Wilkins et al. 1974
			Bd Wt		350M (15% decrease in body weight gain)		
30	Mouse (albino and yellow adipose)	8 hr ad lib (F)	Ocular	108			Bettman 1946
31	Mouse (albino)	1 wk ad lib (F)	Ocular	325			Bettman 1946
32	Mouse (Swiss-Webster)	3 d Gd 10-12 1x/d (GW)	Metabolic	25.5 F		38.3 F (hyperthermia of dams)	Gibson 1973

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
33	Dog (beagle)	1-14 d 1x/d (C)	Cardio	12.5 F		25 (abnormal ECG readings; increased heart rate)	Kaiser 1964
			Gastro	5.0 M	12.5 F (emesis)		
			Metabolic	12.5 F		25 ("markedly" increased respiration rate and body temperature)	
34	Dog (NS)	once (C)	Metabolic		20 (body temperature increased 0.9 °C)		Tainter and Cutting 1933a
35	Rabbit (NS)	8 hr (F)	Ocular	41			Bettman 1946
36	Chicken (NS)	once (GO)	Ocular	6		11 (cataract formation)	Buschke 1947
37	Quail (Bobwhite)	8 d ad libitum (F)	Gastro		33.6 F (diarrhea)		Dominguez et al. 1993
			Bd Wt	33.6 F	56.1 F (mean weight loss of 13%)		
			Metabolic			33.6 F (metabolic rate 23 to 41% higher than control)	
38	Duck (White Pekin)	once (GW)	Ocular	15		20 (3/8 developed temporary cataracts)	Gehring and Buerge 1969a
39	Chicken (NS)	13 d ad libitum (F)	Bd Wt	36.3 F	77.9 F (decreased body weight gain of 12%)		Toyomizu et al. 1992
	Neurological						
40	Human	14 d 3x/d (C)				2.3 F (peripheral neuritis, paresthesias)	Anderson et al. 1933

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
41	Human	1-2 wk 1-3x/d (C)				2.45 F (symptoms of peripheral neuritis: tingling and numbness of extremities in 1/35 patients)	Bortz 1934
42	Human	1-4 d 1x/d (C)			4.4 F (headache, fatigue dizziness, exhaustion)		Dintenfass 1934
43	Human	2 wk (C)				1.86 F (polyneuritis)	Hitch and Schwartz 1936
44	Human	1-8 wk 7 d/wk 3 x/d (C)			4.3 F (complete loss of taste in 1 of 15)		MacBryde and Taussig 1935
45	Human	14 d 1-2x/d (C)				2.66 F (coma, stupor, torpor, confusion)	Masserman and Goldsmith 1934
46	Human	5 d (C)				7 F (headache, weakness, dizziness, coma, hyperemia of spinal cord, pons and medulla, degeneration of ganglion cells)	Poole and Haining 1934
47	Human	14 d 1x/d (C)		1.2			Tainter et al. 1935b
48	Mouse (Swiss-Webster)	3 d Gd 10-12 1x/d (GW)		25.5 F	38.3 F (hyperexcitability of dams)		Gibson 1973

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Developmental						
49	Mouse (Swiss-Webster)	3 d Gd 10-12 1x/d (GW)		38.3			Gibson 1973
50	Mouse (CD-1)	5 d Gd 8-12 1x/d (GW)		125			Kavlock et al. 1987
	INTERMEDIATE EXPOSURE						
	Death						
51	Human	46 d 1x/d (C)				1.03 F (death)	Goldman and Haber 1936
52	Rat (white)	21-24 d ad lib (F)				350 M (4/10 died in 21 days; 6/10 sacrificed as moribund on day 24)	Spencer et al. 1948
53	Rat (white)	94 d ad lib (F)				420 M (6/6 died within 94 days)	Tainter 1938
	Systemic						
54	Human	51-62 d (C)	Gastro		1.94 (nausea)		Bayer and Gray 1935
			Metabolic		1.94 (subjective feeling of heat, excessive sweating)		

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
55	Human	37 d 1x/d (C)	Hemato	2.32 F			Beinhauer 1934
			Hepatic		2.32 F (palpable and tender liver)		
			Renal			2.32 F (moderate albuminuria)	
			Derm			2.32 F (severe pruritis involving entire body)	
			Bd Wt		2.32 F (4.5 kg reduction in body weight in 37 days)		
56	Human	3-13 wk 7 d/wk 1x/d (C)	Metabolic		3.5 (increased basal metabolic rate +23%)		Cutting et al. 1934
57	Human	20 d 3x/d (C)	Hemato			3.5 F (agranulocytosis)	Davidson and Shapiro 1934
			Hepatic			3.5 F (impaired liver function on bromsulphalein test)	
58	Human	182 d 1x/d (C)	Resp		3.3 F (elevated respiratory rate,24 breaths/minute)		Epstein and Rosenblum 1935
			Cardio		3.3 F (elevated pulse, 106 beats/min)		
			Hemato	3.3 F			
			Bd Wt			3.3 F (loss of 20% of body weight)	
			Metabolic			3.3 F (increased basal metabolic rate of 82%)	

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
59	Human	46 d 1x/d (C)	Resp			1.03 F (dyspnea, elevated respiratory rate, cyanosis, vascular congestion)	Goldman and Haber 1936
			Cardio			1.03 F (tachycardia, irregular heart beats)	
			Gastro			1.03 F (necrosis and ulceration of small intestine)	
			Hemato			1.03 F (severe neutropenia, severe agranulocytosis)	
			Hepatic			1.03 F (severe fatty changes)	
			Renal			1.03 F (hemorrhagic nephritis)	
			Endocr			1.03 F (extensive vascularization of spleen and pituitary; goiter in thyroid)	
			Metabolic			1.03 F (body temperature 105.6 °F, excessive perspiration)	
60	Human	118 d (C)	Ocular			3.14 F (cataracts)	Horner et al. 1935
			Bd Wt		3.14 F (individual weight loss of 9.6 kg)		
61	Human	41-49 d (C)	Derm		2.99 M (urticaria)		Hunt 1934
			Metabolic		2.99 M (excessive perspiration)		
62	Human	35 d 2-4x/d (C)	Resp		3.97 F (elevated respiratory rate- 30/ minute)		Imerman and Imerman 1936
			Cardio		3.97 F (elevated pulse rate - 108/minute)		
			Hemato			3.97 F (agranulocytosis)	
			Renal			3.97 F (albuminuria)	
			Metabolic			3.28 F (body temperature 102.8 °F, excessive perspiration)	

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
63	Human	7 wk 7 d/wk 1x/d (C)	Cardio	3			Looney and Hoskins 1934
			Bd Wt		3 (average weight loss of 0.36 kg/week)		
			Metabolic			3 (basal metabolic rate increased 50%)	
64	Human	1-8 wk 7 d/wk 3 x/d (C)	Cardio			4.3 F (abnormalities on ECGs in 3 of 6 tested)	MacBryde and Taussig 1935
			Gastro		4.3 F (gastrointestinal disturbances and vomiting 5/15)		
			Musc/skel		4.3 F (loss of strength and endurance on exercise tests in 4 of 4 tested)		
			Hepatic		4.3 F (increased phenoltetraiodo-phthalein retention above pretreatment values and above normal range in 3 of 3 tested at 3-8 weeks)		
			Renal	4.3 F			
			Derm		4.3 F ("quite severe" skin rashes 3/15)		
			Endocr		4.3 F (decreased glucose tolerance in 4 of 4 tested at 3-4 weeks)		
			Metabolic			4.3 F (basal metabolic rate +30 to +70%, excessive sweating)	
65	Human	21-112 d 1-3 x/d (C)	Musc/skel		3.53 F (weakness and arthritic pains)		Nadler 1935
			Derm		3.89 F (pruritic rash)		
			Bd Wt		3.53 F (16% loss of body weight)	3.99 F (25% loss of body weight)	

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
66	Human	2 mo 3x/d (C)	Cardio		4.29 F (heart palpitations)		Rank and Waldeck 1936
			Ocular			4.29 F (bilateral cataracts)	
			Metabolic		4.29 F (excessive perspiration)		
67	Human	22-89 d 1x/d (C)	Resp		3 (increased respiratory rate by 10/min)		Simkins 1937a, Simkins 1937b
			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)	
			Derm		2.3 (urticaria)		
			Ocular			3 (cataract)	
			Bd Wt		3 (loss of 0.95 kg/wk)		
			Metabolic		3 (increase in basal metabolic rate by 11% per 100mg daily dose)		
68	Human	2-50 wk 7 d/wk 1x/d (C)	Hepatic	3.5			Tainter et al. 1934a

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
69	Human	88 d 1x/d (C)	Cardio	4.0			Tainter et al. 1935b
			Hemato	4.0			
			Derm			4.0 (skin reaction in 23/170, sometimes severe)	
			Ocular			4.0 (cataracts in 1/170)	
			Bd Wt		4.0 (weight loss of 0.64 kg/week, total weight loss 7.8 kg)		
			Metabolic			4.0 (estimated increased basal metabolic rate +38%, increased perspiration, sometimes causing discomfort)	
70	Human	1-18 mo (C)	Ocular			3.6 (cataracts)	Whalman 1936
			Bd Wt		3.6 (average weight loss of 17 kg)		
71	Rat (Sprague-Dawley)	30 d ad lib (F)	Endocr		350M (decreased thyroid weights, decreased pituitary weights)		Bakke and Lawrence 1965
			Bd Wt		350M (18% decrease in body weights)		
			Metabolic		350M (increased body temperature)		
72	Rat (Sherman)	4 wk ad lib (F)	Bd Wt	59			Kaiser 1964
73	Rat (NS)	24-26 d ad lib (F)	Bd Wt			110 (30% decrease in body weight)	Pugsley 1935
			Metabolic			110 (30-85% increase in oxygen consumption)	

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
74	Rat (NS)	6 mo ad lib (F)	Resp	50 M			Spencer et al. 1948
			Cardio	50 M			
			Gastro	50 M			
			Hemato	50 M			
			Musc/skel	50 M			
			Hepatic	50 M			
			Renal	50 M			
			Ocular	50 M			
			Bd Wt	25 M	50M (17% decrease in body weight gain)		
75	Rat (NS)	94 d ad lib (F)	Ocular	420 M			Tainter 1938
			Bd Wt	84 M		420 M (body weight gain decreased by 93%)	
76	Rat (NS)	58-173 d ad lib (F)	Ocular	50			Tainter and Borley 1938
			Bd Wt		50 ("significant" weight loss)		
77	Mouse (yellow adipose)	6 mo ad lib (F)	Ocular			130 (3/40 developed cataracts)	Bettman 1946
78	Mouse (albino and black)	11 mo ad lib (F)	Ocular			130 (1 of 20 albino developed cataracts)	Bettman 1946

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
79	Dog (NS)	7-12x over 45-77 d (C)	Resp	20			Tainter and Cutting 1933b
			Cardio	20			
			Gastro	20			
			Hepatic	20			
			Renal	20			
			Bd Wt	20			
			Metabolic	10	15 (body temperature increased by greater than 1 °C)	20 (body temperature increased by greater than 2 °C)	
80	Dog (NS)	6 mo 6 d/wk 1x/d (C)	Resp	10 M			Tainter et al. 1934b
			Cardio	10 M			
			Gastro	10 M			
			Hemato	10 M			
			Musc/skel	10 M			
			Hepatic	10 M			
			Renal	10 M			
			Bd Wt	10 M			
81	Gn pig (NS)	21-37 d ad lib (F)	Ocular	80			Tainter and Borley 1938
	Immunological/Lymphoreticular						
82	Dog (NS)	6 mo 6 d/wk 1x/d (C)		10 M			Tainter et al. 1934b

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Neurological						
83	Human	182 d 1x/d (C)				3.3 F (peripheral neuritis)	Epstein and Rosenblum 1935
84	Human	46 d (C)				1.03 F (delirium, unconciousness)	Goldman and Haber 1936
85	Human	41-49 d (C)			3.38 F (loss of taste)		Hunt 1934
86	Human	35 d 2-4x/d (C)			3.97 F (extreme malaise, headaches)		Imerman and Imerman 1936
87	Human	1-8 wk 7 d/wk 3x/d (C)			4.3 F (complete loss of taste 1/15)		MacBryde and Taussig 1935
88	Human	21-112 d 1-3x/d (C)				3.53 F (peripheral neuritis)	Nadler 1935
89	Human	22-89 d 1x/d (C)				3 (peripheral neuritis, weakness, loss of taste)	Simkins 1937a,b
90	Human	88 d 1x/d (C)				4.0 (symptoms of peripheral neuritis [sensory] in 18/170)	Tainter et al. 1935b

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
91	Dog (NS)	7-12x over 45-77 d (C)		20			Tainter and Cutting 1933b
92	Dog (NS)	6 mo 6 d/wk 1x/d (C)		10 M			Tainter et al. 1934b
	Reproductive						
93	Human	182 d 1x/d (C)				3.3 F (miscarriage)	Epstein and Rosenblum 1935
94	Human	22-89 d 1x/d (C)				3 F (altered menstrual cycles, amenorrhea)	Simkins 1937a,b
95	Rat (NS)	6 mo ad lib (F)		50 M		350 M (testicular atrophy)	Spencer et al. 1948
96	Dog (NS)	6 mo 6 d/wk 1x/d (C)		10 M			Tainter et al. 1934b
	CHRONIC EXPOSURE						
	Death						
97	Rat (white)	lifetime ad lib (F)				60 M (approximately 50% decrease in median lifespan)	Tainter 1938

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Systemic						
98	Human	16-18 mo (C)	Ocular			2 F (cataracts)	Horner et al. 1935
			Bd Wt			2 F (>30% loss of body weight)	
99	Rat (white)	lifetime ad lib (F)	Resp	60 M			Tainter 1938
			Cardio	60 M			
			Hepatic	60 M			
			Renal	60 M			
			Ocular	60 M			
			Bd Wt	20 M		30 M (25% decrease in body weight gain)	

TABLE 2-1. Levels of Significant Exposure to Dinitrophenol - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Reproductive						
100	Rat (white)	lifetime ad lib (F)		60 M			Tainter 1938

[a] The number corresponds to entries in Figure 2-1.

[b] Used to derive an acute oral minimal risk level (MRL) of 0.01 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for sensitive populations and 10 for use of a LOAEL).

Ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; d = day(s); Derm = dermal; ECG = electrocardiogram; Endocr = endocrine; F = female; (F) = food; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect; M = male; mo = month; Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect; NS = not specified; Resp = respiratory; wk = week(s); x = times

NOTE: In case reports of humans who took 2,4-dinitrophenol (2,4-DNP) for weight reduction, 2,4-DNP was available both in its free form or as a sodium salt. (The chemical formula for 2,4-DNP is $C_6H_4N_2O_5$ and its molecular weight is 184.11; the chemical formula for the sodium salt is $C_6H_3(NO_2)_2(ONa)H_2O$ and its molecular weight is 224.11.) In studies that explicitly stated that 2,4-DNP was taken in the form of the sodium salt, appropriate adjustments for molecular weight differences were used to calculate the dose of free 2,4-DNP. Otherwise, it was assumed that 2,4-DNP was taken in the free form. Furthermore, in some cases in which body weights were not reported, the standard reference body weight for humans (70 kg) was used to calculate dose. As people taking 2,4-DNP for weight reduction were generally obese, use of 70 kg would result in an overestimate of dose.

FIGURE 2-1. Levels of Significant Exposure to Dinitrophenol – Oral

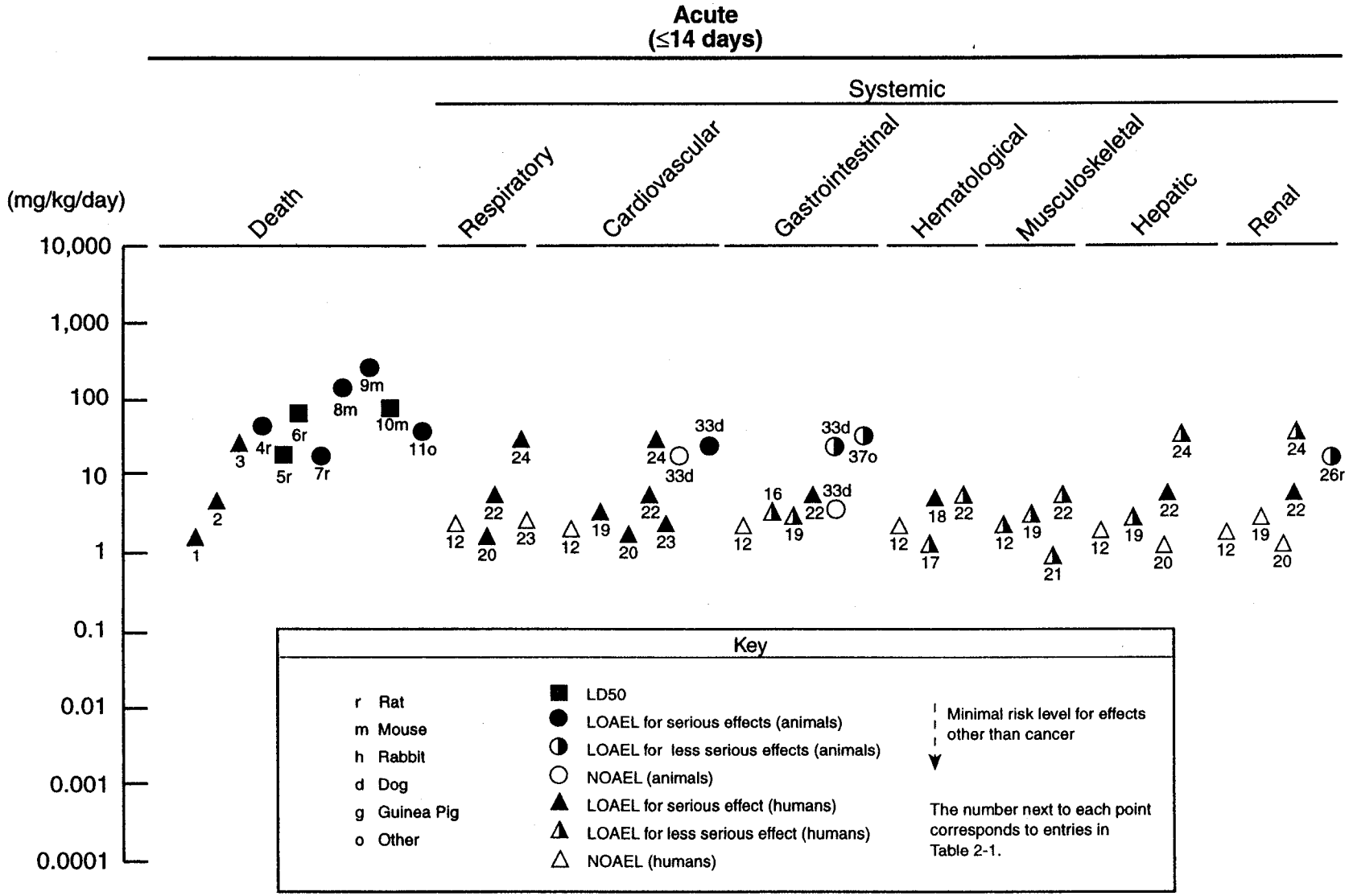


FIGURE 2-1. Levels of Significant Exposure to Dinitrophenol – Oral (continued)

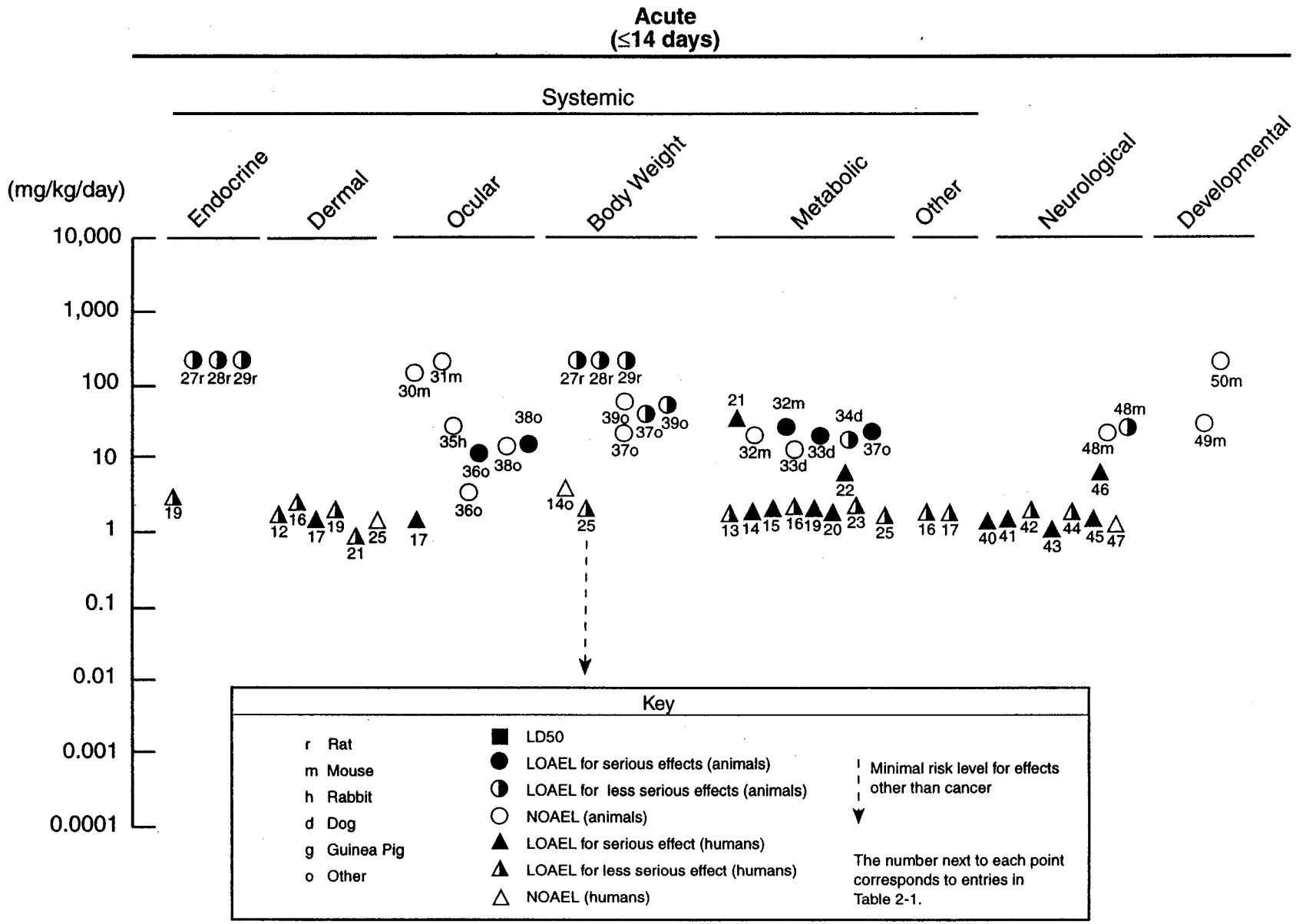


FIGURE 2-1. Levels of Significant Exposure to Dinitrophenol – Oral (continued)

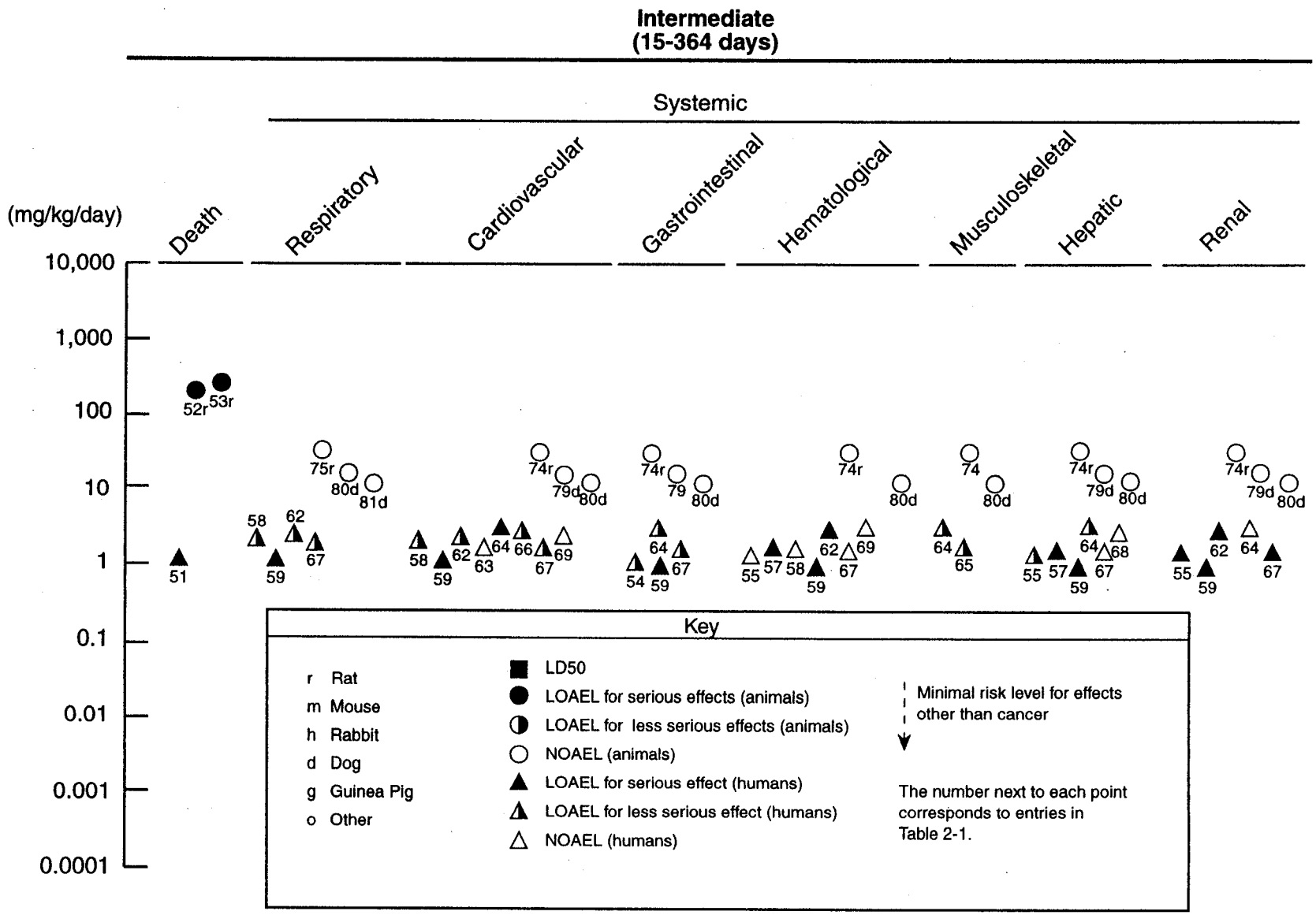


FIGURE 2-1. Levels of Significant Exposure to Dinitrophenol – Oral (continued)

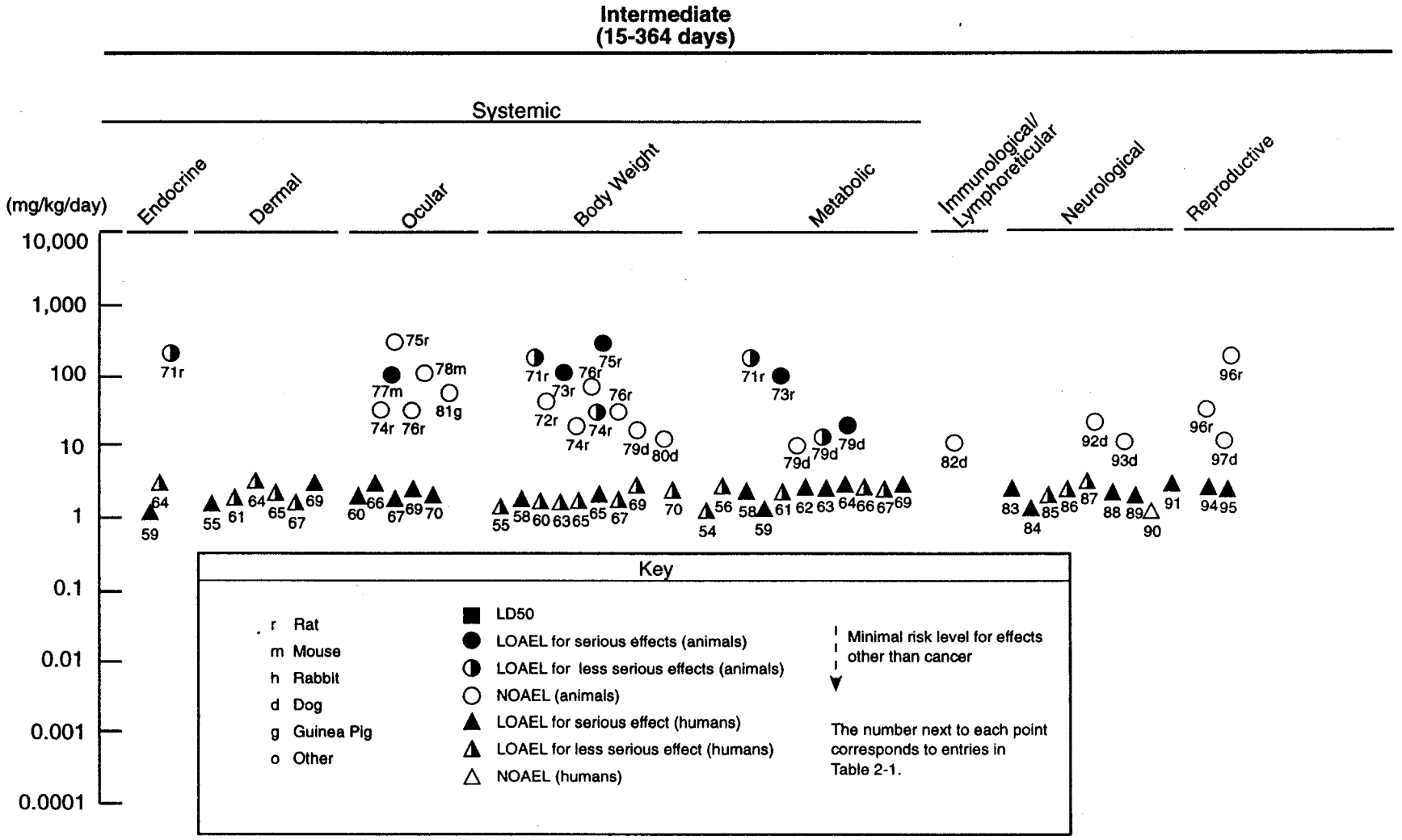
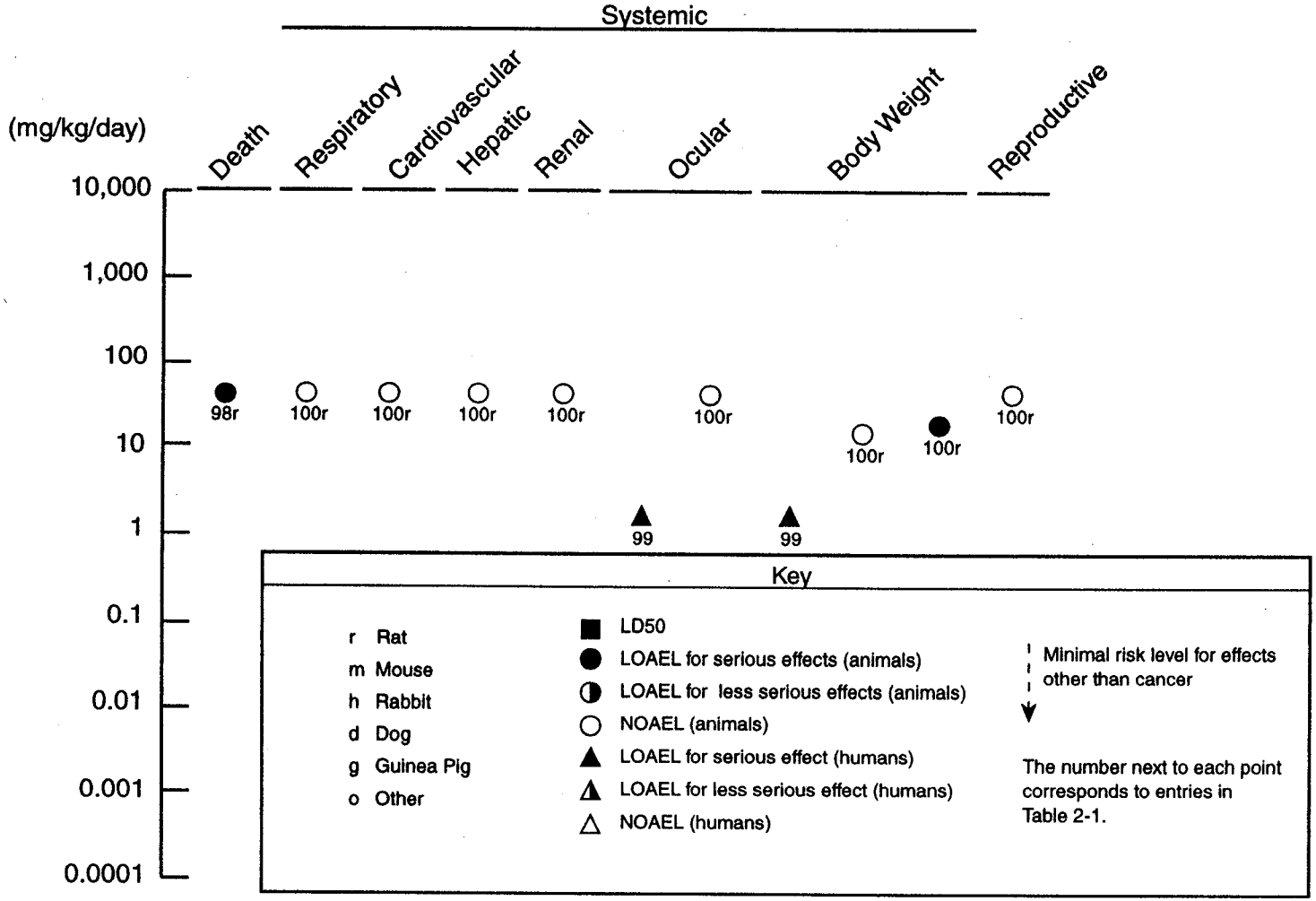


FIGURE 2-1. Levels of Significant Exposure to Dinitrophenol – Oral (continued)

Chronic
(≥ 365 days)



the 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. A clinical study in which obese patients took capsules of the sodium salt of 2,4-DNP at 3.5 mg/kg/day 2,4-DNP for 4-12 days, however, showed no change in vital capacity (Stockton and Cutting 1934). Elevated respiratory rates and dyspnea have also been reported in patients who took, 1.03-3.97 mg/kg/day 2,4-DNP for intermediate durations (Epstein and Rosenblum 1935; Goldman and Haber 1936; Imerman and Imerman 1936; Simkins 1937a, 1937b). The patient who took 1.03 mg/kg/day died, and upon autopsy, vascular congestion was found in the lungs (Goldman and Haber 1936). A patient who took 2.3 mg/kg/day 2,4-DNP for 14 days and had severe dermatological reactions did not exhibit dyspnea (Anderson et al. 1933).

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 (l-2 per group) given 20 mg/kg/day via capsule 7-12 times in 45-77 days (Tainter and Cutting 1933b), or dogs (3 per dose group) exposed via capsules to 5 or 10 mg/kg/day for 6 months (Tainter et al. 1934b).

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).

Cardiovascular Effects. Effects on pulse rate, heart rate, and blood pressure are common findings in people who took 2,4-DNP for weight reduction. A case report and a clinical study of 2,4-DNP reported greatly increased pulse rates at high acute doses (>l0 mg/kg [exact doses not specified] and 46 mg/day followed by another 46 mg/kg 1 week later) (Cutting et al. 1933; Tainter and Wood 1934). This effect is known to be secondary to the elevation of body temperature and basal metabolic rate by 2,4-DNP. In a case of a woman who died after taking 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days, the heart rate prior to death was very rapid, suggestive of auricular fibrillation (Poole and Haining 1934). Autopsy revealed marked segmentation and fragmentation of the cardiac muscles. A psychiatric patient was given sodium 2,4-DNP in an experimental study to determine whether 2,4-DNP would be beneficial in treating depression (Masserman and Goldsmith 1934). Over the course of 14 days, she had been given 2.66 mg/kg/day 2,4-DNP. She died after her pulse

increased to 148 beats per minute and respirations to 48 per minute, her temperature rose to 102 °F, she became comatose, and blood pressure fell from 144/68 to 36/0. Autopsy revealed slight scarring of the tricuspid and mitral valves, hypertrophy of the right ventricle, and small scattered fatty deposits in the aorta. However, because autopsy was delayed for 4 days, no conclusions regarding histopathological lesions could be made. In another case, a woman took 3-5 tablets/day of 2,4-DNP for several months before discontinuing its use for 3 months after which she resumed taking 5 tablets/day for 1 week (Lattimore 1934). After the resumption of dosing, she became ill and subsequently died. Although her pulse was normal upon physical examination, autopsy revealed myocarditis, which was considered to be the cause of death. Information was insufficient to calculate a dose. In addition, 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 3.5 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. The episodes of peripheral vasodilation may have been a cooling response to the pyretic effects of 2,4-DNP. An intermediate-duration clinical study of 6 patients treated for 1-8 weeks with 2,4-DNP at 4.3 mg/kg/day showed definite changes in the electrocardiograms of 3 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 2 patients at 2 weeks after cessation of treatment. Elevations in pulse rates were common findings in people taking 2,4-DNP for intermediate durations. Pulse rates of 106-136 beats per minute were reported in patients taking 1.03-3.97 mg/kg/day (Epstein and Rosenblum 1935; Goldman and Haber 1936; Imerman and Imerman 1936). The individual taking 1.03 mg/kg/day was a young girl who subsequently died after being admitted to the hospital; her pulse was 136 beats per minute and heart sounds were irregular (Goldman and Haber 1936). 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). Insufficient information was provided to calculate the dose. A woman taking 4.29 mg/kg/day 2,4-DNP for 2 months complained of heart palpitations while taking the drug (Rank and Waldeck 1936). 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. A pulse rate as high as 126 beats per minutes was recorded in a woman who took an indeterminate dose intermittently for a year (Imerman and Imerman 1936). In two clinical studies in obese patients given 4 mg/kg/day 2,4-DNP for 88 days (Tainter et al. 1935b) 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. A case report of a patient who took 2.3 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).

Increased heart rate (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). 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 (l-2 per group) given 20 mg/kg/day via capsule 7-12 times in 45-77 days (Tainter and Cutting 1933b), or dogs (3 per dose group) exposed via capsules to 5 or 10 mg/kg/day for 6 months (Tainter et al. 1934b).

Gastrointestinal Effects. Gastrointestinal effects, such as nausea, vomiting, and diarrhea, were common findings in people who took 2,4-DNP for weight reduction. Gastrointestinal disturbances and vomiting occurred in 5 of 15 patients who ingested 2,4-DNP at 4.3 mg/kg/day for l-8 weeks; the duration of treatment for the affected patients was not specified (MacBryde and Taussig 1935). In a case report, a woman took 3-5 tablets/day of 2,4-DNP for several months, discontinued its use for 3 months then resumed taking 5 tablets/day for 1 week (Lattimore 1934). After the resumption of dosing, she became ill and subsequently died. Nausea and vomiting occurred on the day she died. Information was insufficient to calculate a dose. In another fatal case, a woman who was taking 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days vomited on the way to the hospital (Poole and Haining 1934). Upon autopsy, the stomach mucosa was edematous and hemorrhagic, and the glandular epithelium was disintegrated. 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 the case of the young girl who died after taking 1.03 mg/kg/day for 46 days, vomiting occurred after the last dose (Goldman and Haber 1936). Upon autopsy and microscopic examination, no pathological changes were found in the stomach, but the small intestine contained numerous focal hemorrhagic necroses. 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). The effects on the gastrointestinal tract and the pharynx appear to be due to a local irritating or necrotizing effect of 2,4-DNP. 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.

Adult beagles fed capsules containing 2,4-DNP daily at 12.5 mg/kg/day (1 female for 14 days), 25 mg/kg/day (2 females for 1 day, 1 male for 14 days), or 125 mg/kg/day (1 female for 1 day) displayed emesis; however, no emesis was observed at 5 mg/kg/day (1 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 (l-2 per group) given 20 mg/kg/day via capsule 7-12 times in 45-77 days (Tainter and Cutting 1933b), or dogs (3 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).

Hematological Effects. There have been eight reported cases of agranulocytosis (a syndrome characterized by marked decrease in the number of granulocytes, lesions of the throat and other mucous membranes, and fever; also called granulocytopenia, malignant neutropenia, agranulocytic angina) in patients ingesting 2,4-DNP or its sodium salt for weight reduction (Horner 1942). These effects occurred in acute, intermediate, and chronic durations of treatment. Cases of agranulocytosis were reported for women who ingested 5.7 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 3.97 mg/kg/day 2,4-DNP for 35 days (Imerman and Imerman 1936), and an obese young girl who took 1.03 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 1.9 mg/kg/day, increasing to 2.8 mg/kg/day and then to 4.6 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 0.62 mg 2,4-DNP/kg/day, increasing to 2.5 mg/kg/day 2,4-DNP over 5 weeks and then to 3.8 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 2.9 mg/kg/day increasing to 4.3 mg/kg/day for a total duration of ≈6 weeks (Dameshek and Gargill 1934). All of these patients were taking the drug under the care of physicians. 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 was 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). She also had mild anemia. It was not clear whether she was taking the drug under a physician's supervision.

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), and an autopsy of a woman who died after taking 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days revealed that the splenic pulp was filled with blood (Poole and Haining 1934).

Several 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 2.3 mg/kg/day for 1 week (Anderson et al. 1933), 2.32 mg/kg/day for 37 days (Beinhauer 1934), or 3.3 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. 1935b).

Rats exposed to 5-50 mg/kg/day 2,4-DNP in the diet for 6 months had no hematological abnormalities with respect to erythrocyte count, hemoglobin concentration, total leukocyte count, or differential count; in addition, no abnormalities were observed in rats with respect to total cell and nucleated cell counts in the bone marrow (Spencer et al. 1948). Food consumption was not reported in this study, and doses were estimated using standard reference values for food consumption by rats (EPA 1986b). Dogs (3 per dose group) exposed to 5 or 10 mg/kg/day 2,4-DNP via capsules for 6 months had no hematological abnormalities with respect to hemoglobin concentration, red blood cell (RBC) count, oxygen capacity and fragility of RBCs, total leukocytes, and differential counts; histological examination of bone marrow revealed no abnormalities (Tainter et al. 1934b).

Musculoskeletal Effects. Exercise tests revealed considerable loss of strength and reduced endurance in a limited number of obese patients who ingested 4.3 mg/kg/day of 2,4-DNP for l-8 weeks (MacBryde and Taussig 1935). Details of testing methods and results were not reported. The loss of strength and reduced endurance may be related to uncoupling of oxidative phosphorylation by 2,4-DNP, rather than a direct effect of 2,4-DNP on muscle tissue. A woman who subsequently died after taking 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days complained of pain in the arms and legs on the fifth day (Poole and Haining 1934). Weakness in the legs and arthritic or rheumatoidlike pains in the arms and fingers were experienced by 4 women who had been taking 2,4-DNP at doses of 0.91 or 1.45 mg/kg/day for 8 days or 3.89 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 (see Section 2.2.2.4). 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 2.3 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Anderson et al. 1933). The pains subsided within 4-5 days, but swelling and tenderness persisted in the left wrist and fingers. The authors suggested that 2,4-DNP exacerbated her arthritis.

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 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).

Hepatic Effects. Slight detachment of the liver cells from one another with no apparent change in endothelial cells was seen during histopathological examination of tissues from a man who died after ingesting the sodium salt of 2,4-DNP at a dose estimated at 46 mg/kg 2,4-DNP, followed by an additional 46 mg/kg 1 week later (Tainter and Wood 1934). His temperature reached 105.7 °F before death and 115 °F after death. The authors commented that the pathology findings were similar to those seen in heat stroke. In other fatal cases, disintegration of hepatocytes in the periphery of the lobules and granular cytoplasm and pyknotic nuclei in periportal cells were seen 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 were found in the liver of a woman who took an indeterminate dose of 2,4-DNP for 1 week (Lattimore 1934), and severe fatty changes were found in the liver of a young girl who took 1.03 mg/kg/day of 2,4-DNP for 46 days (Goldman and Haber 1936). Whether these hepatic effects represented preexisting conditions is not known. Compromised liver function, leading to decreased ability to metabolize 2,4-DNP (see Section 2.3.3), may have been a contributing factor in these fatal cases. A palpable and tender liver was observed in a patient who took 2.32 mg/kg/day 2,4-DNP for 37 days (Beinhauer 1934). No tests of liver function were performed on this patient. Impaired liver function as measured by a bromsulphalein test was observed in an obese woman who took 3.5 mg/kg/day 2,4-DNP as the sodium salt for 20 days (Davidson and Shapiro 1934). Liver function was not tested before 2,4-DNP treatment; therefore, it is unclear whether this was a preexisting condition or a consequence of 2,4-DNP treatment. Liver function, as assessed by the icteric index (a calorimetric estimation of bilirubin in the serum by comparison with the absorbance of a standard solution of potassium dichromate which is no longer used clinically) (Lichtman 1953) and Van den Bergh test, was not affected in a woman who took 2.3 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Anderson et al. 1933). The Van den Bergh test is a test for 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 (Berkow and Fletcher 1992; Schroeder et al. 1990). The test is not particularly sensitive, but it is a well-established one. Currently, a modification of the test is used, in which the diazotized serum is compared with a standard solution of diazotized bilirubin (Gennaro et al. 1984). The icteric index was normal in a psychiatric patient who subsequently died after being given 2.66 mg/kg/day 2,4-DNP as the sodium salt (Masserman and Goldsmith 1934). Upon autopsy, no gross evidence of liver damage was found, but microscopic examination was inconclusive due to autolysis because autopsy was delayed by 4 days. In clinical studies, the icteric index of 17 patients who ingested 3.5 mg/kg/day 2,4-DNP as the sodium salt for 2-50 weeks did not provide evidence of liver damage, compared with

the icteric index of an unspecified number of "nonmedicated patients" (Tainter et al. 1934a). The bilirubin content of blood serum in 45 patients given 3.5 mg/kg/day 2,4-DNP was elevated in only 2 patients, neither of whom had other clinical evidence of liver disturbance. 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, a yellow discoloration of the skin and sclerae was observed in 5 patients, but the icteric index was normal in each case (Masserman and Goldsmith 1934). Information was insufficient to calculate a dose. Since 2,4-DNP itself is yellow, it is possible that the noted discoloration was a direct effect of the drug rather than a sign of hepatic damage. In obese patients given 2,4-DNP at 4.3 mg/kg/day for 1-8 weeks, increased dye (phenoltetraiodophthalein) retention (above pretreatment values and above the normal range) was seen in 3 of 5 patients tested at l-2 weeks of treatment and in 3 of 3 patients tested at 3-8 weeks of treatment (MacBryde and Taussig 1935). This test measures the ability of the liver to remove this dye from the blood after intravenous injection (Cole et al. 1928). Blood samples are taken 0.5 and 1 hour after injection, and the concentration of phenoltetraiodophthalein in the serum is compared with a standard. In addition, the gall bladder can be X-rayed for presence of the dye to determine the excretory function of the liver; this was not done by MacBryde and Taussig (1935). Other clinical tests relevant to liver function (icteric index, serum bilirubin, galactose tolerance test, urinary urobilinogen) were normal (MacBryde and Taussig 1935). The galactose tolerance test is a measure of the ability of the liver to produce glycogen. The elimination of >3 grams of galactose in the urine over a 5-hour period after administration of 40 grams of galactose to a fasting individual indicates hepatic dysfunction (Gennaro et al. 1984). 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 bromsulphalein (BSP) retention tests revealed no evidence of liver damage in the 14 or 15 patients to whom the tests were given (Simkins 1937a, 1937b). The BSP test measures the ability of the liver to remove this dye from the blood after intravenous injection. It is a sensitive measure of liver disease in the absence of hyperbilirubinemia (which interferes with BSP measurement) (Berkow and Fletcher 1992). Retention of BSP in the blood is an indication of decreased blood flow, biliary obstruction, or hepatic cell damage. However, the use of BSP is currently limited because of toxic side effects.

No significant difference in levels of serum glutamic-pyruvate transaminase (SGPT, also known as alanine aminotransferase [ALAT]) was observed in six mice (strain not specified) treated once by gavage with 22.5 mg/kg/day 2,4-DNP (Robert 1986). Two dogs repeatedly fed capsules containing 2,4-DNP at dose levels ≤20 mg/kg, with "recovery periods" of ≈5 days between doses, followed by a

"fatal dose" (dose level not reported) had normal results with respect to liver function tests and gross and microscopic histology of the liver (Tainter and Cutting 1933b). A 12% decrease in relative liver weight was observed in rats fed 0.2% 2,4-DNP (420 mg/kg/day) in the diet *ad libitum* for 9 days (England et al. 1973). The authors reported that the rats also lost 12% of their initial body weight; however, food consumption was not reported in this study. The significance of the observed effect on the liver is questionable because the rats were probably starving. Other studies have indicated that rats exposed to dietary concentrations ≥0.2% 2,4-DNP had greatly reduced food consumption and growth, and quickly died (Spencer et al. 1948; Tainter 1938). 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). This study was very poorly reported. 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 (3 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).

Thus, while some evidence of hepatic effects was found in some patients taking 2,4-DNP, the possibility of pre-existing liver pathology could not be determined. The lack of hepatic effects in animals suggests that the liver is not a sensitive target organ for the toxicity of 2,4-DNP.

Renal Effects. Mild nephrotic changes were seen during histopathological examination of tissues from a man who died after ingesting the sodium salt of 2,4-DNP in a dose estimated at 46 mg/kg 2,4-DNP, followed by an additional 46 mg/kg 1 week later (Tainter and Wood 1934). His temperature reached 105.7 "F before death and 115 "F after death. The authors commented that the clinical and pathology findings were similar to those seen in heat stroke. 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 glomernlus, 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 glomernli 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 1.03 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 2.66 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. Similarly, the blood nonprotein nitrogen level was normal in a woman who took 2.3 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Anderson et al. 1933). 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 3 patients over a period of 8 weeks while they underwent treatment with 4.3 mg/kg/day 2,4-DNP showed no changes; the data were not provided (MacBryde and Taussig 1935). The phenolsulfonphthalein (also known as phenol red) test measures the ability of the kidney to excrete the dye after intravenous or intramuscular injection. A decrease in excretion of the dye from normal (about 50-70% is normally excreted in 2 hours) is a general indicator of kidney dysfunction (Gennaro et al. 1984; Hook and Hewitt 1986). Moderate and marked albuminuria was found in 2 women who took 2.32 mg/kg/day (Beinhauer 1934) or 3.97 mg/kg/day 2,4-DNP (Imerman and Imerman 1936) for 37 or 35 days, respectively. Kidney function as determined by phenolsulfonphthalein retention was normal in the woman who took 2.32 mg/kg/day (Beinhauer 1934). 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 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). Information was insufficient to calculate a dose.

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). 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). This study was very poorly reported. 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 was observed in the nuclei of the epithelial cells, but the glomeruli were essentially normal. 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 of 14 and 2 of 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 (3 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). Rats exposed to 60 mg/kg/day 2,4-DNP in the diet for life had gross and histological findings in the kidney comparable to the control group (Tainter 1938).

Endocrine Effects. 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 5 of 8 patients after l-2 weeks of treatment and in 4 of 4 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 3.2 mg/kg/day 2,4-DNP for 2 days (Castor and Beierwaltes 1956). Thyroidal I^{131} uptake and fecal and urinary I^{131} excretion, tested in two of these subjects, did not appear to be affected. Hence, the toxicological significance of this finding is unclear. 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; Wilkens et al. 1974). In these studies, rats were exposed for 7-30 days to dietary 2,4-DNP at a concentration of 0.2%. In one study, the authors indicated that a “small amount” of feed was consumed by rats treated with DNP and implied that this amount was 20% of normal consumption (Bakke and Lawrence 1965); the other studies did not discuss feed consumption (England et al. 1973; Maayan 1968; Wilkens et al. 1974). The thyroid studies all report extremely rapid body weight loss (as much as 1% of body weight per day), implying that the animals were starving and/or wasting away; however, similarly starved control groups were not used. Investigation of subtle end points 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 were considered inadequate to estimate the end points addressed (Bakke and Lawrence 1965; England et al. 1973; Maayan 1968; Wilkens et al. 1974).

Dermal Effects. Skin lesions were common findings in people taking 2,4-DNP. 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 (Nadler 1935). However, the rashes reappeared when they resumed taking 2,4-DNP at the same dose. A woman who took 4.4 mg/kg/day 2,4-DNP for 4 days developed a rash on her chest (Dintenfass 1934). Severe skin lesions developed in two women who took 1.86 mg/kg/day 2,4-DNP (Hitch and Schwartz 1936) or 2.3 mg/kg/day 2,4-DNP as the sodium salt (Anderson et al. 1933) for 14 days. 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 1.2 mg/kg/day 2,4-DNP as the sodium salt of 2,4-DNP for an average of 14 days (Tainter et al. 1935b). Serious skin reactions (not otherwise specified) were observed in 3 of 15 obese patients taking 4.3 mg/kg/day 2,4-DNP for l-8 weeks; the duration of 2,4-DNP treatment for the affected patients was not specified (MacBryde and Taussig 1935). In case reports of people taking 2,4-DNP for longer durations, pruritus developed in a woman who took 3.89 mg/kg/day for 21 days (Nadler 1935), urticaria was seen in one or all of 2 women and 1 man who took 2.99-3.38 mg/kg/day for 41-49 days (Hunt 1934); urticaria developed in 1 patient (sex not specified) taking 2.3 mg/kg/day for 110 days (Simkins 1937a, 1937b), and transient pruritic spots were observed in a woman who had been taking 100-200 mg of 2,4-DNP intermittently for 1 year (Imerman and Imerman 1936). A severe case of pruritus involving the entire body was described for a woman who took 2.32 mg/kg/day 2,4-DNP for 37 days (Beinhauer 1934). 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. 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 4.0 mg/kg/day 2,4-DNP from sodium 2,4-DNP for an average of 88 days (Tainter et al. 1935b). The treatment regimen involved an initial dose of 1.2 mg/kg/day 2,4-DNP, usually for 1 week, increasing to

2.4 mg/kg/day for several weeks, then to 3.5 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 ≥3.5 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 5.27 mg/kg/day 2,4-DNP for 20 days, no skin lesions were observed (Grant and Schube 1934).

Ocular Effects. Cataracts developed in a small percentage of patients who took 2,4-DNP or sodium 2,4-DNP as a weight reduction aid for acute, intermediate, and chronic durations; 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 2,4-DNP was withdrawn from medical use. Representative case reports that provided doses at which cataracts developed are recorded in Table 2-1. These doses ranged from 1.86 to 4.29 mg/kg/day 2,4-DNP. 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. 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 1.86 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 4.0 mg/kg/day 2,4-DNP from sodium 2,4-DNP for an average of 88 days was 1 of 170 (Tainter et al. 1935b); 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 to study 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 cornea1 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 cornea1 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 to 420 mg/kg/day 2,4-DNP in the feed did not develop cataracts (Tainter 1938). The authors indicated, however, that these rats failed to eat or grow and all died between days 5 and 94 of treatment. 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, as discussed in Section 2.4 (Ocular Effects), 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 B_2-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). However, in a later study, guinea pigs were fed a vitamin C-deficient diet, while control animals received a vitamin C-deficient diet plus injection of 2 mg of ascorbic acid; both groups were exposed to ≈40 mg/kg/day 2,4-DNP in their diets for 4-57 consecutive days (Ogino and Yasukura 1957). In the test group, 4, 13, and 17 consecutive days of exposure produced cataracts in each of 3 animals. No cataracts were observed in control animals receiving the same dose of 2,4-DNP for 28-57 consecutive days. The author concluded that in guinea pigs there is a clear relationship between vitamin C deficiency and 2,4-DNP-induced cataracts (Ogino and Yasukura 1957). As discussed in Section 2.8.3, antioxidants, such as vitamin C and vitamin E, seem to have a protective effect against radiation-induced and senile cataracts.

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 1986b); 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 (Robbins 1944; Horner 1942). 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 l-l.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 and body weight 16-30 days and 400-800 grams) 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. A study in mice indicated that peak blood levels of metabolites of 2,4-DNP (2-amino-4-nitrophenol and 4-amino-2-nitrophenol) were reached within the first half-hour after dosing, indicating rapid metabolism (Robert and Hagardorn 1985) (see Section 2.3.4). However, ducks and mice are not closely related species, and no data were located regarding the metabolism of 2,4-DNP in ducks, so it is unknown whether metabolism in ducks was complete before the 30-minute time point. Ducks exposed to 0.25% sodium salt of 2,4-dinitro-

phenol in the diet all developed bilateral cataracts after 1 day of treatment (Spencer et al. 1948). Dose levels were not reported in this study, and no standard reference values of daily intake were available for these species (EPA 1986b); therefore, doses were not calculated.

The species that are sensitive and insensitive to the cataractogenesis of 2,4-DNP appear to be the same as those sensitive and insensitive to the cataractogenesis of other agents (see Section 2.4 on Ocular Effects).

Body Weight Effects. Weight loss has been reported in humans in studies of acute- and intermediate-duration. 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 3.53 mg/kg/day 2,4-DNP for 7-16 days, the average weight loss during 2,4-DNP treatment was ≈2 pounds (0.92 kg) (Cutting and Tainter 1933). The type of diet did not appear to influence the degree of weight loss. Thirty-seven obese patients who took sodium 2,4-DNP at 1.2 mg/kg/day 2,4-DNP for an average of 14 days had an average weight loss of 0.43 kg/week (Tainter et al. 1935b). 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. Obese patients taking 4.3 mg/kg/day 2,4-DNP for 1-8 weeks lost an average of 0.80-1.14 kg/week on diets of 20 calories/kg body weight (MacBryde and Taussig 1935). This finding also applies to both acute and intermediate exposure. Schizophrenic patients with low pretreatment basal metabolic rates lost an average of 2.5 kg during 7 weeks of treatment with 3-4 mg/kg/day 2,4-DNP, even though some were given supplemental feedings to minimize weight loss (Looney and Hoskins 1934). The average weight loss for obese patients who took an average of 4.0 mg/kg/day 2,4-DNP for an average of 88 days was 0.64 kg/week, with an average total loss of 7.8 kg (Tainter et al. 1935b). These patients had not been losing weight at the time treatment began and were instructed to continue the same food intake as before treatment.

In an experimental study, a group of 20 obese patients was placed on a calorie-restricted diet and given 1.94 mg/kg/day 2,4-DNP (Bayer and Gray 1935). Within 51 days, 13 of the patients lost an average of 5.4 kg. Another group of 3 obese patients was given 2.35 mg/kg/day but failed to restrict their diets. Within 62 days, one of these patients lost 4.1 kg, but the other 2 gained weight. In an extensive clinical study, 59 patients were given 3 mg/kg/day 2,4-DNP as the sodium salt for 37-89 days (Simkins 1937a, 1937b). Twelve of these patients lost no weight, but 47 lost an average of 5 kg at an average rate of 0.95 kg/week. In a compilation of case reports, 19 of 27 patients taking

an average of 3.6 mg/kg/day 2,4-DNP lost an average of 17 kg over periods of 1-18 months (Whalman 1936). The average weight loss in 13 psychiatric patients given an indeterminate dose of sodium 2,4-DNP for 3-4 months was 0.42 kg/week (Masserman and Goldsmith 1934). A number of individual case reports have also described substantial weight losses in patients taking 2,4-DNP. Doses ranged from 1.03 to 3.99 mg/kg/day, and durations ranged from 21 days to 18 months (Beinhauer 1934; Epstein and Rosenblum 1935; Goldman and Haber 1936; Horner et al. 1935; Nadler 1935).

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). An 18% decrease in body weight gain was observed in rats exposed to 350 mg/kg/day 2,4-DNP for 30 days (Bakke and Lawrence 1965). Food consumption was not reported in these studies; however, the observed body weight loss was probably related to starvation, in addition to inability to use body fuel reserve efficiently. 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 death were observed in weanling rats exposed to 0.2% 2,4-DNP (350 mg/kg/day) in the diet (Spencer et al. 1948). Food consumption and body weight gain in rats exposed to 59 mg/kg/day 2,4-DNP in the diet for 4 weeks were similar to those of controls (Kaiser 1964). After 24-26 days of treatment with 110 mg/kg/day 2,4-DNP in the diet, rats' body weights were 70% of their initial body weights; the author indicated that food consumption was normal (Pugsley 1936). Decreased body weight gains of 9% and 17% were observed in rats exposed for 6 months to 25 and 50 mg/kg/day, respectively (Spencer et al. 1948); food consumption was not reported in this study, and doses were estimated using standard reference values for food intake by rats (EPA 1986b). No change in body weight was observed in 3 male dogs fed 10 mg/kg/day in capsules for 6 months (Tainter et al. 1934b). Rats exposed in the diet to 30, 40, and 60 mg/kg/day 2,4-DNP for their lifetime had final body weights 25% less than those of controls, while food consumption was similar to that of controls; no effect on body weights or food consumption were observed at doses ≤20 mg/kg/day (Tainter 1938).

Rats fed high doses of 2,4-DNP displayed a strong taste aversion, refused to eat, and quickly died (Spencer et al. 1948; Tainter 1938). In longer-term studies, significant decreases in body weight parameters without concurrent decreases in food consumption were observed in rats fed dietary 2,4-DNP at doses (30-110 mg/kg/day) similar in magnitude to those producing increased mortality in a lifetime study (60 mg/kg/day) (Pugsley 1936; Spencer et al. 1948; Tainter 1938). In the animal

studies, body weight parameters generally were not affected by long-term exposure to dietary doses ≤20 mg/kg/day. In studies where food consumption was not decreased by dietary 2,4-DNP, body weight loss probably resulted from an increase in basal metabolic rate, as a result of uncoupling of oxidative phosphorylation by 2,4-DNP.

Two dogs repeatedly fed capsules containing 5, 10, 15, 17.5, or 20 mg/kg/day 2,4-DNP, with "recovery periods" of ≈5 days between doses, did not have changes in body weight (Tainter and Cutting 1933b).

In a study involving female chickens fed 2,4-DNP-containing feed from age 7 to 20 days, body weight gain (which increased from 127 grams to 560 grams in control animals) was not significantly affected at doses of 16.5 and 36.3 mg/kg/day (Toyomizu et al. 1992). At 77.9 mg/kg/day 2,4-DNP, body weight gain was decreased approximately 12%. Feed consumption was slightly increased at 16.5 mg/kg/day, but was not significantly affected at doses of 36.3 and 77.9 mg/kg/day. Feed efficiency (grams of body weight gained divided by grams of feed consumed) was unaffected at 16.5 and 36.3 mg/kg/day, but fell from 65% in control chicks to 59% in chicks consuming 77.9 mg/kg/day. Total body fat as a percentage of body weight was unaffected at 16.5 mg/kg/day, but fell from 12.7% in control chicks to 10.2% in chicks treated at 36.3 mg/kg/day and 9.4% at 77.9 mg/kg/day. Total protein as a percentage of body weight was unaffected by 2,4-DNP treatment. The body weight of female bobwhite quail aged 22-26 weeks fed 2,4-DNP-containing feed for 8 days (Dominguez et al. 1993) was unaffected at a dose of 33.6 mg/kg/day, but fell approximately 13% in quail that consumed 56.1 mg/kg/day. These authors also reported no effect of 2,4-DNP on feed consumption except for a reduction on the first two 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. Control birds and birds consuming 33.6 mg/kg/day appeared normal in all respects.

Metabolic Effects. The characteristic effects of 2,4-DNP are elevation of the basal metabolic rate (often measured indirectly as oxygen consumption), elevation of body temperature and increased perspiration (humans). 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 oxygen consumed per unit time per square meter of body surface area. Surface area was estimated by an empirical formula that took into account the subject's height and weight. Data was obtained as liters O_2 consumed/hour/m^3. This value was compared to a standard table that gave average basal metabolic rates for sex and age groups from hundreds of determinations. Female metabolic rates were slightly lower than males, and metabolic rate declined with age. Basal metabolic rate was expressed as a percentage of the average value from the table; values of -10% to +10% were considered normal because of natural variability.

The threshold dose for elevation of basal metabolic rate has not been established for acute exposure of humans. In studies of 2,4-DNP with 1 obese patient per dose regimen, exposure to 1 mg/kg/day for ≈6 days resulted in an increase in basal metabolic rate of 12% (but this conclusion of the author is not readily verified by analysis of the figure displaying the data), single or repeated doses of 2 mg/kg/day resulted in increases of 25% to 27%, and repeated doses of 3 mg/kg/day in an obese patient with severe hypothyroidism (myxedema) resulted in increases of 35 to 42% (Dunlop 1934). The basal metabolic rate increased by 38% in a psychiatric patient who subsequently died after being given 2.66 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Masserman and Goldsmith 1934). In an experimental study, in which 4 volunteers were placed on various diets (balanced, high carbohydrate, high fat, or high protein) and given an average dose of 3.53 mg/kg/day 2,4-DNP for 7-16 days, increases in basal metabolic rates of 27 to 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 3.2 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 3.5 mg/kg/day 2,4-DNP from sodium 2,4-DNP for l-2 weeks (Cutting et al. 1934); 23% in subjects (most of whom had low (≤15%) pretreatment basal metabolic rates and obesity/ hypothyroidism) given 3.5 mg/kg/day 2,4-DNP from sodium 2,4-DNP 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 4.3 mg/kg/day 2,4-DNP for l-8 weeks and was maintained throughout the treatment period; pretreatment values were

not reported (MacBryde and Taussig 1935). An 82% increase in basal metabolic rate was measured in a patient who received 3.3 mg/kg/day 2,4-DNP over a period of 182 days (Epstein and Rosenbloom 1935). In one clinical study in which patients were treated with the sodium salt of 2,4-DNP at an average dose of 4.0 mg/kg/day 2,4-DNP for an average of 88 days, the estimated increase in basal metabolic rate corresponding to this average dose was 38% (Tainter et al. 1935b). Average increases in basal metabolic rate as a function of dose (with number of determinations in parentheses) were 0.2% (37) before treatment, 13% (1) at 1.2 mg/kg/day 2,4-DNP, 20% (10) at 2.3 mg/kg/day 2,4-DNP, 31% (16) at 3.5 mg/kg/day 2,4-DNP, 50% (16) at 4.7 mg/kg/day 2,4-DNP, and 56% (8) at 5.9 mg/kg/day 2,4-DNP. These values represent an average increase of ≈11% for each 100 mg (1.17 mg/kg) increase in dose (Tainter et al. 1935b). The number of patients tested per dose level and duration at each dose level was not specified. Whether the value at 1.2 mg/kg/day actually represents an increase is uncertain, because it represents only one determination in one patient, whose pretreatment basal metabolic rate was not reported. The data exhibit a dose-severity relationship. Another clinical study also reported an average increase in basal metabolic rate of ≈11% per each 100 mg/day increase in dose in 66 patients given an average dose of 3 mg/kg/day for 22-89 days (Simkins 1937a, 1937b). Although not clearly established, increases in basal metabolic rate of 10% or less do not appear to be toxicologically significant and are not considered adverse. However, increases of 10% to 29% result in increased body temperature that may be adverse, and increases of 30% or more may result in severe pyrexia, and hence represent a serious adverse effect. The LOAEL for this effect would appear to be l-l.2 mg/kg/day for both acute and intermediate exposure.

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 even at the lowest dosages, 1.0 mg/kg/day and 1.2 mg/kg/day 2,4-DNP; these symptoms and signs became much more severe at higher dosages. 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). In this fatal case, body temperature rose to 105.7 °F (40.9 °C) and was measured at 115 °F (46.1 °C) shortly after death. The pyretic effects of 2,4-DNP have been well documented in case reports and studies of people taking the drug for acute, intermediate, and chronic durations. In fatal cases, a body temperature of 102 °F (38.9 °C) was recorded just before death in a psychiatric patient who was given 2.66 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Masserman and Goldsmith 1934); a temperature of 101.8 °F (38.8 °C) and

profuse perspiration were found in a woman who died after taking 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days (Poole and Haining 1934); and profuse perspiration was noted on the day of death of a woman who took an indeterminate dose for 1 week (Lattimore 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). A group of 13 obese patients given 3.5 mg/kg/day for 4-12 days experienced sensations of warmth and excessive perspiration (Stockton and Cutting 1934). In an experimental study, in which 4 volunteers were placed on various diets (balanced, high carbohydrate, high fat, or high protein) and given an average dose of 3.53 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).

In a fatal case, a young girl who had' taken 1.03 mg/kg/day 2,4-DNP for 46 days had a body temperature of 105.6 °F (40.9 °C) before admission to a hospital (Goldman and Haber 1936). Side effects experienced by 23 obese patients taking 1.94 or 2.35 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 2.99-4.29 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).

Two dogs repeatedly fed capsules containing 5, 10, 15, 17.5, or 20 mg/kg/day 2,4-DNP, with "recovery periods" of ≈5 days between doses, had dose-related increases in body temperature, with increases of >l and 2 °C observed at dose levels of 15 and 20 mg/kg/day, respectively (Tainter and Cutting 1933b). The authors indicated that repeated dosing did not seem to affect the observed changes in temperature, implying that the dogs recovered completely between dosings. Dogs fed single doses of 20 and 30 mg/kg 2,4-DNP via capsules had average maximal increases in body temperature of 0.9 and 1.5 °C (Tainter and Cutting 1933a). 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 l0-12 (Gibson 1973).

Acute exposure to 10 mg/kg/day 2,4-DNP caused slight elevations in body temperatures of dogs; higher elevations in body temperature were associated with exposure levels of 15 mg/kg/day (0.9-1.2 °C) and 20 mg/kg/day (0.7-2.5 °C) (Tainter and Cutting 1933a, 1933b). The pyretic effect of 2,4-DNP is related to the heat produced following uncoupling of oxidative phosphorylation.

A 30-85% increase in oxygen consumption was observed in mice exposed to 110 mg/kg/day in the diet for 26 days (Pugsley 1936). 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 a recent study which focused on the effect of 2,4-DNP on metabolic rate (Dominguez et al. 1993), bobwhite quail hens were exposed to doses of 2,4-DNP of 33.6 and 56.1 mg/kg/day (as calculated from feed consumption) over an &day period. The birds were housed in respirometers designed to continuously monitor exchange of oxygen and carbon dioxide from which metabolic rates were estimated. Birds receiving 33.6 mg/kg/day had dark period (nighttime) metabolic rates 31-41% higher than corresponding control values and light period (daytime) metabolic rates 23-32% higher than controls. Birds receiving 56.1 mg/kg/day had dark period metabolic rates 48-77% higher than controls and light period metabolic rates 41-67% higher than controls. It was calculated that at the 33.6 mg/kg/day dose, the birds expended 32% more energy over 8 days than controls, and at the 56.1 mg/kg/day dose, the birds expended 60% more energy than control birds. When the birds in this study were returned to normal feed, their metabolic rates returned to normal.

Other Systemic Effects. 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 drumheads,

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.

2.2.2.3 Immunological and Lymphoreticular Effects

Eight cases of agranulocytosis were reported in people who took 2,4-DNP as a diet pill. These reports are discussed in the Hematological Effects section of Section 2.2.2.2.

Whether the dermal effects (urticarial and macropapular rashes discussed in Section 2.2.2.2) of 2,4-DNP are related to sensitization is unclear. As described in Section 2.2.2.2, Dermal Effects, a woman who had been taking 2.32 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 authors, however, did not comment on whether they considered her condition to be due to sensitization. Twelve subjects whose patch, scratch, and intradermal tests for sensitivity to the sodium salt of 2,4-DNP 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. 1935b). 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 (3 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.

2.2.2.4 Neurological Effects

2,4-DNP appears to affect both the central and peripheral nervous systems. A woman who took 4.4 mg/kg/day 2,4-DNP complained of headache and weakness within a few hours after taking the first dose (Dintenfass 1934). These symptoms became more severe and, after the fourth dose, she experienced extreme fatigue and extreme dizziness. In a fatal case, a woman who took 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days complained of headache, weakness, and dizziness (Poole and Haining 1934). She became comatose on the way to the hospital and subsequently died. Autopsy revealed hyperemia of the spinal cord, pons, and medulla, slight degeneration of ganglion cells in the pons, and capillaries distended with blood. A psychiatric patient given 2.66 mg/kg/day 2,4-DNP as the sodium salt for 14 days became confused, torpid, stuporous and comatose, and subsequently died (Masserman and Goldsmith 1934). Because autopsy was delayed for 4 days, the histological examination was inconclusive due to autolysis. A young girl who died after taking 1.03 mg/kg/day 2,4-DNP for 46 days initially complained of weakness and malaise, became delirious and occasionally euphoric, and showed diminished knee-jerk reflex (Goldman and Haber 1936). She was admitted to the hospital 2 days later, where she became unconscious and died. Autopsy revealed no pathological changes in the cortex, medulla, cerebellum, pons, or proximal portion of the spinal cord. A patient taking 3.97 mg/kg/day 2,4-DNP for 35 days complained of headache and extreme malaise, and a patient taking an undetermined dose of 2,4-DNP intermittently for 1 year was semiconscious and occasionally irrational (Imerman and Imerman 1936).

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 listlessness, indifference, mild depression, or lethargy (Masserman and Goldsmith 1934). No psychological change was found for 8 patients, lethargy and depression increased in severity in 4 patients, while alertness increased and depression decreased in 6 patients. Discontinuation of the drug resulted in marked retrogression in three of the 6 patients that benefitted from the treatment.

No symptoms of peripheral neuritis were reported by 37 patients who took the sodium salt of 2,4-DNP at an estimated dose of 1.2 mg/kg/day 2,4-DNP for an average of 14 days (Tainter et al. 1935b). 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. 1935b). The treatment regimen involved an initial dose of 1.2 mg/kg/day 2,4-DNP with small

increases over time, as noted in the discussion of Dermal Effects in Section 2.2.2.2. The neurological effects occurred only among the 100 patients who took ≥3.5 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 4.3 mg/kg/day 2,4-DNP for l-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 fifth to seventh 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 1.86 to 3.53 mg/kg/day, and durations ranged from 10 days to several months (Anderson et al. 1933; Bortz 1934; Epstein and Rosenblum 1935; Hitch and Schwartz 1936; Hunt 1934; Nadler 1935). Pathological examination of the brain and spinal cords of dogs who received 20 mg doses of 2,4-DNP/kg periodically over 45-77 days (7-12 times) showed "no damage" (Tainter and Cutting 1933b).

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). The lower dose (25.5 mg/kg/day) apparently did not produce maternal toxicity. Dogs (3 per dose group) exposed via capsules 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 et al. 1934b). The highest NOAEL value and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-1.

2.2.2.5 Reproductive Effects

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 2.32 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 patient who had been taking 3.3 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, she was hospitalized for profuse vaginal bleeding, and no evidence of a fetus was found. The authors concluded the 2,4-DNP caused a premature separation of the placenta, resulting in miscarriage. In an extensive clinical study of 159 patients taking 3 mg/kg/day 2,4-DNP as the sodium salt for 22-89 days, altered menstrual cycles or amenorrhea developed in 15 of an unspecified number of women (Simkins 1937a, 1937b). In addition, 18 of an unspecified number of women experienced excessive menstrual edema. The author noted that most menstrual disorders common in obese women can be corrected by proper diet, but because the menstrual changes in many of the women in the study were so marked and occurred so soon after 2,4-DNP dosing (i.e., before any significant weight loss), he concluded that the altered menstrual cycles were due to a direct action of 2,4-DNP.

No gross or histological evidence of treatment-related testicular 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 (3 per dose group) exposed via capsules to 5 or 10 mg/kg/day for 6 months (Tainter et al. 1934b). However, 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.

The NOAEL values and all LOAEL values in each reliable study for reproductive effects in each species for intermediate duration are recorded in Table 2-l and plotted in Figure 2-1.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to 2,4-DNP.

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, then sacrificed on gestation day 19 for evaluation of developmental toxicity (Gibson 1973). The study measured important developmental toxicity end points, including teratogenicity. The authors indicated that the high dose produced overt toxicity (hyperexcitability and hyperthermia) in dams (data not presented), but no deaths. The percentage resorption increased, but

the effect was not significant or dose-related; no other abnormalities were observed. Thus, treatment of mice at a dose level producing maternal toxicity did not result in adverse developmental effects (Gibson 1973). This study is limited because dosing only occurred during the first part of organogenesis. In another study, CD-l mice were treated by gavage with 125 mg/kg/day 2,4-DNP on gestation days 8-12 and were sacrificed following birth of the offspring to determine percent resorptions (Kavlock et al. 1987). Maternal body weight change was not significantly ($p>0.05$) different from controls. Two dams each died in the control (40 mice) and treatment groups (30 mice). The percent of live litters and resorptions and the numbers and weights of live offspring on postnatal days 1 and 3 were not significantly different ($p>0.05$) from controls. Teratogenicity was not evaluated. This study was designed as a screening test, so that numerous compounds could be evaluated for developmental toxicity at low cost. Thus, there were limitations in the study, including exposure during only a portion of organogenesis, measurement of a small number of end points, and no discussion of the results; potential reasons for the high mortality rate in the control group were not discussed. In a study of white rats that were dosed for 8 days prior to mating, during pregnancy, and during lactation (Wulff et al. 1935), it was not clear 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. Furthermore, this study did not investigate teratogenic end points, and no statistical analysis of data was performed. Despite these limitations, the data indicate that 2,4-DNP is fetotoxic in rats. 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. The mortality rate for pups during lactation was 30.9% for the treated rats and 13.4% for the control groups; thus, the number of young reared per litter was less (3.01) for rats dosed with 2,4-DNP than for the control groups (5.07).

The NOAELs for developmental effects in mice are recorded in Table 2-1 and plotted in Figure 2-l.

2.2.2.7 Genotoxic Effects

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

Some chemical mutagens and carcinogens bind covalently to deoxyribonucleic acid (DNA) and inhibit DNA synthesis. DNA synthesis (as determined by rate of uptake of tritiated thymidine given as a 30-minute pulse, 3.5 hours after drug administration) was measured in testicular cells of male Swiss mice treated once by gavage with 0 or 20 mg/kg 2,4-DNP (Friedman and Staub 1976). The rate of

DNA synthesis in testicular cells was essentially the same in treated and untreated mice. The authors concluded that 2,4-DNP was not genotoxic under these experimental conditions. 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 ^{14}C-thymidine injected 16 hours before drug administration) was measured in testicular cells of mice treated by gavage with a single dose of 0 or 30 mg/kg 2,4-DNP (Seiler 1981). The rate of DNA synthesis in testicular cells of mice treated with 2,4-DNP was 55% less than that of untreated mice. Based on further *in vitro* experiments, the author claimed that the inhibition of DNA synthesis by 2,4-DNP was due to some other mechanism than genotoxicity. It is likely that the 2,4-DNP-induced decrease in DNA synthesis *in vivo* resulted from the effects of 2,4-DNP on energy-dependent cellular processes in testicular cells, rather than from a genotoxic effect.

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to 2,4-DNP.

2.2.3 Dermal Exposure

Two studies involving occupational exposure to 2,4-DNP are discussed under Inhalation Exposure; exposure may have occurred by the dermal as well as the inhalation routes. Studies of dermal exposure to 2,4-DNP in animals are limited to one lethality study, two dermal irritation studies, and two initiation-promotion studies.

No studies were located regarding health effects in humans or animals after dermal exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to 2,4-DNP.

Guinea pigs were exposed dermally to l00-1,000 mg/kg 2,4-DNP for 4 hours (Spencer et al. 1948). No mortality was observed at 100 or 200 mg/kg. The lowest dose causing mortality was 300 mg/kg (20%), and the lowest dose causing 100% mortality was 700 mg/kg.

The LOAEL value for death in guinea pigs is recorded in Table 2-2.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, ocular, body weight, or metabolic effects in humans or animals after dermal exposure to 2,4-DNP.

Dermal Effects. No studies were located regarding dermal effects in humans after dermal exposure to 2,4-DNP.

Studies regarding 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.

The LOAEL values for dermal irritation in rabbits are recorded in Table 2-2.

TABLE 2-2. Levels of Significant Exposure to Dinitrophenol - Dermal

Species/ (Strain)	Exposure/ Duration/ Frequency/	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
ACUTE EXPOSURE						
Death						
Gn pig (NS)	once 4 hr				300 mg/kg (1/5 died)	Spencer et al. 1948
Systemic						
Rabbit (NS)	6x	Derm		4% (moderate hyperemia, edema, and denaturation)		Dow Chemical Co. 1940
INTERMEDIATE EXPOSURE						
Systemic						
Rabbit (white)	4 wk 5 d/wk	Derm		3% (mild hyperemia, edema, and exfoliation of skin)		Spencer et al. 1948

d = day(s); Derm = dermal; Gn pig = guinea pig; hr = hour(s); LOAEL = lowest-observable-adverse-effect level; NOAEL = no-observable-adverse-effect level; wk = week(s); x = times.

2.2.3.3 Immunological and Lymphoreticular Effects

Information on immunological effects in humans after dermal exposure to 2,4-DNP is limited. Three methods of skin testing were performed on 157 people, 117 of whom were patients with hay fever, asthma, or urticaria (Matzger 1934). For the patch test, 10 mg of sodium 2,4-DNP was applied to the forearm or back under waxed paper. For the scratch test, 2 mg sodium 2,4-DNP in saline or 2 drops of a 2% aqueous solution was rubbed on a scarification. For the intradermal test, 0.01-0.02 mL of 0.001%, 0.01%, 0.1%, or 1% sodium 2,4-DNP was introduced in the upper arm. In the indirect or passive transfer test, blood serum from a patient with a violent clinical reaction to DNP was introduced intradermally in nonallergic subjects. After 24 hours, the sites of passive transfer were tested intradermally with 2,4-DNP. The direct tests and the passive transfer test were negative.

No studies were located regarding immunological effects in animals after dermal exposure to 2,4-DNP.

No studies were located regarding the following health effects in humans or animals after dermal exposure to 2,4-DNP:

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding cancer in humans after dermal exposure to 2,4-DNP.

Female Sutter mice (initial age 2-3 months) received a single initiating dose of 0.3% 9,10-dimethyl-1,2-benzanthracene (DMBA; 25 μL) in acetone applied to a shaved area of the back (Boutwell and

Bosch 1959). Then, 281 mg/kg 2,4-DNP (25 µL of solution containing 20% 2,4-DNP in acetone) was applied to the same area twice weekly for 12 weeks (time-weighted average [TWA] dose = 80 mg/kg/day). The survival rate was 100%. No evidence of skin papillomas or carcinomas was observed. In animals similarly treated with phenol, 13% mortality was observed, and 58% and 5% of the survivors had papillomas and carcinomas, respectively. Treatment with phenol alone also resulted in the development of skin tumors, but at a slower rate and a lower incidence. A single application of DMBA did not result in skin tumors. The authors concluded that the introduction of nitro groups into the phenol ring destroyed the promoting effect of phenol, and that 2,4-DNP was not effective as a tumor promotor. 2,4-DNP was not tested as an initiator.

A series of skin-painting experiments was done on female Swiss mice (initial weight 20-25 grams), with compounds applied to a shaved area of the back (Stenback and Garcia 1975). In the first experiment, 10 µg of 7,12-dimethylbenz(a)anthracene (DMBA) in acetone was applied to the back twice a week for 30 weeks. Skin tumors developed in 23 of 30 mice (18 of 30 had papillomas and 12 of 30 had squamous cell carcinomas). Similar treatment with acetone alone resulted in no skin tumors. Thus, DMBA was a complete carcinogen in this experiment. In other experiments, a single dose of 100 µg DMBA was applied to the back, followed by application of other compounds or a combination of other compounds twice a week for 50 weeks. In the experiments relevant to 2,4-DNP, papillomas occurred in 6 of 30 mice (20%) receiving DMBA followed by 2,4-DNP plus croton oil; 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. Croton oil, which contains a variety of phorbol esters, is a known promoter. No squamous cell carcinomas were induced by any of these treatments. The experiment did not include a group treated with DMBA followed by 2,4-DNP alone. However, since the incidence of papillomas was essentially no different or lower in mice receiving DMBA followed by 2,4-DNP and acetone, compared with mice receiving DMBA followed by acetone alone, and in mice receiving DMBA followed by 2,4-DNP and croton oil, compared with mice receiving DMBA followed by croton oil alone, 2,4-DNP did not appear to be a promoter for DMBA. 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. Thus, 2,4-DNP had no significant influence on DMBA initiation of tumors promoted by croton oil.

2.3 TOXICOKINETICS

The toxicokinetics of 2,4-DNP in humans and animals have not been studied systematically. The available data from human case reports and experimental animal studies indicate that 2,4-DNP is readily absorbed by the oral and inhalation routes, and possibly by the derrnal route. Some evidence about distribution is available suggesting that a portion of the 2,4-DNP in the blood is bound to serum proteins and that the unbound fraction enters organs such as the eye. 2,4-DNP is rapidly metabolized via reduction of the nitro groups; the parent compound and metabolites are excreted in the urine.

2,4-DNP is an uncoupler of oxidative phosphorylation. In humans or animals exposed to 2,4-DNP, the energy produced from the Krebs cycle is not stored in adenosine triphosphate (ATP), but is released as heat. This short-circuiting of metabolism results in the characteristic clinical signs of increased basal metabolic rate, oxygen consumption, perspiration, and body temperature. Elevated environmental temperatures may compromise the body's ability to dissipate the heat.

Limited toxicokinetic and mechanism data for the other DNP isomers indicate that these isomers also uncouple oxidative phosphorylation but that, with the exception of 2,6-DNP, they are eliminated much more rapidly than is 2,4-DNP.

2.3.1 Absorption

No studies were located regarding absorption in humans or animals after any route of exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

2.3.1.1 Inhalation Exposure

No quantitative data were located regarding absorption in humans after inhalation exposure to DNPs. A metabolite of 2,4-DNP, 2-amino-4-nitrophenol, was commonly detected by the Derrien test in the urine of workmen (women were generally not employed in dangerous processes) exposed via inhalation to vapor and airborne dust of 2,4-DNP and by direct contact of the skin with the solid chemical in the munitions industry in France (Perkins 1919). Exposure may have occurred by the dermal and possibly oral routes, as well as by inhalation. In addition, examination of the blood, unspecified organs, and urine of workmen in this industry who died from exposure to 2,4-DNP

revealed the presence of 2,4-DNP and its metabolites; quantitative data were not provided (Perkins 1919). Despite its limitations, the study provides some evidence of absorption from inhalation exposure.

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). Workroom air levels of 2,4-DNP, determined subsequent to the death, were "normally" ≥40 mg/m^3. This may underestimate breathing-zone levels, and significant dermal absorption was thought to have occurred following deposition of the chemical on the skin. Hence, no conclusions regarding fraction absorbed can be drawn from this study, but it does provide additional evidence of absorption from the inhalation route.

No studies were located regarding absorption in animals after inhalation exposure to 2,4-DNP.

2.3.1.2 Oral Exposure

The data regarding absorption in humans after oral exposure are limited. 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-dinitrophenol and estimation of the total body burden, assuming the drug was evenly distributed between blood and tissues, gave a body burden of ≈2.72 grams 2,4-DNP, which corresponds to 3.31 grams of the sodium salt of 2,4-dinitrophenol 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 additional information is provided by animal studies. 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 (Robert and Hagardom 1983). A highly specific capillary gas chromatography (GC)-mass spectrometry (MS) technique was used to measure serum concentrations of 2,4-DNP at 1, 3, 6, 12, and 24 hours after dosing. The data were best represented by a two-compartment open model, and the analysis was performed accordingly. Fractional absorption was not determined. In an additional 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, 1, 2, 4, 6, 9, 12, 24, 48, and 96 hours following a single gavage dose of 22.5 mg/kg (Robert and Hagardom 1985). Maximum values for the plasma concentration of 2,4-DNP were seen at 0.5 and 1.0 hours after dosing, giving additional evidence of rapid absorption.

The time course of plasma concentrations of 2,4-DNP following oral administration to dogs (1 per dose) at 5, 12.5, 25, or 125 mg/kg indicated substantial absorption by the first sampling period, 0.5 hours (Kaiser 1964). Peak plasma levels were attained in 0.5-4 hours after dosing. In general, plasma levels tended to be higher in dogs that received higher doses. The 24-hour urine collections were analyzed for 2,4-DNP but not for metabolites; results were highly variable, raising the suspicion that collection may have been incomplete in some instances. Hence, the excretion data do not provide a reliable estimate of the fraction of the dose absorbed.

2.3.1.3 Dermal Exposure

The 1917 and 1918 records from two French munitions factories show that the percentage of positive Derrien tests (for the metabolite 2-amino-4-nitrophenol) tended to rise during the warm months of the year (Perkins 1919). One of these factories also provided records of the percentage of clinical cases, which, although much lower than the percentage of positive Derriens, roughly paralleled the Derrien results. The increases in percentages of positive Derrien tests and clinical cases during the warmer months provide support for the idea that dermal absorption may be an important route of entry to the body, since greater exposure of the skin would be expected during the warmer months. Alternatively, the higher ambient temperatures may have caused greater loss of body water through sweating, resulting in more concentrated urine, which could result in a greater percentage of positive Derrien tests. The higher ambient temperatures would also tend to exacerbate the effects of 2,4-DNP.

Similarly, the details of two fatal cases of 2,4-DNP in the U.S. chemical industry suggest that dermal absorption may have been a contributing factor. Two workers exposed to mists and dust of 2,4-DNP for a few months developed signs of toxicity; following treatment and rest, and then a return to the job during warmer weather, they 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; it may also have exacerbated the effects by contributing to hyperpyrexia and increased pulse produced by 2,4-DNP.

No studies were located regarding the rate or extent of absorption in animals after dermal exposure to 2,4-DNP. However, the death of 1 of 5 guinea pigs dermally exposed to 300 mg/kg (Spencer et al. 1948) suggests that dermal absorption occurred.

2.3.2 Distribution

No studies were located regarding distribution in humans or animals after any route of exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

2.3.2.1 Inhalation Exposure

Examination of the blood and unspecified organs of workmen who died from exposure to 2,4-DNP in the munitions industry in France revealed the presence of 2,4-DNP and its metabolites (Perkins 1919), indicating distribution to the tissues. Analysis of the organs (not specified) of two workmen who died following exposure to 2,4-DNP in the U.S. chemical industry, however, did not demonstrate the presence of the chemical or metabolites, although 2,4-DNP and 2-amino-4-nitrophenol were detected in the urine of one worker (urine was not tested in the other case) (Gisclard and Woodward 1946). Because one report (Perkins 1919) did not provide details of extraction and analytical methods, reasons for the discrepancy in results cannot be determined. In both studies, exposure may have occurred by the dermal as well as the inhalation routes.

No studies were located regarding distribution in animals after inhalation exposure to 2,4-DNP.

2.3.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to 2,4-DNP.

Limited information is available regarding distribution in animals after oral exposure to 2,4-DNP. 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 Hagardorn 1983). Serum and tissue levels of the parent compound were quantitated by a highly specific capillary GC-MS method at l-24 hours postdosing. Although concentrations of 2,4-DNP were much lower in liver and kidney than in serum, similar half-times for absorption ($t_{1/2}$ = 0.50-0.62 hours) and for the fast (alpha) ($t_{1/2}$ = 1.00-1.20 hours) phase of biphasic elimination in all 3 tissues were determined. However, terminal (beta) elimination phase from kidney was very slow ($t_{1/2}$ = 76.2 hours) compared with the beta phase in liver ($t_{1/2}$ = 8.7 hours) and in serum ($t_{1/2}$ = 7.7 hours). The similar half-times for absorption and biphasic elimination in all three tissues (except terminal elimination phase in kidney) indicated that rapid exchange of 2,4-DNP occurred among these sites. 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 (1 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.

2.3.2.3 Dermal Exposure

Distribution data from occupational exposure studies of 2,4-DNP are discussed in Section 2.3.2.1.

No studies were located regarding distribution in animals after dermal exposure to 2,4-DNP.

2.3.2.4 Other Routes of Exposure

In coordination with toxicity studies (Gehring and Buerge 1969a) (see Section 2.4, 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). Accordingly, animal models of higher (ducklings) and intermediate (immature rabbits) susceptibility were compared with those of low susceptibility (mature rabbits). 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 types. The concentration of 2,4-DNP in the ocular compartments appears to be more important than the elimination rates in determining susceptibility of 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 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 mature rabbits. Since the immature rabbits and ducklings are susceptible to 2,4-DNP-induced cataracts while mature rabbits are not, this result shows a correlation between local concentration of 2,4-DNP and cataract formation.

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. The ducklings eliminated 2,4-DNP from eye compartments more rapidly, however, than did immature rabbits, which may account for the faster disappearance of cataracts in ducklings than in immature rabbits. The results of this study indicate that differences in sensitivity among these three animal types may be attributable to the attainment and maintenance of higher levels of 2,4-DNP in the eye in immature rabbits than in mature rabbits, with ducklings intermediate to the other two. Although this study provides some evidence that 2,4-DNP may be the cataractogenic agent, the study did not address the possible role of metabolites in cataractogenesis.

2.3.3 Metabolism

No studies were located regarding metabolism in humans or animals after any route of exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

Some information regarding metabolism of 2,4-DNP in humans is available from cases of occupational 2,4-DNP poisoning and from a case involving ingestion of the chemical as a diet pill. The limitations of these studies include the relative lack of specificity of the methods employed to detect or quantify 2,4-DNP and its metabolites. 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 compounds found in urine were 2,4-DNP, 2-amino-4-nitrophenol, 4-amino-2-nitrophenol, 2,4-diaminophenol, and other unidentified nitrogen compounds, which may have been glucuronide conjugation products (NRC 1982). In cases of serious 2,4-DNP poisoning, the presence of large amounts of 2-amino-4-nitrophenol in the urine were found and were the basis of a-particular test called the Derrien test, used as an indicator of exposure to 2,4-DNP. This test is a color reaction for aminonitrophenols, with the formation of a characteristic red wine to violet color for 2-amino-4-nitrophenol and a more or less yellow-orange color for 4-amino-2-nitrophenol constituting a positive test. Quantitation apparently was limited to visual inspection for color intensity. The test is not highly specific, as picramic acid also yields a red wine to violet color. The test cannot detect diaminophenols because the ability to produce the colored reaction depends on the NO_2 group in aminonitrophenols. Mild cases of intoxication produced positive Derrien tests; when the urine gave a positive Derrien that increased in intensity day by day or remained fairly high, acute intoxication frequently developed. The percentage of positive Derriens did not appear to correlate with race (“white, yellow, black”) (Perkins 1919).

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 2,4-DNP and 50 mg/L of 2-amino-4-nitrophenol (Gisclard and Woodward 1946).

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).

Additional information regarding metabolism of 2,4-DNP is available from studies in animals. 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). The total dose given (probably to all the rabbits combined) was 30 grams (30,000 mg), and the total amount of 2,4-diaminophenol isolated from 10 liters of urine (presumably collected from all the dosed rabbits throughout the study) was 50 mg from 20 liters of urine. Hence, the yield of this urinary metabolite was 0.17% of the dose. The metabolite was extracted from the urine, purified, and identified by its properties, including melting point, nitrogen analysis, absorption curve, and various color reactions. Limitations of the study include the lack of adequate reporting of dose, route, and number of animals, relative lack of specificity in the identification methods available at the time, and the lack of experiments to quantitate losses of metabolite during the extraction and purification processes. Accordingly, only tentative conclusions regarding the identity of the metabolite as 2,4-diaminophenol and the percentage of the dose metabolized to this metabolite can be drawn from this study.

2,4-DNP and two of its metabolites, 2-amino-4-nitrophenol and 4-amino-2-nitrophenol, were monitored in plasma of mice at 0.5, 1, 2, 4, 6, 9, 12, 24, 48, and 96 hours following a single gavage dose of 22.5 mg/kg using a highly specific capillary GC/MS technique (Robert and Hagardom 1985). The authors concluded that the amount of 2-amino-4-nitrophenol formed 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. Plasma concentrations of these two metabolites reached their highest levels within the first half hour after dosing, indicating rapid metabolism. The results demonstrate that 2-amino-4-nitrophenol is the major circulating metabolite of 2,4-DNP and that 4-amino-2-nitrophenol is also a significant circulating metabolite. The authors mentioned that they could not analyze for 2,4-diaminophenol because of its reactivity with oxygen to form reactive quinones that could not be extracted and chromatographed by their method.

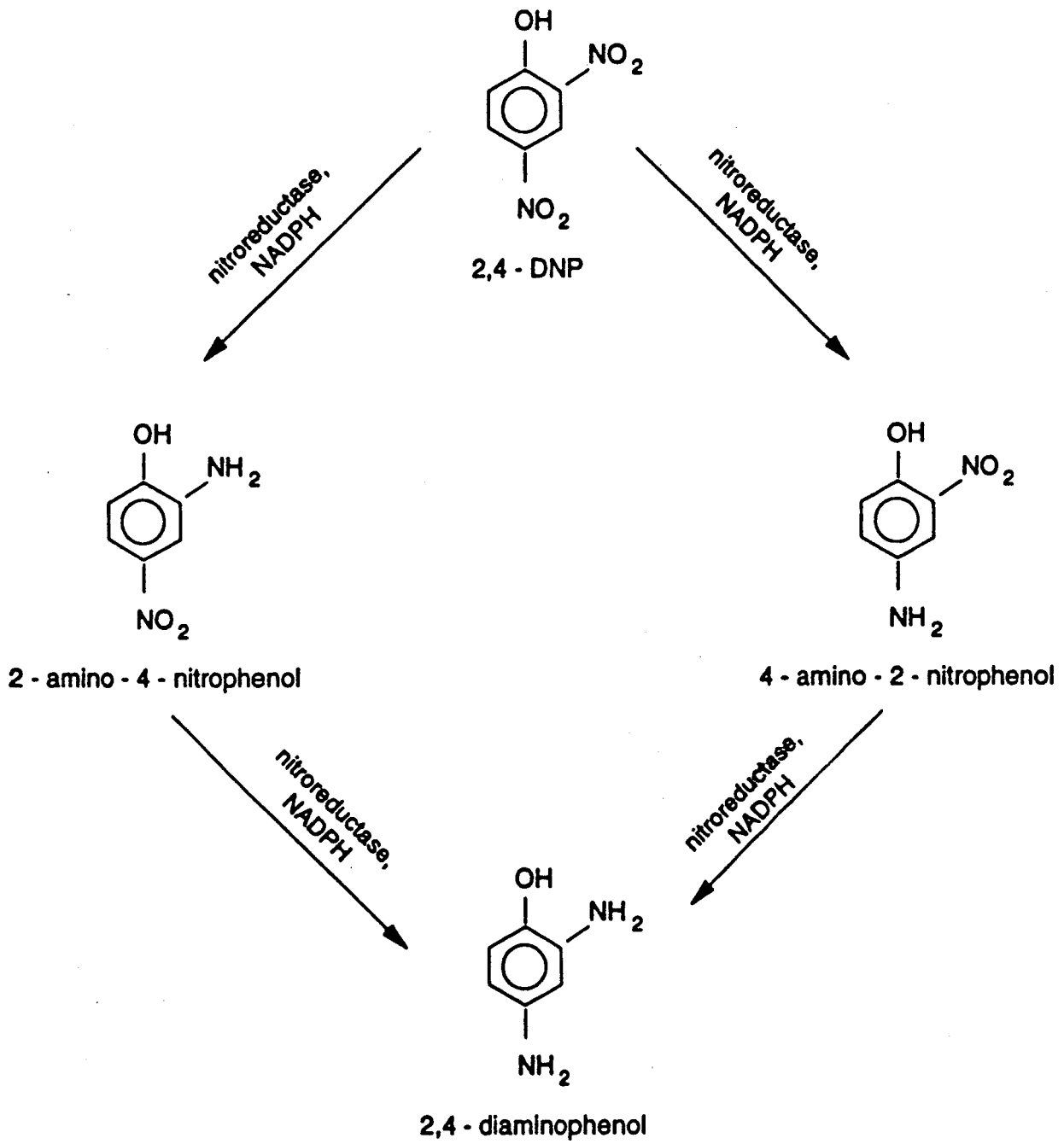
Analysis of urine for 2,4-DNP and its aminonitrophenol metabolites following a single subcutaneous injection of 20 mg/kg 2,4-DNP into rats revealed only parent compound and 2-amino-4-nitrophenol (Parker 1952). 4-Amino-2-nitrophenol was not detected. The urine was collected over the 24-hour period following dosing.

An *in vitro* study of 2,4-DNP metabolism using rat liver homogenates identified 2-amino-4-nitrophenol and 4-amino-2-nitrophenol as metabolic products, with the 4-amino-2-nitrophenol present in greater abundance (Parker 1952). An additional ether-insoluble metabolite was tentatively identified as 2,4-diaminophenol. When 2-amino-6nitrophenol 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 that liver homogenate metabolized 2,4-DNP at a higher rate than did other tissue homogenates. In the rat, enzyme activities (mg 2,4-DNP metabolized per 100 grams wet weight of tissue) of other tissue homogenates 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 activity was found in the blood of rats or rabbits.

A more 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 for 23%, and 2,4-diaminophenol for ≈1% of the total amine metabolites produced (Eiseman et al. 1972). Even under suboptimal conditions, 2-amino-4-nitrophenol was the predominant metabolite. The proposed metabolic pathway for 2,4-DNP is presented in Figure 2-2.

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 in the cytosol was enhanced by

FIGURE 2-2. Proposed Metabolic Pathway for 2,4-Dinitrophenol



Adapted from Eiseman et al. 1972; Parker 1952; Robert and Hagardorn 1985

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 $FADH_2$ or NADPH in the presence of oxygen (Kamm and Gillette 1963). Reduction of [^{14}C]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 be excreted in the urine (NRC 1982).

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

2.3.4 Excretion

No studies were located regarding excretion in humans after any route of exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

Limited information was located regarding excretion in animals after other routes of exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP and is discussed in Section 2.3.4.4.

2.3.4.1 Inhalation Exposure

In humans exposed to 2,4-DNP by inhalation, both the parent compound and metabolites appear to be excreted in the urine. 2,4-DNP and its metabolites have been detected in the urine of workmen who died from exposure to 2,4-DNP in the munitions industry in France; quantitative exposure or urinary

data were not provided (Perkins 1919). In addition, a yellow staining of the skin was observed after workers perspired profusely, indicating that 2,4-DNP was excreted in the sweat. As discussed in Section 2.3.1.1, a metabolite of 2,4-DNP, 2-amino-6nitropheno1, was commonly detected in the urine of such workmen; quantitative data were not provided (Perkins 1919). In a case of fatal occupational 2,4-DNP poisoning in the U.S., 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.

No studies were located regarding excretion in animals after inhalation exposure to 2,4-DNP.

2.3.4.2 Oral Exposure

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 of parent compound 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.

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 Hagardorn 1983). Serum and tissue levels of parent compound were quantitated by a highly specific capillary GC-MS method at l-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 an additional 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 Hagardorn 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 ($t_{1/2}$) 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.

In dogs (1 per dose) that received 1, 12.5, or 25 mg/kg/day 2,4-DNP, 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. Greater amounts of 2,4-DNP were excreted after the first than after subsequent doses. Excretion of metabolites was not investigated.

Species differences in elimination of 2,4-DNP do not appear to be large (less than two-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).

2.3.4.3 Dermal Exposure

Excretion data from occupational exposure studies of 2,4-DNP are discussed in Section 2.3.4.1.

No studies were located regarding excretion in animals after dermal exposure to 2,4-DNP.

2.3.4.4 Other Routes of Exposure

Analysis of urine 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 (Parker 1952). 4-Amino-2-nitrophenol was not detected. The urine was collected over the 24-hour period following dosing.

Absolute elimination rate constants for 2,4-DNP from blood in a limited study in rats, rabbits, guinea pigs, and mice were 0.122, 0.22, 0.135, and 0.21 $hours^{-1}$, respectively, following a single unspecified intraperitoneal dose of 2,4-DNP (Lawford et al. 1954).

In a limited study using single large intraperitoneal doses, half-times for elimination of the 6 dinitrophenol isomers, 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNP, were roughly estimated in rats at 12.5, 225.0, 13.0, 210.0, 11.5, and 2.1 minutes, and in mice, 2.7, 54.0, 3.3, 238.0, 3.5, and 2.7 minutes, respectively (Harvey 1959). The small number of sampling times, short duration of sampling, and the rough estimation of half-lives severely limit any conclusions that could be drawn from this study, except that 2,4- and 2,6-DNP appear to be eliminated more slowly by both species than the other isomers.

2.3.5 Mechanisms of Action

2,4-DNP appears to be readily absorbed from the respiratory and gastrointestinal tracts (Gisclard and Woodward 1946; Kaiser 1964; Perkins 1919; Robert and Hagardom 1983; Tainter and Wood 1934). Some evidence suggests significant absorption through the skin as well (Gisclard and Woodward 1946; Perkins 1919). 2,4-DNP is relatively lipophilic with a pKa of 4.09 (see Chapter 3), and therefore is likely to be rapidly absorbed by passive diffusion of the un-ionized form from acidic compartments like the stomach. Another factor favoring the rapid absorption of 2,4-DNP is its small molecular weight (184.1 daltons). Molecules with a molecular weight below 600 daltons can permeate cell membranes through aqueous channels (Benz et al. 1980) regardless of their ionization state.

Tissue analysis of cases of fatal occupational 2,4-DNP poisoning demonstrated the presence of 2,4-DNP and/or its metabolites in the organs and tissues (Perkins 1919). One oral study in animals demonstrated higher concentrations of 2,4-DNP and its metabolites in serum than in liver or kidney (Robert and Hagardom 1983).

A study in rabbits, with supporting *in vitro* experiments, suggests 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 than in immature rabbits. In addition, the mature rabbit eliminated 2,4-DNP more rapidly from semm 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 in the eye.

2,4-DNP appears to be metabolized to less toxic metabolites (primarily aminonitrophenols) that are excreted in the urine (Davidson and Shapiro 1934; Gisclard and Woodward 1946; Ogino and Yasukura

1957; Perkins 1919; Robert and Hagardorn 1985). The mechanism of excretion may be passive diffusion, in part, although there is some evidence from one *in vitro* study of active secretion of 2,4-DNP by the renal organic acid transport process (Bemdt and Grote 1968).

Limited information from a study in animals indicates that the 2,3-, 2,5-, 3,4-, and 3,5- isomers may be eliminated from the body much more rapidly than are 2,4- and 2,6-DNP (Harvey 1959).

2,4-DNP was demonstrated to be an uncoupler of oxidative phosphorylation in an *in vitro* study (Loomis and Lipmann 1948). 2,4-DNP prevented phosphorylation without affecting, or with a slight stimulation of, oxidation. During the Krebs cycle, 2,4-DNP uncouples oxidative phosphorylation from electron transport by carrying protons across the inner mitochondrial membrane, thereby dissipating the pH gradient and membrane electrochemical potential and preventing the formation of adenosine triphosphate (ATP) (Stryer 1988). During this uncoupling, electron transport from NADH to oxygen proceeds normally, but the energy produced, which is normally stored in high-energy phosphate bonds in ATP, is released as heat. The small amount of ATP produced directly from glycolysis is not affected. *In vitro* studies further demonstrated and investigated the ability of 2,4-DNP to uncouple oxidative phosphorylation (Ilivicky and Casida 1969; Muscatello et al. 1975; Pinchot 1967; Weinbach and Garbus 1969). 2,4-DNP increased the rate of oxygen uptake and changed the electron microscopic appearance of rat liver mitochondria *in vitro* from an expanded configuration to a condensed state, with the ultrastmctural change occurring in concert with the functional changes of uncoupling (Muscatello et al. 1975). All energy-dependent biochemical processes thus are likely to be affected. Many of the clinical observations of 2,4-DNP toxicity, such as elevated basal metabolic rate or oxygen consumption, elevated respiration and pulse rates, increased perspiration, increased body temperature in humans and animals are related to the uncoupling of oxidative phosphorylation. When heat production exceeds the organism's capacity to dissipate heat, fatal hyperthermia may result (Murphy 1986).

The uncoupling of mitochondrial electron transport from oxidative phosphorylation 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 (Trayhum 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).

Because 2,4-DNP uncouples oxidative phosphorylation but does not interfere with glycolysis in most tissues, one would not expect 2,4-DNP to affect lens metabolism. However, 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 some time after treatment is terminated and may not be reversible. This phenomenon has not been fully explained.

Other DNP isomers have also been demonstrated to uncouple oxidative phosphorylation in isolated rat liver mitochondria (Burke and Whitehouse 1967). The relative potencies of the DNPs in uncoupling were (in declining order): 3,5- > 2,4- > 2,6- ≈3,4- > 2,3- ≈2,5-DNP. This order is not congruent with limited acute toxicity data in animals (Harvey 1959), but in the whole animal, elimination and other processes may play a deciding role.

2.4 RELEVANCE TO PUBLIC HEALTH

DNPs are released to the environment primarily during their manufacture and use, and from waste disposal sites. They can also form in the atmosphere through chemical reactions, can be removed from air by dry or wet deposition on soil, and can leach into groundwater. Other than in workplace air, DNPs have not been measured in the ambient air. No monitoring data for drinking water in the United States are available. No regulations by the Occupational Safety and Health Administration (OSHA), recommendations by the National Institute for Occupational Safety and Health (NIOSH), or information regarding current workroom levels of DNPs were located. Their occurrence and levels in food or total diet have not been documented. People with potentially high exposures to DNPs are

occupational groups and people who live near factories that make or use them, or near hazardous waste sites that contain DNPs. 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. Another possible source of exposure is ingestion of contaminated soil by children. However, the extent of exposure for residents around waste sites has not been documented. The toxicity of 2,4-DNP has been shown to increase at high environmental temperatures; therefore, susceptibility to the toxic effects may be greater for workers at high workroom temperatures or in the general population at high environmental temperatures.

The available human and animal data suggest that 2,4-DNP is readily absorbed by the oral and inhalation routes. There is some evidence suggesting significant absorption through the skin as well. In the blood, 2,4-DNP is transported both free and bound to serum proteins. There appears to be no tendency to accumulate in the body, although a pre-existing condition of compromised liver function might lead to a diminished ability of the liver to metabolize 2,4-DNP to its less toxic, readily excreted metabolites (primarily aminonitrophenols), perhaps resulting in some retention. Data regarding 2,3-, 2,5-, 2,6-, 3,4-, and 3,5-DNP are limited to parenteral studies in animals, which indicate that, with the exception of 2,6-DNP, the other isomers are eliminated much more rapidly than is 2,4-DNP.

The great majority of data regarding toxicity of DNPs pertained to 2,4-DNP administered by the oral route. Limited data were available regarding occupational exposure of humans and dermal exposure of animals to 2,4-DNP. No studies were located regarding toxicity in humans exposed to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP. With the exception of oral studies of 2,6-DNP cataractogenesis in chickens, toxicity data for these isomers in animals were limited to exposure by the parenteral routes of administration. 2,4-DNP was prescribed as a weight reduction drug in the 1930s; therefore, numerous case reports and clinical studies regarding the oral toxicity of this drug in humans are available (Tainter et al. 1935b, Simkins 1937b). 2,4-DNP has not been legally used as a weight reduction drug since the 1930s so widespread exposure via ingestion of diet pills no longer occurs. However, in the early 1980s a physician in Texas administered 2,4-DNP to patients at his diet clinic (Kurt et al. 1986). After many complaints of side effects, a state court ordered the physician to cease distributing 2,4-DNP to his patients. Acute, intermediate, and chronic oral exposure of humans to 2,4-DNP results in increased basal metabolic rate, increased perspiration, sensation of warmth, weight loss, and, at higher levels of exposure, increased heart and respiratory rates and body temperature. Limited evidence from dermal and inhalation studies indicates that these effects are not route dependent. These effects are related to

the uncoupling of mitochondrial electron transport from oxidative phosphorylation by 2,4-DNP, which results in the release of energy as heat, rather than storage in the high energy phosphate bonds of ATP (Loomis and Lipmann 1948). The uncoupling of oxidative phosphorylation has the potential to affect all tissues and organs. In humans, the increased basal metabolic rate and related end points are observed at approximately the same exposure levels regardless of whether the duration of exposure is acute, intermediate, or chronic. Individual susceptibility to many of the effects of 2,4-DNP varies widely. The reason that some people are more susceptible may be due to pre-existing conditions, such as liver or kidney disease (Perkins 1919). These effects of 2,4-DNP are acute; onset is rapid (over several hours), and in spite of the danger of mortality from large increases in basal metabolic rate, cessation of exposure to 2,4-DNP often leads to a complete recovery. Limited data on 2,3-, 2,5-, 2,6-, 3,4-, and 3,5-DNP indicate that these compounds appear to have equivalent or less potential for increasing basal metabolic rate, in comparison to 2,4-DNP.

In addition to the above effects, a small percentage of humans ingesting 2,4-DNP or its sodium salt for weight loss developed peripheral neuritis (Nadler 1935), life-threatening agranulocytosis (Hoffman et al. 1934), serious skin reactions (MacBryde and Taussig 1935), or cataracts leading to blindness (Whalman 1936). Most of these effects were not observed in other mammalian species exposed orally, although cataracts were induced in yellow adipose mice and in guinea pigs on a vitamin C-deficient diet. There does not seem to be a most sensitive target organ in animals; however, in humans, the hematopoietic, ocular and nervous systems seem to be more sensitive than other body organ systems. 2,4-DNP inhibits mitochondrial function and affects all cells in the body. The increased sensitivity of certain human organ systems to 2,4-DNP suggests that their proper function is critically dependent on optimal ATP production.

Limited information from a few case reports of people who took 2,4-DNP suggests that it may affect female reproductive organs and menses, but this evidence is not conclusive. No studies were located regarding developmental effects in humans. Evidence of developmental toxicity was reported in two studies administering 2,4-DNP parenterally to animals. Decreases in fetal body weight and length, and an increase in early fetal resorptions were reported in rats that received 2,4-DNP subcutaneously (Goldman and Yakcovac 1964). A small effect on fetal body weight and length was seen in mice that received 2,4-DNP intraperitoneally, but no other developmental effects were observed (Gibson 1973). Two oral gavage studies on animals reported no developmental toxicity at exposure levels producing maternal toxicity (Gibson 1973; Kavlock et al. 1987). In another oral gavage study, treatment of

female rats before mating and during gestation and lactation resulted in a high incidence of stillborn pups and high mortality of pups during lactation (Wulff et al. 1935). However, this study did not evaluate teratogenicity. Since these studies were inadequate with respect to experimental protocol or reporting of protocol and results, the potential for 2,4-DNP to produce developmental effects has not been adequately addressed. Adequate reproductive toxicity studies in animals were not located.

2,4-DNP has been tested for genotoxicity in several *in vivo* and numerous *in vitro* assays; in general, the compound was negative for genotoxicity. However, genotoxic data do indicate a potential for mutagenicity of 2-amino-4-nitrophenol, 4-amino-2-nitrophenol, and 2,4-diaminophenol, which are metabolites of 2,4-DNP. No studies were located regarding cancer in humans after exposure to any isomer of DNP. 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.

Minimal Risk Levels for DNPs

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.

Inhalation MRLs

No Marls have been derived for inhalation exposure to 2,4-DNP because data for all durations are insufficient. 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 were located regarding health effects in animals after inhalation exposure to 2,4-DNP.

Oral MRLs

- An MRL of 0.01 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to 2,4-DNP.

The MRL for acute-duration oral exposure of 0.01 mg/kg/day was derived from the lowest observed adverse effect level (LOAEL) of 1.2 mg/kg/day identified in 37 humans who took 2,4-DNP for weight reduction for an average of 14 days (Tainter et al. 1935b). The adverse effects seen at this dose were a sensation of warmth, increased perspiration, and body weight loss of 0.43 kg/week. These patients were not losing weight at the time they began 2,4-DNP treatment and had been instructed to continue the same diets as before treatment. These results are consistent with the uncoupling of oxidative phosphorylation by 2,4-DNP and resultant increases in basal metabolic rate and loss of body weight. No dermal effects, cataracts, hematological effects or symptoms of peripheral neuritis occurred in these patients at this dosage over this time period. The MRL was derived by dividing the 1.2 mg/kg/day dose from the Tainter study by an uncertainty factor of 10 for human variability and again by 10 because a level of acute oral exposure which caused no adverse effects (NOAEL) could not be located in the literature.

Most of the toxic effects of 2,4-DNP are related to its ability to uncouple oxidative phosphorylation from electron transport, resulting in greatly diminished production of ATP, with the energy dissipated as heat, which can lead to fatal hyperthermia. All energy-dependent biochemical processes are likely to be affected, with toxicity resulting in any organ system. Furthermore, the local metabolic poisoning may exacerbate other pre-existing diseases. The most sensitive and proximate indicators are increased basal metabolic rates, increased body temperature, increased pulse, and body weight loss, which alone do not necessarily lead to toxicity (unless a large acute dose is taken and pyrexia develops). In a few people, oral ingestion of 2,4-DNP at doses above 1.2 mg/kg/day for longer periods of time led to serious toxic effects including cataracts, agranulocytosis, peripheral neuritis and serious dermatological conditions.

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 well with true metabolic rates when expressed as liters oxygen consumed per unit time per square meter of body surface area. Surface area was estimated by an empirical formula that took into account the subject's height and weight. Data was obtained as liters O_2 consumed/hour/m^2. This value was compared to a standard table that gave average basal metabolic rates for sex and age group from hundreds of determinations. Female metabolic rates were slightly lower than males, and metabolic rate declined

with age. Basal metabolic rate was expressed as a percentage of the average value from the table; values of -10% to +10% were considered normal because of natural variability.

The 37 patients in the MRL study were part of a larger clinical trial of sodium 2,4-DNP involving 170 patients who ingested an average of 4.0 mg/kg/day 2,4-DNP for an average of 88 days (Tainter et al. 1935b). In this trial, the initial dose of 1.2 mg/kg/day 2,4-DNP was increased in small increments at intervals ≥1 week until a target rate of weight loss was reached (1-1.5 kg/week). Basal metabolism was measured in some of these patients, pretreatment values were not reported, but there was a statistically significant dose-response relationship between increase in metabolic rate and increasing dosages of 2,4-DNP. Several additional experimental and clinical studies conducted at 3.2-4.3 mg/kg/day 2,4-DNP for acute (Castor and Beierwaltes 1956; Cutting and Tainter 1933; Cutting et al. 1934; MacBryde and Taussig 1935) and intermediate durations (Castor and Beierwaltes 1956; Cutting et al. 1934; Looney and Hoskins 1934; MacBryde and Taussig 1935; Simkins 1937a, 1937b) provide results on basal metabolic rate and body weight in good agreement with those obtained at the higher dosage levels in the study by Tainter et al. (1935b). Increases in basal metabolic rate are reversible after cessation of dosing. The threshold region for effects on basal metabolic rate and body weight has not been investigated as extensively as higher dose levels producing these effects. Although not clearly established, increases in basal metabolic rate of 10% or less do not appear to be toxicologically significant and are not considered adverse. However, increases of 10-29% result in increased body temperature that may be adverse, and increases of 30% or more may result in severe pyrexia, and therefore represents a serious adverse effect.

Dermal reactions (itching and/or urticarial or maculopapular lesions) were seen in 23 and sensory peripheral neuritis was seen in 18 of the 100 patients who took ≥3.5 mg/kg/day 2,4-DNP for at least 6 weeks in the study by Tainter et al. (1935b). Skin lesions were common in people taking 2,4-DNP (Anderson et al. 1933; Beinhauer 1934; Dintenfass 1934; Hitch and Schwartz 1936; Hunt 1934; Imerman and Imerman 1936; Nadler 1935; Simkins 1937a, 1937b) for acute to chronic durations. A patient who took 1.86 mg/kg/day 2,4-DNP for 14 days (Hitch and Schwartz 1936) developed a serious dermal reaction that worsened and resulted in hospitalization for severe exfoliating dermatitis eight months later. This patient recovered after two months. The lowest dose reported in the reviewed case histories occurred at 0.91 mg/kg/day for 8 days (Nadler 1935), but individual susceptibility varied widely.

Cataracts developed in a small percentage of patients who took 2,4-DNP as a weight reduction aid for acute, intermediate-, and chronic-durations. The case report literature regarding cataractogenesis of 2,4-DNP 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). Representative case reports that provided doses show that cataracts developed in the patients at doses ranging from 1.86 to 4.29 mg/kg/day 2,4-DNP, but no correlation with duration of exposure can be established. Cataract development at the lowest dose occurred eight months after exposure at 1.86 mg/kg/day 2,4-DNP for 14 days ceased (Hitch and Schwartz 1936). Again, individual susceptibility to 2,4-DNP cataractogenesis appears to vary widely.

Three case histories reported death from agranulocytosis due to ingestion of 2,4-DNP for intermediate durations (Dameshek and Gargill 1934; Goldman and Haber 1936; Silver 1934). In only one of these cases was enough information provided to calculate a dose (1.03 mg/kg/day); autopsy of this case revealed severe fatty changes in the liver and severe histological changes in other organs (Goldman and Haber 1936). Whether the fatty liver was a pre-existing condition is not known. Compromised liver function, with resultant reduced ability to metabolize 2,4-DNP, could have contributed to the severe effects and death of this individual. Agranulocytosis was also reported in other individuals who recovered after discontinuation of dosing for acute or intermediate durations (Dameshek and Gargill 1934; Davidson and Shapiro 1934; Hoffman et al. 1934; Imerman and Imerman 1936). Doses in these cases ranged from 3.5 to 5.7 mg/kg day 2,4-DNP for 14-35 days.

Peripheral neuritis has been described in patients taking 2,4-DNP orally at therapeutic doses for weight reduction for acute to intermediate durations (Anderson et al. 1933; Bortz 1934; Epstein and Rosenblum 1935; Hitch and Schwartz 1936; Hunt 1934; Nadler 1935; Simkins 1937a, 1937b; Tainter et al. 1935b). No correlations between this effect and dose or duration could be discerned, again indicating wide variation in individual susceptibility. Doses at which peripheral neuritis was observed ranged from 1.86 to 3.53 mg/kg/day 2,4-DNP. Neuritis at the lowest dose occurred eight months after exposure at 1.86 mg/kg/day 2,4-DNP for 14 days ceased (Hitch and Schwartz 1936).

The MRL of 0.01 mg/kg/day for acute duration oral exposure should be protective against the toxic effects of 2,4-DNP. The more serious effects that have been associated with 2,4-DNP use (neuritis, cataract, agranulocytosis, dermal reactions) generally occurred in patients who either took doses two to five-fold higher than the 1.2 mg/kg/day from which the MRL is derived or, took 2,4-DNP for much

longer periods of time than the 14 day or less period covered by the MRL. One case of serious toxicity was located (Hitch and Schwartz 1936) that involved a patient who took 1.86 mg/kg/day 2,4-DNP for 14 days and discontinued dosing because of a dermal reaction. Over the next eight months, this condition grew worse until she was hospitalized with severe exfoliating dermatitis. While in the hospital she also developed symptoms of neuritis and bilateral cataracts, which were not present on admission. Hematological tests were normal except for a slight secondary anemia. This individual may have had an idiosyncratic reaction to 2,4-DNP, dermal reactions in other patients were reversed when 2,4-DNP was discontinued. Oral exposure to 2,4-DNP at 0.01 mg/kg/day would be unlikely to result in effects on ATP production in the mitochondria, which is the mechanism of action for 2,4-DNP-induced toxicity.

No MRL has been derived for intermediate- or chronic-duration oral exposure to 2,4-DNP because no human studies of chronic duration reporting reliable doses were located, and data from humans exposed orally for intermediate durations indicate that effects do not seem to be correlated with dose or duration. One patient who took 1.03 mg/kg/day for 46 days (Goldman and Haber 1936) was hospitalized with fever and vomiting. Severe agranulocytosis was observed and the patient subsequently died. Autopsy revealed severe fatty changes in the liver suggesting that this patient had a pre-existing liver condition that may have made her more susceptible to 2,4-DNP toxicity. A chronic-duration study in animals (Tainter 1938) is available and identifies a NOAEL of 20 mg/kg/day for reduced body weight. At 30 mg/kg/day, body weight gain was reduced 25%. No respiratory, cardiovascular, hepatic, renal, or dermal/ocular effects were seen at $\leq$60 mg/kg/day, although a 50% decrease in life span was seen at 60 mg/kg/day. Use of the rat NOAEL to derive a chronic oral MRL is not recommended because this NOAEL is higher than the acute LOAEL identified in humans for reduced body weight and metabolic effects. The available data for all durations shows that animals are much less sensitive than humans to the effects of 2,4-DNP and are not good models for predicting doses that would result in adverse effects in humans.

The U.S. Environmental Protection Agency (EPA) has established a reference dose (RfD) of 0.002 mg/kg/day over a lifetime for protection against cataracts. The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (Barnes and Dourson 1988). The EPA identified a LOAEL for cataracts of 2 mg/kg/day based on a compilation of case reports (Horner 1942). The RfD was derived by dividing

by uncertainty factors of 10 for human variation, 10 for extrapolation of a LOAEL to a hypothetical no effect level, and 10 for extrapolation of intermediate-duration exposure to chronic exposure. The MRL for 2,4-DNP (0.01 mg/kg/day) is higher than the RfD because it was derived for the acute duration and no extrapolation for longer durations was necessary.

Death. Occupational exposure of humans to 2,4-DNP has resulted in death (Gisclard and Woodward 1946; Perkins 1919). This exposure included airborne vapor, mists, dust, and direct dermal contact with the solid form of 2,4-DNP, indicating that exposure probably occurred via inhalation, dermal, and possibly oral routes. Death from occupational exposure to 2,4-DNP appeared to occur at a greater rate in workers having alcoholism or liver or kidney disease (Perkins 1919). Case studies reported death in humans ingesting 2,4-DNP and its sodium salt (doses expressed here as mg/kg 2,4-DNP) in capsules. Ingestion of 46 mg/kg twice at an interval of 1 week resulted in hyperpnea, chest pain, extreme elevation of body temperature and pulse rate, then death; death in this case probably resulted from the pyretic effects of 2,4-DNP (Tainter and Wood 1934). In another fatal case, similar effects and coma preceded the death of a psychiatric patient given 2.66 mg/kg/day for 14 days (Masserman and Goldsmith 1934). A woman who ingested 7 mg/kg/day for 5 days also had similar effects, became comatose, and died (Poole and Haining 1934). Severe histopathological lesions were found in her lungs, kidneys, liver, heart, spleen, stomach, brain, and spinal cord. Death from agranulocytosis (see Hematological Effects) was described in three case reports of individuals ingesting 2.9-4.3, 1.03, and 0.62-3.8 mg/kg/day 2,4-DNP, respectively, for ≈6-7 weeks as prescribed by their doctors (Dameshek and Gargill 1934; Goldman and Haber 1936; Silver 1934). However, no deaths from agranulocytosis or other causes were reported in numerous clinical studies of humans ingesting up to 5.27 mg/kg/day for acute or intermediate durations (Castor and Beierwaltes 1956; Cutting et al. 1934; Grant and Schube 1934; Looney and Hoskins 1934; MacBryde and Taussig 1935; Simkins 1937a, 1937b; Stockton and Cutting 1934; Tainter et al. 1935a, 1935b), suggesting that large variations in sensitivity to 2,4-DNP may exist among humans. The basis for these differences in sensitivity is not known. One possibility is that the differences may be related to the ability of the liver and other tissues to metabolize 2,4-DNP to a less toxic form or to exacerbation of pre-existing impaired organ function. No studies were located regarding death in humans after exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

No studies were located regarding death in animals after inhalation exposure to any of the DNP isomers, or after oral or dermal exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP. A large database

regarding death in animals after oral administration of 2,4-DNP supports the data on 2,4-DNP induced mortality in humans. Single-dose, oral LD_{50} values for 2,4-DNP were 30 mg/kg for rats of unspecified age (Dow Chemical Co. 1950), 71 mg/kg for weanling rats, and 72 mg/kg for weanling mice (Kaiser 1964). The cause of death in animals treated once by gavage with 2,4-DNP was generally attributed to the pyretic effect of 2,4-DNP; rats exposed to potentially lethal doses of 2,4-DNP either died within a few hours or recovered completely (Kaiser 1964; Spencer et al. 1948). There was no clear difference in the lethality of 2,4-DNP among animal species tested (rats, mice, and dogs), and usually death did not occur from single gavage doses ≤10 mg/kg. A comparison of the lethality of 2,4-DNP after intraperitoneal or subcutaneous administration supports the contention that interspecies (rats, mice, rabbits, and dogs) differences in lethality of 2,4-DNP are not substantial (Harvey 1959; Tainter and Cutting 1933b). One study suggested that 2,4-DNP was more lethal to adult mice than to weanling mice (Bettman 1946). A clear difference in lethality exists between administration of 2,4-DNP by continuous intermittent feeding, with no increased mortality in rats after 110 mg/kg/day for 30 days (Pugsley 1935) or 40 mg/kg/day for life (Tainter 1938), but 100% mortality in rats after a single gavage dose of 60-100 mg/kg) (Dow Chemical Co. 1940; Kaiser 1964; Spencer et al. 1948), and 100% mortality in dogs after an intravenous dose of 5 mg/kg (Davies et al. 1991). This difference in toxicity may be explained by higher systemic blood levels of the parent compound after bolus administration. The oral data in humans concern bolus (capsule) administration, usually l-3 times a day with meals. Exposure of humans to 2,4-DNP near NPL sites is probably intermittent. Dermal exposure to 200, 300, and 700 mg/kg 2,4-DNP for 4 hours resulted in deaths of 0%, 20%, and 100% of guinea pigs, respectively, indicating that 2,4-DNP is absorbed through the skin (Spencer et al. 1948). The cause of increased mortality in animal studies after oral administration of 2,4-DNP was either attributed to the pyretic effects of 2,4-DNP or was not discussed; no deaths from agranulocytosis were reported in animals.

The lethality of the different isomers of DNP can be compared by examining data from parenteral routes. The LD_{50} values after intraperitoneal administration of 2,4-, 2,6-, 3,5-, 3,4-, 2,5-, and 2,3-DNP in rats were 35, 38, 45, 98, 150, and 190 mg/kg, respectively, and in mice were 36, 45, 50, 112, 273, and 200 mg/kg, respectively (Harvey 1959), indicating little difference in species sensitivity to the various isomers. These differences in lethality among the isomers could result from differences in mechanism of action, and different metabolism and elimination factors for the isomers. In an *in vitro* study, the relative potencies of the six isomers in uncoupling oxidative phosphorylation in rat liver mitochondria were 3,5- > 2,4- > 2,6- = 3,4- > 2,3- = 2,5-DNP (Burke and Whitehouse 1967). The

half-life for elimination of the isomers from the blood of rats was 225, 210, 13, 12.5, 11.5, and 2.1 minutes for 2,4-, 2,6-, 2,5-, 2,3-, 3,4-, and 3,5-DNP, respectively (Harvey 1959). Thus, the relatively high toxicity of 2,4- and 2,6-DNP might be explained by a greater ability to uncouple oxidative phosphorylation at the cellular level and a longer half-life in the blood. Intraperitoneal administration of the six isomers to mice exposed at air temperatures of 39-41 °C greatly increased the lethality of 2,4-DNP (100% mortality at 5 mg/kg) and 2,6-DNP (100% mortality at 10 mg/kg/day) compared to mice maintained at room temperature; however, lethality of the other isomers did not change significantly, suggesting that mortality from 2,4- and 2,6-DNP results from the pyretic effects of those two isomers (Harvey 1959). A comparison of the toxicities of compounds administered by the intraperitoneal route may not be relevant to oral human exposure, because intraperitoneal administration bypasses first pass metabolism by the gastrointestinal tract and liver; however, LD_{50} values for 2,4-DNP were similar between oral, intraperitoneal, and intravenous administration in rats (60-72 mg/kg) and mice (52-72 mg/kg) (Kaiser 1964).

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in death. Death from exposure in the ambient environment seems unlikely, based on limited information.

Systemic Effects. No studies were located regarding systemic effects in humans after exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP or in animals after inhalation exposure to any of the DNP isomers. Studies of systemic effects of DNPs in animals after dermal exposure were limited to two dermal irritation studies on 2,4-DNP.

Respiratory Effects. Occupational exposure to 2,4-DNP did not cause specific damage to the lungs; pulmonary edema was noted in fatal cases but was thought to be secondary to vasomotor effects (Perkins 1919). Increased respiratory rates were observed in workers exposed to 2,4-DNP (Perkins 1919), after single oral doses >10 and 46 mg/kg (Cutting et al. 1934; Tainter and Wood 1934), 7 mg/kg/day for 5 days (Poole and Haining 1934), or 2.66 mg/kg/day for 14 days (Masserman and Goldsmith 1934). This increase in respiration is secondary to 2,4-DNP-induced uncoupling of oxidative phosphorylation, leading to elevation of basal metabolic rate and body temperature. Three of these cases were fatalities (Masserman and Goldsmith 1934; Poole and Haining 1934; Tainter and Wood 1934). Autopsy of one of the cases revealed hyperemic and hemorrhagic lungs, congestion of alveolar walls, and edema in alveoli. Elevated respiratory rates and dyspnea were also observed in

people who took 1.03-3.97 mg/kg/day for intermediate durations (Goldman and Haber 1936; Imerman and Imerman 1936; Simkins 1937a, 1937b). The patient who took 1.03 mg/kg/day died, and vascular congestion was found in the lungs upon autopsy (Goldman and Haber 1936). A patient who took 2.3 mg/kg/day 2,4-DNP for 14 days and had severe dermatological reactions did not exhibit dyspnea (Anderson et al. 1933).

Respiratory effects in animals after oral exposure to 2,4-DNP were similar to those observed in humans. Increased respiratory rates were observed in dogs, rats, and mice following acute bolus administration of 2,4-DNP (Kaiser 1964). Parenteral administration of 2,4-DNP to rats intravenously and rabbits subcutaneously resulted in substantial increases in respiratory rate, tidal volume, and minute ventilation (Tainter and Cutting 1933a; Takehiro et al. 1979). No gross or histological evidence of treatment-related pulmonary damage was reported following long-term treatment of rats in the diet (Spencer et al. 1948) or dogs via capsules at 50-60 and 10 mg/kg/day 2,4-DNP, respectively (Tainter 1938; Tainter et al. 1934b). Thus, the effects of 2,4-DNP on respiration in humans and animals appear to be a consequence of an increase in basal metabolic rate; no evidence of progressive damage to the pulmonary system was observed in animals exposed to 2,4-DNP for long durations.

Effects of the six dinitrophenol isomers on minute volume, respiratory rate, and tidal volume were studied in rats injected intraperitoneally at dose ranges relative to their LD_{50} values (Grant 1959). The parameters were increased by all six isomers, but the greatest increases were seen with 2,4-DNP. Minute volumes were increased 60% by 2,4-DNP; 47% by 2,3-DNP; 32% by 2,5-DNP; 23% by 3,4-DNP; 21% by 2,6-DNP; and 11% by 3,4-DNP. Respiratory rates were increased 16% by 2,4-DNP; 12% by 2,3-DNP; 11% by 3,4-DNP; 9% by 2,5-DNP; 5% by 2,6-DNP; and 1.5% by 3,5-DNP. Tidal volumes were increased 37% by 2,4-DNP; 31% by 2,3-DNP; 21% by 2,5-DNP; 15% by 2,6-DNP; 10% by 3,4-DNP; and 9% by 3,5-DNP. This study was conducted to determine whether effects on oxygen consumption and carbon dioxide output as determined in a study by Cameron (1958) (see Metabolic Effects) were due to metabolic disturbances at the cellular level or to hyperventilation. The author concluded that since there was no relationship between increased ventilation and carbon dioxide output, the increase in carbon dioxide output was not caused by hyperventilation but was instead due to the effect of 2,4-DNP on cellular respiration. Other studies regarding respiratory effects in animals exposed parenterally to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP were limited to an indirect assessment of basal metabolic rates measured by oxygen consumption and carbon dioxide output; these data are discussed in the section on Metabolic Effects.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in respiratory effects. Respiratory effects from exposure in the ambient environment seem unlikely, based on limited information.

Cardiovascular Effects. No studies were located regarding cardiac effects in humans after inhalation exposure to any isomer of DNP. Increased pulse rates were reported for humans ingesting high doses (>10 mg/kg and 46 mg/kg) of 2,4-DNP in capsules, but the effect was probably secondary to increased basal metabolic rate and body temperature (Cutting et al. 1933; Tainter and Wood 1934). Similarly, an increase in venous pressure (measured directly in the median cubital vein) noted at 3.5 mg/kg/day for up to 12 days appeared to be a compensatory mechanism for the maintenance of normal blood pressure while the body attempted to dissipate heat through peripheral vasodilation (Stockton and Cutting 1934). In a clinical study, persistent abnormal electrocardiograms were observed starting at 2 weeks of treatment in 3 of 6 patients treated with 4.3 mg/kg/day 2,4-DNP for 6-8 weeks (MacBryde and Taussig 1935). Elevated pulse rates were common findings in people who took 2,4-DNP for acute or intermediate durations (Epstein and Rosenblum 1935; Goldman and Haber 1936; Imerman and Imerman 1936; Poole and Haining 1934). Doses ranged from 1.03 to 7 mg/kg/day, but effects did not correlate with the measured pulse rates. Autopsy and histological examination in one fatal case revealed marked segmentation and fragmentation of the cardiac muscles (Poole and Haining 1934), and autopsy in another fatal case 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). Myocarditis was considered the cause of death of a woman who took an indeterminate dose of 2,4-DNP for 1 week (Lattimore 1934). A case report of a patient who took 2.3 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).

Elevated heart rates and highly abnormal electrocardiograms were observed in dogs fed capsules containing 25 mg/kg/day for 1-14 days or 125 mg/kg for 1 day (Kaiser 1964). Increased heart rates were reported following intravenous administration of 2,4-DNP in rats at 6 mg/kg (Takehiro et al. 1979) and dogs at 36 mg/kg (Kaiser 1964). In the dogs, intravenous infusion of 2,4-DNP resulted in an increased heart rate from ≈120 beats per minute preinfusion to 220 beats per minute at ≈50 minutes during infusion (Kaiser 1964). Thereafter, the heart rate declined, and death ensued at 84-85 minutes during infusion. No gross or histological evidence of treatment-related cardiac damage was reported after long-term treatment of rats in the diet (Spencer et al. 1948) or dogs via capsules at 50-60 and

10 mg/kg/day 2,4-DNP, respectively (Tainter 1938; Tainter et al. 1934b). Thus, acute effects of 2,4-DNP on cardiac function are adverse and potentially life-threatening in animals and humans; however, animal studies suggest that long-term exposure to nonfatal levels of the compound does not result in progressive damage to the heart.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in cardiovascular effects. Cardiovascular effects from exposure in the ambient environment seem unlikely, based on limited information.

Gastrointestinal Effects. Nausea and vomiting were reported in occupationally exposed humans (Perkins 1919) and in 5 of 15 patients ingesting 4.3 mg/kg 2,4-DNP for l-8 weeks (MacBryde and Taussig 1935). Nausea, vomiting, diarrhea, and heartburn were among the gastrointestinal effects experienced by people ingesting 2,4-DNP (Bayer and Gray 1935; Goldman and Haber 1936; Lattimore 1934; Poole and Haining 1934; Simkins 1937a, 1937b). Doses ranged from 1.03 to 7 mg/kg/day but effects did not correlate with duration. In a fatal case, autopsy revealed edema and hemorrhage in the stomach and disintegration of the glandular mucosa (Poole and Haining 1934). In another fatal case, no pathological changes were found in the stomach, but numerous focal hemorrhagic necroses were found in the small intestine (Goldman and Haber 1936). In addition, a woman who took 4.4 mg/kg/day for 4 days experienced pharyngitis after the first dose that worsened after repeated doses (Dintenfass 1934). The effects on the gastrointestinal tract and pharynx appear to be due to local necrosis or irritation by 2,4-DNP. 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).

Vomiting was observed in dogs exposed to 12.5-125 mg/kg/day 2,4-DNP for 1-14 days, but not at 5 mg/kg/day for 14 days. No gross or histological evidence of treatment-related damage to the gastrointestinal tract was reported after long-term treatment of rats in the diet or dogs via capsules at 50 and 10 mg/kg/day 2,4-DNP, respectively (Spencer et al. 1948; Tainter et al. 1934b). Thus, vomiting appears to be an acute effect of 2,4-DNP on the gastrointestinal tract; however, long-lasting effects (lesions) were not reported, even at doses producing mortality.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in gastrointestinal effects. Gastrointestinal effects from exposure in the ambient environment seem unlikely, based on limited information.

Hematological Effects. No studies were located regarding hematological effects in humans after inhalation exposure to any DNP isomer. Agranulocytosis (a syndrome characterized by marked decrease in the number of granulocytes, lesions of the throat and other mucous membranes, and fever [also called granulocytopenia, malignant neutropenia, agranulocytic angina]) was reported in 8 patients treated orally with 2,4-DNP or its sodium salt in capsules, either acutely or for intermediate durations (Horner 1942). Agranulocytosis resulting in death was reported in 3 patients ingesting 1.03 mg/kg/day (Goldman and Haber 1936), 2.9-4.3 mg/kg/day 2,4-DNP (Dameshek and Gargill 1934), or 0.62-3.8 mg/kg/day 2,4-DNP (Silver 1934) for ≈6-7 weeks. Other patients recovered following discontinuance of the dmg (Dameshek and Gargill 1934; Davidson and Shapiro 1934; Hoffman et al. 1934; Imerman and Imerman 1936). Agranulocytosis was also reported in other individuals who recovered after discontinuation of dosing for acute or intermediate durations (Dameshek and Gargill 1934; Davidson and Shapiro 1934; Hoffman et al. 1934; Imerman and Imerman 1936). Doses in these cases ranged from 3.5 to 5.7 mg/kg day 2,4-DNP for 14-35 days. Most case reports and clinical studies that examined hematological end points found no effects (Anderson et al. 1933; Beinhauer 1934; Epstein and Rosenblum 1935; Masserman and Goldsmith 1934; Simkins 1937a, 1937b; Tainter et al. 1935b); however, slight anemia was reported in two cases (Hitch and Schwartz 1936; Imerman and Imerman 1936).

No evidence of hematological abnormalities was observed in rats treated with 50 mg/kg/day 2,4-DNP in the diet for 6 months (Spencer et al. 1948) or in dogs treated via capsules to 10 mg/kg/day 2,4-DNP; analysis of bone marrow in the dogs revealed no abnormalities (Tainter et al. 1934b). The mechanism for 2,4-DNP induced agranulocytosis in humans is not known. Abnormalities in leukocyte or differential counts were not observed in animal studies evaluating hematological end points.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in hematological effects. Hematological effects from exposure in the ambient environment seem unlikely, based on limited information.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after inhalation exposure to any DNP isomer. Strength and endurance were adversely affected in some patients ingesting 4.3 mg/kg/day 2,4-DNP for l-4 weeks (MacBryde and Taussig 1935). This effect may be related to the uncoupling of oxidative phosphorylation by 2,4-DNP, rather than a specific

adverse effect of 2,4-DNP on muscle tissue. Rheumatoid or arthritic-like pains were experienced by five women taking 2,4-DNP for weight reduction (Anderson et al. 1933; Nadler 1935). Doses ranged from 0.91 to 3.53 mg/kg/day and durations ranged from 8 to 105 days; however, doses did not correlate with symptoms. One woman had a history of chronic hypertrophic arthritis of the cervical spine and knees, but because arthritic-like pains developed in her fingers and all large joints while taking 2,4-DNP, it was thought that the drug exacerbated her arthritis (Anderson et al. 1933). These pains may be related to the development of peripheral neuritis (Nadler 1935) (see Neurological Effects).

In support of a nonspecific action on muscle, no gross or histological evidence of treatment-related damage to muscle or skeletal tissue was reported following long-term treatment of rats in the diet or dogs via capsules at 50 and 10 mg/kg/day 2,4-DNP, respectively (Spencer et al. 1948; Tainter et al. 1934b). Thus, the effects of 2,4-DNP on muscle tissue appear to be related to the uncoupling action of the chemical and are probably not progressive.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in musculoskeletal effects. Musculoskeletal effects from exposure in the ambient environment seem unlikely, based on limited information.

Hepatic Effects. Workers who died from exposure to 2,4-DNP had no consistent microscopic lesions or changes in the liver (Perkins 1919). Some evidence of impaired liver function (increased phenoltetraiodophthalein retention) was observed in one clinical study of a limited number of patients ingesting capsules containing 2,4-DNP at a dosage of 4.3 mg/kg/day for l-8 weeks (MacBryde and Taussig 1935). In fatal cases, histological examination revealed slight detachment of liver cells (Tainter and Wood 1934), disintegration of hepatocytes, granular cytoplasm and pyknotic nuclei (Poole and Haining 1934), necrosis of hepatocytes and hemorrhage in the liver (Lattimore 1934), and severe fatty changes in the liver (Goldman and Haber 1936) in patients taking oral doses of 2,4-DNP for acute to intermediate durations. Tests for liver function, such as icteric index (a measure of serum bilirubin), the Van den Bergh test, and the bromsulphalein retention test, in other case reports and clinical studies of people exposed orally found no evidence of impaired damage (Anderson et al. 1933; Masserman and Goldsmith 1934; Simkins 1937a, 1937b).

Animal data suggest that the liver is not a particularly sensitive target organ for oral exposure to 2,4-DNP. No abnormal liver function or gross or histological evidence of treatment-related liver damage was observed after long-term treatment of rats in the diet (Spencer et al. 1948) or dogs via capsules at 50-60 and 10 mg/kg/day 2,4-DNP, respectively (Tainter 1938; Tainter et al. 1934b). Rats that died after single dose gavage treatment with 2,4-DNP had kidney, liver, and spleen damage (Spencer et al. 1948). However, in another acute study no histological abnormalities were observed in the liver of dogs after several oral treatments, including a fatal dose (Tainter and Cutting 1933b). The effect of six isomers of DNP on bile secretion, BSP (bromsulphalein) retention, and rectal temperature was studied in anesthetized dogs (Pugh and Stone 1968). 2,4-DNP was administered at single intravenous doses of 1-5 mg/kg, while the other isomers were administered at doses of 4-6 mg/kg. 2,4-DNP produced a significant dose-related increase in rectal temperature (1.3 °C at 1 mg/kg and 3.9 °C at 5 mg/kg/day), while the other isomers produced no significant increase. Bile secretion was increased by 75% at 1 mg/kg, 200% at 2 mg/kg, 133% at 3 mg/kg, 132% at 4 mg/kg, and 153% at 5 mg/kg 2,4-DNP. At each dose, this choleresis persisted throughout the 2-hour sampling period. Bile secretion increased by 54% with 5 mg/kg 3,4-DNP, 51% with 6 mg/kg 2,3-DNP, 50% with 3 mg/kg 2,5-DNP, 28% with 6 mg/kg 3,5-DNP, and 13% with 4 mg/kg 2,6-DNP. The choleresis produced by the other isomers did not persist. 2,4-DNP decreased BSP excretion by 13% at 1 mg/kg and by 17% at 2 mg/kg, but increased BSP excretion by 10% at 3 mg/kg, by 8% at 4 mg/kg, and by 16% at 5 mg/kg. The other isomers at 4-6 mg/kg all decreased BSP excretion. The choleretic effect of 2,4-DNP may be related to its ability to uncouple oxidative phosphorylation. Thus, the liver does not appear to be a particularly sensitive target organ for 2,4-DNP toxicity in humans or animals exposed orally.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in hepatic effects. Hepatic effects from exposure in the ambient environment seem unlikely, based on limited information.

Renal Effects. Workers who died from exposure to 2,4-DNP had no consistent microscopic lesions or changes in the kidney (Perkins 1919). However, histological examination of kidneys from people who died after ingesting 2,4-DNP revealed mild nephrosis in a man who took 46 mg/kg followed by an additional 46 mg/kg 1 week later (Tainter and Wood 1934); cloudy swelling, pyknosis in the renal tubules, edema in interstitial tissue, distention of capillary and arterial loops in the glomerulus, and hemorrhage in a woman who took 7 mg/kg/day for 5 days (Poole and Haining 1934); marked

destruction of the renal tubule epithelial lining with hemorrhage into the glomeruli in a woman who took an undetermined dose for 1 week (Lattimore 1934); and hemorrhagic nephritis in a girl who took 1.03 mg/kg for 46 days (Goldman and Haber 1936). In nonfatal cases, albuminuria was found in 2 women who took 2.32 mg/kg/day (Beinhauer 1934) or 3.97 mg/kg/day (Imerman and Imerman 1936) for 37 or 35 days, respectively, and in 6 of 15 people for whom urinalysis was performed in an extensive clinical study of 159 patients taking 3 mg/kg/day for 22-89 days (Simkins 1937a, 1937b). Kidney function, as assessed by phenolsulfonphthalein retention, was not affected in the case reported by Beinhauer (1934) or in the 15 patients tested by Simkins (1937a, 1937b). Repeated tests of renal function on 3 patients treated with 4.3 mg/kg/day 2,4-DNP over a period of 8 weeks indicated no changes, but quantitative data were not provided (MacBryde and Taussig 1935).

Animal data regarding the toxicity of 2,4-DNP to the kidney is equivocal. Rats exposed to 5-50 mg/kg/day 2,4-DNP in the diet for 6 months had no gross or histological evidence of kidney damage (Spencer et al. 1948). BUN (blood urea nitrogen) was greatly elevated in 2 of 14 and 2 of 9 rats exposed to 25 and 50 mg/kg/day, respectively, but the mean values in each group were similar to the controls (Spencer et al. 1948). Dogs exposed to 5 or 10 mg/kg/day 2,4-DNP in 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). Gross and histological findings in the kidneys of rats exposed to 60 mg/kg/day 2,4-DNP in the diet for life were comparable to those of the control group (Tainter 1938). Rats treated once by gavage with 20 mg/kg 2,4-DNP displayed very mild tubular necrosis in 5 of 16 kidneys examined; no statistical analysis of the data was reported (Arnold et al. 1976). The kidneys, livers, and spleens of rats that died after a single gavage dose treatment with 2,4-DNP were damaged (Spencer et al. 1948). However, in another acute study no histological abnormalities were observed in the kidneys of dogs after several oral treatments that resulted in death (Tainter and Cutting 1933b). Thus, animal data suggest that slight renal damage may occur following non-lethal doses of 2,4-DNP. The human data suggest that albuminuria may occur in nonfatal cases, and profound kidney damage may occur in fatal cases.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in renal effects. Renal effects from exposure in the ambient environment seem unlikely, based on limited information.

Endocrine Effects. No studies were located regarding endocrine effects in humans or animals after inhalation exposure to any of the DNP isomers.

Little information is available regarding endocrine effects in humans exposed 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 observed in one clinical study in 5 of 8 patients after l-2 weeks of treatment and in 4 of 4 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 3.2 mg/kg/day 2,4-DNP for 2 days (Castor and Beierwaltes 1956). Thyroidal I^{131} excretion did not appear to be affected. Hence the toxicological significance of this finding is unclear.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in endocrine effects. Endocrine effects from exposure in the ambient environment seem unlikely, based on limited information.

Dermal Effects. No studies were located regarding dermal effects in human or animals after inhalation exposure to any of the DNP isomers. The lowest oral dose causing dermal effects in the reviewed case histories occurred at 0.91 mg/kg/day for 8 days (Nadler 1935), but individual susceptibility varied widely. Skin reactions were observed in some of the patients in two clinical studies in which patients ingested 4.3 mg/kg/day or an average of 4.0 mg/kg/day for intermediate durations (MacBryde and Taussig 1935; Tainter et al. 1935b). Some patients experienced itching with no rashes, while others had visible skin lesions (urticarial or maculopapular); in some cases, the reaction was considered severe. Skin lesions (pruritic, maculopapular, or urticarial) were common in people who ingested 2,4-DNP for weight reduction for acute to chronic durations (Anderson et al. 1933; Beinhauer 1934; Dintenfass 1934; Hitch and Schwartz 1936; Hunt 1934; Imerman and Imerrnan 1936; Nadler 1935; Simkins 1937a, 1937b). In some cases, the lesions were severe, covering the entire body surface (Anderson et al. 1933; Beinhauer 1934; Hitch and Schwartz 1936). In one case,

the lesions were characterized by severe exfoliating dermatitis, with redness, edema, oozing of serum, and crusting (Hitch and Schwartz 1936). In another case, the pruritus was characterized by swelling of 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 (Beinhauer 1934). The skin reaction generally disappears after discontinuation of treatment. Dermal effects were not observed in patients taking 1.2 mg/kg/day 2,4-DNP as the sodium salt of 2,4-DNP for an average of 14 days (Tainter et al. 1935b) or in 13 non-obese men experimentally dosed with 5.27 mg/kg/day for 20 days (Grant and Schube 1934). No evidence of skin reactions was reported in animal studies following oral administration of 2,4-DNP for acute-, intermediate-, or chronic-durations. 2,4-DNP was slightly to moderately irritating to the skin after a 3-4% solution was applied to the shaved abdomen of guinea pigs (Dow Chemical Co. 1940; Spencer et al. 1948).

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in dermal effects. Dermal effects from exposure in the ambient environment seem unlikely, based on limited information.

Ocular Effects. No studies were located regarding ocular effects in humans or animals after inhalation exposure to any of the DNP isomers. Bilateral, irreversible cataracts, progressing to total blindness, were observed in a small percentage of patients who ingested 2,4-DNP or sodium 2,4-DNP for acute, intermediate, or chronic durations. Cataract formation is the main reason 2,4-DNP was banned from use for weight control by the Food and Drug Administration. At least 164 cases of cataract development were reported in patients taking the drug for weight loss (Hill 1936; Hitch and Schwartz 1936; Horner 1942; Horner et al. 1935; Rank and Waldeck 1936; Rodin 1936; Simkins 1937a, 1937b; Tainter et al. 1935b; Whalman 1936). Representative case reports that provided doses show that cataracts developed in the patients at doses ranging from 1.86 to 4.29 mg/kg/day 2,4-DNP, but no correlation with duration of exposure can be established. Cataract development at the lowest dose occurred 9 months after exposure at 1.86 mg/kg/day 2,4-DNP for 14 days ceased (Hitch and Schwartz 1936). Again, individual susceptibility to 2,4-DNP cataractogenesis appears to vary widely. The cataracts developed very rapidly while the patient was still taking 2,4-DNP or after the treatment had ended; many of the affected patients were too young for the occurrence of senile cataracts. In some cases, marked swelling of the lens occurred that occasionally caused acute secondary glaucoma. A genetic predisposition to 2,4-DNP cataractogenesis has been suggested (Buschke 1947; Hessing 1937).

2,4-DNP-induced cataract formation was not generally observed in other mammals. No cataracts were observed following acute oral exposure to 2,4-DNP in mice and rabbits, and in longer-term studies on black mice, dogs, and rats, even at dose levels that caused death (Bettman 1946; Spencer et al. 1948; Tainter 1934b, 1938). One of 20 albino mice developed cataracts after an 11-month exposure in feed to 130 mg/kg/day 2,4-DNP (Bettman 1946). However, several factors can apparently increase the sensitivity of some mammals to 2,4-DNP-induced cataract formation, including abnormal diets (vitamin C deficiency in guinea pigs) (Ogino and Yasukura 1957), strain (yellow adipose mice) (Bettman 1946), and age (newborn rabbits) (Gehring and Buerge 1969a). In addition, chicks and ducks developed cataracts within hours to days after oral exposure to 2,4-DNP (Bettman 1946; Buschke 1947; Gehring and Buerge 1969a; Robbins 1944). The progression of development of cataracts in birds was considered "remarkably similar" to that reported in humans exposed to 2,4-DNP (Horner 1942; Robbins 1944). Unlike cataracts in humans, however, the cataracts in birds were transient, usually regressing within days to weeks after development. Injection of 2,4-DNP directly into the posterior chamber of the eyes of ducks or rabbits produced a dose-related increased incidence of cataracts, sometimes within 10-20 minutes after injection (Bettman 1946; Gehring and Buerge 1969a). The authors suggested that the rapidity of the cataract formation following intraocular injection indicated that the parent compound was the toxic agent in cataract formation, and that cataracts are formed only after concentration of 2,4-DNP in the aqueous fluid "bathing" the lens attained a threshold (Gehring and Buerge 1969a). Age-related susceptibility to cataract formation was observed in rabbits injected intraperitoneally with 2,4-DNP. The ED_{50} values were 6.6 and 32 mg/kg in 10- and 62-day-old rabbits, respectively. Cataract formation did not occur in 90-day old rabbits at any intraperitoneal dose of 2,4-DNP. The authors suggested that increased sensitivity of newborn rabbits to 2,4-DNP may result from decreased ability to metabolize substances and a general increased permeability of the blood-ocular fluid barrier to substances. The species that are sensitive and insensitive to the cataractogenesis of 2,4-DNP appear to be the same as those sensitive and insensitive to the cataractogenesis of other agents. For example, no cornea1 opacities or cataracts were produced in rats fed 2,4-dinitro-*o*-cresol (DNOC), 2-sec-butyl-4,6-dinitrophenol, 2-cyclohexyl-4,6-dinitrophenol, or 2-cyclohexyl-4,6-dinitrophenol for 6 months, while cataracts developed in ducks within l-2 days on diets of DNOC and within 4-6 days on diets of 2-sec-butyl-4,6-dinitrophenol (Spencer et al. 1948). In addition, cataracts were induced within l-5 hours in chickens given a single oral dose of DNOC (Buschke 1947). 2,6-Dibromo-4-nitrophenol, 2,4-aminoanisole, 2,4-dinitrophenetole, 2-chloro-4,6-dinitrophenol, and 2,4-dinitro-5chlorophenol were also active cataractogenic agents in chickens. Cataracts were induced in guinea pigs by dermal application or subcutaneous injection of acetone, but

no cataracts developed in rabbits after dermal application of acetone (Rengstorff et al. 1972; Rengstorff and Khafagy 1985). Acetone is not known to cause cataracts in humans; therefore, no suitable animal model to study cataract development in humans after exposure to chemical agents was identified in these studies. Humans are apparently more sensitive than other mammals to the cataract forming effects of 2,4-DNP. However, only a small percentage of humans ingesting 2,4-DNP developed cataracts. Increased sensitivity of some humans to 2,4-DNP induction of cataract formation may be related to metabolism and distribution of 2,4-DNP, especially to the levels of the parent compound in the ocular fluid. Genetic factors may also play a role.

As discussed in Section 2.3.5, the uncoupling of mitochondrial electron transport from oxidative phosphorylation with resultant decreased production of ATP by 2,4-DNP is also related to the cataractogenesis of 2,4-DNP. In most animal species, the energy needs for the lens are met principally by anaerobic glycolysis, <30% by oxidative phosphorylation (Kuck 1970; Trayhurn and van Heyningen 1971). Energy in the form of ATP, along with an active Na^+/K^+-activated ATPase, are required for the transport of these cations across the lens to maintain proper ionic balance. Interference with this active transport mechanism can result in increased sodium in the lens, disruption of the ionic balance between the lens and aqueous humor, and subsequent cataract formation. Because 2,4-DNP uncouples oxidative phosphorylation but does not interfere with glycolysis in most tissues, 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 DNP and are reversible (Buschke 1947). However, in humans, cataracts can occur some time after treatment is terminated and may not be reversible. This phenomenon has not been fully explained.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in ocular effects. Ocular effects from exposure in the ambient environment are possible, based on limited information. However, exposure to 2,4-DNP at high workroom or environmental temperatures may increase human susceptibility.

Body Weight Effects. Weight loss is a well-documented effect of exposure to 2,4-DNP in both humans and animals. In a case of occupational exposure (Perkins 1919), one of the earliest symptoms was weight loss to the point of excessive thinness after several months of exposure. Body weight loss

and metabolic effects are likely the most sensitive symptoms of exposure to 2,4-DNP. A LOAEL of 1.2 mg/kg/day for body weight loss was identified in humans who took sodium 2,4-DNP for weight reduction for an average of 14 days (Tainter et al. 1935b). These patients were not losing weight at the time they began 2,4-DNP treatment and had been instructed to continue on the same diets as before treatment. The average weight loss was 0.43 kg/week. These 37 patients were part of a larger clinical trial of sodium 2,4-DNP involving 170 patients who ingested an average of 4.0 mg/kg/day for an average of 88 days (Tainter et al. 1935b). In this trial, the initial dose of 1.2 mg/kg/day was increased in small increments at intervals $\geq$1 week, and weight loss was shown to be dose related. Several additional experimental and clinical studies conducted at 3.2-4.3 mg/kg/day 2,4-DNP for acute-duration exposure (Cutting and Tainter 1933; Cutting et al. 1934; MacBryde and Taussig 1935) and intermediate-duration exposure durations (Cutting et al. 1934; Looney and Hoskins 1934; MacBryde and Taussig 1935; Simkins 1937a, b) provide results on body weight in good agreement with those obtained at the higher dosage levels in the study by Tainter et al. (1935b).

Decreased body weight gain (18%) was observed in rats exposed for 6 months to 50 mg/kg/day (Spencer et al. 1948). Rats exposed via diet to 30 mg/kg/day 2,4-DNP for their lifetimes had final body weights 25% less than those of controls, while food consumption was similar to that of controls; no effect on body weights or food consumption was observed at doses ≤20 mg/kg/day (Tainter 1938). No change in body weight was observed in 3 male dogs fed 10 mg/kg/day in capsules for 6 months (Tainter et al. 1934b). Body weight loss (12%) was observed in bobwhite quail exposed to 2,4-DNP via diet (Dominguez et al. 1993) over 8 days at a dose of 56.1 mg/kg/day. Body weight was unaffected by a dose of 33.6 mg/kg/day. Birds on the 56.1 mg/kg/day dose had greatly decreased subcutaneous and visceral body fat. Chicks fed 2,4-DNP via diet showed a body weight gain reduction of approximately 13% at a dose of 77.9 mg/kg/day (Toyomizu et al. 1992). Doses of 16.5 and 36.3 mg/kg/day had no effect on body weight gain. Thus, animals may be less sensitive to body weight changes produced by ingesting 2,4-DNP than humans are. No studies were located regarding body weight changes in animals exposed to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in body weight effects associated with increased basal metabolic rates (e.g., body weight loss, increased body temperature). Body weight effects from exposure in the ambient environment are possible, based on limited information. However, exposure to 2,4-DNP at high workroom or environmental temperatures may increase human susceptibility.

Metabolic Effects. The characteristic effects of 2,4-DNP in humans are elevation of the basal metabolic rate (often measured indirectly as oxygen consumption), elevation of body temperature, and increased perspiration. These effects are related to the action of this chemical as an uncoupler of oxidative phosphorylation and are well documented for oral exposure (during the use of 2,4-DNP and its sodium salt as weight reduction drugs). There is enough documentation from occupational exposure (Perkins 1919; Gisclard and Woodward 1946) to indicate these effects would occur regardless of route of exposure. The threshold for elevation of basal metabolic rate from ingested 2,4-DNP has not been established for humans, but increases appear to start at ≈1-1.2 mg/kg/day, the level at which related effects, such as weight loss, also become apparent (Dunlop 1934; Tainter et al. 1935b). Although not clearly established, increases in basal metabolic rate of 10% or less do not appear to be toxicologically significant and are not considered adverse. However, increases of 10% to 29% result in increased body temperature that may be adverse; increases of 30% or more may result in severe pyrexia, and hence represent a serious adverse effect.

Clear and striking increases in basal metabolic rate are seen at dose levels of ≈3-4.2 mg/kg/day (Castor and Beierwaltes 1956; Cutting and Tainter 1933; Cutting et al. 1934; Looney and Hoskins 1934; MacBryde and Taussig 1935; Masserman and Goldsmith 1934; Simkins 1937a, 1937b; Tainter et al. 1935b). At these doses, effects on body weight are more marked, and symptoms of warmth and increased perspiration tend to become uncomfortable. Again, this is the case regardless of whether exposure is acute or intermediate in duration. Actual increases in body temperature did not become apparent in humans at single doses between 5 and 10 mg/kg, but increases ≥3 °C were seen with single doses >10 mg/kg (exact doses not specified) (Cutting et al. 1933).

A number of toxic end points related to increased basal metabolic rate were reported in animals treated orally with 2,4-DNP, including increased body temperature and respiration rate. No studies were located regarding the effects related to increased basal metabolic rate in animals after inhalation or dermal exposure to 2,4-DNP. Studies regarding effects of 2,3-, 2,5-, 2,6-, 3,4-, and 3,5-DNP related to increased basal metabolic rate were limited to those exposing animals parenterally.

Acute oral exposure to 2,4-DNP produced small increases in body temperature in dogs at doses as low as 10 mg/kg/day, with larger increases observed at 15 mg/kg/day (0.9-1.2 °C) and 20 mg/kg/day (0.7-2.5 °C) (Tainter and Cutting 1933a, 1933b). Dose-related changes in body temperature were observed in rats, rabbits, and dogs treated once parenterally with 2,4-DNP; the pyretic effects in dogs

following parenteral exposure to 2,4-DNP were similar in magnitude to those after oral administration of 2,4-DNP at similar exposure levels (Tainter and Cutting 1933a, 1933b). Thus, pyretic effects in acute studies in animals were observed at doses higher than those producing pyretic effects in humans.

Intraperitoneal injection of the 6 DNP isomers to mice exposed to air at temperatures of 39-41 °C greatly increased lethality (as determined by LD_{50} values) of 2,4- and 2,6-DNP, compared to mice maintained at room temperature; the lethality of 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP did not change significantly (Harvey 1959). This suggests that mortality from 2,4- and 2,6-DNP results from the pyretic effects of those two isomers. Essentially no effect on body temperature was observed in pigeons injected once intramuscularly with 40 mg/kg 2,5-DNP (Tainter and Cutting 1933b). Similar treatment of pigeons with 2,6-DNP produced a dose-related large increase in body temperature; the pyretic effect of 2,6-DNP in pigeons was much less than that of 2,4-DNP (Tainter and Cutting 1933b). The pyretic effect of 2,4-DNP (and potentially 2,6-DNP) is related to the heat produced following uncoupling of oxidative phosphorylation from electron transport in mitochondria.

Increased oxygen consumption (30-85%) was reported in rats receiving 110 mg/kg/day 2,4-DNP in the diet (Pugsley 1936). In rats injected intravenously with 6 mg/kg 2,4-DNP, oxygen consumption increased by ≈50% (Takehiro et al. 1979). In rats injected intraperitoneally with 2,4- and 2,5-DNP, oxygen consumption increased at 10 mg/kg (17%) and 100 mg/kg (23%), respectively; no increases in oxygen consumption were observed in rats injected intraperitoneally with 2,3-, 2,6-, 3,4-, or 3,5-DNP (Harvey 1959). In guinea pigs injected intraperitoneally with 20 mg/kg 2,4-, 2,6-, and 3,5-DNP, oxygen consumption increased by 37%, 13%, and 19%, respectively; no increases in oxygen consumption were observed in guinea pigs injected intraperitoneally with 2,3-, 2,5-, or 3,4-DNP (Harvey 1959).

In animals, 2,4-DNP uncouples oxidative phosphorylation, resulting in increased basal metabolic rate and body temperature (Burke and Whitehouse 1967; Harvey 1959; Tainter 1938; Tainter and Cutting 1933a, 1933b; Takehiro et al. 1979; Dominguez et al. 1993). Limited evidence from an *in vitro* study and parenteral administration to rats, mice, and guinea pigs indicates that 2,6-DNP may also uncouple oxidative phosphorylation, resulting in increasing basal metabolic rate and body temperature (Burke and Whitehouse 1967; Harvey 1959). Data from an *in vitro* study and parenteral studies in rats, mice, and guinea pigs regarding lethality, uncoupling of oxidative phosphorylation, and oxygen consumption suggest a similar mechanism of action for 3,5-DNP (Burke and Whitehouse 1967; Harvey 1959). In

another study, carbon dioxide output, oxygen consumption, and body temperature were measured in rats treated intraperitoneally with the six isomers of DNP (Cameron 1958). Only 2,4-DNP caused significant increases in all three measures. 3,4-DNP increased carbon dioxide output, but had no effect on oxygen consumption. 2,5-DNP decreased oxygen consumption, but had no effect on carbon dioxide output. 2,3-DNP, 2,6-DNP, and 3,5-DNP had no effect on either measurement. 2,3-DNP, 2,5-DNP, and 2,6-DNP significantly decreased body temperature (by 2.3-4.7 °C). In dogs injected intravenously with 2,4-DNP, 2,5-DNP, or 2,6-DNP, all three isomers increased oxygen consumption and pulmonary ventilation, but 2,4-DNP had the most profound effects, followed by 2,6-DNP (Levine 1977). Although 2,3-, 2,5-, and 3,4-DNP were shown to uncouple oxidative phosphorylation *in vitro,* the effects of these DNP isomers on basal metabolic rate in animals were not clear (Burke and Whitehouse 1967; Cameron 1958; Harvey 1959; Levine 1977).

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in metabolic effects associated with increased basal metabolic rates (e.g., body weight loss, increased body temperature). Metabolic effects from exposure in the ambient environment are possible, based on limited information. Exposure to 2,4-DNP at high workroom or environmental temperatures may increase human susceptibility.

Other Systemic Effects. Two case reports described hearing impairment in patients taking 2,4-DNP for weight reduction. The hearing impairment was secondary to a reactive exudation in the middle ear in a woman who developed severe skin lesions over 100% of her body (Hitch and Schwartz 1936) or to congestion and inflammation of the pharynx (Dintenfass 1934), rather than to nerve impairment.

Immunological and Lymphoreticular Effects. Dermal effects (urticarial and macro-papular rashes discussed in Section 2.2.2.2) have been reported in humans exposed to 2,4-DNP. A woman who developed a severe skin reaction over her entire body after ingesting 2,4-DNP had a mildly positive reaction to a 1:2 dilution and a negative reaction to a 1:10 dilution of 2,4-DNP in a contact skin test (Beinhauer 1934). Three subjects testing negative for dermal sensitization to 2,4-DNP developed a strong urticarial skin reaction following oral administration of the compound, but had no recurrence when they resumed dosing (Matzger 1934). Other studies have noted that some patients who experienced dermal effects were able to resume treatment with no further difficulties and, in some, the rash disappeared while they were still in treatment (Bortz 1934; Tainter et al. 1935b). Patch tests, scratch tests, intradermal tests, and passive transfer tests with 2,4-DNP were performed on

158 people, 117 of whom had hay fever, asthma or urticaria (Matzger 1934). All four tests were negative. This evidence argues against dermal sensitization as a mechanism for the dermal effects.

No evidence exists for skin reactions in animals exposed to 2,4-DNP by non-dermal routes. No gross or histological evidence of treatment-related damage to the spleen, bone marrow, or lymph nodes was reported following long-term treatment of rats via the diet or dogs via capsules at 50-60 and 10 mg/kg/day 2,4-DNP, respectively (Spencer et al. 1948; Tainter et al. 1934b).

Agranulocytosis was reported in several patients who took 2,4-DNP as diet pills. Reports of these cases are discussed above under Hematological Effects.

No studies were located regarding immunological effects in humans or animals after any route of exposure to DNP isomers other than 2,4-DNP.

Neurological Effects. 2,4-DNP appears to affect both the central and peripheral nervous systems. Symptoms of central nervous toxicity, such as fatigue and agitation, have been experienced in people occupationally exposed to 2,4-DNP (Gisclard and Woodward 1946; Perkins 1919). In some workers who died, coma or collapse preceded death. Symptoms of central nervous toxicity (headache, weakness, extreme fatigue, dizziness, euphoria, irrationality, confusion, stupor) have also been experienced by some people who ingested 2,4-DNP for acute to chronic durations (Dintenfass 1934; Goldman and Haber 1936; Imerman and Imerman 1936; Masserman and Goldsmith 1934; Poole and Haining 1934). Coma usually preceded death in fatal cases (Goldman and Haber 1936; Masserman and Goldsmith 1934; Poole and Haining 1934). Autopsy of a woman who died after ingesting 7 mg/kg/day for 5 days revealed hyperemia of the spinal cord, pons, and medulla, and degeneration of ganglion cells (Poole and Haining 1934). However, no pathological lesions were found in the cortex, medulla, cerebellum, pons, or spinal cord of a young girl who became unconscious and died after taking 1.03 mg/kg/day for 46 days (Goldman and Haber 1936). In psychiatric patients with clinical depression, administration of 2,4-DNP resulted in no psychological changes in some patients, an increase in depression in other patients, but an increase in alertness and decreased depression in still others (Masserman and Goldsmith 1934).

Peripheral neuritis, which is characterized by sensations of numbness, “pins and needles,” heat or cold, heightened sensation of pain, loss of taste, and numbness or tingling of the tongue, has been described

in patients taking 2,4-DNP orally at therapeutic doses for weight reduction for acute to intermediate durations (Anderson et al. 1933; Bortz 1934; Epstein and Rosenblum 1935; Hunt 1934; Nadler 1935; Simkins 1937a, 1937b). Doses at which peripheral neuritis was observed ranged from 1.86 to 3.53 mg/kg/day 2,4-DNP. The lowest dose associated with neuritis occurred in a patient who had ceased taking 2,4-DNP eight months earlier (Hitch and Schwartz 1936). Some of the people who experienced loss of taste recovered this sense while dosing continued (Simkins 1937a, 1937b), suggesting the development of tolerance, but the mechanism is unknown. The arthritic-like pains experienced by some patients (see Musculoskeletal Effects) may be related to the development of peripheral neuritis (Nadler 1935). Symptoms of sensory peripheral neuritis were observed 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, but not in those ingesting the sodium salt of 2,4-DNP at an estimated dose of 1.2 mg/kg/day 2,4-DNP for an average of 14 days (Tainter et al. 1935b).

No adequate animal studies testing for neurological end points were located. Pregnant mice treated by gavage with 38.3 mg/kg/day 2,4-DNP during gestation displayed hyperexcitability (Gibson 1973). Dogs (3 per dose group) exposed via capsules to 5 or 10 mg/kg/day 2,4-DNP for 6 months had no gross or histological evidence of brain or spinal cord damage (Tainter et al. 1934b). No studies were located regarding neurological effects in humans or animals after any route of exposure to DNP isomers other than 2,4-DNP.

It is not known whether current workroom levels of 2,4-DNP or levels around hazardous waste sites are high enough to result in neurological effects. Neurological effects from exposure in the ambient environment seem unlikely, based on limited information.

Reproductive Effects. No studies were located regarding reproductive effects in humans after inhalation or dermal exposure to any isomer of DNP. Three case reports and a clinical study of women taking 2,4-DNP orally for weight reduction suggest that 2,4-DNP may affect the female reproductive system (Beinhauer 1934; Epstein and Rosenblum 1935; Goldman and Haber 1936; Simkins 1937a, 1937b), but this limited information is inconclusive. Autopsy of a 13-year-old girl who died revealed a small and infantile uterus and numerous follicular cysts in the ovary; menses had not yet begun (Goldman and Haber 1936). In addition, physical examination of a woman revealed fibroid degeneration of the uterus and a cystic left ovary (Beinhauer 1934). Whether these were

preexisting conditions is not known. A miscarriage (Epstein and Rosenblum 1935) and altered menstrual cycles (Simkins 1937a, 1937b) were attributed to the ingestion of 2,4-DNP.

No adequate animal studies testing for reproductive end points were located. However, no gross or histological evidence of treatment-related damage to testes was reported following long-term treatment of rats via the diet up to 50 mg/kg/day (Spencer et al. 1948) or dogs via capsules at 50-60 and 10 mg/kg/day 2,4-DNP (Tainter 1938; Tainter et al. 1934b). Testicular atrophy was noted in rats receiving 350 mg/kg/day (Spencer et al. 1948). This finding is questionable, however, because the rats appeared to be starving.

No studies were located regarding reproductive effects in animals after inhalation or dermal exposure to 2,4-DNP or after any route of exposure to the other isomers of DNP. Information is insufficient to predict whether reproductive effects could occur in humans exposed to 2,4-DNP under any exposure scenario.

Developmental Effects. No studies were located regarding developmental effects of any DNP isomer in humans.

Increased incidence of stillborn pups and pup mortality during lactation was reported in rats treated by gavage with at least 20 mg/kg/day, prenatally for 8 days, throughout gestation, and during lactation (Wulff et al. 1935). Evidence of developmental toxicity was reported in rats injected subcutaneously (decreased fetal weight and length, more early resorptions) (Goldman and Yakovac 1964), and mice injected intraperitoneally (decreased fetal weight and length) (Gibson 1973) with 2,4-DNP. Two oral gavage studies reported no developmental toxicity at exposure levels producing maternal toxicity; these studies were inadequate with respect to experimental protocol or reporting of protocol and results (Gibson 1973; Kavlock et al. 1987).

No studies were located regarding developmental effects in animals after inhalation or dermal exposure to 2,4-DNP or after any route of exposure to the other isomers of DNP. Information is insufficient to predict whether developmental effects could occur in humans exposed to 2,4-DNP under any exposure scenario.

Genotoxic Effects. No studies were located regarding genotoxicity in humans after exposure to 2,4-DNP.

2,4-DNP has been tested for genotoxicity in several in viva 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 2-3 and 2-4). 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 ^{14}C-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 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. Mice were injected intraperitoneally with 0.25, 0.50, and 1 mL of a saturated solution of 2,4-DNP, 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.

In *in vitro* studies of prokaryotic organisms, 2,4-DNP was negative for reverse mutations using one or more standard stains of *S. 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 (see Toxicity of Dinitrophenol Metabolites below). The S9 fraction contains both microsomal and soluble enzymes that metabolize 2,4-DNP to amino

Table 2-3. Genotoxicity of Dinitrophenols *In Vivo*

Species (test system)	End point	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)	–	Freidman and Staub 1976	2,4-DNP

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

Table 2-4. Genotoxicity of Dinitrophenols *In Vitro*

Species (test system)	End point	Result: With activation	Result: Without activation	Reference	Isomer
Prokaryotic organisms:					
Salmonella typhimurium					
TA98	Reverse mutation	No data	–	Chiu et al. 1978	2,4,-DNP
TA100		No data	–		
S. typhimurium	Reverse mutation			Garner and Nutman 1977	2,4-DNP
TA1538		–	–		
S. typhimurium	Reverse mutation			Anderson and Styles 1978	2,4-DNP
TA98		–	No data		
TA100		–	No data		
TA1535		–	No data		
TA1538		–	No data		
S. typhimurium	Reverse mutation			Kleinhofs and Smith 1976	2,4-DNP
TA1530		No data	–		
S. typhimurium	Reverse mutation			Probst et al. 1981	2,4-DNP
TA98		–	–		
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
TA1538		–	–		
G46		–	–		
C7036		–	–		
D3052		–	–		
S. typhimurium	Reverse mutation			De Flora 1981	2,4-DNP
TA98		–	–		
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
TA1538		–	–		
S. typhimurium	Reverse mutation			Kawai et al. 1987	
TA98		–	(+)		2,4-DNP
TA100		–	(+)		2,4-DNP

Table 2-4. Genotoxicity of Dinitrophenols *In Vitro* (continued)

Species (test system)	End point	Result: With activation	Result: Without activation	Reference	Isomer
S. typhimurium	Reverse mutation			Kawai et al. 1987	
TA98		+	+		2,3-DNP
TA100		+	+		2,3-DNP
S. typhimurium	Reverse mutation			Kawai et al. 1987	
TA98		+	+		2,5-DNP
TA100		+	+		2,5-DNP
S. typhimurium	Reverse mutation			Kawai et al. 1987	
TA98		–	–		2,6-DNP
TA100		–	–		2,6-DNP
S. typhimurium	Reverse mutation			Kawai et al. 1987	
TA98		–	–		3,4-DNP
TA100		+	+		3,4-DNP
S. typhimurium TA1535/pSK1002	DNA damage (induction of sister chromatid exchange response)	–	–	Nakamura et al. 1987	2,4-DNP
Escherichia coli	Reverse mutation			Probst et al. 1981	2,4-DNP
WP2		–	–		
WP2(uvrA-)		–	–		
E. coli	Reverse mutation			Demerec et al. 1951	2,4-DNP
B/Sd-4/1,3,4,5		No data	+		
B/Sd-4/3,4		No data	+		
E. coli K-12(lamdba)	Phage induction	No data	–	Heinemann and Howard 1964	2,4-DNP
Eukaryotic organisms:					
Mammalian cells:					
Chinese hamster ovary cells V79	DNA damage (alkali elution)	–	–	Swenberg et al. 1976	2,4-DNP

Table 2-4. Genotoxicity of Dinitrophenols *In Vitro* (continued)

Species (test system)	End point	Result		Reference	Isomer
		With activation	Without activation		
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 ATP pools.

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid

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- and 2,5-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).

Using *Esherichia 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. 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.

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 end point 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 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).

Cancer. No studies were located regarding cancer in humans after exposure to any DNP isomer.

No studies were located regarding cancer in animals after inhalation or oral exposure to any isomer of DNP. 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); in one of the studies, the authors concluded that the introduction of nitro groups into the phenol ring destroyed the promoting effect of phenol.

Thus, 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.

Toxicity of Dinitrophenol Metabolites. Since dinitrophenols are metabolized in the body (Davidson and Shapiro 1934; Eiseman et al. 1972; Gisclard and Woodward 1946; Okino and Yasukura 1957; Perkins 1919; Robert and Hagardorn 1985), exposure to DNPs also results in exposure to DNP metabolites. While information on the metabolism of DNPs is limited to 2,4-DNP, it is likely that the metabolism of the other DNP isomers would also be carried out by a nitroreductase and result in aminonitrophenols and diaminophenols. Table 3-3 in Chapter 3 lists the parent DNPs and their likely metabolites.

No studies were located regarding human toxicity for any DNP metabolite. Toxicity information in animals and genotoxicity studies are available for three DNP metabolites that are used in hair dye

formulations (IARC 1993a, 1993b; NCI 1978; NTP 1988a, 1988b): 2-amino-4-nitropheno1, 4-amino-2-nitrophenol, and 2-amino-5nitrophenol (a potential metabolite of 2,5-DNP). Only genotoxicity information is available for 2,4-diaminophenol. This information will be summarized below; readers needing more information are advised to consult the listed references. Genotoxicity information is summarized in Tables 2-5 and 2-6.

The LD_{50} of 2-amino-4-nitrophenol has been reported to be 2,400 mg/kg body weight in rats after oral administration (Lloyd et al. 1977). The toxicity of 2-amino-4-nitrophenol was studied by corn oil gavage 5 days per week in Fischer 344/N rats and B6G3Fl mice of each sex for 15 days, 13 weeks, and 2 years (NTP 1988a). During the 15-day study all rats and mice administered 5,000 or 2,500 mg/kg 5 days a week, and all female rats and mice plus 2 of 5 male mice administered 1,250 mg/kg 5 days a week died before the completion of the study. One of 5 female mice administered 313 mg/kg 5 days a week died before the end of the study. Although the final mean body weights of surviving rats and mice were comparable to vehicle controls, diarrhea was observed in all groups of treated rats except those administered 313 mg/kg 5 days a week, the lowest dose in the study. In the 13-week study, all rats receiving 1,000 mg/kg 5 days a week, and 2 of 10 males and 2 of 10 female rats receiving 500 mg/kg 5 days a week, died before completion of the study. All male and most female mice administered 500 mg/kg 5 days a week died. A 10% reduction in final mean body weight of the male rats administered 500 mg/kg 5 days a week was observed. Diarrhea and lethargy were reported in rats administered 500 or 1,000 mg/kg 5 days a week. Other effects at these doses were mineralization of the renal cortex and degeneration of the renal tubular epithelium. In the 2-year study, survival of male rats receiving 250 mg/kg 5 days a week was much lower than the vehicle control survival, while the female rat and mouse survival in both sexes was comparable to control. An 8-10% reduction in body weight in male rats and a 17% increase in body weight in female mice were observed. Soft stools, occasional diarrhea, and pigmentation of the small and large intestine were detected in all rats. Male rats had a higher incidence of nephropathy, digestive ulcers, and erosive lesions of the gastrointestinal tract.

2-Amino-4-nitrophenol was mutagenic in *Salmonella 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

Table 2-5. Genotoxicity of Dinitrophenol Metabolites *In Vivo*

Species (test system)	End point	Results	Reference	Metabolite
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-nitrophenol

Table 2-6. Genotoxicity of Dinitrophenol Metabolites *In Vitro*

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

Table 2-6. Genotoxicity of Dinitrophenols Metabolites *In Vitro* (continued)

Species (test system)	End point	Result		Reference	Metabolite
		With activation	Without activation		
S. typhimurium	Reverse mutation			Shahin et al. 1982	2-a-5np
TA98		+	+		
TA100		–	(+)		
TA1535		–	+		
TA1537		+	+		
TA1538		+	+		
S. typhimurium	Reverse mutation			Ames et al. 1975	2-a-5np
TA1538		No data	+		
S. typhimurium	Reverse mutation			Chiu et al. 1982	2-a-5np
TA98		No data	+		
TA100		No data	–		
S. typhimurium	Reverse mutation			Zeiger et al. 1987	2-a-5np
TA98		+	+		
TA100		(+)	(+)		
TA1535		–	–		
TA1537		(+)	(+)		
S. typhimurium	Reverse mutation			Garner and Nutman 1977	4-a-2np
TA98		+	+		
TA1538		+	+		
S. typhimurium	Reverse mutation			Zeiger et al. 1987	4-a-2np
TA97		+	+		
TA98		+	+		
S. typhimurium	Reverse mutation			Shahin et al. 1982	4-a-2np
TA98		–	–		
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
TA1538		–	–		

Table 2-6. Genotoxicity of Dinitrophenols Metabolites *In Vitro* (continued)

Species (test system)	End point	Result		Reference	Metabolite
		With activation	Without activation		
S. typhimurium 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
Chinese hamster ovary cells	Sister chromatid exchange	+	+	NTP 1988a	2-a-4np
Chinese hamster ovary cells	Sister chromatid aberrations	+	+	NTP 1988a	2-a–4np
Chinese hamster ovary cells	Sister chromatid aberrations	+	+	Anderson et al. 1990	2-a-4np
Mouse lymphoma L518Y cells	Gene mutation	No data	+	NTP 1988b	2-a-5np
Chinese hamster ovary cells	Sister chromatid exchange	+	+	NTP 1988b	2-a-5np
Chinese hamster ovary cells	Chromosomal aberrations	+	+	NTP 1988b	2–a–5np
Rat F344 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

+ = positive result; (+) = weakly positive or equivocal result; – = negative 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

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).

A 2-year carcinogenesis study has been conducted where Fischer 344/N rats and B6C3Fl mice of each sex received 0, 125, or 250 mg/kg body weight 2-amino-4-nitrophenol by gavage 5 days a week (NTP 1988a). Colon carcinoma in a male rat receiving 250 mg/kg 5 days a week was reported; however, no other digestive tract neoplasms were detected. Renal tubular cell hyperplasia and renal cortical adenomas also occurred in male rats. Low-dose male rats had a higher incidence of preputial gland adenomas or carcinomas than the vehicle controls. The incidence of clitoral gland neoplasms was decreased in female rats. Evidence for carcinogenicity was described as “some evidence” for male rats and “no evidence” for female rats and both sexes in mice.

4-Amino-2-nitrophenol was administered in the diet to groups of 5 Fischer 344/N rats of both sexes at doses of 0-6,810 ppm (0-540 mg/kg/day) and to groups of 5 B6C3Fl mice of both sexes at doses of 0-4,640 ppm (0-788 mg/kg/day) for 6 weeks (NCI 1978). There were no deaths in the rats or mice, nor were there any changes in the mean body weights of the dosed animals in comparison with controls, other than an approximate 20% weight depression in female rats fed concentrations of 3,160 ppm (253 mg/kg/day) or greater. No gross pathologic changes were noted. In rats and mice given feed containing 1,250 or 2,500 ppm (100 or 200 mg/kg/day) 4-amino-nitrophenol for 2 years, mean body weights and survival were not affected by the test chemical (NCI 1978). Deposition of pigment occurred in the lamina propria of the small intestine in over 90% of the rats and the mice, but did not occur in control animals.

Commercial grade 4-amino-2-nitrophenol was reported to be mutagenic in *3. 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 and TA98 (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 rats and mice receiving feed containing 1,250 ppm and 2,500 ppm (100 and 200 mg/kg/day) 4-amino-2-nitrophenol (NCI 1978), male Fischer 344/N rats had a statistically significant increase of transitional-cell carcinomas of the urinary bladder over control rats. Carcinomas of the bladder also occurred in one low-dose female and two high-dose female rats but in none of the control group. In B6C3Fl mice, no tumors occurred in dosed groups of males or females at incidences that were significantly higher than the control group.

The LD_{50} of 2-amino-5-nitrophenol in rats has been reported to be greater than 4,000 mg/kg body weight by oral administration (Burnett et al. 1977). During 16-day studies (NTP 1988b), groups of 5 Fischer 344/N rats of each sex received doses ranging from 0 to 2,500 mg/kg 5 days a week and groups of 5 B6C3Fl mice of each sex received doses ranging from 0 to 5,000 mg/kg 5 days a week by gavage in corn oil. A dose-related reduction of survival was observed in the female mice. In 13-week studies, groups of 10 Fischer 344/N rats and 10 B6C3Fl mice received doses ranging from 0 to 1,600 mg/kg 5 days a week. A dose-related reduction in survival was observed in the rats. Rats receiving 400-1,600 mg/kg 5 days a week and mice receiving 1,600 mg/kg 5 days a week had acute and chronic perivasculitis of the vessels of the caecum and the colon. In 2-year studies using the same doses as the 13-week study, acute and chronic inflammation of the caecum and colon were observed in low- and high-dose male rats, high-dose female rats, and high-dose male mice. This inflammation was accompanied by the accumulation of an orange, granular pigment in the submucosa of the intestine. Focal ulceration of the intestinal mucosa was often present.

In a phage induction test for mutagenicity in *E. coli,* 2-amino-5-nitrophenol was mutagenic without activation (Kvelland 1985). In *Salmonella 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).

In 2-year studies conducted by the National Toxicology Program (NTP 1988b), male Fischer 344/N rats receiving 100 mg/kg 2-amino-5-nitrophenol 5 days a week had an increase in pancreatic cell adenomas. Female rats dosed at 0, 100, and 200 mg/kg 5 days a week showed no evidence of carcinogenicity, as did mice of both sexes dosed at 0, 400, and 800 mg/kg 5 days a week. Evidence of carcinogenicity was judged to be “some evidence” for male rats and “no evidence” for female rats and both sexes of mice (NTP 1988b).

2,4-Diaminophenol was reported to be mutagenic only with activation in *S. typhimurium* strain TA1538 (Dybing and Thorgeirsson 1977). Another report (Kawai eta 1. 1987) stated that 2,4-diaminophenol was mutagenic, but did not produce further information.

Little information on the toxicokinetics of DNP metabolites is available. 4-Amino-2-nitrophenol in an acetone vehicle was rapidly absorbed through monkey skin (Bronaugh and Maibach 1985). 2-Amino-4-nitrophenol in mixtures resembling hair dye formulations was only slowly absorbed through rat skin (Hofer et al. 1982).

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 for 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. There are no chronic-duration studies in animals treated with 2,4-DNP that have assessed carcinogenicity. It appears unlikely that exposure to DNP metabolites as a result of exposure to 2,4-DNP would cause systemic effects. It is not known whether metabolites of 2,4-DNP are genotoxic or can cause cancer in humans. 2-Amino-4-nitrophenol and 2-amino-5-nitrophenol have been designated as "not classifiable as to their carcinogenicity to humans" (IARC 1993a, 1993b).

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1982).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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 (NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to DNPs are discussed in Section 2.5.1.

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 1982). 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 effects caused by DNPs are discussed in Section 2.5.2.

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 2.7, Populations That Are Unusually Susceptible.

2.5.1 Biomarkers Used to Identify or Quantify Exposure to Dinitrophenols

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 Hagardorn 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. 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. This test is still listed as diagnostic for 2,4-DNP intoxication (Duke Poison Control Center 1995), although no reports of its clinical use in recent years were located in the literature. m-Dinitrobenzene is metabolized to 2-amino-4-nitrophenol (Parke 1961) and would also give a positive Derrien test. The total amount or concentration of 2,4-DNP and its principal metabolite(s) would probably be a better indicator of exposure than either alone; however, this requires specialized laboratory measurement.

2,4-Dinitroanisole is metabolized in the body to 2,4-DNP. The possibility of 2,4-dinitroanisole exposure should be considered if 2,4-DNP is found in blood or urine (Hayes 1982).

Yellow staining of the skin or sclera rarely occurs with 2,4-DNP (in contrast with 4,6-dinitro-*o*-cresol) and is due to the color of 2,4-DNP.

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

2.5.2 Biomarkers Used to Characterize Effects Caused by Dinitrophenols

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. 1935b). 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 like 2,4-DNP 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.

No studies correlating blood or urine levels of 2,4-DNP and its metabolites with toxic effects in humans were located. Higher levels of 2,4-DNP were found in the aqueous and vitreous humor and lens of animals susceptible to 2,4-DNP cataractogenesis; elimination from serum of nonsusceptible animals was more rapid than from that of susceptible animals (Gehring and Buerge 1969b). 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.

In one study in humans, an increase in galactose tolerance and in phenoltetraiodophthalein retention (indicating impaired liver function) was seen at an oral dose of 4.3 mg/kg/day 2,4-DNP for acute- or intermediate-exposure durations (MacBryde and Taussig 1935).

Additional information regarding biomarkers for effects can be found in OTA (1990) and CDC/ATSDR (1990). A more detailed discussion of the health effects caused by 2,4-DNP can be found in Section 2.2 of Chapter 2.

2.6 INTERACTIONS WITH OTHER SUBSTANCES

The belief that alcoholics are more susceptible to the toxicity of 2,4-DNP during occupational exposure (Perkins 1919) may indicate an interaction with ethanol (and possibly other alcohols) or it may simply be a function of the compromised physiological state of alcoholics. 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, and, therefore, the metabolic effect of 2,4-DNP enhances the clearance of ethanol (Eriksson et al. 1974). Because 2,4-DNP is known to augment the rate of respiration and perspiration, ≈2.7-8.2% of the initial dose of ethanol was also eliminated by expiration and cutaneous evaporation in the rat (Israel et al. 1970).

In an attempt to determine the best treatment regimen for mice given intraperitoneal doses of 4,6-dinitro-*o*-cresol (DNOC), which, like 2,4-DNP, uncouples oxidative phosphorylation and is hyperthermic, the effect of hypothermic dinitrophenols (i.e., 2,3-DNP, 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 hypothermic DNPs were given immediately after DNOC, mortality was 60% after 2,3-DNP, 100% after 2,5-dinitrophenol, and 50% after 3,4-dinitrophenol. 2,4-DNP, which, like DNOC, is hyperthermic, afforded no protection of the mice.

In isolated perfused rat livers, 2,4-DNP caused a depletion of the mitochondrial calcium pool, without altering the extramitochondrial calcium pool (Kleineke and Ming 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, Section 2.8.3) 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; Ellenhom 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 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.

4,6-Dinitro-*o*-cresol (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 2.3.5, 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.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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 arid 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 we expect 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. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

Experience with occupationally exposed populations in the munitions industry in France in 1917-1918 indicated that workers with renal or hepatic disease or with alcoholism were especially susceptible to 2,4-DNP toxicity (Perkins 1919). Increased numbers of clinical cases of poisoning were seen during the warmer months of the year (Perkins 1919; Gisclard and Woodward 1946), but it is uncertain whether this finding was related to greater exposure of 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 hypertheimia, persons with the genetic predisposition may be more susceptible to the hyperthermic effects of 2,4-DNP.

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).

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 B_2 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 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 B_2 (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 polyvinylchloride (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.

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.

No studies were located regarding unusually susceptible populations in regard to the other isomers of DNP.

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and experimental research concerning methods for reducing toxic effects of exposure to DNPs. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposure to DNPs. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

The available information on methods for reducing toxic effects is concerned with 2,4-DNP. The other DNP isomers do not appear to have been a clinical problem and are not discussed in the reports dealing with this topic.

2.8.1 Reducing Peak Absorption Following Exposure

Because 2,4-DNP is readily absorbed from the respiratory tract, little can be done to reduce its absorption other than removal of the person from the contaminated area to prevent further exposure (Bronstein and Currance 1988).

2,4-DNP is relatively lipophilic and has a pK_a of 4.09 (see Chapter 3) and, therefore, may be absorbed by passive diffusion by the upper gastrointestinal tract where the pH is low and DNP is non-ionized. Administration of bicarbonate to increase the pH of the upper gastrointestinal tract might reduce the absorption of 2,4-DNP, but no information regarding this possible treatment was located. In cases of

acute ingestion, measures that have been suggested to slow or prevent absorption within the first few hours include ingestion of water (Bronstein and Currance 1988), ipecac to induce emesis, gastric lavage, activated charcoal, and a cathartic such as magnesium sulfate (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Stutz and Janusz 1988). The use of emetics, however, has been discouraged by other authors (Bronstein and Currance 1988). Gastric lavage with large quantities of sodium bicarbonate solution has also been recommended (National Safety Council 1988).

For dermal exposure, removal of contaminated clothes and decontamination of the skin (and eyes) with copious amounts of water is frequently recommended (Bronstein and Currance 1988; Haddad and Winchester 1990; Stutz and Janusz 1988).

2.8.2 Reducing Body Burden

No studies or recommendations were located for reducing the body burden of 2,4-DNP. Elimination from the body appears to be rapid, except possibly in cases of compromised liver function. Whether procedures such as dialysis would result in significant reductions of body burden is uncertain, because there is evidence from animal studies that a portion of the circulating 2,4-DNP is bound to serum proteins (Gehring and Buerge 1969b).

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

2,4-DNP uncouples oxidative phosphorylation from electron transport by carrying protons across the inner mitochondrial membrane, thereby dissipating the pH gradient and membrane electrochemical potential and preventing the production of ATP (see Section 2.3.5). Electron transport from NADH to oxygen proceeds normally, but the energy produced is dissipated as heat, accounting for the pyrexic effects of 2,4-DNP. It is also by this mechanism that 2,4-DNP exerts its other toxic effects, since ATP is required for most energy-dependent biochemical processes. The hyperpyrexia and lethality of 2,4-DNP have been significantly diminished in animals by pretreatment of the animals with haloperidol (Gatz and Jones 1972). Further *in vitro* experiments suggested that haloperidol interfered (probably indirectly by an action at the mitochondrial membrane) with the uncoupling of oxidative phosphorylation by 2,4-DNP. Hence, haloperidol may be a candidate for further investigation as an antidote to 2,4-DNP.

Rapid cooling of the body (using a hypothermia blanket or ice) is suggested to control hyperpyrexia (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). The protective effect of cooling the body with water after 2,4-DNP administration is documented in animals (Harvey 1959). The excessive perspiration noted in many of the human studies and case reports can contribute to dehydration; administration of fluids would be appropriate to counteract this effect.

Glucose administration may also be useful since glycolysis would be the main source of ATP production in 2,4-DNP poisoned cells.

Salicylates and anticholinergics are contraindicated because they may potentiate the action of 2,4-DNP (Brody 1956; Ellenhom and Barceloux 1988; Haddad and Winchester 1990). Although salicylate may increase cell viability in tissues that have been exposed to 2,4-DNP (Nishihata et al. 1988a, 1988b) (see Section 2.6), salicylate in high doses can also uncouple oxidative phosphorylation, increase respiratory rates, and produce hyperthermia in humans (Brody 1956), and possibly exaggerate these signs in persons acutely exposed to 2,4-DNP.

2,4-DNP induced cataracts in young people when it was given as a diet pill in the 1930s. Data from an *in vitro* study suggest that vitamin E delays or minimizes development of radiation-induced cataracts in isolated rat lenses (Ross et al. 1983). Data from an epidemiological study involving adults over 55 years of age suggest that daily vitamin E or vitamin C supplementation or a combination of the two antioxidants reduced senile cataract risk (Robertson et al. 1989). In another study involving adults aged 40-70 years, high plasma concentrations of at least two antioxidant vitamins reduced the risk of senile cataracts (Jacques et al. 1988). 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 vitamin C deficiency made the guinea pigs susceptible to 2,4-DNP cataractogenesis. Therefore, if the mechanisms of induction of 2,4-DNP-induced and senile cataracts are similar, antioxidant vitamins may protect people from 2,4-DNP-induced cataracts. Whether vitamin therapy would be beneficial in treating 2,4-DNP-induced cataracts is not known.

2.9 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 the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of DNPs.

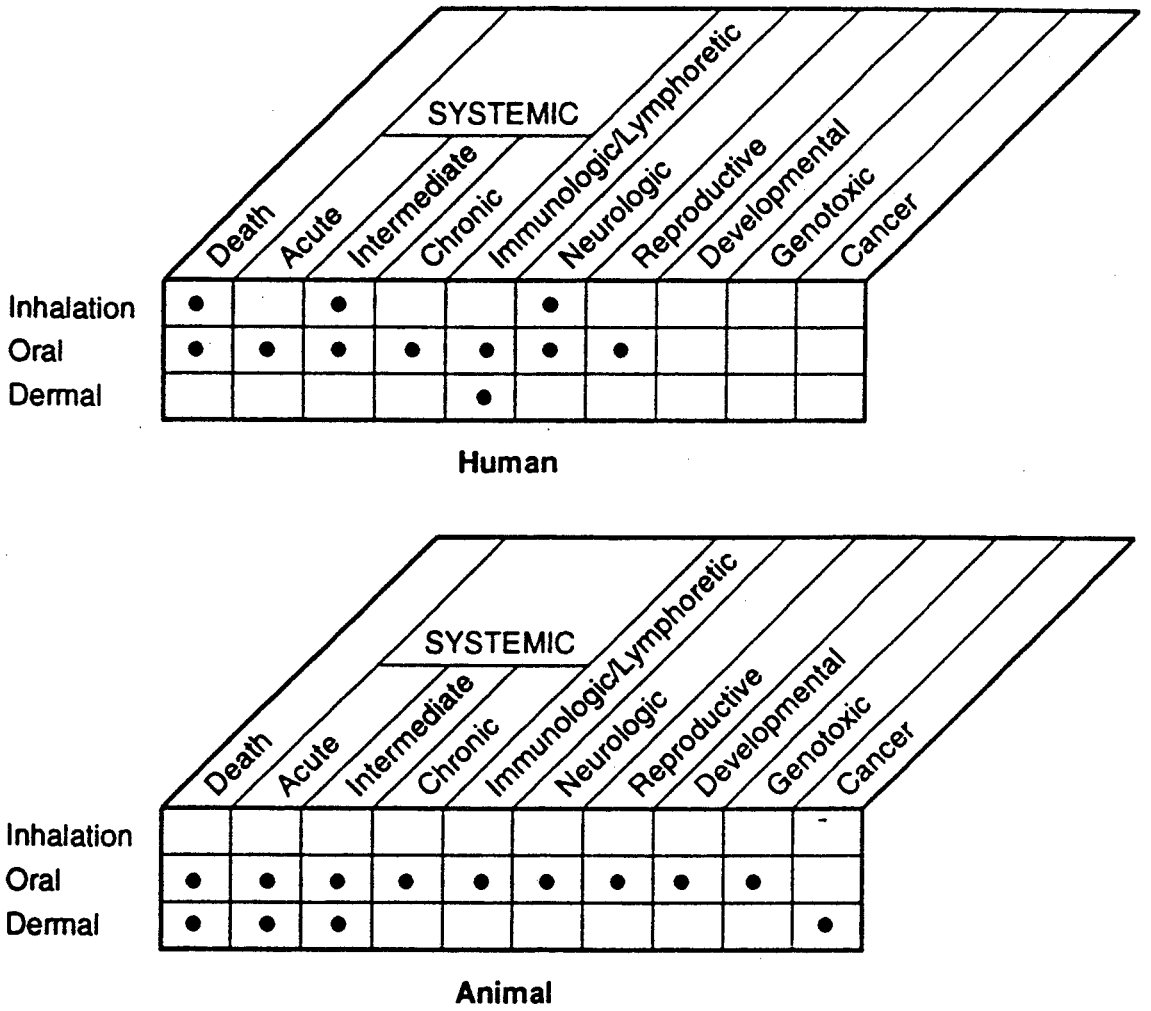
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean 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.

2.9.1 Existing Information on Health Effects of Dinitrophenols

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 2,4-DNP are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of DNPs. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs." 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.

As shown in Figure 2-3, data exist regarding death, systemic effects, and neurological effects in humans after occupational exposure to 2,4-DNP for intermediate durations. The occupational exposure appeared to involve primarily inhalation with some contribution from dermal contact. The systemic effect categories for which some data were available were respiratory, gastrointestinal, hepatic, renal, body weight, and metabolic. Data also exist regarding death, systemic effects of acute-, intermediate-, and chronic-duration exposure, and immunologic and neurologic effects in humans after oral exposure

FIGURE 2-3. Existing Information on Health Effects of 2,4-Dinitrophenol



● Existing Studies

to 2,4-DNP. The systemic effect categories for which some data were located were respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, metabolic, and other (hearing). The ocular effects consisted of cataract formation after acute-, intermediate- or chronic-duration oral exposure to 2,4-DNP.

Most of the data regarding health effects in humans after oral exposure to 2,4-DNP come from case reports and clinical studies of the use of 2,4-DNP and sodium 2,4-DNP as diet pills in the 1930s. Although these studies have limitations, they provide a useful and reasonably consistent picture of dose-effect relationships for acute- and intermediate-duration exposure.

No data were located regarding health effects in animals after inhalation exposure to 2,4-DNP. Animal data exist regarding death, systemic effects of acute-, intermediate, and chronic-duration exposure, and immunologic, neurologic, developmental, reproductive, and genotoxic effects after oral exposure. The systemic effect categories for which some data were available were respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, and metabolic.

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).

2.9.2 Identification of Data Needs

Acute-Duration Exposure. No studies were located regarding health effects in humans after inhalation or dermal exposure to 2,4-DNP for acute durations. Therefore, data are not available to derive an acute-duration inhalation MRL.

In humans exposed via acute oral ingestion, death has occurred from the chemical's pyretic effects following what appeared to be intentional ingestion of overdoses (Tainter and Wood 1934). Other patients have died in a comatose condition after taking 2,4-DNP for acute durations (Masserman and Goldsmith 1934; Poole and Haining 1934).

Information on health effects in humans after both acute-duration and intermediate-duration oral exposure to 2,4-DNP indicates that the characteristic effects of 2,4-DNP for this route are increased basal metabolic rate and perspiration, weight loss, a sensation of warmth, and, at higher dosages, increased pulse, respiratory rate, and body temperature (Castor and Beierwaltes 1956; Cutting and Tainter 1933; Cutting et al. 1933, 1934; Dintenfass 1934; Dunlop 1934; Lattimore 1934; MacBryde and Taussig 1935; Masserman and Goldsmith 1934; Poole and Haining 1934; Tainter et al. 1935b). These effects are related to the uncoupling of mitochondrial oxidative phosphorylation by 2,4-DNP. A LOAEL for these effects has been identified on the basis of weight loss and characteristic signs of metabolic toxicity, but a NOAEL is not defined. An acute oral MRL of 0.01 mg/kg/day was derived from the LOAEL of 1.2 mg/kg/day 2,4-DNP observed for weight loss (Tainter et al. 1935b). Respiratory effects (increased respiratory rate) appear to be secondary to the increased basal metabolic rate and body temperature (Cutting et al. 1934; Masserman and Goldsmith 1934; Tainter and Wood 1934). Some of the cardiovascular effects (increased pulse rate) noted in humans are secondary to the elevated metabolic rate, but abnormal electrocardiograms were seen in patients treated with relatively high dosages of 2,4-DNP (MacBryde and Taussig 1935) suggesting the heart may be a target organ. A death was attributed to myocarditis due to acute oral ingestion of 2,4-DNP (Lattimore 1934), and segmentation and fragmentation of cardiac muscles were found in another fatal case (Poole and Haining 1934). There is evidence of gastrointestinal effects (nausea and vomiting, hemorrhage, edema, and degeneration) (Lattimore 1934; MacBryde and Taussig 1935; Poole and Haining 1934), liver effects (Lattimore 1934; MacBryde and Taussig 1935; Tainter and Wood 1934), and musculoskeletal effects (probably secondary to uncoupling of oxidative phosphorylation or peripheral neuritis) (Anderson et al. 1933; MacBryde and Taussig 1935; Nadler 1935; Poole and Haining 1934); the available data also suggest that the kidney is a target organ in fatal cases due to acute oral exposure (Lattimore 1934; Poole and Haining 1934). In addition, a case report has attributed agranulocytosis (a syndrome characterized by marked decrease in the number of granulocytes, lesions of the throat and other mucous membranes, and fever; also called granulocytopenia, malignant neutropenia, or agranulocytic angina) to acute-duration ingestion of a relatively high dosage of 2,4-DNP (Goldman and Haber 1936; Hoffman et al. 1934; Silver 1934). Dermal effects and cataracts have also been observed in humans ingesting 2,4-DNP for acute durations (Anderson et al. 1933; Dintenfass 1934; Hitch and Schwartz 1936). Hearing impairment has been found to be a complication of severe dermal lesions (Hitch and Schwartz 1936) or pharyngitis (Dintenfass 1934) in humans who ingested 2,4-DNP for acute durations.

Animal lethality data are available for the oral route in a number of species; clear differences in sensitivity were not seen among species. Death was generally attributed to the pyretic effect of 2,4-DNP. Increases in body temperature occurred in animals given 2,4-DNP (Tainter and Cutting 1933a, 1933b) at relatively high doses; in general, the increases were less pronounced than in humans. Highly abnormal electrocardiograms were seen in dogs at high dosage levels (Kaiser 1964). A few acute-duration oral studies in animals have investigated histopathological effects (Arnold et al. 1976; Spencer et al. 1948; Tainter and Cutting 1933b), but results were not consistent or conclusive. Some evidence of renal effects was seen in two rat studies (Arnold et al. 1976; Spencer et al. 1948) but not in a dog study (Tainter and Cutting 1933b). Hematological effects have not been investigated in animals after acute-duration oral exposure.

A single-exposure dermal study in guinea pigs reported death from dermal administration (Spencer et al. 1948). Hyperemia, edema, and denaturation of the skin was reported in rabbits after dermal exposure (Dow Chemical Co. 1940).

Additional acute studies in animals to better define the threshold region for acute-duration oral exposure would be useful. The animal data currently available, particularly for intermediate-duration exposure, suggest that the tested species may not be the best models for human response to this chemical because effects observed in animals occurred at much higher doses than the doses taken by humans who developed adverse effects. Test animals, especially small mammals and birds, have significantly higher metabolic rates than humans, which they maintain by consuming more food per kilogram body weight. A more suitable model for 2,4-DNP-induced metabolic toxicity might be larger animals, for example, swine or large dogs. However, the most sensitive toxic end points for humans (body weight loss and metabolic effects) can be reproduced in most test animals, as can the serious end point of cataracts in birds and rabbits.

Although human data regarding acute-duration inhalation and dermal exposure are not available, data from intermediate-duration occupational exposure indicate that effects related to uncoupling of oxidative phosphorylation and gastrointestinal effects probably are not route-specific. Acute-duration inhalation and dermal studies in animals could be conducted to determine dose-response relationships for these routes.

Intermediate-Duration Exposure. Occupational exposure to 2,4-DNP resulted in death from pyrexia in some cases, and fatigue, elevation of body temperature, increased respiratory rate, profuse perspiration, and weight loss in some of the workers (Gisclard and Woodward 1946; Perkins 1919). Exposure occurred by the inhalation and also probably by the dermal route. Gastrointestinal symptoms (nausea, vomiting) were also noted (Perkins 1919). Autopsy of the fatal cases did not reveal any characteristic lesions, other than pulmonary edema, which was thought to be secondary to vasomotor effects. Hence many of the effects of 2,4-DNP in humans do not appear to be route-specific. No exposure levels were reported in these studies.

No inhalation studies in animals exposed to 2,4-DNP for intermediate durations were located. Therefore, information in humans and animals exposed by inhalation is insufficient to derive an intermediate-duration inhalation MRL.

In general, the effects in humans that are obvious consequences of the uncoupling of oxidative phosphorylation appear to occur with the same intensity at the same oral dose levels during intermediate-duration oral exposure (Bayer and Gray 1935; Castor and Beierwaltes 1956; Cutting et al. 1933, 1934; Dunlop 1934; Epstein and Rosenblum 1935; Goldman and Haber 1936; Horner et al. 1935; Hunt 1934; Imerman and Imerman 1936; Looney and Hoskins 1934; MacBryde and Taussig 1935; Nadler 1935; Rank and Waldeck 1936; Simkins 1937a, 1937b, Tainter et al. 1935b) as during acute-duration exposure. As for acute-duration exposure, the threshold for these effects is not welldefined; no NOAEL is available. Case reports and clinical studies of people ingesting 2,4-DNP for intermediate durations have identified the lungs (Goldman and Haber 1936; Simkins 1937a, 1937b), cardiovascular system (Epstein and Rosenblum 1935; Goldman and Haber 1936; MacBryde and Taussig 1935; Masserman and Goldsmith 1934; Rank and Waldeck 1936; Simkins 1937a, 1937b), gastrointestinal tract (Bayer and Gray 1935; Goldman and Haber 1936; MacBryde and Taussig 1935; Simkins 1937a, 1937b), hematological system (agranulocytosis) (Davidson and Shapiro 1934; Goldman and Haber 1936), musculoskeletal system (MacBryde and Taussig 1935; Nadler 1935), liver (Goldman and Haber 1936, MacBryde and Taussig 1935), kidney (Goldman and Haber 1936; Simkins 1937a, 1937b), skin (Hunt 1934; MacBryde and Taussig 1935; Simkins 1937a, 1937b; Tainter et al. 1935b), and eyes (Hill 1936; Horner et al. 1935; Rank and Waldeck 1936; Simkins 1937a, 1937b; Tainter et al. 1935b) as target organs of the effects of 2,4-DNP. The effects on these organs and systems observed after intermediate-duration oral exposure are comparable to those observed after acute-duration oral exposure. Comparison of doses for intermediate-duration with doses for acute-duration

exposure reveals no correlation of toxicity with dose or exposure duration. Therefore, no intermediate-duration oral MRL was derived. Less well-documented effects of intermediate-duration oral exposure include a decrease in serum protein-bound iodine and a decrease in glucose tolerance (Castor and Beierwaltes 1956; MacBryde and Taussig 1935).

The animal data for intermediate duration oral exposure also show decreased body weight, but at more than 10-fold higher doses than in humans, as well as increased oxygen consumption; histopathological effects were generally absent (Pugsley 1936; Spencer et al. 1948; Tainter et al. 1934b). No hematological, dermal, neurological, or ocular effects were seen (Spencer et al. 1948; Tainter et al. 1934b). Cataracts in animals after oral exposure have been demonstrated only in a special strain of mice, in vitamin C deficient guinea pigs, and in chicks and ducks and were transient and/or histologically different from the human cataracts (Bettman 1946; Ogino and Yasukura 1957; Robbins 1944). Hence, the animal data indicate that animals may be much less sensitive and may not respond in the same manner to 2,4-DNP as do humans. However, cataracts were induced in immature rabbits injected intraperitoneally with 2,4-DNP (Gehring and Buerge 1969a). Hyperemia, edema, and exfoliation of the skin were reported in rabbits exposed dermally to 2,4-DNP for 4 weeks (Spencer et al. 1948).

Additional studies to better define the threshold region for intermediate-duration inhalation, oral, and dermal exposure would be useful, but the animal data currently available suggest that tested species may not be suitable models for human response to this chemical. Studies to identify a suitable animal model may be worthwhile. Further studies on the cataractogenesis of 2,4-DNP in immature rabbits may provide a useful animal model for humans.

Chronic-Duration Exposure and Cancer. No studies were located regarding toxicity in humans after inhalation or dermal exposure or in animals after inhalation or dermal exposure to 2,4-DNP for chronic durations. Case reports of humans who ingested 2,4-DNP for chronic durations have identified cardiovascular, hematological, renal, dermal, and ocular effects and weight loss, increases in body temperature, and increases in basal metabolic rates (Horner et al. 1935; Imerman and Imerman 1936) similar to those observed after acute- and intermediate-duration exposures. Again, comparison of doses taken for chronic durations with doses taken for acute or intermediate durations reveals no correlation between dose and duration and health effects observed. A study was located assessing death and systemic effects in rats after dietary exposure to 2,4-DNP for life (Tainter 1938). Although

100% mortality was observed at the highest exposure level, the rats may have died from starvation. Decreased life span and growth were observed at lower exposure levels; the cause for the decrease in life span was not determined. In spite of gross and histological examination of most tissues and organs, no target organs for 2,4-DNP toxicity were identified in this study (Tainter 1938). The paucity of chronic data in humans and animals precludes the derivation of chronic-duration inhalation and oral MRLs. Chronic duration inhalation, oral, and dermal testing in animals may be useful because the most likely human exposure at this time might be long-term exposure at workplaces or hazardous waste sites. The effects of impaired mitochondrial function over long periods of time are unknown. If a suitable animal model were identified, further chronic studies might be worthwhile.

No studies were located regarding cancer in humans after inhalation, oral, or dermal exposure, or in animals after inhalation or oral exposure to 2,4-DNP. 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). No signs of cancer were reported in studies exposing rats and dogs to 2,4-DNP for intermediate or chronic durations; however, these studies were designed to evaluate systemic toxicity, rather than carcinogenicity (Spencer et al. 1948; Tainter et al. 1934b; Tainter 1938). In addition, 2,4-DNP was negative for genotoxicity in most in *vivo* and *in vitro* studies (see Section 2.9.2, Genotoxicity). While there is essentially no positive evidence for 2,4-DNP induced genotoxicity or cancer in humans and animals, and negative evidence exists with respect to cancer and genotoxicity in animals, the metabolites of 2,4-DNP (2-amino-4-nitrophenol, 4-amino-2-nitrophenol, and 2,4-diaminophenol) appear to be mutagenic. 2-Amino-4-nitrophenol (NTP 1988a) and 4-amino-2-nitrophenol (NC1 1978) show some evidence of carcinogenicity in male rats. For this reason, carcinogenicity studies with 2,4-DNP may be justified.

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); 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 indicates that the metabolites of 2,4-DNP (2-amino-nitrophenol, 4-amino-2-nitrophenol, and 2,4-diaminophenol) are mutagenic in *S. typhimurium* (Gamer 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, which are hypothermic rather than hyperthermic like 2,4-DNP, and thus may have a different mechanism of action (Harvey 1959), would be useful to better determine their potential genotoxicity.

Reproductive Toxicity. No studies were located regarding reproductive toxicity in humans after inhalation or dermal exposure, or in animals after inhalation or dermal exposure to 2,4-DNP. Three case reports and a clinical study of women taking 2,4-DNP orally for weight reduction suggest that 2,4-DNP may affect the female reproductive system (Beinhauer 1934; Epstein and Rosenblum 1935; Goldman and Haber 1936; Simkins 1937a, 1937b). The effects included a small and infantile uterus and numerous follicular cysts in the ovary (Goldman and Haber 1936) and a fibroid degeneration of the uterus and a cystic left ovary (Beinhauer 1934). Whether these were preexisting conditions is not known. A miscarriage (Epstein and Rosenblum 1935) and altered menstrual cycles (Simkins 1937a, 1937b) were attributed to the ingestion of 2,4-DNP. No gross or histological evidence of treatment-related damage to the testes was reported following intermediate- and chronic-duration exposure of rats in the diet or intermediate exposure of dogs via capsules to 2,4-DNP; however, the protocol for

examining reproductive organs was not reported, and may not have been adequate to assess reproductive toxicity (Spencer et al. 1948; Tainter 1938; Tainter et al. 1934b). In addition, only male animals were used in these studies. Intermediate- and chronic-duration studies specifically assessing effects on reproductive organs in male and, particularly, female animals of two species after inhalation and oral exposure to 2,4-DNP would be useful. Positive findings on effects on reproductive organs of female animals would help determine whether the effects seen in human females were actually due to 2,4-DNP exposure. Furthermore, if reproductive effects are confirmed, multigeneration studies by both routes would provide information on reproductive function.

Developmental Toxicity. No studies were located regarding developmental toxicity in humans after inhalation, oral, or dermal exposure or in animals after inhalation or dermal exposure to 2,4-DNP. Increased incidence of stillborn pups and pup mortality during lactation was reported in female rats treated by gavage with 2,4-DNP prenatally for 8 days, and throughout gestation and lactation (Wulff et al. 1935). Two other oral gavage studies on mice reported no developmental toxicity at exposure levels producing maternal toxicity; however, these studies only exposed dams during the first 3-4 days of organogenesis (Gibson 1973; Kavlock et al. 1987). Evidence of developmental toxicity (decreased fetal body weight and crown-rump length) was reported in rats receiving 2,4-DNP by subcutaneous injection and in mice injected intraperitoneally with 2,4-DNP; no teratogenic effects were noted in these studies (Gibson 1973; Goldman and Yakovac 1964). Because evidence of developmental toxicity was observed after parenteral administration in two species, further testing would be useful to determine whether developmental effects occur with environmentally relevant routes of exposure.

Immunotoxicity. The available studies did not focus on immunotoxicity. Agranulocytosis was reported in some people who took 2,4-DNP for intermediate durations (Davidson and Shapiro 1934; Goldman and Haber 1936). Whether the dermal reactions (including severe urticarial or macropapular lesions) seen in humans after acute-, intermediate-, and chronic-duration oral exposure involve sensitization is unclear. These reactions sometimes disappeared during continuing 2,4-DNP treatment or were not seen again in patients who discontinued 2,4-DNP after experiencing dermal effects and then resumed treatment at the same or higher doses (Bortz 1934; Tainter et al. 1935b). A woman who developed a severe skin reaction over her entire body after ingesting 2,4-DNP had a mildly positive reaction to a 1:2 dilution and a negative reaction to a 1:10 dilution of 2,4-DNP in a contact skin test (Beinhauer 1934). Patch tests, scratch tests, intradermal tests, and passive transfer tests with 2,4-DNP performed on 158 people, 117 of whom had hay fever, asthma or urticaria, were all negative (Matzger

1934). Such reactions were not reported in the occupational exposure studies, which involved inhalation and dermal exposure (Gisclard and Woodward 1946; Perkins 1919).

No histopathological effects were seen in the spleen, bone marrow, or lymph nodes of animals after intermediate-duration oral exposure (Spencer et al. 1948; Tainter et al. 1934b). No studies were located regarding immunological effects in animals after inhalation or dermal exposure to 2,4-DNP. A battery of immune function tests may be useful in determining whether the immune system is affected by exposure to 2,4-DNP.

Neurotoxicity. 2,4-DNP appears to affect both the central and peripheral nervous systems. Symptoms of central nervous toxicity, such as fatigue and agitation, have been experienced by people occupationally exposed to 2,4-DNP (Gisclard and Woodward 1946; Perkins 1919). Headache, weakness, extreme fatigue, dizziness, euphoria, irrationality, confusion, and stupor have also been experienced by some people who ingested 2,4-DNP for acute to chronic durations (Dintenfass 1934; Gisclard and Woodward 1946; Goldman and Haber 1936; Imerman and Imerman 1936; Masserman and Goldsmith 1934; Perkins 1919; Poole and Haining 1934). The autopsy of a woman who died after ingesting 2,4-DNP revealed histopathological lesions in the brain and spinal cord (Poole and Haining 1934). Peripheral neuritis has been described in patients taking 2,4-DNP orally at therapeutic doses for weight reduction for acute to intermediate durations (Anderson et al. 1933; Bortz 1934; Epstein and Rosenblum 1935; Hunt 1934; Nadler 1935; Simkins 1937a, 1937b).

Studies in animals did not specifically investigate neurological end points. An intermediate-duration oral study in dogs found no gross or histological evidence of damage to the brain or spinal cord (Tainter et al. 1934b). A battery of neurological and behavioral studies in animals might provide further information on this end point and its relative sensitivity.

Epidemiological and Human Dosimetry Studies. No epidemiology studies of workers or other populations exposed to 2,4-DNP were located. A study describing occupational experience with 2,4-DNP in the French munitions industry in the early 1900s (Perkins 1919), a case report of occupational 2,4-DNP overexposure in the U.S. (Gisclard and Woodward 1946), and numerous case reports and clinical studies regarding the use of 2,4-DNP as a diet pill in humans in the 1930s (Castor and Beierwaltes 1956; Cutting et al. 1933, 1934; Dameshek and Gargill 1934; Davidson and Shapiro 1934; Dunlop 1934; Hoffman et al. 1934; Looney and Hoskins 1934; MacBryde and Taussig 1935;

Silver 1934; Tainter et al. 1935b) are available and indicate that the end points related to the uncoupling of oxidative phosphorylation are the most sensitive. These end points 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. 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. In the occupational studies, comparisons were made with conditions under which worker exposure to 2,4-DNP was greatly reduced, and in the clinical studies, with before-treatment and after-treatment measurements on the same patients. Workers currently exposed to 2,4-DNP and people who live or work near waste sites contaminated with 2,4-DNP could be studied to define relationships among exposure, blood and urine levels of parent compounds and metabolites, and the sensitive effects.

Biomarkers of Exposure and Effect.

Exposure. 2,4-DNP and metabolites have been monitored in body fluids 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 Hagardorn 1983, 1985). The predominant compounds in blood and urine appear to be unchanged 2,4-DNP and 2-amino-4-nitrophenol. Systematic attempts to correlate levels of 2,4-DNP or its metabolites in blood or urine with exposure levels have not been made. The total amount or concentration of 2,4-DNP and its principal metabolite(s) would probably be a better indicator of exposure than either alone. It would be useful to investigate this possibility.

Effect. 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.

No studies correlating blood or urine levels of 2,4-DNP and its metabolites with toxic effects in humans were located. This approach may be worth investigating since limited information from

parenteral animal studies indicates that, at least for ocular effects, there is a correlation between levels of 2,4-DNP in the eye and the development of cataracts; rate of elimination from serum was also inversely related to cataract development (Gehring and Buerge 1969b).

Absorption, Distribution, Metabolism, and Excretion. Limited data suggest that absorption of high single doses from the gastrointestinal tract may be fairly complete in humans (Tainter and Wood 1934). The attainment of maximal increases in basal metabolic rate within one hour of ingestion of 2,4-DNP in clinical studies provides indirect evidence of rapid absorption (Cutting et al. 1933; Dunlop 1934). Evidence of distribution consists of the detection of parent compound and metabolites in the tissues of fatal cases of occupational exposure (inhalation and probably dermal) to 2,4-DNP (Perkins 1919). Detection of 2,4-DNP and metabolites in the urine of a patient who ingested 2,4-DNP and in workers exposed to 2,4-DNP occupationally (Davidson and Shapiro 1934; Perkins 1919) shows that this is a significant route of excretion. The major blood, tissue, and urinary forms of DNP following occupational and oral exposure of humans appear to be the parent compound and 2-amino-4-nitrophenol (Davidson and Shapiro 1934; Perkins 1919; Tainter and Wood 1934). There are no human data to suggest major differences by route and duration of exposure, but the database is meager. Studies in animals treated orally with 2,4-DNP have identified 2-amino-4-nitrophenol, 4-amino-2-nitrophenol (Robert and Hagardom 1985), and possibly 2,4-diaminophenol (Ogino and Yasukura 1957) in the urine. *In vitro* studies using rat liver homogenates suggested that the enzyme responsible for the reduction of 2,4-DNP to 2-amino-4-nitrophenol and 4-amino-2-nitrophenol is a nitroreductase located primarily in the cytosol (Eiseman et al. 1972). A small amount of 2,4-diaminophenol was also forrned. Another *in vitro* study suggested that 2,4-diaminophenol was formed from the further reduction of aminonitrophenols by the same nitroreductase (Parker 1952). Identification of 2,4-diaminophenol in the urine of humans exposed to 2,4-DNP would help to clarify whether these pathways are similar in humans.

While half-lives of absorption after oral administration have been estimated at 0.5 hour in rats (Robert and Hagardom 1983), and plasma monitoring data from a limited study in dogs also suggest rapid absorption from the gastrointestinal tract (Kaiser 1964), the fraction absorbed has not been determined, and no studies were located that provide a total accounting of excretion. Tissue levels of 2,4-DNP in liver and kidney were found to be much lower than in serum of rats after a single oral dose of 2,4-DNP, but metabolites were not monitored (Robert and Hagardom 1983). No evidence of accumulation or saturation phenomena was seen during plasma monitoring of dogs for parent

compound in a daily dosing experiment with three 2,4-DNP dose levels, but the data were limited (Kaiser 1964).

In addition, no studies were located regarding absorption, distribution, metabolism, or excretion in animals after inhalation or dermal exposure to 2,4-DNP. Therefore, pharmacokinetic studies in animals exposed to 2,4-DNP by the inhalation, oral, and dermal routes would be useful in determining 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. 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, as noted previously. Based on the limited available data from oral and occupational exposure in humans and from oral administration in animals, no striking differences in absorption, metabolites, or excretion among species are evident. Differences in elimination rates among rats, rabbits, guinea pigs, mice, and monkeys following a single oral dose were not large (less than two-fold difference among species) on the basis of a single limited study (Lawford et al. 1954). Parenteral studies investigating differences in sensitivity to 2,4-DNP cataractogenesis among various animal models concluded that the concentration of 2,4-DNP in the target organ is a critical factor in sensitivity to cataractogenesis (Gehring and Buerge 1969a, 1969b). This conclusion was based on the finding that differences in elimination from serum and from compartments of the eye and in the blood-aqueous humor barrier appeared to account for differences in sensitivity among animals of different ages and species. In addition, intraocular injection of 2,4-DNP rapidly produced cataracts in otherwise insensitive animals (Gehring and Buerge 1969a). No studies to corroborate this by the oral, inhalation, or dermal route were located.

Methods for Reducing Toxic Effects. Since 2,4-DNP is a small (molecular weight <500 daltons) relatively lipophilic weak acid and has a pK_a of 4.09, it is probably absorbed by passive diffusion in the stomach and lungs. Little can be done to reduce its absorption from inhalation exposure. Removal of contaminated clothes and thorough washing of the skin is suggested in cases of dermal exposure (Bronstein and Currance 1988; Haddad and Winchester 1990; Stutz and Janusz 1988). The usual methods of dilution (ingestion of water) and removal (via emesis, gastric lavage, activated charcoal, use of cathartics) have been suggested to slow or prevent absorption within the first few

hours of ingestion (Bronstein and Currance 1988; Ellenhom and Barceloux 1988; Haddad and Winchester 1990; Stutz and Janusz 1988). Substances that neutralize the acidic gastric environment (e.g., bicarbonate) could be tested to determine whether they would effectively reduce the gastrointestinal absorption of 2,4-DNP. These methods are suitable for acute-duration high-dose scenarios. 2,4-DNP and its metabolites have been detected in the tissues of fatal cases of occupational exposure (Perkins 1919). 2,4-DNP was present in liver and kidney of orally dosed rats at levels lower than those in serum; the data indicated rapid exchange of 2,4-DNP among serum and these tissues (Robert and Hagardom 1983). Distribution appears to involve the exchange between blood and tissues of 2,4-DNP that is not bound to serum proteins; this evidence comes from a parenteral study in animals (Gehring and Buerge 1969b). 2,4-DNP is rapidly eliminated in animals; data for humans were not located. Because of the binding to serum proteins, it is uncertain whether procedures such as dialysis would significantly aid in reducing body burden. The hyperpyrexia and lethality of 2,4-DNP have been significantly diminished in rats by pretreatment with haloperidol (Gatz and Jones 1972). Further in vitro experiments suggested that haloperidol interfered (probably indirectly by an action at the mitochondrial membrane) with the uncoupling of oxidative phosphorylation by 2,4-DNP. Hence haloperidol may be a candidate for further investigation as an antidote to 2,4-DNP. Rapid cooling of the body (using a hypothermia blanket or ice) has been suggested to control hyperpyrexia resulting from high doses of 2,4-DNP (Bronstein and Currance 1988; Ellenhom and Barceloux 1988; Haddad and Winchester 1990). The protective effect of cooling the body with water following 2,4-DNP administration is documented in animals (Harvey 1959). Avoidance of hot environments and use of air conditioning would be useful in less severe exposures. The excessive perspiration noted in many of the human studies and case reports can contribute to dehydration; administration of fluids would be appropriate to counteract this effect.

2.9.3 Ongoing Studies

E. Hunter of the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, is conducting a study investigating the effect of 2,4-DNP and other metabolic toxins on embryonic development in the mouse.