3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

3.2 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 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 B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

The primary focus of this document is information on 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT. However, some information on technical-grade DNT (Tg-DNT) is also provided. Tg-DNT contains approximately 76% 2,4-DNT, 19% 2,6-DNT, and <5% 3,4-, 2,3-, and 2,5-DNT. For each route of exposure (inhalation, oral, and dermal), human data will be discussed first. Available data for individual isomers (2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT, in that order) will be presented followed by data for Tg-DNT. Studies conducted in experimental animals will be discussed thereafter, with data on the individual isomers preceding data for Tg-DNT.

3.2.1 Inhalation Exposure

Most of the data on health effects associated with exposure of humans to DNTs are from studies of workers exposed to 2,4-, 2,6-, or Tg-DNT. Exposure monitoring of workers in the past has generally been inadequate; consequently, few dose-response data based on human exposure to DNT isomers are available. No data were identified regarding inhalation exposure of humans to 2,3-, 2,5-, 3,4-, or 3,5-DNT. Regarding available animal data, one study was located that examined the acute inhalation toxicity of 2,6-DNT in experimental animals. No studies were located regarding inhalation exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNT in experimental animals.

Human exposure to chemicals in an occupational setting can occur via multiple routes: inhalation, dermal, and inadvertent ingestion (Hamill et al. 1982). Although the low vapor pressure of DNTs makes inhalation of vapors unlikely, it can occur when contaminated particulate material is in the air. In

addition, some dermal exposure is probable, and some ingestion may also occur as the result of eating or smoking without prior handwashing.

3.2.1.1 Death

In a retrospective cohort mortality study of 457 munitions workers who were exposed to either 2,4-DNT or Tg-DNT at two geographically different U.S. manufacturing plants, significant increases in death rates due to ischemic heart disease and residual diseases of the circulatory system were found (standardized mortality ratios [SMRs] of 126 and 143; 95% confidence intervals [CIs] of 65–234 and 112–179, respectively) (Levine et al. 1986a). Residual diseases of the circulatory system include congestive heart failure, cardiac arrest, and arteriosclerosis. The workers had been exposed to unreported concentrations of either 2,4-DNT (98% pure) or Tg-DNT for periods ranging from 30 days to >5 years (Levine et al. 1986a). Cigarette smoking was not taken into account in this study, but the study authors suggested that it may not have been a risk factor because mortality from lung cancer was less than expected. Among workers at both plants, there appeared to be a latency period of >15 years for a significant increase in mortality due to ischemic heart disease. There also appeared to be a relationship between heart disease and the intensity of exposure to DNTs. No statistical increase was found in death due to cancer, either from malignant neoplasms as a whole or from individual cancers, although the statistical power of the study was insufficient to detect anything but gross changes in the death rate due to cancer.

The Levine et al. (1986a) retrospective cohort mortality study was limited by small cohort size, and thus, the study had diminished power to detect an effect. As a result, the finding of elevated mortality from heart disease among workers in two plants from different parts of the United States linked only by exposure to DNTs is unusual. Workers in the United States generally have lower rates of heart disease than the general population because of the "healthy worker effect." At both plants, mortality from ischemic heart disease during the first 15 years following cohort entry was less than expected, and mortality increased only in later years. Suggestive, but not significant, is evidence of a relationship between heart disease and duration and intensity of exposure, also reported by Levine et al. (1986a). No studies were identified with respect to mortality after inhalation exposure of humans to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

In an acute-duration study, no mortality was observed in male or female F344 rats (5/sex/group) exposed nose-only to 2,6-DNT as a vapor at 26 mg/m^3 for 6 hours and observed 14 days after dosing (CMA 1991). In the same study, groups of male and female rats exposed to 2,6-DNT at 0, 196, 473, or 694 mg/m^3 as an

aerosol exhibited mortality at ≥196 mg/m^3. Two of five males (and no females) died at 196 mg/m^3; all males and three of five females died at 694 mg/m^3. Rats that died showed evidence of lung congestion and had increased relative lung weights compared to controls. An LC_{50} of 0.43 mg/L was identified in rats, with LC_{50} values for males and females of 0.24 and 0.66 mg/L, respectively.

No studies were located regarding death in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 3,4-, or 3,5-DNT.

3.2.1.2 Systemic Effects

No studies were located regarding dermal, ocular, endocrine, or body weight effects in humans or animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT. No studies were located regarding respiratory effects in humans after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT or in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 3,4-, or 3,5-DNT.

Respiratory Effects. No studies were identified with respect to respiratory effects after inhalation exposure of humans to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

In an acute-duration study, groups of male and female rats were exposed to 2,6-DNT at 0, 196, 473, or 694 mg/m^3 as an aerosol for 6 hours and observed 14 days after dosing (CMA 1991). Rats exposed to 2,6-DNT as an aerosol at ≥196 mg/m^3 exhibited signs of respiratory distress (exaggerated respiratory movements) for several days following exposure; recovery from this effect occurred by day 5 of the observation period. Rats that died showed evidence of lung congestion and had increased relative lung weights compared to controls.

No studies were identified with respect to respiratory effects after inhalation exposure of animals to 2,3-, 2,4-, 2,5-, 3,4-, or 3,5-DNT.

Cardiovascular Effects. No studies were identified with respect to cardiovascular effects after inhalation exposure of humans to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Levine et al. (1986a) reported a significant increase in deaths from diseases of the circulatory system in workers involved in the manufacture and processing of 2,4-DNT and/or Tg-DNT. The preponderance of circulatory disease deaths was due to ischemic heart disease. The SMR was significantly elevated for

ischemic heart disease when compared to the U.S. male population and to persons living in the area. When workers were divided by exposure duration, the significant increase in cardiovascular deaths was only observed in workers exposed for at least 5 months. Although the study did not control for cigarette smoking, a known risk factor for heart disease, the investigators noted that the study did not find significant increases in lung cancer or disease deaths.

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

Gastrointestinal Effects. Vomiting and nausea were mentioned as health complaints in a survey of male workers involved in the production of smokeless gunpowders during World War II (McGee et al. 1947). The exposure concentrations of 2,4-DNT were not specified. The investigators noted that prior to improving industrial hygiene practices, a much higher incidence of gastrointestinal symptoms was found. Since exposure to other compounds cannot be ruled out, attribution of these symptoms to DNT cannot be verified.

No studies were identified with respect to gastrointestinal effects after inhalation exposure of humans to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT. No studies were located regarding gastrointestinal effects in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Hematological Effects. Several hematological effects, including anemia and cyanosis, were found in male workers employed by a munitions factory during World War II (McGee et al. 1947). In some cases, there were increases in leukocyte count, which may be related to prolonged exposure to DNT. The study authors presumed that the exposure concentrations to 2,4-DNT were relatively high because of the relatively primitive industrial hygiene practices at that time. Although 36 of 154 workers were anemic in the earlier study and 73 of 714 workers were anemic in the follow-up study, no control groups were used as a basis for comparison. Because of possible exposure to other compounds, lack of work histories, lack of exposure monitoring, lack of a control population, and small cohort size, the results obtained are equivocal and may be best used as qualitative descriptions of symptoms. Marked cyanosis and other incapacitating symptoms were reported after exposure to unspecified concentrations of Tg-DNT in a study of French workers in a DNT production plant during World War I (Perkins 1919). It is assumed that workers were exposed to high concentrations of Tg-DNT via both inhalation and dermal pathways, since the processes described involved direct handling of large amounts of Tg-DNT without protective equipment.

No studies were identified with respect to hematological effects after inhalation exposure of humans to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

In an acute-duration study, male and female F344 rats (5/sex/group) were exposed to 2,6-DNT as a vapor at 0 or 26 mg/m^3 or as an aerosol at 0, 196, 473, or 694 mg/m^3 for 6 hours and observed 14 days after dosing (CMA 1991). Blood was analyzed for serum methemoglobin concentration 24 hours prior to dosing and 1, 24, and 48 hours and 7 days after dosing. Although the study authors noted slight increases in serum methemoglobin in 2,6-DNT-exposed animals in the first 24 hours after exposure, these changes were not clearly dose-related and were not statistically significantly different from control animals. No other hematological end points were evaluated.

No studies were located regarding hematological effects in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 3,4-, or 3,5-DNT.

Musculoskeletal Effects. Joint pain, especially in the knees, and other incapacitating symptoms were found in unspecified numbers of French workers in a plant that produced DNTs during World War I (Perkins 1919). No exposure concentrations were reported, but it is assumed that they were high because of the direct handling of large amounts of Tg-DNT without protective equipment, which also suggests that the workers were exposed dermally. However, because exposure to other compounds cannot be ruled out and no control data are available, caution must be used when interpreting these results.

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

Hepatic Effects. A study of 714 workers at a munitions plant found that 29 experienced liver tenderness (McGee et al. 1947). Other factors, such as alcohol consumption, may account for these results which should be viewed with caution because of the lack of control data, lack of information on exposure concentrations, and possible multiple chemical exposure. Medical surveys of 52 male workers exposed to Tg-DNT in a chemical plant that manufactured toluenediamine (TDA) revealed no differences in hepatic blood chemistry profiles (NIOSH 1982). Air samples contained concentrations ranging from 0.026 to 0.890 mg/m^3 Tg-DNT (mean 0.207 mg/m^3).

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

Renal Effects. No effects were observed on either of the renal parameters (blood urea nitrogen [BUN], creatinine) monitored in blood chemistry in a medical survey of 52 male workers exposed to Tg-DNT in a chemical plant that manufactured TDA (NIOSH 1982). Exposure concentrations in air samples taken for this study ranged from 0.026 to 0.890 mg/m^3 Tg-DNT (mean 0.207 mg/m^3). The study was limited by a small exposure population and lack of historical individual exposure monitoring.

Evidence of tubular and/or glomerular damage (alterations in urinary protein excretion patterns) was found among a cohort of approximately 160 workers at a copper mine exposed to Tg-DNT explosives (Brüning et al. 2001). Dividing the workers into exposure categories resulted in a significant dose-related trend for the incidence of tubular and/or glomerular damage among the workers without renal cell cancer or urothelial cancer. Approximately 80% of the 25 workers in the very high exposure category had evidence of renal damage. Dose-related increases in the excretion of α1-microglobulins and glutathione-S-transferase-α were also observed suggesting proximal tubule damage. The lack of effect on glutathione-S-transferase-π levels suggested a lack of damage to the distal tubules.

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

3.2.1.3 Immunological and Lymphoreticular Effects

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

3.2.1.4 Neurological Effects

Dizziness and headache were reported by Perkins (1919) in a study of French workers exposed to Tg-DNT at a production plant during World War I. Although no exposure concentrations were reported, it is assumed that the workers were exposed to high concentrations of Tg-DNT via both inhalation and dermal pathways, since the manufacturing processes required workers to handle large amounts of Tg-DNT without protective equipment. Exposure to chemicals other than DNT in this environment could not be ruled out. Health effects of munitions workers exposed to unspecified levels of what was presumed to be 2,4-DNT were studied by McGee et al. (1947). Neurological signs reported by these

workers included headache, dizziness, and pain, numbness, and tingling in the extremities. The 2,4-DNT exposure concentrations were not specified, but were considered by these authors to be relatively high as a result of the lack of safety practices.

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

3.2.1.5 Reproductive Effects

Studies of men occupationally exposed to Tg-DNT at DNT and TDA plants showed no significant differences in sperm counts or morphology, follicle stimulating hormone (FSH) levels, or incidence of miscarriage in their wives compared to controls (Hamill et al. 1982; NIOSH 1982). In the NIOSH (1982) study, Tg-DNT concentrations ranged from 0.026 to 0.890 mg/m^3 (mean 0.207 mg/m^3). Interpretation of these studies is somewhat confounded by the lack of distinction between DNT and TDA exposure and the lack of information regarding exposure concentration in the Hamill et al. (1982) study. The limitations of these studies are similar (small exposure populations and the lack of individual exposure monitoring) and limit the ability of the studies to detect adverse effects.

No significant effects on the fertility of workers occupationally exposed to Tg-DNT have been found in several studies (Hamill et al. 1982; Levine et al. 1985a; NIOSH 1982). However, Levine et al. (1985a) estimate that only a 50–70% reduction in fertility could have been detected in the worker population that they studied.

One study by the CDC (1981) noted that sperm counts were decreased by >50% in workers in a Kentucky chemical plant exposed to DNTs and TDA compared to workers not exposed to these chemicals. The study was limited because of multiple chemical exposures and the small numbers of workers examined. Thirty workers participated in the study: 9 currently exposed, 12 previously exposed, and 9 with no history of exposure to DNTs/TDA.

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

3.2.1.6 Developmental Effects

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

3.2.1.7 Cancer

The mortality of a cohort of 4,989 men who worked at least 5 months in a munitions facility was analyzed to determine whether DNT exposure was associated with an increased risk of cancer of the liver and biliary tract (Stayner et al. 1993). Workers were considered exposed if they had worked at least 1 day on a job with probable exposure to DNT. In this study, a significant increase in hepatobiliary cancer mortality (standard rate ratio [SRR]=3.88, 95% CI 1.04–14.41) was observed among DNT-exposed workers compared to non-exposed control workers. However, no significant changes were noted when compared to the U.S. population, the SRR for hepatobiliary cancer being 2.67 (95% CI 0.98–5.83; p=0.052). No quantitative data were available on the DNT exposure of these men. This study is limited by the small numbers of hepatobiliary cancer cases, small numbers of workers with long term exposure to DNT, and possible exposure of the workers to other chemicals. However, no significant increases in mortality from malignant neoplasms as a group or from particular cancers (liver, lung, gallbladder, kidney, and connective tissues) were observed in workers occupationally exposed to 2,4-DNT and/or Tg-DNT (Levine et al. 1986b). Exposures were not quantified and the cohort was small. The study authors estimated that an 8-fold increase in liver and gallbladder cancer in exposed workers would be necessary in order to be detected at the p=0.05 level; thus, the statistical analysis was not strong enough to detect small increases in cancer.

Among 500 workers in an underground copper mine where Tg-DNT-containing explosives were extensively used, increases, as compared to the German national cancer registry, in the incidence of renal cell cancer and urothelial cancer incidence were observed (Brüning et al. 1999). However, when the workers were divided by exposure categories, no apparent relationship between renal cell cancer incidence and exposure category was found. In contrast, four of the six workers with urothelial cancer cases were in the high exposure group (one was in the lowest group and the other was in the highest exposure group). The genotype distribution of several polymorphic xenobiotic enzymes, including *N*-acetyltransferase 2 and glutathione-S-transferases M1 (GSTM1) and T1 (GSTT1), was examined in the 14 workers with renal cell cancer and 6 workers with urothelial cancer. The genotype distribution among the renal cell cancer cases did not differ from the normal German population. Similarly, the distribution of GSTM1 and GSTT1 genotypes for the urothelial cancer cases was similar to the German population.

However, all of the urothelial cancer cases were found to be “slow acetylators” compared to a 58% distribution in the German population.

Harth et al. (2005) reported three cases of urothelial carcinoma of the urinary bladder among 60 workers exposed to Tg-DNT at a German explosive manufacturing facility. The investigators noted that this cancer rate was 15.9 times higher than the expected incidence in the federal state.

Seidler et al. (2014a) examined the cancer incidence through 2005 in a cohort of 16,441 male copper miners who worked between 1953 and 1990 in one of two German mines that used Tg-DNT explosives. The mean duration of employment was 6.8 years. Compared with the cancer incidence in the general population of the region in Germany where the mines were located (Saxony-Anhalt), the cancer incidences in the miners were not significantly increased for any cancer other than lung cancer (standardized incidence ratio [SIR] = 1.29, 95% CI 1.13–1.46). Moderate, but not statistically significant, increases in the incidences of kidney and bladder cancer (SIRs of 1.25 and 1.41 for kidney and bladder cancer, respectively, among workers with >20 years of employment with exposure to Tg-DNT) were seen when the data were stratified by duration of employment with Tg-DNT exposure. A follow-up case-cohort study of this group, with follow-up through 2006, compared the Tg-DNT exposure history (by job exposure matrix) of 109 renal cancer cases with that of 999 randomly chosen cohort members (Seidler et al. 2014b). The Cox proportional hazard ratio (HR) was elevated, but not significantly, for high inhalation exposure to Tg-DNT (HR 1.36, 95% CI 0.84–2.21). When combined across medium and high exposure categories for both dermal and inhalation exposure, the HR for renal cancer was significantly increased (HR 2.12, 95% CI 1.03–4.37).

No studies were located regarding cancer in humans following inhalation exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNT or in animals following inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

3.2.2 Oral Exposure

No studies were located regarding health effects in humans following oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT. However, it is assumed that oral ingestion could be a secondary route for occupationally exposed humans.

In one series of studies performed at the U.S. Army Public Health Command (Lent et al. 2012a; USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f), groups of male Sprague-Dawley rats were

administered one of the DNT isomers (2,3-DNT, 2,4-DNT, 2,5-DNT, 2,6-DNT, 3,4-DNT, or 3,5-DNT) by gavage in corn oil vehicle for 14 days at doses ranging from 1/128th or 1/64th of the reported LD_{50} value to 1/4 or 1/2 LD_{50}. The observed isomer-specific differences in hematological, hepatic, renal, neurological, and reproductive effects are discussed at the end of each corresponding subsection of Section 3.2.2.

3.2.2.1 Death

No studies were located regarding death in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

2,4-DNT is lethal to experimental animals after oral administration. Animals generally developed cyanosis and ataxia after dosing. In general, rats are more sensitive than mice to the lethal effects of 2,4-DNT. The LD_{50} values that have been determined for rats after gavage dosing with 2,4-DNT range from 270 to 650 mg/kg (U.S. Army 1975, 1978a; Vernot et al. 1977); in mice, LD_{50} values were reported to be between 1,340 and 1,954 mg/kg after 2,4-DNT administration (U.S. Army 1975, 1978a; Vernot et al. 1977). In female Sprague-Dawley rats (5/group) administered a single dose of 2,4-DNT (in 5% v/v DMSO in corn oil) via gavage at 398 mg/kg and observed for 24 or 48 hours after dosing, two of five animals and one of five animals died within 24 and 48 hours, respectively (Deng et al. 2011). No deaths occurred in rats administered 2,4-DNT at 5, 50, 99, or 198 mg/kg. In a dominant lethal study by Lane et al. (1985), 8 of 15 male Sprague-Dawley rats died after receiving five daily doses of 240 mg/kg 2,4-DNT. No deaths were reported when male and female Sprague-Dawley rats were fed 78 or 82 mg/kg/day 2,4-DNT, respectively, in the diet for 14 days (McGown et al. 1983). When male Sprague-Dawley rats were given gavage doses of 284 mg/kg/day for 14 days, all six exposed rats died (Lent et al. 2012a; USAPHC 2011b).

Death has been reported after intermediate- and chronic-duration exposure to 2,4-DNT in numerous studies. One of eight male and eight of eight female CD rats died after 3–13 weeks of ingesting 2,4-DNT in the diet (Lee et al. 1985; U.S. Army 1978b). Concentrations in the feed causing these deaths were equivalent to doses of 93 and 145 mg/kg/day in males and females, respectively. Death has also been reported in rodents fed concentrations equivalent to doses of 371–413 mg/kg 2,4-DNT in the diet for up to 6 months (Hong et al. 1985; Kozuka et al. 1979; U.S. Army 1978b). No treatment-related deaths were reported in rats fed up to 16.5 mg/kg/day 2,4-DNT or mice fed up to 28.5 mg/kg/day 2,4-DNT for 4 weeks, or in rats fed up to 22 mg/kg/day 2,4-DNT or mice fed up to 76 mg/kg/day 2,4-DNT for

78 weeks (NCI 1978). In a 3-generation reproductive study, there appeared to be an increased incidence of death among F_0 dams during parturition after receiving 45.3 mg/kg/day 2,4-DNT in the diet for 6 months (U.S. Army 1979). These deaths were associated with prolonged parturition, hemorrhage, and placental retention. However, because these effects were also seen to a lesser extent in control animals, it may be that the effects of 2,4-DNT simply enhanced effects caused by the advancing age of the dams (U.S. Army 1979).

In a 13-week study, some dogs fed 25 mg/kg/day became moribund after ≥22 days and had to be terminated, whereas no treatment-related deaths were reported in dogs fed 5 mg/kg/day (Ellis et al. 1985; U.S. Army 1978b). In addition to severe weight loss, severe neurological effects and histopathological changes were found in these animals, including vacuolization and focal gliosis in the cerebellum and perivascular hemorrhages in the cerebellum and brain stem, as well as peripheral neuropathy, testicular degeneration, and biliary hyperplasia. In a 24-month study of dogs, the administration of 10 mg/kg/day 2,4-DNT by capsule caused death within 6 months, but no deaths were reported at 1.5 mg/kg/day; clinical signs prior to death were similar to those reported in the 13-week study (Ellis et al. 1985; U.S. Army 1979). Decreased longevity was reported in 1–2-year studies of CD rats at average daily intakes as low as 3.9 mg/kg/day (males) and 5.1 mg/kg/day (females), and of CD-1 mice at 898 mg/kg/day (Hong et al. 1985; Lee et al. 1985; U.S. Army 1978b, 1979).

The experimental data are more limited for 2,6-DNT than for 2,4-DNT. After administration of 2,6-DNT, LD_{50} values have been reported to range from 180 to 795 mg/kg in rats and from 621 to 807 mg/kg in mice (U.S. Army 1975, 1978a; Vernot et al. 1977). Complete lethality was reported in female Sprague-Dawley rats (5/group) administered a single dose of 2,6-DNT via gavage (in 5% v/v DMSO in corn oil) at 398 mg/kg (Deng et al. 2011). No deaths occurred in animals administered 5–199 mg/kg and observed for 2 days after dosing. Mortality was reported in a dose-range finding study of male rats given oral doses of 200 mg/kg/day 2,6-DNT in corn oil for 3 days; no rats died at 100 mg/kg/day (Rothfuss et al. 2010). The maximum tolerated dose (MTD) of 2,6-DNT corresponding to 100% survival of A/J mice after 6 doses over a 2-week period was 250 mg/kg (Schut et al. 1983). No male Sprague-Dawley rats died when groups of six rats were given 14 consecutive daily gavage doses up to 134 mg/kg/day 2,6-DNT (Lent et al. 2012a; USAPHC 2011d).

All six male rats exposed to oral doses of 33 mg/kg/day 2,6-DNT in corn oil for 29 consecutive days survived the treatment (Rothfuss et al. 2010). Intermediate-duration studies have shown an increase in mortality of mice and dogs after 2,6-DNT administration. After feeding 51 mg/kg/day 2,6-DNT to male

Swiss albino mice in the diet for up to 13 weeks, 8 of 16 of these animals died; 6 of 16 females fed 55 mg/kg/day 2,6-DNT also died (U.S. Army 1976). No treatment-related deaths were reported when rats were fed up to 155 mg/kg/day 2,6-DNT for the same duration (U.S. Army 1976). Two of eight dogs treated with 20 mg/kg 2,6-DNT by capsule died in a 13-week study (U.S. Army 1976). Thus, dogs seem to be the most sensitive of the three species to intermediate-duration oral 2,6-DNT exposure.

Vernot et al. (1977) reported LD_{50} values for 2,3- DNT of 1,120 mg/kg/day in male Sprague-Dawley rats and 1,070 mg/kg/day in male CF-1 mice. No cause of death or additional information was reported. Exposure to 550 mg/kg/day 2,3-DNT via gavage for 14 days resulted in the deaths of 6/6 male Sprague-Dawley rats; no deaths occurred at doses of ≤275 mg/kg/day (Lent et al. 2012a; USAPHC 2011a).

For 2,5-DNT, LD_{50} values of 710 and 1,230 mg/kg/day, respectively, were reported in male Sprague-Dawley rats and male CF-1 mice (Vernot et al. 1977); no additional information was provided. One rat in a group of six male Sprague-Dawley rats died after 14 consecutive daily gavage doses of 308 mg/kg/day 2,5-DNT (Lent et al. 2012a; USAPHC 2011c).

Lent et al. (2012a; USAPHC 2011e, 2011f) provide the only acute-duration lethality data for 3,4- and 3,5-DNT. When groups of six male Sprague-Dawley rats received gavage doses of 3,4-DNT up to 227 mg/kg/day for 14 days, there were no deaths. In contrast, 3,5-DNT was lethal to 1/6 rats at a dose of 39 mg/kg/day and to all rats at doses of 77 and 155 mg/kg/day.

Administration of up to 150 mg/kg/day Tg-DNT for 14 days was lethal to 6 of 13 pregnant F344 rats when administered by gavage during gestation (Jones-Price et al. 1982), yet this same concentration of Tg-DNT fed in the diet for 30 days did not kill any of the same strain of rats in another study (Hazleton Laboratories 1977). Decreased survival was found in CDF rats fed 35 mg/kg/day Tg-DNT for 52 weeks or 14 mg/kg/day Tg-DNT for 104 weeks (Hazleton Laboratories 1982).

All LOAEL values from each reliable study for death in each species and duration category for 2,3-, 2,4-DNT, 2,5-, 2,6-, 3,4-, and 3,5-DNT are recorded in Tables 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively, and plotted in Figures 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively.

Table 3-1 Levels of Significant Exposure to 2,3-Dinitrotoluene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	1 x/d 14 d (G)				550 M (6/6 died)	Lent et al. 2012a 2,3-DNT	
2	Rat (Sprague-Dawley)	once (G)				1120 M (LD50)	Vernot et al. 1977 2,3-DNT	
3	Mouse (CF-1)	once (G)				1070 M (LD50)	Vernot et al. 1977 2,3-DNT	
Systemic								
4	Rat (Sprague-Dawley)	1 x/d 14 d (G)	Cardio	275 M			Lent et al. 2012a 2,3-DNT	
			Hemato		275 M[b] (extramedullary hematopoiesis and lymphoid hyperplasia of spleen, lymphoid depletion)			
			Hepatic	275 M				
			Renal		275 M (trace tubular dilatation and lymphocytic infiltration)			
			Bd Wt	275 M				
Neurological								
5	Rat (Sprague-Dawley)	1 x/d 14 d (G)		275 M			Lent et al. 2012a 2,3-DNT	

Table 3-1 Levels of Significant Exposure to 2,3-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
6	Rat (Sprague-Dawley)	1 x/d 14 d (G)		275 M			Lent et al. 2012a 2,3-DNT	No effects on testes or epididymides weight or histopathology.

a The number corresponds to entries in Figure 3-1.

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.09 mg/kg/day; the MRL was derived by dividing the LOAEL of 275 mg/kg/day by an uncertainty factor of 3000 (10 for use of a LOAEL, 10 for animal-to-human extrapolation, 10 for human variability, and 3 for database limitations).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); (G) = gavage; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; x = time(s)

Figure 3-1 Levels of Significant Exposure to 2,3-Dinitrotoluene - Oral

Acute (≤14 days)

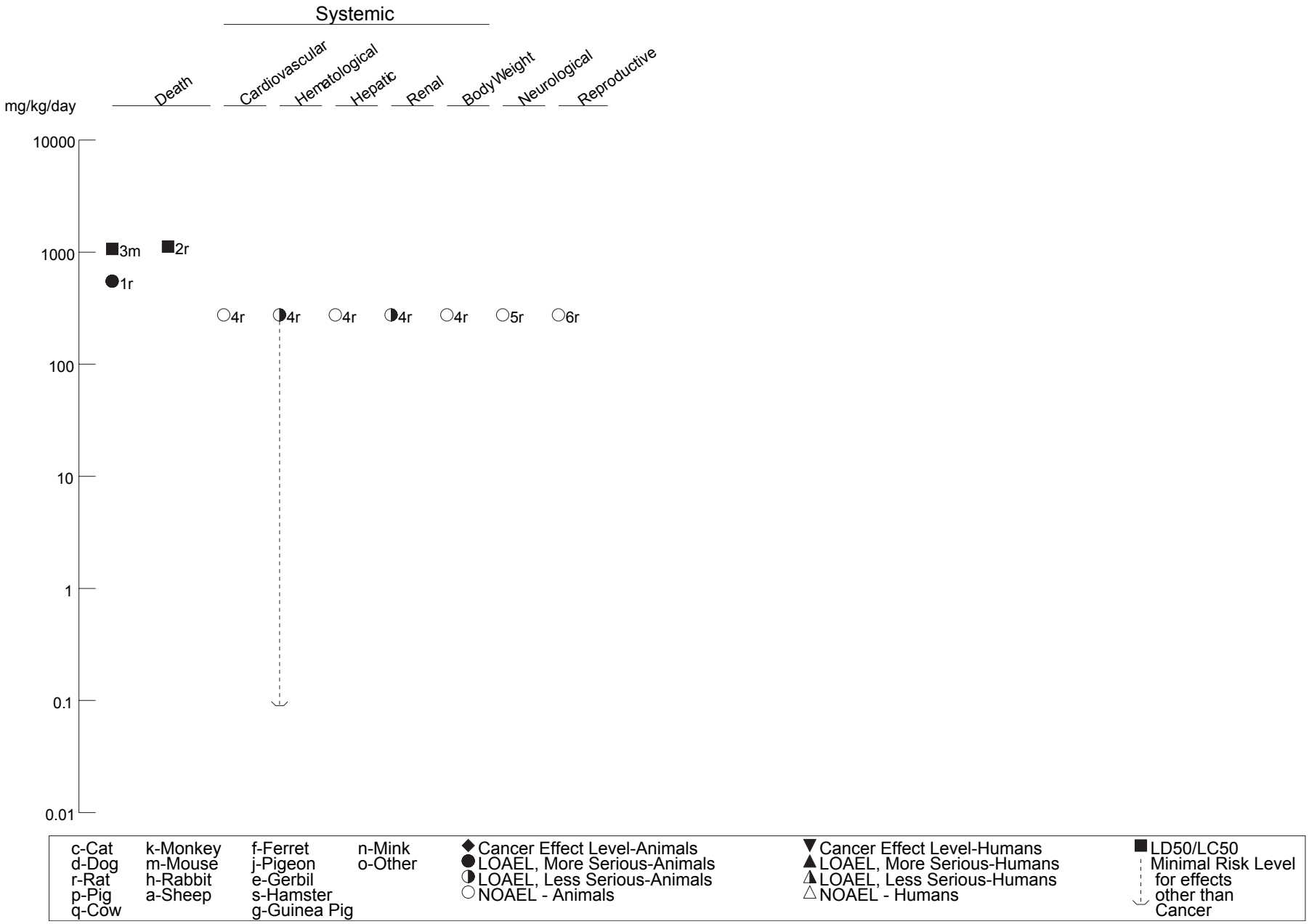


Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	5 d 1 x/d (GO)				240 M (8/15 died)	Lane et al. 1985 2,4-DNT	
2	Rat (Sprague-Dawley)	1 x/d 14 d (G)				284 M (6/6 died)	Lent et al. 2012a 2,4-DNT	
3	Rat (CD)	once (GO)				568 M (LD50) 650 F (LD50)	U.S. Army 1975; U.S. Army 1978a 2,4-DNT	
4	Rat (Sprague-Dawley)	once (G)				270 M (LD50)	Vernot et al. 1977 2,4-DNT	
5	Mouse (Swiss albino)	once (GO)				1954 M (LD50) 1340 F (LD50)	U.S. Army 1975 2,4-DNT	
6	Mouse (CF-1)	once (G)				1630 M (LD50)	Vernot et al. 1977 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
7	Rat (Sprague-Dawley)	once (GO)	Hepatic	50 F	99 F Significantly decreased levels of serum albumin (13% lower than controls)		Deng et al. 2011 2,4-DNT	The NOAEL for renal effects is based on the absence of effects on levels of creatinine or urea in the serum.
			Renal	398 F				
			Bd Wt	198 F	398 F Decreased body weight gain (1 to 4 g compared to 17 g for controls)			
8	Rat (Sprague-Dawley)	5 d 1 x/d (GO)	Hemato		60 M (slight cyanosis)		Lane et al. 1985 2,4-DNT	
			Bd Wt	180 M	240 M (weight loss)			

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
9	Rat (Sprague-Dawley)	1 x/d 14 d (G)	Cardio	142 M			Lent et al. 2012a 2,4-DNT	
			Hemato	36 M	71 M (mild to moderate extramedullary hematopoiesis of the spleen)			
			Hepatic	18 M	36 M (trace-to-mild single cell necrosis and glycogen deposition)			
			Renal	142 M				
			Bd Wt	71 M	142 M (terminal Bd Wt 12% lower than control)			

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
10	Rat (Sprague-Dawley)	14 d ad lib	Resp	260.9 M 272.7 F			McGown et al. 1983 2,4-DNT	
			Cardio	260.9 M 272.7 F				
			Gastro	260.9 M 272.7 F				
			Hepatic		78.3 M (increased alanine aminotransferase and cholesterol) 81.8 F (increased cholesterol)			
			Renal		78.3 M (hyaline droplet formation) 81.8 F (hyaline droplet formation)			
			Dermal	260.9 M 272.7 F				
			Ocular	260.9 M 272.7 F				

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
11	Mouse (C57BL/6N)	14 days daily (G)	Hemato	134 F			Wilbanks et al. 2014 2,4-DNT	No change in hematocrit, reticulocyte percent, or relative spleen weight.
			Musc/skel		134 F (swim-to-exhaustion time decreased by 82% relative to controls)			
			Bd Wt		134 F (2.7-fold greater body weight loss than control)			
Immuno/ Lymphoret								
12	Rat (Sprague-Dawley)	14 d ad lib		260.9 M 272.7 F			McGown et al. 1983 2,4-DNT	
Neurological								
13	Rat (Sprague-Dawley)	1 x/d 14 d (G)		142 M			Lent et al. 2012a 2,4-DNT	
14	Dog (Beagle)	12 d 1 x/d (C)		5[b]		25 (incoordination, stiffness, abnormal gait)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
15	Rat (Sprague-Dawley)	5 d 1 x/d (GO)		60 M		180 M (decreased fertility)	Lane et al. 1985 2,4-DNT	
16	Rat (Sprague-Dawley)	1 x/d 14 d (G)		71 M	142 M (decreased absolute and relative testes weight and decreased absolute epididymis weight; tubular degeneration, multinucleated giant cell formation, and interstitial atrophy of the testes)		Lent et al. 2012a 2,4-DNT	
17	Rat (Sprague-Dawley)	14 d ad lib				78.3 M (decreased thickness of spermatogenic sperm layers)	McGown et al. 1983 2,4-DNT	
18	Mouse DBA/2J	2 d 1 x/d (G)				250 M (decreased fertility)	Soares and Lock 1980 2,4-DNT	
INTERMEDIATE EXPOSURE								
Death								
19	Rat (Wistar)	6 mo ad lib (F)				371 M (71% died)	Kozuka et al. 1979 2,4-DNT	
20	Rat (CD)	4 or 13 wk ad lib (F)				93 M (1/8 died) 145 F (8/8 died)	Lee et al. 1985 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
21	Rat (CD)	3 or 6 mo ad lib (F)				45.3 F (increased incidence of death during parturition)	U.S. Army 1979 2,4-DNT	
22	Mouse (CD-1)	4 or 13 wk ad lib (F)				413 (2/16M, 2/16F died)	Hong et al. 1985; U.S. Army 1978b 2,4-DNT	
23	Dog (Beagle)	4 or 13 wk 1 x/d (C)				25 (5/8 died)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	
24	Dog (Beagle)	6 mo 1 x/d (C)				10 M (4/6 died)	U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
Systemic								
25	Rat (Sprague-Dawley)	3 wk ad lib (F)	Bd Wt	76.7 M	156.4 M (10% decrease body weight)		Bloch et al. 1988 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
26	Rat (Wistar)	6 mo ad lib (F)	Hemato		371 M (increased methemoglobin)		Kozuka et al. 1979 2,4-DNT	
			Hepatic		371 M (increased relative liver weight; increased SGOT, LDH, alkaline phosphatase, acid phosphatase, triglycerides, glucose; formation of puruloid matter)			
			Renal	371 M				
			Bd Wt			371 M (41% decrease body weight)		
27	Rat (Fischer- 344)	6 or 26 wk ad lib (F)	Bd Wt		27 M (11% decrease body weight)		Leonard et al. 1987 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
28	Rat (CD)	4 or 13 wk ad lib (F)	Hemato	34 M 38 F	93 M (reticulocytosis; hemosiderosis) 108 F (reticulocytosis; hemosiderosis)	266 M (anemia) 145 F (anemia)	U.S. Army 1978b, Lee et al. 1985 2,4-DNT	
			Hepatic	266 M 145 F				
			Renal	266 M 145 F				
			Bd Wt			34 M (75% decrease body weight gain with decreased food consumption) 38 F (94% decrease body weight gain with decreased food consumption)		
29	Rat (CD)	3 or 6 mo ad lib	Bd Wt			34.5 M (23-25% decrease in body weight) 45.3 F (10-23% decrease in body weight)	U.S. Army 1979 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
30	Mouse (CD-1)	4 or 13 wk ad lib (F)	Hemato	137 M 147 F	413 M 468 F (mild anemia, reticulocytosis)		Hong et al. 1985; U.S. Army 1978b 2,4-DNT	
			Hepatic	47 M 147 F		137 M 468 F (mild hepatocellular dysplasia)		
			Renal	413 M 468 F				
			Bd Wt			413 M 468 F (body weight loss with decreased food consumption)		

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
31	Dog (Beagle)	4 or 13 wk 1 x/d (C)	Hemato	5		25 (anemia, Heinz bodies)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	
			Hepatic	25				
			Renal	25				
32	Dog (Beagle)	9 mo 1 x/d (C)	Hemato	1.5 M 1.5 F 0.2 F[c]	10 M (increased Heinz bodies and methemoglobin) 10 F (decreased erythrocyte count, hemoglobin, and hematocrit; increased reticulocytes and Heinz bodies) 1.5 F (increased methemoglobin)		U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
Immuno/ Lymphoret								
33	Rat (Wistar)	6 mo ad lib (F)			371 M (increased relative spleen weight)		Kozuka et al. 1979 2,4-DNT	
34	Dog (Beagle)	4 or 13 wk 1 x/d (C)		25			Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Neurological								
35	Rat (Wistar)	6 mo ad lib (F)				347 M (humpback incoordination)	Kozuka et al. 1979 2,4-DNT	
36	Rat (CD)	4 or 13 wk ad lib (F)		34 M 108 F		93 M 145 F (demyelination of cerebellum and brain stem; widespread and stiff-legged gait)	U.S. Army 1978b, Lee et al. 1985 2,4-DNT	
37	Mouse (CD-1)	4 or 13 wk ad lib (F)		413 M 468 F			Hong et al. 1985; U.S. Army 1978b 2,4-DNT	
38	Dog (Beagle)	4 or 13 wk 1 x/d (C)		5		25 (incoordination, abnormal gait, paralysis)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	
Reproductive								
39	Rat (Sprague-Dawley)	3 wk ad lib (F)			76.7 M (multinucleated spermatids, mild irregularity of basal lamina, vacuolation and lipid accumulation in Sertoli cells)	153.4 M (extensive degeneration of spermatids and spermatocytes; ultrastructural changes in Sertoli cells; 63% decrease sperm count)	Bloch et al. 1988 2,4-DNT	
40	Rat (Wistar)	6 mo ad lib (F)				371 M (testicular atrophy)	Kozuka et al. 1979 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
41	Rat (CD)	13 wk ad lib (F)		9.3 M		93 M (decreased fertility)	U.S. Army 1978b, Lee et al. 1985 2,4-DNT	
42	Rat (CD)	4 or 13 wk ad lib (F)		34 M		93 M (severe decrease in spermatogenesis)	U.S. Army 1978b, Lee et al. 1985 2,4-DNT	
43	Rat (CD)	3 or 6 mo ad lib (F)				34.5 M 45.3 F (decreased fertility; difficult parturition)	U.S. Army 1979 2,4-DNT	
44	Rat (CD)	3 or 6 mo ad lib (F)		34.5 M		45 M (decreased fertility; severe atrophy/degeneration of seminiferous tubules)	U.S. Army 1979 2,4-DNT	
45	Mouse (CD-1)	4 or 13 wk ad lib (F)		137 M 468 F		413 M (mild degeneration of seminiferous tubules)	Hong et al. 1985; U.S. Army 1978b 2,4-DNT	
46	Mouse (albino-Swiss)	4 wk (C)		295 M		1032 M (decreased fertility index)	U.S. Army 1978b 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
47	Dog (Beagle)	4 or 13 wk 1 x/d (C)		5 M 25 F		25 M (testicular degeneration, decreased spermatogenesis)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	
Developmental								
48	Rat (CD)	3 or 6 mo ad lib (F)		5.1 F		45.3 F (difficult parturition)	U.S. Army 1979 2,4-DNT	
CHRONIC EXPOSURE								
Death								
49	Rat (CD)	1-2 yr ad lib (F)				3.9 M 5.1 F (decreased survival)	U.S. Army 1978b; U.S. Army 1979; Lee et al. 1985 2,4-DNT	
50	Mouse (CD-1)	24 mo ad lib (F)				898 (decreased survival)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	
Systemic								
51	Rat (Fischer- 344)	52 wk ad lib (F)	Hepatic			27 M (hepatocellular degeneration and vacuolation; basophilic and acidophilic foci of cellular alteration)	Leonard et al. 1987 2,4-DNT	
			Bd Wt			27 M (25% body weight decrease)		

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
52	Rat (Fischer- 344)	78 wk ad lib (F)	Bd Wt	8 M 8.8 F		20 M 22 F (25% decrease body weight)	NCI 1978 2,4-DNT	
53	Rat (CD)	1-2 yr ad lib (F)	Hemato	0.6 M 5.1 F	3.9 M (decreased RBC count)	34.5 M (anemia) 45.3 F (anemia)	U.S. Army 1978b, 1979; Lee et al. 1985 2,4-DNT	
			Renal	34.5 M 45.3 F				
			Bd Wt	3.9 M 5.1 F		34.5 M (30% decrease body weight with decreased food consumption) 45.3 F (27% decrease body weight with decreased food consumption)		
54	Mouse (C57BL/6N)	78 wk ad lib (F)	Bd Wt	14.4 M	72 M (18% decrease in body weight gain) 15.2 F (11% decrease in body weight gain)	76 F (24% decrease in body weight gain)	NCI 1978 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
55	Mouse (CD-1)	24 mo ad lib (F)	Hemato	95		898 (anemia; reticulocytosis; Heinz bodies)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	
			Hepatic	95 F		14 M (hepatocellular dysplasia) 898 F (hepatocellular dysplasia)		
			Renal			14 M (cystic dysplasia; toxic nephropathy)		
			Bd Wt	14 M 95 F	95 M (16% decrease in body weight)	898 F (20% decrease in body weight)		
56	Dog (Beagle)	24 mo 1 x/d (C)	Hemato	0.2	1.5 (methemoglobinemia, anemia)		U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
			Hepatic	1.5	10 Biliary hyperplasia			
			Renal	10				
57	Dog (Beagle)	12 mo 1 x/d (C)	Hemato		0.2[d] F (increased reticulocytes)		U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
Neurological								
58	Rat (CD)	1-2 yr ad lib (F)				34.5 M (wide-spread and stiff-legged gait) 45.3 F (wide-spread and stiff-legged gait)	US Army 1978b, 1979; Lee et al. 1985 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
59	Mouse (CD-1)	24 mo ad lib		95		898 (stiff-legged gait, hyperactivity)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	
60	Dog (Beagle)	24 mo 1 x/d (C)		0.2		1.5 (loss of hindquarter control, convulsions)	U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
Reproductive								
61	Rat (CD)	1-2 yr ad lib (F)		3.9 M	34.5 M Atophy of seminiferous tubules, aspermatogenesis		U.S. Army 1978b, 1979; Lee et al. 1985 2,4-DNT	
62	Rat (CD)	1-2 yr ad lib (F)			34 M		U.S. Army 1978b; U.S. Army 1979; Lee et al. 1985 2,4-DNT	
63	Mouse (CD-1)	24 mo ad lib		95 F		14 M (decreased spermatogenesis and degenerative change; testicular atrophy)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	
						898 F (ovarian atrophy; nonfunctioning follicles)		

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
64	Dog (Beagle)	24 mo 1 x/d (C)		10 M			U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
Cancer								
65	Rat (Fischer- 344)	78 wk ad lib (F)				7.5 M (CEL: skin and subcutaneous fibroma) 22 F (CEL: mammary fibroadenoma)	NCI 1978 2,4-DNT	
66	Rat (CD)	1-2 yr ad lib (F)				34.5 M (CEL: hepatocellular carcinoma; mammary and skin tumors) 45.3 F (CEL: hepatocellular carcinoma, mammary and skin tumors)	U.S. Army 1978b, 1979; Lee et al. 1985 2,4-DNT	

Table 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
67	Mouse (CD-1)	24 mo ad lib				95 M (CEL: renal solid carcinoma, cystic papillary carcinoma and adenoma, cystic adenoma)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	

a The number corresponds to entries in Figure 3-2.

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.05 mg/kg/day; the MRL was derived by dividing the NOAEL of 5 mg/kg/day by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

c Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.007 mg/kg/day; the MRL was derived by dividing the BMDL1SD of 0.67 mg/kg/day by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

d Used to derive a chronic-duration oral minimal risk level (MRL) of 0.001 mg/kg/day; the MRL was derived by dividing the BMDL1SD of 0.12 mg/kg/day by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; (GO) = gavage in oil; Hemato = hematological; LD50 = lethal dose, 50% kill; LDH = ; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; RBC = red blood cell; Resp = respiratory; SGOT = ; x = time(s); wk = week(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

Acute (≤14 days)

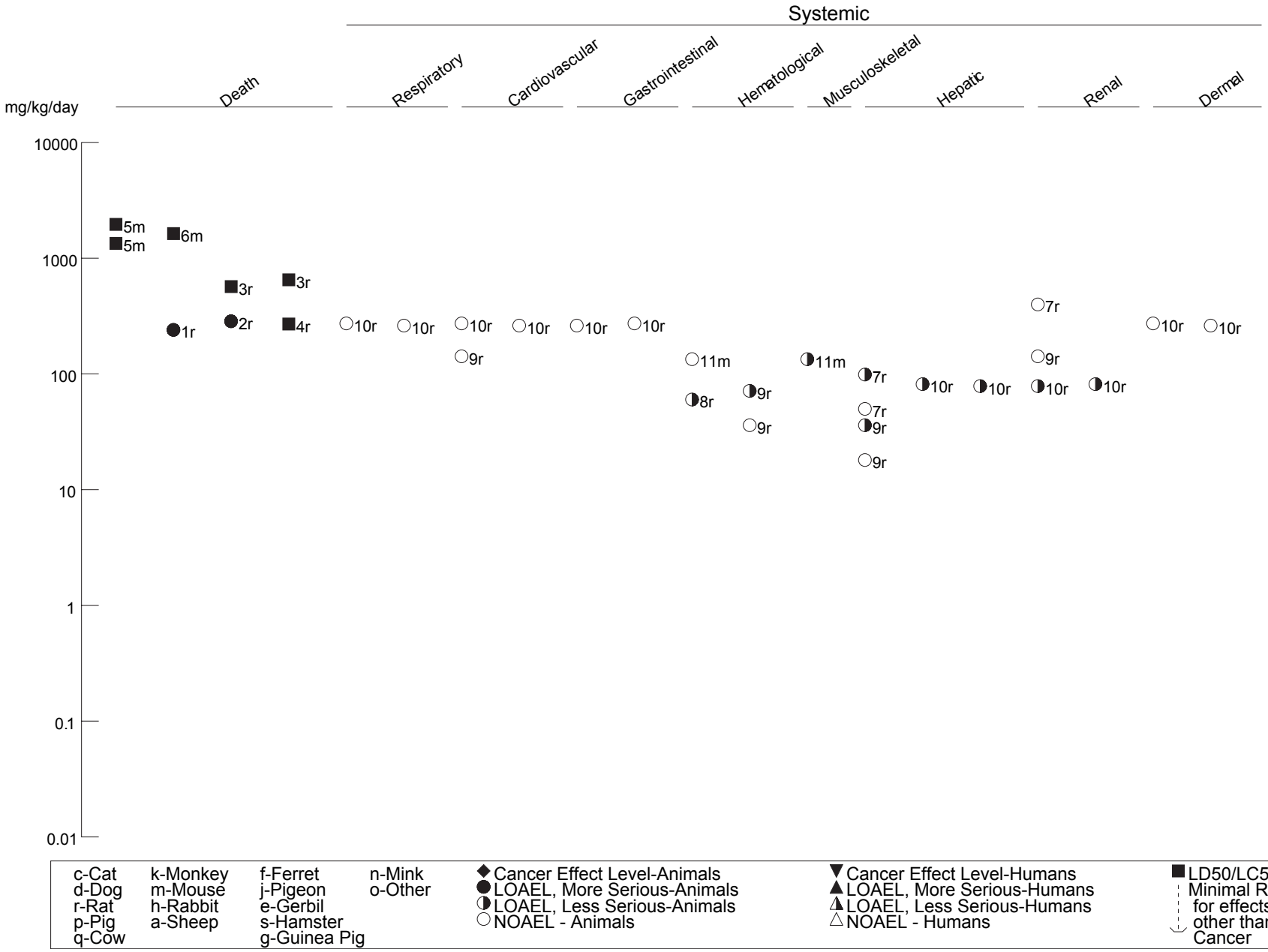


Figure 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral *(Continued)*

Acute (≤14 days)

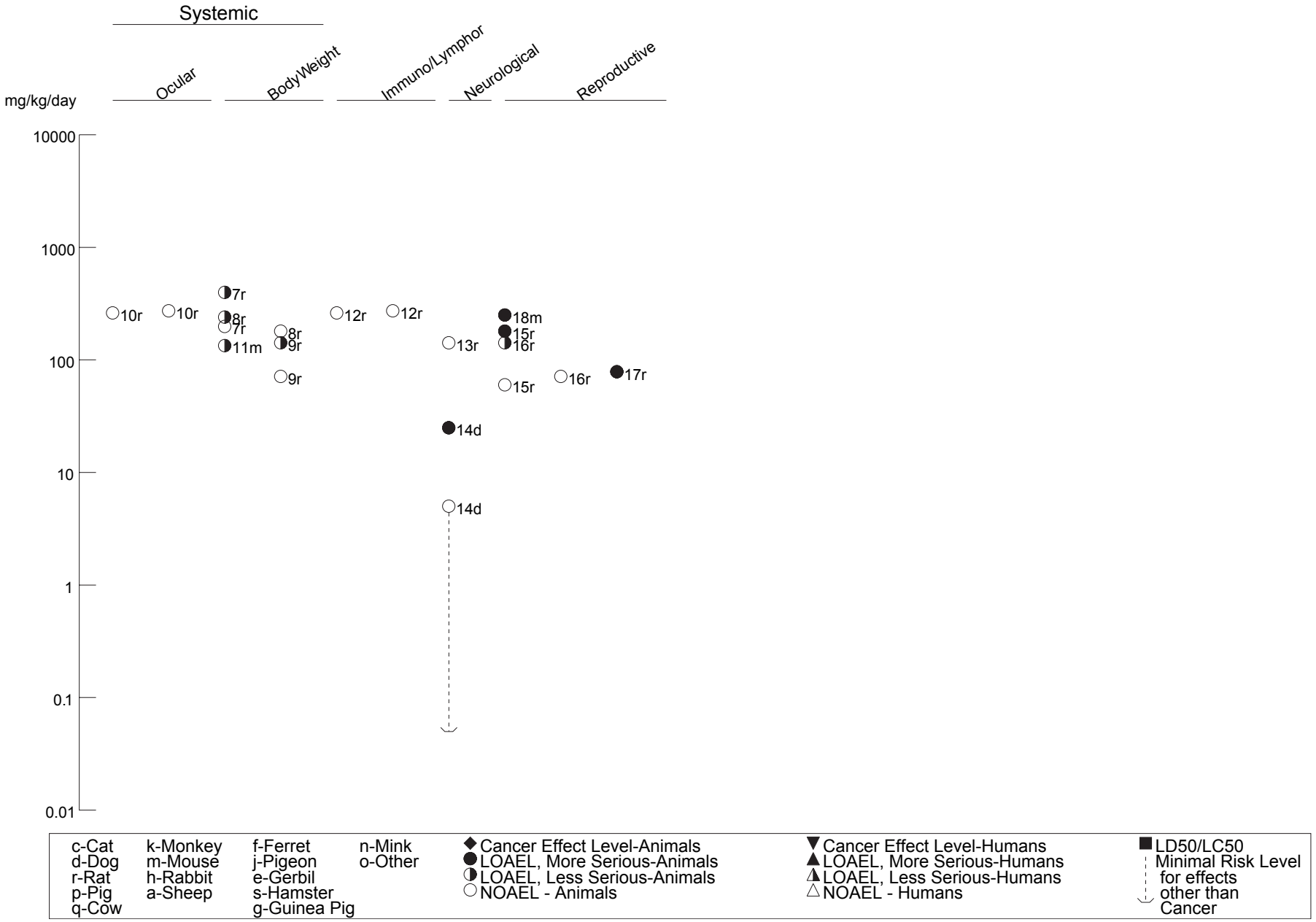


Figure 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral *(Continued)*

Intermediate (15-364 days)

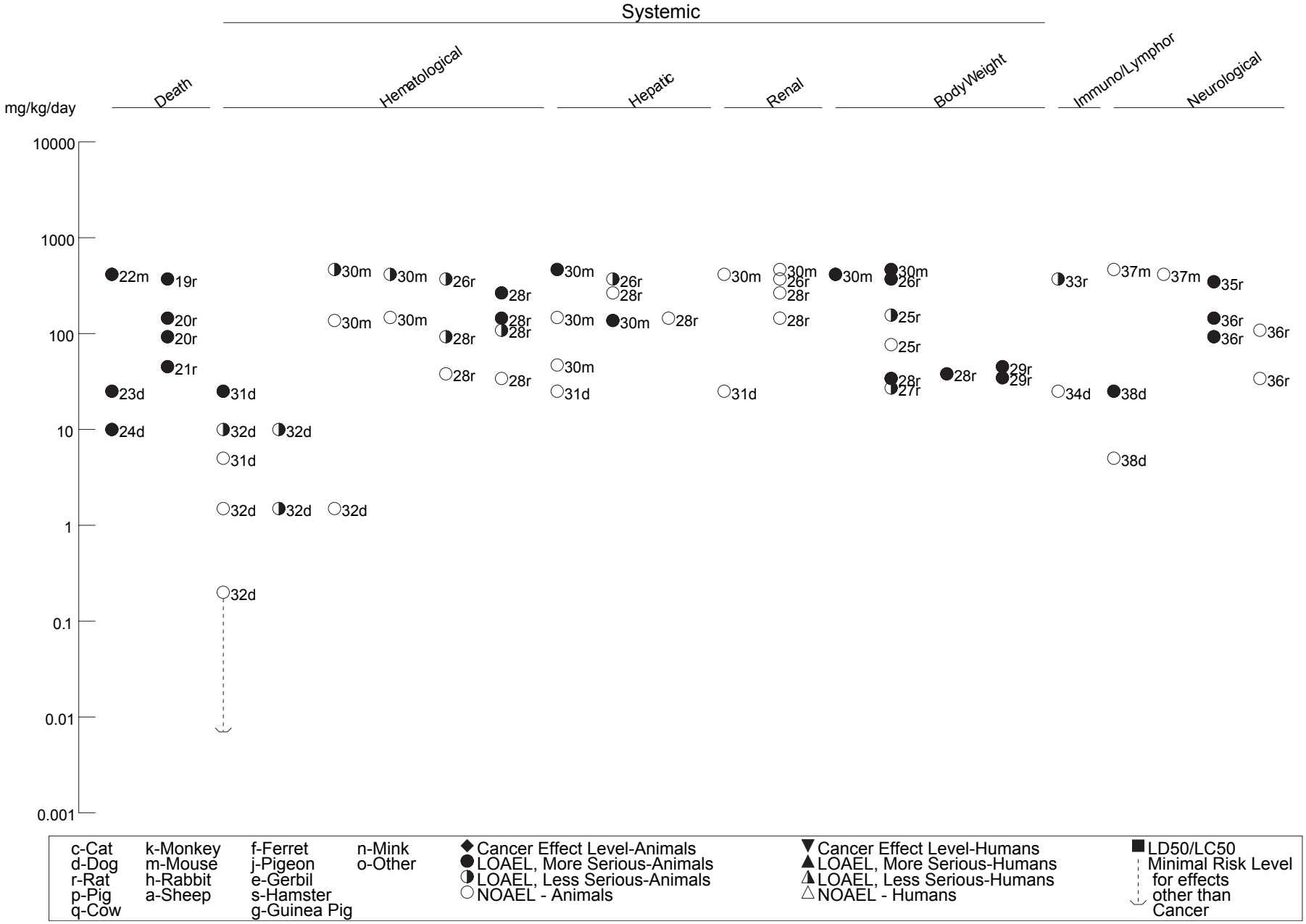
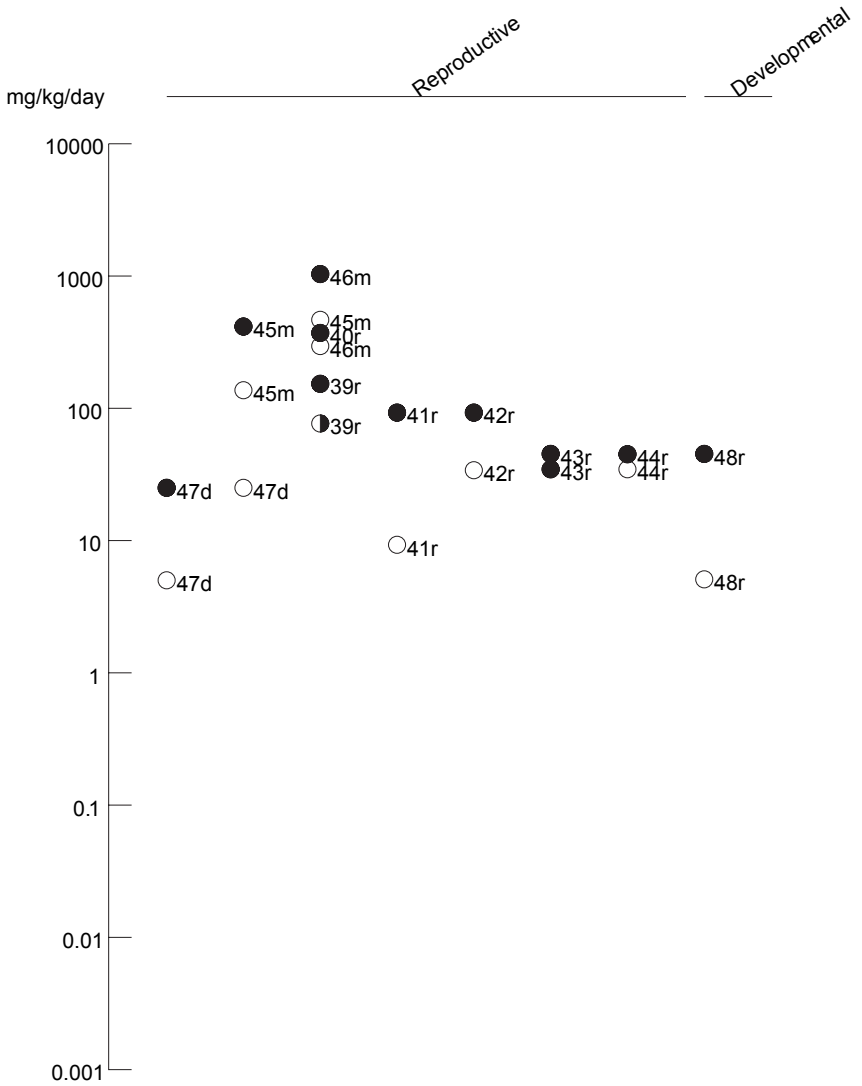


Figure 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral *(Continued)*

Intermediate (15-364 days)



c-Cat, d-Dog, r-Rat, p-Pig, q-Cow; k-Monkey, m-Mouse, h-Rabbit, a-Sheep; f-Ferret, j-Pigeon, e-Gerbil, s-Hamster, g-Guinea Pig; n-Mink, o-Other

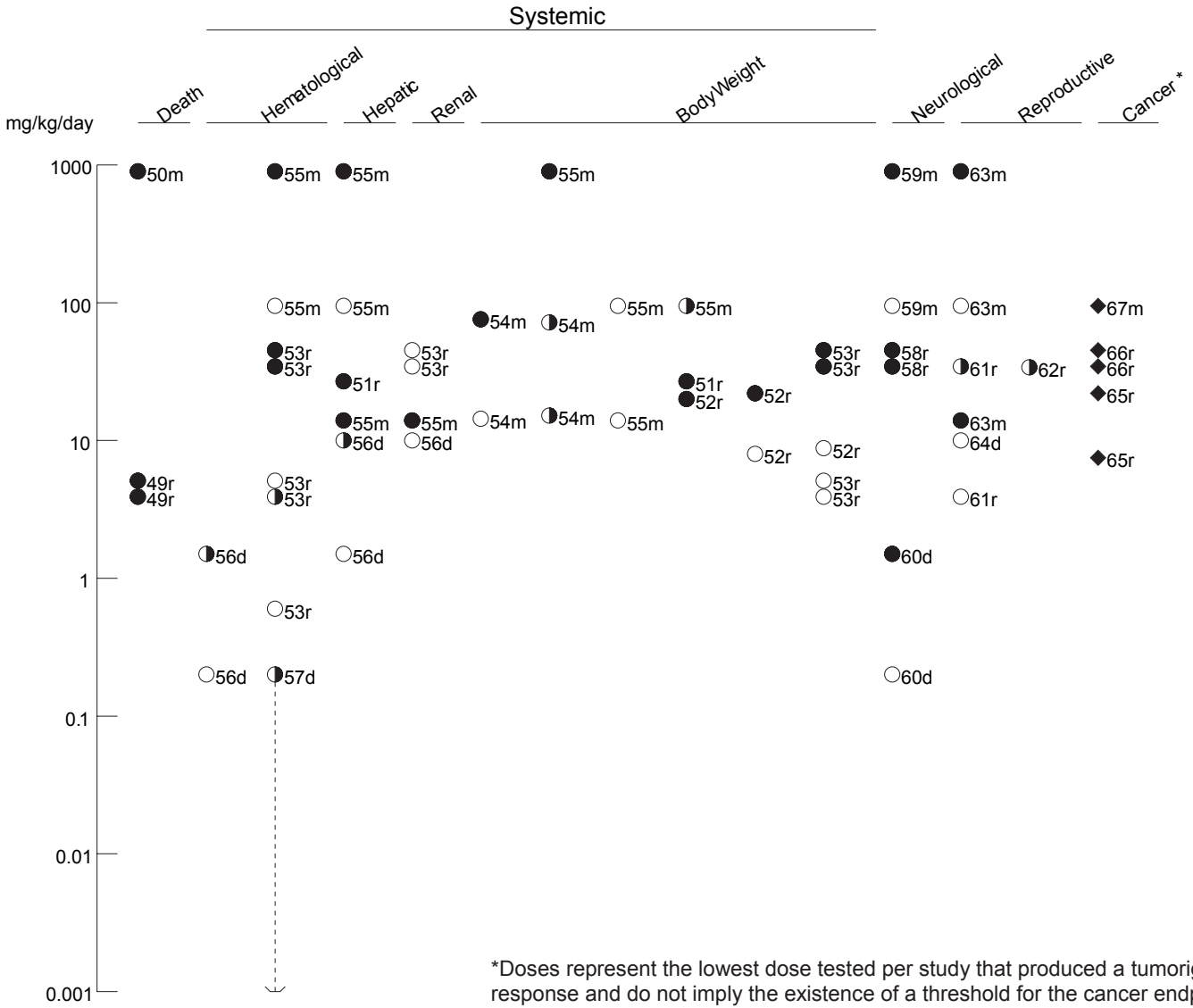
◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-2 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat, d-Dog, r-Rat, p-Pig, q-Cow; k-Monkey, m-Mouse, h-Rabbit, a-Sheep; f-Ferret, j-Pigeon, e-Gerbil, s-Hamster, g-Guinea Pig; n-Mink, o-Other

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Table 3-3 Levels of Significant Exposure to 2,5-Dinitrotoluene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	1 x/d 14 d (G)				308 M (1/6 died)	Lent et al. 2012a 2,5-DNT	
2	Rat (Sprague-Dawley)	once (G)				710 M (LD50)	Vernot et al. 1977 2,5-DNT	
3	Mouse (CF-1)	once (G)				1230 M (LD50)	Vernot et al. 1977 2,5-DNT	
Systemic								
4	Rat (Sprague-Dawley)	1 x/d 14 d (G)	Cardio	154 M	308 M (increased absolute and relative heart weight; mild-to-moderate fibrosis and trace-to-moderate inflammation)		Lent et al. 2012a 2,5-DNT	
			Hemato		39 M[b] (dark spleen; mild to moderate extramedullary hematopoiesis)			
			Hepatic	308 M				
			Renal	308 M				
			Bd Wt	308 M				
Neurological								
5	Rat (Sprague-Dawley)	1 x/d 14 d (G)		308 M			Lent et al. 2012a 2,5-DNT	

Table 3-3 Levels of Significant Exposure to 2,5-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
6	Rat (Sprague-Dawley)	1 x/d 14 d (G)		308 M			Lent et al. 2012a 2,5-DNT	No effect on testes of epididymides weight or histopathology.

a The number corresponds to entries in Figure 3-3.

b Used to derive an acute-duration oral minimal risk level (MRL) for 2,5-DNT of 0.007 mg/kg/day; the MRL was derived by dividing the BMDL10 of 2.05 mg/kg/day by an uncertainty factor of 300 (10 for animal-to-human extrapolation, 10 for human variability, and 3 for database limitations).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); (G) = gavage; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; x = time(s)

Figure 3-3 Levels of Significant Exposure to 2,5-Dinitrotoluene - Oral

Acute (≤14 days)

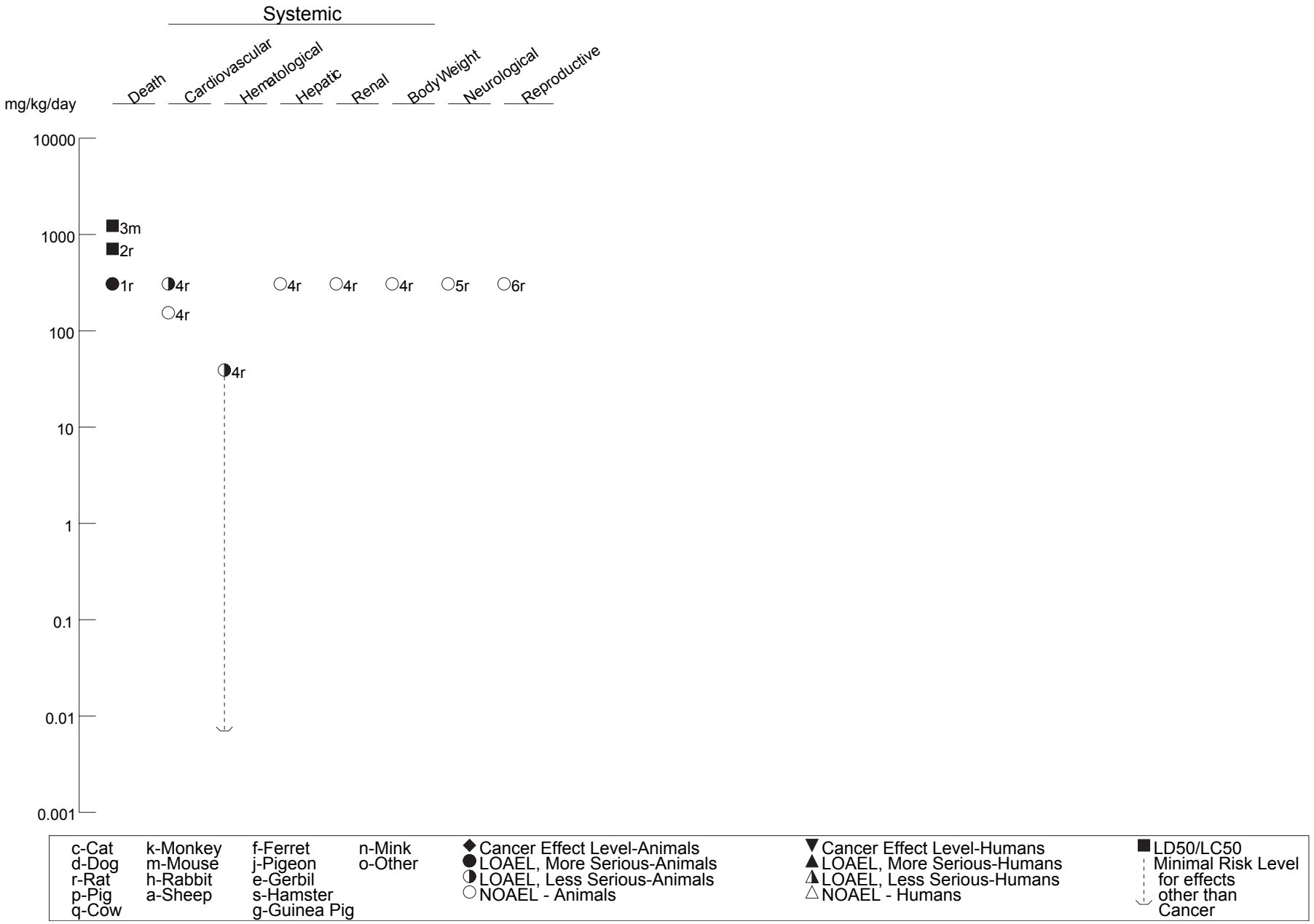


Table 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (CD)	once (GO)				535 M (LD50) 795 F (LD50)	U.S. Army 1975; U.S. Army 1978a 2,6-DNT	
2	Rat (Sprague-Dawley)	once (G)				180 M (LD50)	Vernot et al. 1977 2,6-DNT	
3	Mouse (CD)	once (GO)				621 M (LD50) 807 F (LD50)	U.S. Army 1975 2,6-DNT	
4	Mouse (CF-1)	once (G)				1000 M (LD50)	Vernot et al. 1977 2,6-DNT	
Systemic								
5	Rat (Sprague-Dawley)	once (GO)	Renal	199 F			Deng et al. 2011 2,6-DNT	The NOAEL for renal effects is based on the absence of effects on levels of creatinine or urea in the serum.
			Bd Wt	25 F	50 F Decreased body weight gain (1 to 4 g compared to 17 g for controls)			

Table 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
6	Rat (Sprague-Dawley)	1 x/d 14 d (G)	Cardio	134 M			Lent et al. 2012a 2,6-DNT	
			Hemato		68 M (extramedullary hematopoiesis and lymphoid depletion of the spleen)			
			Hepatic	14 M	35 M (hepatocellular hyperplasia, oval cell hyperplasia, and hepatocellular hypertrophy)			
			Renal		134 M (proximal tubule degeneration and renal tubular basophilia)			
			Bd Wt	14 M	35 M (terminal body weight 10% lower than controls)			
7	Rat (Sprague-Dawley)	1 x/d 3 d (G)	Hepatic		100 M (diffuse hepatocellular hypertrophy)		Rothfuss et al. 2010 2,6-DNT	
			Bd Wt	100 M				
8	Dog (Beagle)	2 wk ad lib (C)	Hemato	4[b]	20 (decreased erythrocytes and increased mean cell hemoglobin)		US Army 1976 2,6-DNT	

Table 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Neurological								
9	Rat (Sprague-Dawley)	1 x/d 14 d (G)		134 M			Lent et al. 2012a 2,6-DNT	
Reproductive								
10	Rat (Sprague-Dawley)	1 x/d 14 d (G)		35 M	68 M (tubular degeneration, multinucleated giant cell formation, and interstitial atrophy of the testes)		Lent et al. 2012a 2,6-DNT	
INTERMEDIATE EXPOSURE								
Death								
11	Rat (Sprague-Dawley)	1 x/d 29 d (G)		33 M			Rothfuss et al. 2010 2,6-DNT	
12	Mouse (Swiss-albino)	4 or 13 wk ad lib (F)				51 M (8/16 died) 55 F (6/16 died)	US Army 1976 2,6-DNT	
13	Dog (Beagle)	4 or 13 wk ad lib (C)				20 F (2/8 died)	US Army 1976 2,6-DNT	
Systemic								
14	Rat (Fischer- 344)	6 or 26 wk ad lib (F)	Bd Wt	7 M		14 M (20% decrease body weight)	Leonard et al. 1987 2,6-DNT	

Table 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	Rat (Sprague-Dawley)	1 x/d 29 d (G)	Hepatic		33 M (hepatocellular hypertrophy, single cell necrosis, bile duct hyperplasia, focal hepatocellular vacuolation)		Rothfuss et al. 2010 2,6-DNT	
			Bd Wt		33 M (decreased body weight)			

Table 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
16	Rat (CD)	4 or 13 wk ad lib (F)	Hemato	7 F	37 F (splenic hemosiderosis; extramedullary hematopoiesis)		U.S. Army 1976 2,6-DNT	
					7 F Increased incidence of extramedullary hematopoiesis of the spleen at 4 weeks			
			Hepatic		35 M (bile duct hyperplasia;hemosiderosi			
					37 F (bile duct hyperplasia; hemosiderosis)			
			Renal	145 M				
				155 F				
			Bd Wt	7	35 M	145 M		
					37 F (decreased body weight gain)	155 F (body weight loss)		

Table 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
17	Mouse (Swiss-albino)	4 or 13 wk ad lib (F)	Hemato	11 M 11 F	51 M (extramedullary hematopoiesis) 55 F (extramedullary hematopoiesis)		US Army 1976 2,6-DNT	
			Hepatic	11	51 M (bile duct hyperplasia) 55 F (bile duct hyperplasia)			
			Renal	289 M 299 F				
			Bd Wt	11		51 M (weight loss) 55 F (weight loss)		
18	Dog (Beagle)	4 or 13 wk ad lib (C)	Hemato		4[c] (mild extramedullary erythropoiesis)		US Army 1976 2,6-DNT	
			Hepatic	4		20 (bile duct hyperplasia; degenerative and inflammatory liver changes)		
			Renal	4	20 (dilated tubules, degenerative foci)			
			Bd Wt	4		20 (body weight loss with decreased food consumption)		

Table 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
19	Rat (CD)	4 or 13 wk ad lib (F)		145 M 155 F			U.S. Army 1976 2,6-DNT	
20	Dog (Beagle)	4 or 13 wk ad lib (C)		20	100 (thymic involution)		US Army 1976 2,6-DNT	
Neurological								
21	Rat (CD)	4 or 13 wk ad lib (F)		145 M 155 F			U.S. Army 1976 2,6-DNT	
22	Mouse (Swiss-albino)	4 or 13 wk ad lib (F)		289 M 299 F			US Army 1976 2,6-DNT	
23	Dog (Beagle)	4 or 13 wk ad lib (C)		4		20 (incoordination, lack of balance)	US Army 1976 2,6-DNT	
Reproductive								
24	Rat (CD)	4 or 13 wk ad lib (F)		7 M 155 F		35 M (decreased spermatogenesis; degeneration of testes)	U.S. Army 1976 2,6-DNT	

Table 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
25	Mouse (Swiss-albino)	4 or 13 wk ad lib (F)		11 M 299 F		51 M (decreased spermatogenesis)	US Army 1976 2,6-DNT	
26	Dog (Beagle)	4 or 13 wk ad lib (C)		4 M 100 F		20 M (testicular degeneration)	US Army 1976 2,6-DNT	
CHRONIC EXPOSURE								
Systemic								
27	Rat (Fischer- 344)	52 wk ad lib (F)	Hepatic			7 M (hepatocellular degeneration, vacuolation; acidophilic and basophilic foci of cellular alteration)	Leonard et al. 1987 2,6-DNT	
			Bd Wt		7 M (18% decrease body weight)			
Cancer								
28	Rat (Fischer- 344)	52 wk ad lib (F)				7 M (CEL: cholangiocarcinoma, hepatocellular carcinoma)	Leonard et al. 1987 2,6-DNT	

a The number corresponds to entries in Figure 3-4.

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.09 mg/kg/day; the MRL was derived by dividing the BMDL1SD of 9.31 mg/kg/day by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

c Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.004 mg/kg/day; the MRL was derived by dividing the LOAEL of 4 mg/kg/day by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for animal-to-human extrapolation and 10 for human variability).

ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); (F) = feed; F = Female; (G) = gavage; (GO) = gavage in oil; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; x = time(s); wk = week(s)

Figure 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral

Acute (≤14 days)

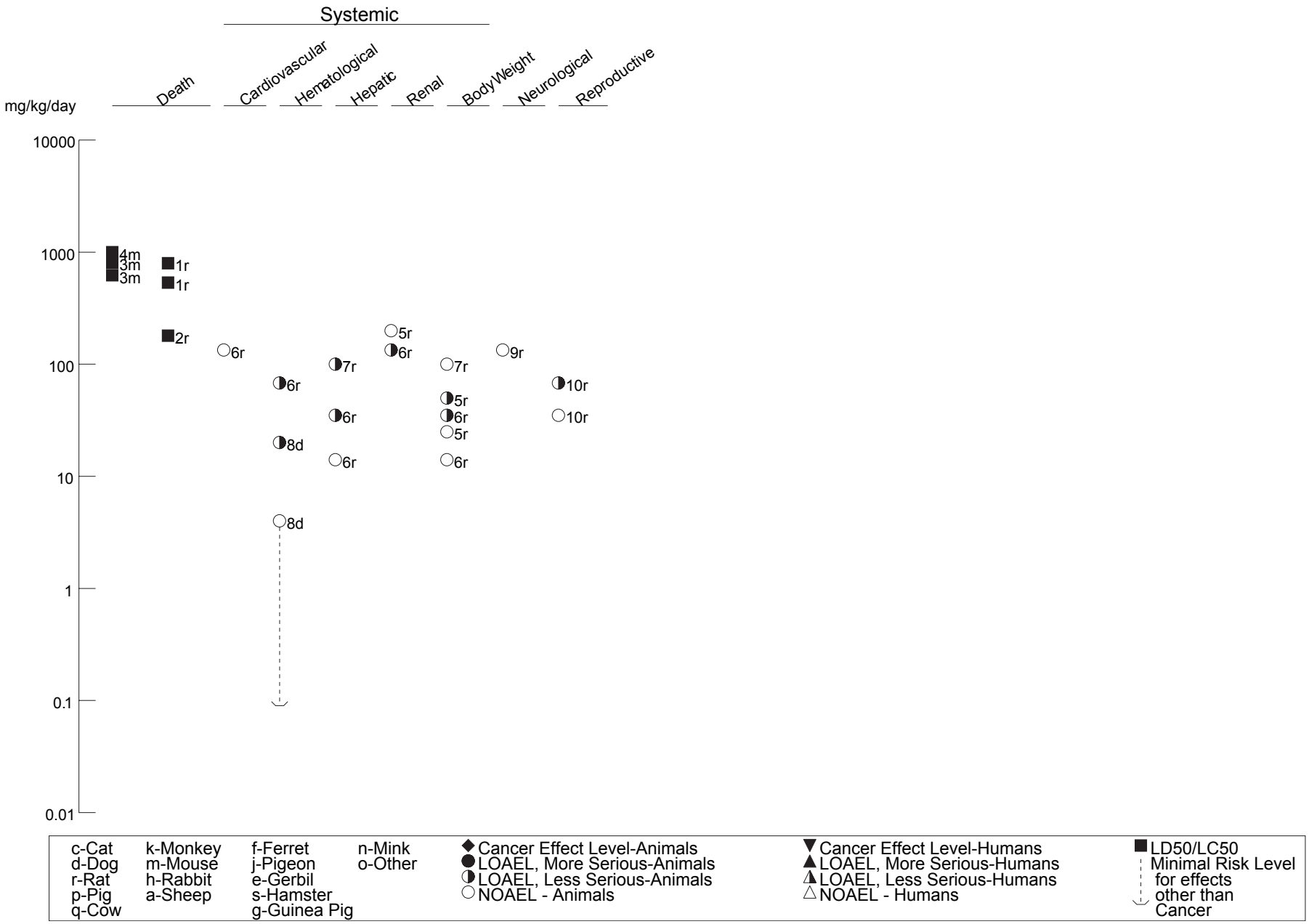


Figure 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral *(Continued)*

Intermediate (15-364 days)

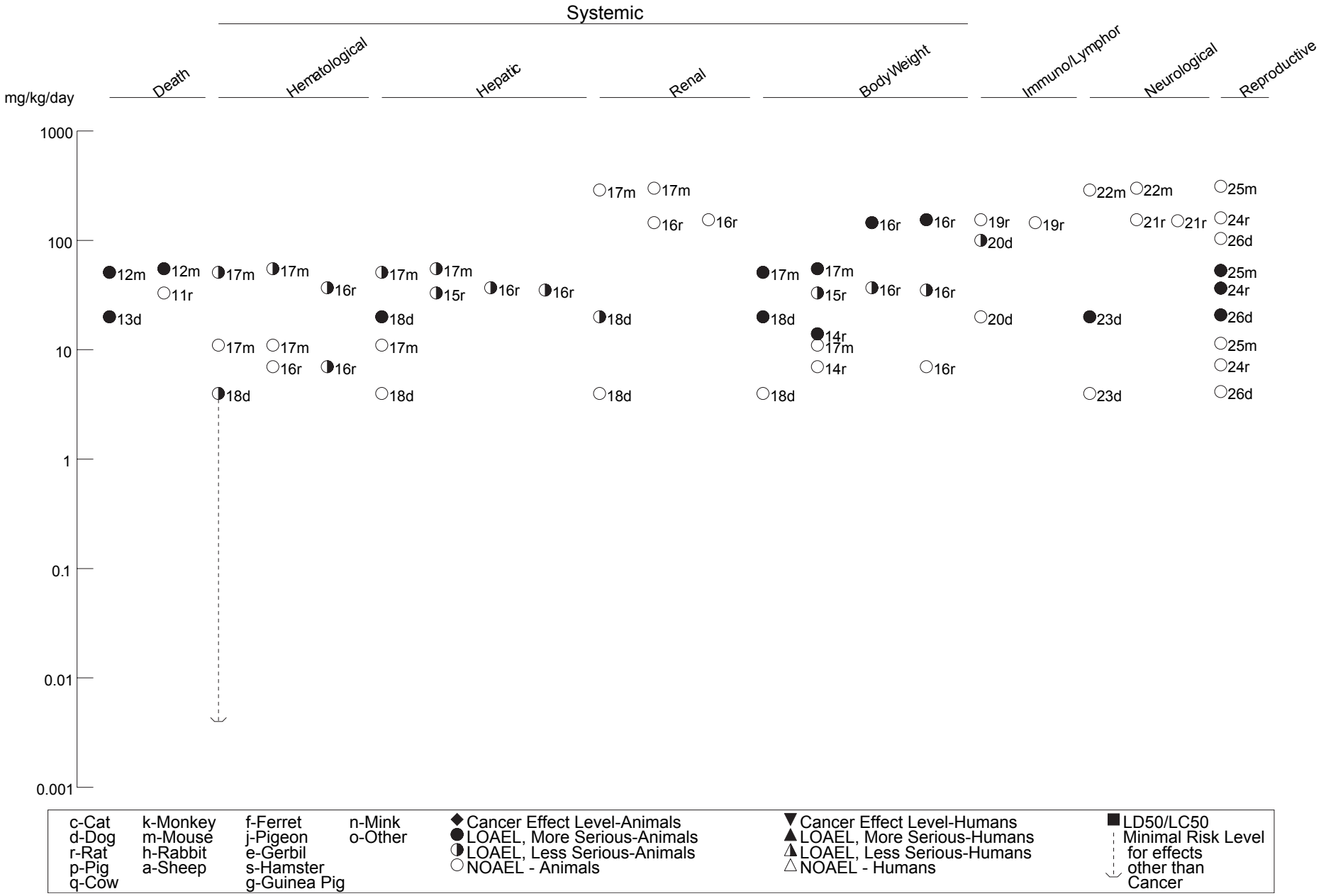
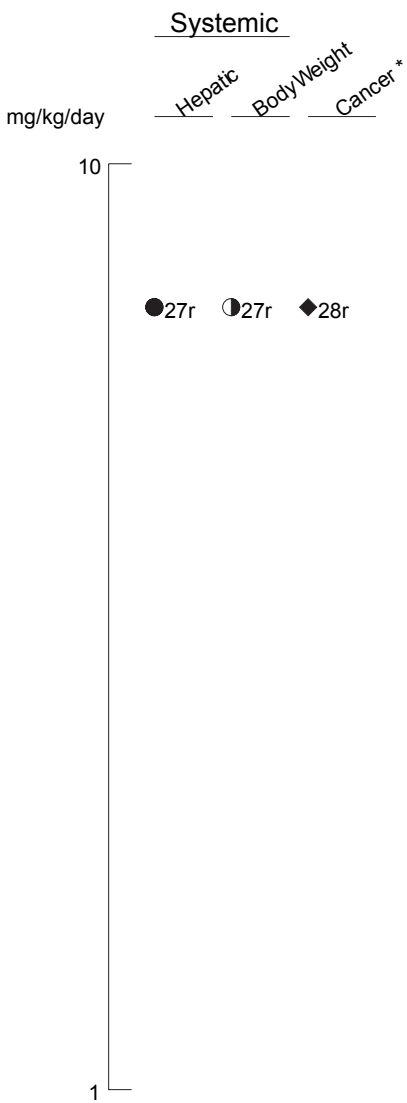


Figure 3-4 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat d-Dog r-Rat p-Pig q-Cow	k-Monkey m-Mouse h-Rabbit a-Sheep	f-Ferret j-Pigeon e-Gerbil s-Hamster g-Guinea Pig	n-Mink o-Other	◆ Cancer Effect Level-Animals ● LOAEL, More Serious-Animals ◑ LOAEL, Less Serious-Animals ○ NOAEL - Animals	▼ Cancer Effect Level-Humans ▲ LOAEL, More Serious-Humans ◭ LOAEL, Less Serious-Humans △ NOAEL - Humans	■ LD50/LC50 Minimal Risk Level for effects other than Cancer

Table 3-5 Levels of Significant Exposure to 3,4-Dinitrotoluene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Systemic								
1	Rat (Sprague-Dawley)	1 x/d 14 d (G)	Cardio	113 M	227 M (myocardial fibrosis, inflammation, and necrosis)		Lent et al. 2012a 3,4-DNT	
			Hemato		57 M[b] (extramedullary hematopoiesis and lymphoid hyperplasia)			
			Hepatic	227 M				
			Renal		227 M (proximal tubule degeneration, renal tubule basophilia, and lymphocytic infiltration)			
			Bd Wt	113 M	227 M (terminal body weight 10% less than controls)			
Neurological								
2	Rat (Sprague-Dawley)	1 x/d 14 d (G)		113 M	227 M (facial twitching, hypoactivity, staring)		Lent et al. 2012a 3,4-DNT	
Reproductive								
3	Rat (Sprague-Dawley)	1 x/d 14 d (G)		227 M			Lent et al. 2012a 3,4-DNT	No effects on testes or epididymides weight or histopathology.

a The number corresponds to entries in Figure 3-5.

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.03 mg/kg/day; the MRL was derived by dividing the BMDL10 of 8.05 mg/kg/day by an uncertainty factor of 300 (10 for animal-to-human extrapolation, 10 for human variability, and 3 for database limitations).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); (G) = gavage; Hemato = hematological; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; x = time(s)

Figure 3-5 Levels of Significant Exposure to 3,4-Dinitrotoluene - Oral

Acute (≤14 days)

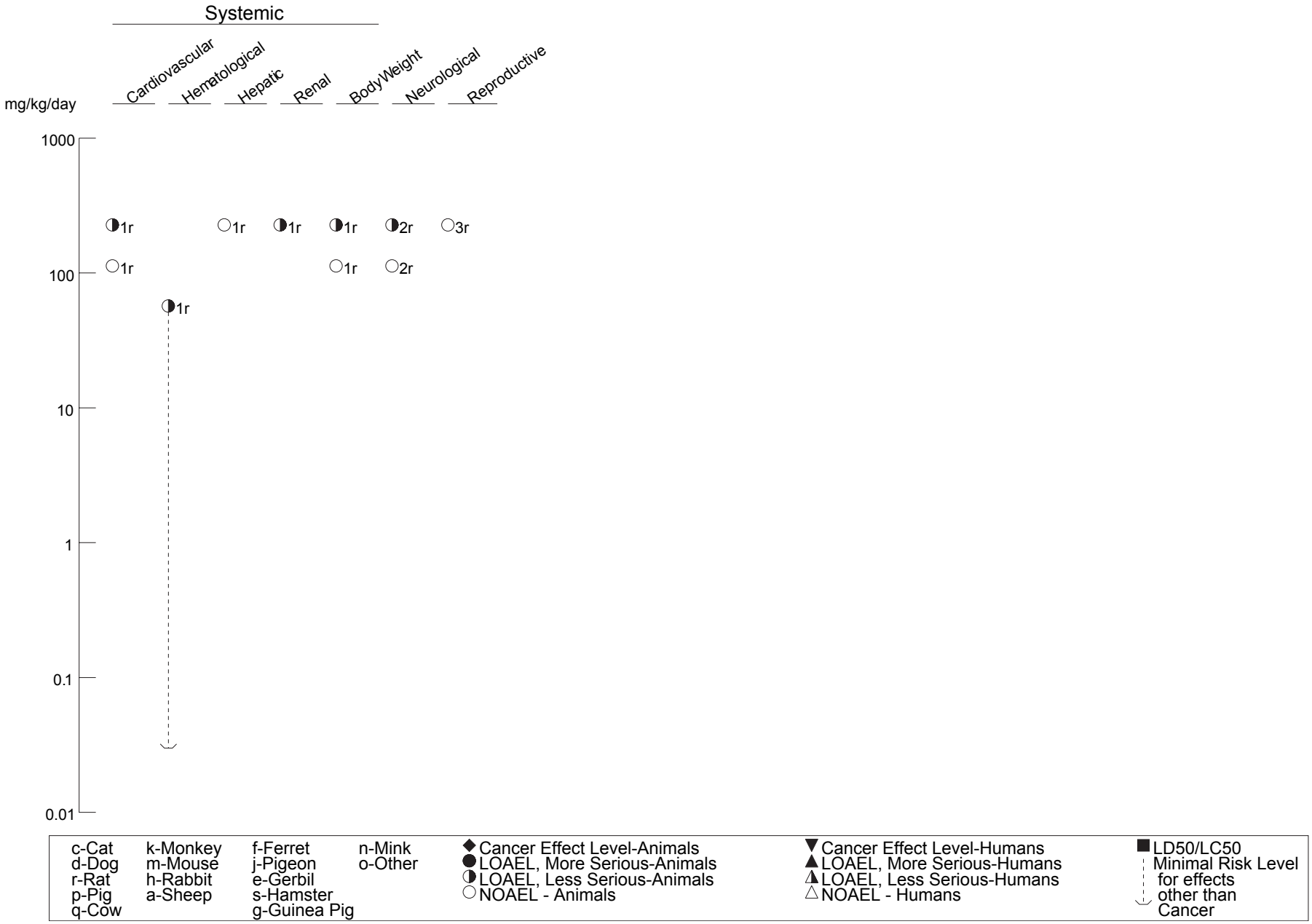


Table 3-6 Levels of Significant Exposure to 3,5-Dinitrotoluene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	1 x/d 14 d (G)				39 M (1/6 died)	Lent et al. 2012a 3,5-DNT	
Systemic								
2	Rat (Sprague-Dawley)	1 x/d 14 d (G)	Cardio	39 M			Lent et al. 2012a 3,5-DNT	
			Hemato	39 M				
			Hepatic	39 M				
			Renal	39 M				
			Bd Wt	39 M				
Neurological								
3	Rat (Sprague-Dawley)	1 x/d 14 d (G)		19 M	39 M (facial twitching and paralysis; inflammatory infiltrates in the brain)		Lent et al. 2012a 3,5-DNT	
Reproductive								
4	Rat (Sprague-Dawley)	1 x/d 14 d (G)		10 M[b]	19 M (small testes; significantly reduced testes weight; tubular degeneration and multinucleated giant cell formation in the testes)		Lent et al. 2012a 3,5-DNT	

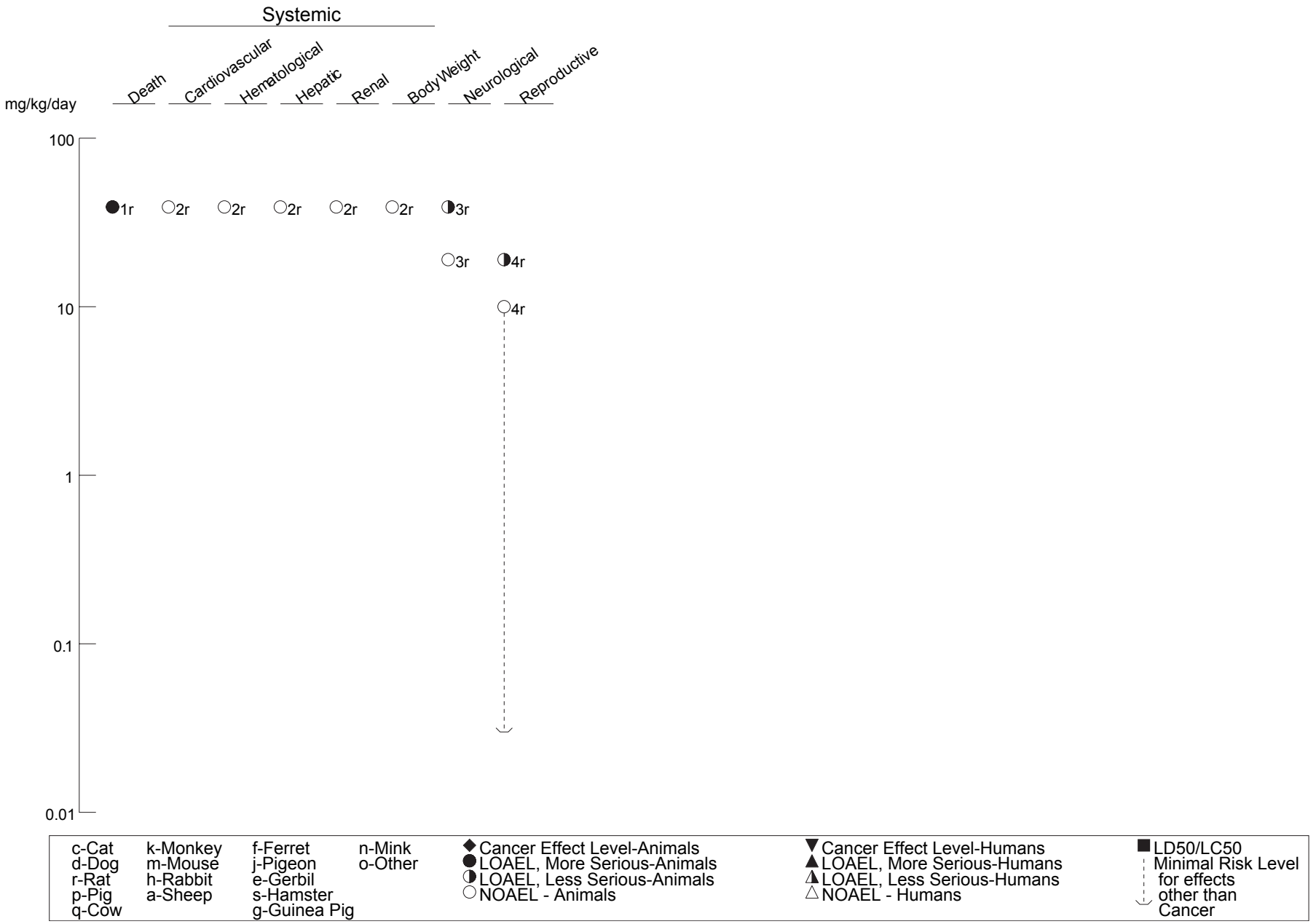
a The number corresponds to entries in Figure 3-6.

b Used to derive an acute-duration oral minimal risk level (MRL) 3,5-DNT of 0.03 mg/kg/day; the MRL was derived by dividing the NOAEL of 10 mg/kg/day by an uncertainty factor of 300 (10 for animal-to-human extrapolation, 10 for human variability, and 3 for database limitations).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); (G) = gavage; Hemato = hematological; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; x = time(s)

Figure 3-6 Levels of Significant Exposure to 3,5-Dinitrotoluene - Oral

Acute (≤14 days)



3.2.2.2 Systemic Effects

The systemic effects observed after oral exposure of humans and animals to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT are discussed below. No studies were located regarding musculoskeletal effects in humans or animals after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

All LOAEL values from each reliable study for systemic effects in each species and duration category for 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT are recorded in Tables 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively, and plotted in Figures 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively.

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

No histopathological effects on the lungs were found when Sprague-Dawley rats were fed 261 mg/kg/day (males) or 273 mg/kg/day (females) 2,4-DNT for 14 days (McGown et al. 1983).

No respiratory system effects were observed when CDF rats were fed 14 mg/kg/day Tg-DNT for 2 years (Hazleton Laboratories 1982). Histopathological examination of the lungs and respiratory tract tissues of rats exposed to 14 mg/kg/day Tg-DNT for 2 years or 35 mg/kg/day for 1 year did not reveal any abnormalities (Hazleton Laboratories 1982).

No studies were located regarding respiratory effects in animals after oral exposures to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Neither changes in heart weight nor microscopic lesions in the heart were observed in male Sprague-Dawley rats receiving gavage doses up to 275 mg/kg/day 2,3-DNT for 14 days (Lent et al. 2012a; USAPHC 2011a).

No histopathological effects on the cardiovascular system were found after Sprague-Dawley rats received 261 mg/kg/day (males) or 273 mg/kg/day (females) 2,4-DNT in the diet for 14 days (McGown et al. 1983). Neither changes in heart weight nor microscopic lesions in the heart were observed in male

Sprague-Dawley rats receiving gavage doses up to 142 mg/kg/day 2,4-DNT for 14 days (Lent et al. 2012a; USAPHC 2011b).

Increased absolute and relative heart weight, along with mild-to-moderate fibrosis in 4/5 animals and trace-to-moderate inflammation in 2/5 animals, were noted in male rats receiving 308 mg/kg/day 2,5-DNT via gavage for 14 days (Lent et al. 2012a; USAPHC 2011c).

One of six male Sprague-Dawley rats exposed to 227 mg/kg/day 3,4-DNT (the highest dose tested) exhibited cardiac lesions consisting of mild myocardial fibrosis, inflammation, and necrosis; heart weight was not affected at this dose or at the lower doses tested (Lent et al. 2012a; USAPHC 2011e). Exposure to 14 days of gavage doses up to 39 mg/kg/day 3,5-DNT did not result in changes in heart weight or microscopic lesions in the hearts of male Sprague-Dawley rats (Lent et al. 2012a; USAPHC 2011f).

At the 26-week interim sacrifice in a 104-week study in which CDF rats were fed 0, 3.5, 14, or 35 mg/kg/day Tg-DNT in the diet, an increased incidence and severity of myocarditis was noted in males at 35 mg/kg/day (Hazleton Laboratories 1982). It was believed that this spontaneous inflammatory condition was exacerbated by ingestion of Tg-DNT in the high-dose animals. Although this condition was also observed at the 55-week sacrifice, it was not observed at 52 or 104 weeks.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

There were no histopathological effects on the gastrointestinal tract of Sprague-Dawley rats fed 261 mg/kg/day (males) or 273 mg/kg/day (females) 2,4-DNT in the diet for 14 days (McGown et al. 1983).

Treatment of rats with up to 35 mg/kg/day Tg-DNT for up to 1 year or 14 mg/kg/day for up to 2 years did not cause any histopathological changes in the gastrointestinal tract (Hazleton Laboratories 1982).

No studies were located regarding gastrointestinal effects in animals after oral exposures to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Hematological effects were noted in virtually all animal studies of oral exposure to DNTs in which circulating blood was examined. The most common findings were methemoglobinemia, anemia, reticulocytosis, and an increase in Heinz bodies. The hematological effects are caused by oxidation of the iron in hemoglobin, producing methemoglobin. Heinz bodies are granules in erythrocytes that are believed to result from denatured hemoglobin. Reticulocytosis, a finding in many animals in these studies, is caused by the increased production of immature erythrocytes (red blood cells) and is seen as a compensatory mechanism in anemia resulting from exposure to 2,4- and 2,6-DNT. This hematotoxic syndrome is a common effect of exposure to aromatic amines and most organic and inorganic nitrates, and it has been implicated for many oxidizing agents (Smith 1996; U.S. Army 1979).

When male Sprague-Dawley rats were treated for 14 days with gavage doses of 275 mg/kg/day 2,3-DNT, hematological effects included extramedullary hematopoiesis and lymphoid hyperplasia of the spleen and lymphoid depletion (Lent et al. 2012a; USAPHC 2011a).

Female Sprague-Dawley rats (5/group) administered 2,4-DNT via gavage (in 5% v/v DMSO in corn oil) and observed for 24 or 48 hours after dosing showed evidence of erythrocytosis, as indicated by significant increases in hemoglobin, hematocrit, and/or erythrocyte and granulocyte counts, at doses ≥99 mg/kg (Deng et al. 2011). Relative to controls, erythrocyte and granulocyte counts were increased by 11 and 552% at 99 mg/kg after 24 hours; elevations in hemoglobin (27–31%) and hematocrit (29–33%) were only statistically significant in rats treated at 198 or 398 mg/kg and evaluated at 48 hours. No significant changes in hematological end points were observed in rats treated with 2,4-DNT at 5 or 50 mg/kg and evaluated 24 or 48 hours after dosing. Development of erythrocytosis 48 hours following a single exposure to 2,4-DNT may be a secondary effect of dehydration, rather than a direct effect on the hematological system; however, no information on drinking water consumption was reported in this study. Slight cyanosis was observed in rats administered 60 mg/kg 2,4-DNT by gavage for 5 days (Lane et al. 1985). No changes in hematological parameters were found in Sprague-Dawley rats fed 261 mg/kg/day (males) or 273 mg/kg/day (females) 2,4-DNT in the diet for 14 days (McGown et al. 1983). In male rats receiving 2,4-DNT by gavage for 14 days, red blood cell count was significantly decreased at 142 mg/kg/day, while extramedullary hematopoiesis and lymphoid hyperplasia of spleen were seen at doses ≥71 mg/kg/day (Lent et al. 2012a; USAPHC 2011b). Hematocrit, percent reticulocytes, and relative spleen weight were not different from controls in C57Bl/6N mice exposed to 134 mg/kg/day 2,4-DNT via gavage for 14 days (Wilbanks et al. 2014). Kozuka et al. (1979) found methemoglobin concentrations increased to 7 times those of controls in the blood of rats fed a time-

weighted average (TWA) dose of 371 mg/kg/day 2,4-DNT in the diet for 6 months. Anemia was observed in a 13-week feeding study in which male and female CD rats were fed 266 and 145 mg/kg/day, respectively, in the diet; milder effects, such as reticulocytosis and hemosiderosis or abnormal pigment in the spleen, were found at 93 and 108 mg/kg/day in males and females, respectively (Lee et al. 1985; U.S. Army 1978b). No hematological effects were observed in males and females administered 34 and 38 mg/kg/day, respectively. Mild anemia (as indicated by decreases in erythrocyte count, hematocrit, or hemoglobin concentration) and concurrent reticulocytosis were also observed in male and female CD-1 mice administered 413 and 468 mg/kg/day 2,4-DNT, respectively, in the diet for 13 weeks (Hong et al. 1985; U.S. Army 1978b). Anemia, accompanied by the presence of Heinz bodies, was observed in beagle dogs given 25 mg/kg/day 2,4-DNT in capsules (Ellis et al. 1985; U.S. Army 1978b).

As part of a 2-year study in Beagle dogs (6/sex/group) exposed to 2,4-DNT at doses of 0.2, 1.5, or 10 mg/kg/day, effects on hematological parameters were evaluated after 3, 6, and 9 months of treatment (U.S. Army 1979). At these intermediate-duration timepoints, hematological effects consistent with development of methemoglobinemia, anemia, and compensatory hematopoiesis (including decreased hemoglobin, hematocrit, and erythrocyte counts, and increased serum methemoglobin and reticulocyte counts) were observed in beagle dogs administered oral 2,4-DNT at 1.5 or 10 mg/kg/day. In female dogs administered 10 mg/kg/day, statistically significant decreases in erythrocyte count, hematocrit, and hemoglobin, a statistically significant increase in reticulocyte count, and the presence of Heinz bodies in serum were observed. Similar hematological effects were observed in female dogs administered 0.2 and 1.5 mg/kg/day, although effects did not reach statistical significance, most likely because the power of the study to detect statistically significant changes was compromised by the small number of dogs per treatment group. However, a clinically significant increase in methemoglobin levels of 225% was observed in female dogs administered 1.5 mg/kg/day; no significant hematological effects were observed at 0.2 mg/kg/day. Although effects at all time points were qualitatively similar, hematological changes observed after 9 months of exposure were more consistent and pronounced than those observed at the 3- and 6-month time periods.

Chronic studies of animals administered 2,4-DNT provide data that strengthen the weight-of-evidence supporting hematological effects. In a 24-month study, hematological effects consistent with development of methemoglobinemia, anemia, and compensatory hematopoiesis (including decreased hemoglobin, hematocrit, and erythrocyte counts, and increased serum methemoglobin and reticulocyte counts) were observed in beagle dogs (6/sex/group) administered oral 2,4-DNT at 1.5 or 10 mg/kg/day for 12 months of continuous dosing (U.S. Army 1979). No significant hematological effects were observed

in dogs administered 2,4-DNT at 0.2 mg/kg/day. After treatment for 18 or 24 months, only slight or no anemia, near normal reticulocyte levels, no Heinz bodies, and minimal amounts of methemoglobin were detected, likely reflective of an adaptive response (Ellis et al. 1985; U.S. Army 1978b).

In a 2-year study (with a 1-year interim sacrifice) in which CD rats were fed 0.6, 3.9, or 34.5 mg/kg/day (males) or 0.7, 5.1, or 45.3 mg/kg/day (females) 2,4-DNT, significant decreases in red blood cell count were found in mid-dose males compared to controls, and anemia (as indicated by further reductions in red blood cell count, decreased hematocrit, decreased hemoglobin, and a compensatory increase in reticulocytes) was found in high-dose animals after 1 year (Lee et al. 1985; U.S. Army 1978b, 1979). No changes in methemoglobin or Heinz bodies were found. CD-1 mice that were administered 14, 95, or 898 mg/kg/day 2,4-DNT in the diet for 24 months were found to be anemic (as shown by significant reductions in erythrocytes and hemoglobin) at the high concentration, with compensatory increases in reticulocytes (Hong et al. 1985; U.S. Army 1979).

Dark spleen and mild-to-moderate extramedullary hematopoiesis were observed in male Sprague-Dawley rats treated for 14 days with gavage doses ≥39 mg/kg/day 2,5-DNT (Lent et al. 2012a; USAPHC 2011c). Dose-related hematology changes were noted, including decreased red blood cells at ≥77 mg/kg/day; increased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) at ≥154 mg/kg/day; and increased red blood cell distribution width (at 77 and 154 mg/kg/day, but not at 308 mg/kg/day). Total white blood cell count was increased at 308 mg/kg/day, resulting from a nonsignificant increase in lymphocytes; in addition, the percent of eosinophils was decreased at this dose (Lent et al. 2012a; USAPHC 2011c).

Female Sprague-Dawley rats (5/group) administered 199 mg/kg 2,6-DNT as a single dose via gavage (in 5% v/v DMSO in corn oil) showed evidence of erythrocytosis, as indicated by statistically significant increases in serum hemoglobin (45%), hematocrit (41%), and erythrocyte (61%) and granulocyte (11-fold) counts 48 hours after dosing (Deng et al. 2011). Increased numbers of reticulocytes (44% higher than controls), which were associated with mature erythrocytes containing Heinz bodies, were also observed in rats administered 199 mg/kg and evaluated at 24 hours. No significant changes in hematological end points were observed in rats administered 2,6-DNT at 5–99 mg/kg and evaluated 24 or 48 hours after dosing. Development of erythrocytosis 48 hours following a single exposure to 2,6-DNT may be a secondary effect of dehydration, rather than a direct effect on the hematological system; however, no information on drinking water consumption was reported in this study. Lent et al. (2012a; USAPHC 2011d) reported hematology changes consisting of significantly reduced hemoglobin and

hematocrit, as well as increased neutrophils, increased monocytes, and increased percentages of neutrophils and lymphocytes, in male rats exposed by gavage to 134 mg/kg/day of 2,6-DNT. Nonsignificant decreases in hemoglobin and hematocrit were seen at all doses in this study (4–134 mg/kg/day). As part of a 13-week study in Beagle dogs (4/sex/group) exposed to 2,6-DNT at doses of 4, 20, or 100 mg/kg/day, effects on hematological parameters were evaluated after 2 weeks of treatment (U.S. Army 1976). Dogs treated at 20 mg/kg/day showed a statistically significant decrease in erythrocyte count (16%) and a significant increase in mean cell hemoglobin (5%) after dosing for 2 weeks. At 100 mg/kg/day, more pronounced changes consistent with development of methemoglobinemia, anemia, and compensatory hematopoiesis were observed; dogs showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and an increase in reticulocyte count after 2 weeks of continuous dosing (U.S. Army 1976).

Subchronic administration (13 weeks) of 2,6-DNT in dogs and rats provide data that strengthen the weight-of-evidence supporting hematological effects. Significant hematological effects were observed in beagle dogs (4/sex/group) after administration of 2,6-DNT at 20 and 100 mg/kg/day, but not 4 mg/kg/day (U.S. Army 1976). Dogs treated at 20 mg/kg/day showed a statistically significant decrease in erythrocyte count (12%) after dosing for 4 weeks. At 100 mg/kg/day, more pronounced changes consistent with development of methemoglobinemia, anemia, and compensatory hematopoiesis were observed; dogs showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and an increase in reticulocyte count after 4 weeks of continuous dosing. Histopathological evaluation of the spleen showed an increased incidence of extramedullary erythropoiesis in dogs treated with 2,6-DNT at 4, 20, or 100 mg/kg/day for 4 or 13 weeks; this effect is an adaptive response to 2,6-DNT-induced methemoglobinemia and anemia.

Subchronic (13-week) administration of 2,6-DNT in CD rats induced changes in hematological parameters (measured at 4, 8, and 13 weeks) indicative of anemia and compensatory hematopoiesis (including significant decreases in erythrocytes, hematocrit, and hemoglobin and increased reticulocytes) at the highest tested dose (145 and 155 mg/kg/day for male and female rats, respectively) only; these effects were most pronounced after treatment for 4 weeks (U.S. Army 1976). No significant hematological changes were observed at 7 and 35 mg/kg/day (males) or 7 and 37 mg/kg/day (females). However, histopathological effects (extramedullary hematopoiesis and/or splenic hemosiderosis), indicative of an adaptive response to anemia and compensatory erythropoiesis, were observed in male and female rats administered 2,6-DNT at doses ≥7 mg/kg/day. Although histopathological effects (extramedullary hematopoiesis) were observed in CD-1 mice administered 2,6-DNT at ≥51 mg/kg/day

(but not 11 mg/kg/day) for 4 or 13 weeks, no statistically significant changes in hematological parameters were seen at levels up to 289 mg/kg/day (males) or 299 mg/kg/day (females). The study authors indicated that some blood samples clotted, making hematological analyses impossible to perform. The small number of animals evaluated likely contributed to the identification of histopathological findings of the spleen in the apparent absence of 2,6-DNT-induced hematological effects. The 2,6-DNT isomer was not tested for hematological end points in studies of chronic duration.

Fourteen-day gavage exposure of male Sprague-Dawley rats to 3,4-DNT resulted in reduced hemoglobin and hematocrit at all doses, with statistically significant reductions at doses of 14, 28, and 113 mg/kg/day; however, the authors indicated that both parameters remained within reference ranges at all doses (Lent et al. 2012a; USAPHC 2011e). Splenic lesions, of trace-to-mild severity, were observed at doses ≥57 mg/kg/day, including extramedullary hematopoiesis and lymphoid hyperplasia.

In the corresponding 14-day rat study of 3,5-DNT, Lent et al. (2012a; USAPHC 2011f) observed no changes in hematology parameters and no splenic lesions at doses up to 39 mg/kg/day.

Hematological changes consistent with those observed in anemia were found in pregnant F344 rats administered 100 mg/kg Tg-DNT by gavage during gestation days 7–20 (Jones-Price et al. 1982). Administration of Tg-DNT to rats in the diet for 4 weeks (Hazleton Laboratories 1977) or 26 weeks (Hazleton Laboratories 1982) resulted in dose- and duration-related adverse effects on hematological parameters. In the 4-week study at 37.5 mg/kg/day, significant increases in reticulocytes and percentage of Heinz bodies were noted in both sexes and significant increases in methemoglobin levels were found in females; anemia was observed at 100 mg/kg/day in both sexes (Hazleton Laboratories 1977). Spleens of rats fed 150 mg/kg Tg-DNT for 30 days in the diet were altered in appearance; these alterations included discoloration, enlargement, and surface irregularity (Hazleton Laboratories 1977). An increased incidence of extramedullary hematopoiesis was noted in the splenic red pulp of male, but not female, rats fed 35 mg/kg/day Tg-DNT in the diet for 52 weeks (Hazleton Laboratories 1982). In rats sacrificed after 26 weeks in a 24-month study, no effects on hematological parameters were observed at 14 mg/kg/day Tg-DNT. However, at 35 mg/kg/day, there were increases in reticulocytes and methemoglobin and decreases in red blood cells along with hemosiderosis and extramedullary hematopoiesis in males, and increases in MCV in females (Hazleton Laboratories 1982). After 1 year, slight-to-moderate myeloid and erythroid hyperplasia was noted in the bone marrow of most male rats treated with 35 mg/kg/day Tg-DNT (Hazleton Laboratories 1982). In a 24-month study in which Tg-DNT was administered to rats in the

diet, anemia was observed at 14 mg/kg/day in males but not in females; the NOAEL for this effect in males was 3.5 mg/kg/day Tg-DNT (Hazleton Laboratories 1982).

The consistent observation of adverse hematological effects following exposure of laboratory animals to DNTs indicates that the blood is a primary target of DNT toxicity. As noted previously and shown in Table 3-7, the various DNT isomers elicited isomer-specific differences in selected hematological parameters following 14 days of gavage treatment of male Sprague-Dawley rats (Lent et al. 2012a; USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f). The 2,4- and 2,5-DNT isomers were the only ones causing decreases in red blood cell counts, with 2,5-DNT being more potent than 2,4-DNT. Increased spleen weight was observed at similar concentrations of 2,4-, 2,5-, and 2,6-DNT. Splenic lesions were observed after acute exposure to all DNT isomers except 3,5-DNT; the available data suggest that the 2,5-DNT isomer is more potent than the other isomers.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, 3,5-, or Tg-DNT or in animals after oral exposure to 2,3-, 2,5-, 2,6-, 3,4-, 3,5-, or Tg-DNT. Swim-to-exhaustion time was decreased by 82% (relative to controls) in C57Bl/6N mice exposed to 134 mg/kg/day 2,4-DNT via gavage for 14 days (Wilbanks et al. 2014).

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

The hepatotoxic effects of DNTs have been consistently observed in animals. The liver appears to be a target organ of DNT toxicity, particularly when administered to rats, but hepatotoxic effects have also been observed in mice and dogs. Hepatic effects of DNTs include liver discoloration and inflammation, alteration of hepatocytes, proliferation of bile duct epithelium, and hyperplastic foci. However, as discussed in Section 3.2.2.7 (Cancer), 2,4-, 2,6-, and Tg-DNT have been shown to induce hepatocellular carcinoma following chronic-duration oral exposure. Thus, hepatic effects observed at less-than-chronic exposure durations or at lower doses may represent early stages of progressive development to hepatic cancer.

In male Sprague-Dawley rats receiving gavage doses of 2,3-DNT for 14 days, mean relative (but not absolute) liver weight was increased at 275 mg/kg/day, but this increase was likely attributable to lower body weight at this dose (Lent et al. 2012a; USAPHC 2011a). Dose-related clinical chemistry changes were not observed, and no histopathology lesions were seen in the liver.

Table 3-7. NOAELs and LOAELs for Hematological Effects Following 14-Day Gavage Dosing of Male Sprague-Dawley Rats with Individual DNT Isomers

Effect	Decreased red blood cell count		Increased spleen weight		Increased incidence splenic lesions	
Effect level (mg/kg/day)	NOAEL	LOAEL	NOAEL	LOAEL	NOAEL	LOAEL
Isomer						
2,3-DNT	275	–	275	–	138	275
2,4-DNT	71	142	36	71	36	71
2,5-DNT	39	77	39	77	19	39
2,6-DNT	134	–	35	68	35	68
3,4-DNT	227	–	227	–	28	57
3,5-DNT	39	–	39	–	39	–

DNT = dinitrotoluene; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

Sources: Lent et al. 2012a; USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f

In female Sprague-Dawley rats (5/group) administered 2,4-DNT via gavage (in 5% v/v DMSO in corn oil) and observed for 48 hours, significantly decreased levels of serum albumin (13–51% lower than controls) were observed at doses ≥99 mg/kg (Deng et al. 2011). Although relative liver weight was also significantly increased at 99 mg/kg, this effect was not observed at 198 or 398 mg/kg. Hepatic sinusoid congestion was observed in rats administered 398 mg/kg 2,4-DNT in the absence of other histopathological effects. Significantly increased (14% higher than controls) relative liver weight was observed in wild type C57Bl/6N mice exposed to 134 mg/kg/day 2,4-DNT via gavage for 14 days; serum triglycerides and glucose levels were not affected (Wilbanks et al. 2014). Increased blood cholesterol was found in male and female Sprague-Dawley rats fed 78 or 82 mg/kg/day 2,4-DNT, respectively, in the diet for 14 days, and increased alanine aminotransferase levels were found in males (McGown et al. 1983). Blood glucose levels trended upward in all male and female groups in this study, but were increased significantly only in females fed 273 mg/kg/day. Trace-to-mild single cell necrosis occurred in the livers of male Sprague-Dawley rats receiving gavage doses of 18, 36, 71, and 142 mg/kg/day 2,4-DNT; vehicle controls and rats exposed to 9 mg/kg/day did not exhibit this effect (Lent et al. 2012a; USAPHC 2011b). In addition, the authors observed trace apoptosis in the livers of 1/6 rats in each of the 36 and 71 mg/kg/day groups, and increases in glycogen deposition at ≥36 mg/kg/day. No clinical chemistry changes were seen at any dose.

Oral administration of 2,4-DNT for 13 weeks to rats (266 or 145 mg/kg/day in males and females, respectively) and dogs (25 mg/kg/day) did not result in liver toxicity (Ellis et al. 1985; U.S. Army 1978b). After 26 weeks of treatment, rats fed 27 mg/kg/day in the diet had significant increases in epoxide hydrolase (EH) activity, which is sometimes considered to be a phenotypic marker of neoplastic nodules; however, hepatocellular lesions did not develop in these animals when treatment was carried through 52 weeks (Leonard et al. 1987). Mild hepatocellular dysplasia was observed in mice fed 137 mg/kg/day (males) or 468 mg/kg/day (females) of 2,4-DNT for 13 weeks (Hong et al. 1985; U.S. Army 1978b).

Hepatic effects have also been observed in laboratory animals following chronic-duration oral exposure to DNTs. However, these effects are often observed in conjunction with the development of hepatocellular carcinoma and may represent precancerous changes. For example, dietary exposure of male rats to 0.6 mg/kg/day 2,4-DNT induced “hepatocellular” alterations; however, neoplastic nodules were noted at this dose level and higher as well (Lee et al. 1985). Hepatocellular degeneration and vacuolation accompanied by acidophilic foci and occasional basophilic foci of cellular alteration were found in F344 rats fed 27 mg/kg/day 2,4-DNT for 52 weeks (Leonard et al. 1987). The incidences of focal areas of

alteration were less in the 2,4-DNT-treated rats than they were in rats similarly treated with 2,6-DNT or Tg-DNT. Wistar rats fed a TWA dose of 371 mg/kg/day 2,4-DNT in the diet for 6 months had increased relative liver weights, formation of puruloid matter, and increased levels of serum glutamic-oxaloacetic transaminase (SGOT), lactate dehydrogenase (LDH), alkaline and acid phosphatase, triglycerides, and blood glucose levels compared to controls (Kozuka et al. 1979). In this study, the levels of serum albumin and the albumin/globulin ratios were decreased. Hepatocellular dysplasia was found in male and female CD-1 mice fed 14 or 898 mg/kg/day 2,4-DNT, respectively, for 24 months (Hong et al. 1985; U.S. Army 1979). Administration of 10 mg/kg/day 2,4-DNT for 24 months resulted in biliary hyperplasia in dogs; this effect was not seen in dogs administered 1.5 mg/kg/day (Ellis et al. 1985; U.S. Army 1979).

When 2,5-DNT was administered to male Sprague-Dawley rats via gavage for 2 weeks at doses up to 308 mg/kg/day, there were no treatment-related adverse effects on serum liver enzyme activity, liver weight, or liver histology (Lent et al. 2012a; USAPHC 2011c).

Increased ALT activity and histopathological changes to the liver were observed in individual female Sprague-Dawley rats administered 50 and 99 mg/kg 2,6-DNT (in 5% DMSO in corn oil) by gavage and observed for 48 hours (Deng et al. 2011). Serum ALT activity was significantly increased 7-fold compared to controls in rats administered 50 and 99 mg/kg 2,6-DNT, but no increase in serum ALT level was observed at lower dose levels (5–25 mg/kg). Histopathological changes to the liver observed in rats administered 2,6-DNT at 50 or 99 mg/kg included congested sinusoids with sloughed hepatocytes and segmented neutrophils, disorganized midzonal regions characterized by infiltration of erythrocytes and hepatocytes with pyknotic nuclei and microvesiculated cytoplasm, and apoptotic hepatocytes (severity of effects not specified). ALT levels were similar to controls and hepatocytes were mostly undamaged in rats administered 2,6-DNT at 199 mg/kg, although evidence of sinusoid congestion, occasional erythrocyte infiltration (with no signs of necrosis), and enlarged nuclei in several hepatocytes were noted. Three consecutive daily oral doses of 100 mg/kg/day 2,6-DNT resulted in mild diffuse hepatocellular hypertrophy in male Sprague-Dawley rats (Rothfuss et al. 2010). After 2 weeks of gavage doses of 68 or 134 mg/kg/day 2,6-DNT, male rats exhibited significant increases in serum ALT and aspartate aminotransferase (AST), as well as a nonsignificant but dose-related increase in alkaline phosphatase (ALP) (Lent et al. 2012a; USAPHC 2011d). Liver lesions consisting of hepatocellular and oval cell hyperplasia and hepatocellular hypertrophy occurred at doses ≥35 mg/kg/day. Additional lesions (increases in mitotic activity, single cell necrosis, and karyocytomegaly) occurred at higher doses of 68 and 134 mg/kg/day (Lent et al. 2012a; USAPHC 2011d).

Intermediate duration exposure to 2,6-DNT also results in liver toxicity. After oral exposure to 33 mg/kg/day 2,6-DNT for 29 consecutive days, male rat livers exhibited hepatocellular hypertrophy, single cell necrosis, bile duct hyperplasia, and focal hepatocellular vacuolation, in the absence of changes in serum liver enzyme levels (Rothfuss et al. 2010). Six weeks of dietary consumption of 7 mg/kg/day 2,6-DNT caused a 380% increase in EH levels in rats but did not increase the level of DT-diaphorase (DTD) (Leonard et al. 1987). In the same study, both of these enzymes were elevated after 6 weeks of treatment with 14 mg/kg/day of 2,6-DNT. Dosing of rats, mice, and dogs with 2,6-DNT for 13 weeks resulted in liver toxicity (U.S. Army 1976). Bile duct hyperplasia was observed in rats fed 35 mg/kg/day and mice fed 51 mg/kg/day 2,6-DNT for 13 weeks (U.S. Army 1976). Liver degeneration and bile duct hyperplasia were observed in dogs dosed with 20 mg/kg/day 2,6-DNT but were not seen in dogs dosed with 4 mg/kg/day (U.S. Army 1976). After 52 weeks of treatment with 7 mg/kg/day 2,6-DNT, hepatocellular degeneration and vacuolation accompanied by acidophilic and basophilic foci of cellular alteration were found in F344 rats (Leonard et al. 1987).

Gavage doses up to 227 mg/kg/day 3,4-DNT did not alter clinical chemistry or liver histology in male Sprague-Dawley rats (Lent et al. 2012a; USAPHC 2011e). Significant increases in relative, but not absolute liver weight were noted at 113 and 227 mg/kg/day, but were likely attributable to reduced body weights at these doses.

In the 14-day gavage study of rats by Lent et al. (2012a; USAPHC 2011f), no exposure-related changes in serum liver enzymes, liver weight, or histopathology were seen at doses up to 39 mg/kg/day of 3,5-DNT.

Irregular liver surfaces were found in male F344 rats fed 37.5 mg/kg/day Tg-DNT for 30 days (Hazleton Laboratories 1977). Hepatocytic necrosis, nonsupportive pericholangitis, and periportal megalocytosis were found in CDF rats fed 14 mg/kg/day for 26 weeks, and when treatment of these animals was extended to 2 years, slight-to-severe biliary cirrhosis was found in males (Hazleton Laboratories 1982). It has been suggested that this latter lesion may be a precursor to cholangiocarcinoma (Hazleton Laboratories 1982). Hepatocytic degeneration and acidophilic and basophilic foci of cellular alteration were observed in F344 rats fed 35 mg/kg/day Tg-DNT in the diet for 52 weeks (Leonard et al. 1987). When administration of Tg-DNT was continued for 24 months, liver discoloration resulted at 3.5 mg/kg/day and liver nodules and malignancies at 14 mg/kg/day (Hazleton Laboratories 1982).

As shown in Table 3-8, 2,4- and 2,6-DNT were the only isomers that elicited hepatic effects in Sprague-Dawley rats following 14 days of gavage treatment (Lent et al. 2012a; USAPHC 2011a, 2011b, 2011c,

Table 3-8. NOAELs and LOAELs for Hepatic Effects Following 14-Day Gavage Dosing of Male Sprague-Dawley Rats with Individual DNT Isomers

Effect	Increased liver weight		Increased serum liver enzyme activity		Increased incidence hepatic lesions	
Effect level (mg/kg/day)	NOAEL	LOAEL	NOAEL	LOAEL	NOAEL	LOAEL
Isomer						
2,3-DNT	275	–	275	–	275	–
2,4-DNT	71	142	142	–	18	36
2,5-DNT	308	–	308	–	308	–
2,6-DNT	134	–	35	68	14	35
3,4-DNT	227	–	227	–	227	–
3,5-DNT	39	–	39	–	39	–

DNT = dinitrotoluene; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

Sources: Lent et al. 2012a; USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f

2011d, 2011e, 2011f). The 2,4-DNT isomer caused increased liver weight (LOAEL=142 mg/kg/day) and increased incidences of single cell necrosis and glycogen deposition (LOAEL=36 mg/kg/day). The 2,6-DNT isomer caused increased serum liver enzyme activity (LOAEL=68 mg/kg/day) and increased incidences of hepatocellular hypertrophy and hyperplasia and oval cell hyperplasia (LOAEL=35 mg/kg/day).

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Exposure to 2,3-DNT by daily gavage for 14 days did not result in serum chemistry changes indicative of renal toxicity, or changes in kidney weight in male rats; however, increased incidences of renal tubular dilatation (2/6 rats) and renal lymphocytic infiltration (4/6) were observed (Lent et al. 2012a; USAPHC 2011a).

Sprague-Dawley rats (5/group) administered 2,4-DNT (in 5% DMSO in corn oil) via gavage at doses up to 398 mg/kg and evaluated at 24 or 48 hours showed no effects on levels of serum creatinine or urea (Deng et al. 2011). Hyaline droplet accumulation in the epithelium of the proximal convoluted tubule was found in both sexes of Sprague-Dawley rats after they were administered 78, 104, 165, or 261 mg/kg/day 2,4-DNT (males) or 82, 109, 173, or 273 mg/kg/day 2,4-DNT (females) in the diet (McGown et al. 1983). Although this effect was observed at all concentrations, there was no dose response evident. The kidney was not a target organ of 2,4-DNT in male rats exposed via gavage for 14 days at doses up to 142 mg/kg/day; serum chemistry and kidney weight were not affected by exposure, nor were there histopathology findings in the kidney (Lent et al. 2012a; USAPHC 2011b). Oral administration of 2,4-DNT to mice (413 mg/kg/day), rats (145 mg/kg/day), and dogs (25 mg/kg/day) for 13 weeks did not result in significant adverse effects in the kidney (Hong et al. 1985; U.S. Army 1978b). Treatment of the same species for 24 months resulted in renal dysplasia in male mice at a dose of 14 mg/kg/day of 2,4-DNT, but no renal effects were observed in rats or dogs dosed with 34.5 mg/kg/day or 10 mg/kg/day, respectively (U.S. Army 1979). Adverse effects in the kidneys of mice included cystic dysplasia in the tubular epithelium, atypical epithelium lining the cysts, and a variety of tumors (Hong et al. 1985). These effects were more pronounced in male mice than in female mice.

Exposure to 2,5-DNT for 14 days at gavage doses up to 308 mg/kg/day did not result in kidney effects in male rats, as assessed by serum chemistry, kidney weight, and histopathology (Lent et al. 2012a; USAPHC 2011c).

Renal tubular degeneration and proximal tubule degeneration were observed in male rats exposed by gavage to 2,6-DNT at 134 mg/kg/day (the highest dose tested) for 14 days (Lent et al. 2012a; USAPHC 2011d).

Female Sprague-Dawley rats (5/group) administered 2,6-DNT (in 5% DMSO in corn oil) via gavage at doses up to 199 mg/kg showed no effects on levels of serum creatinine or urea at 24 or 48 hours (Deng et al. 2011). Dosing of dogs with 20 mg/kg/day 2,6-DNT for 13 weeks resulted in dilated tubules, foci of inflammation, and degeneration of the kidney (U.S. Army 1976). No treatment-related effects on the kidney were found when rats were fed 2,6-DNT for 13 weeks (U.S. Army 1976). The severe renal effects observed after 2,4-DNT administration in mice were not observed when mice were fed 289 mg/kg/day 2,6-DNT for 13 weeks (U.S. Army 1976). However, the renal toxicity of 2,4-DNT in mice was observed only after chronic administration. Chronic studies of 2,6-DNT have not been performed in mice.

After 26 or 52 weeks of dietary consumption of 35 mg/kg/day Tg-DNT, BUN levels were significantly increased in CDF rats (Hazleton Laboratories 1982). Exacerbation of chronic interstitial nephritis that was also observed in controls was observed at 14 mg/kg/day Tg-DNT in a chronic study in rats (Hazleton Laboratories 1982).

Renal effects were observed in male rats exposed to 3,4-DNT by gavage for 14 consecutive days (Lent et al. 2012a; USAPHC 2011e). These effects consisted of proximal tubule degeneration, renal tubule basophilia, and lymphocytic infiltration at the highest dose tested (227 mg/kg/day); however, renal histopathology was not examined at lower doses.

In the corresponding study of 3,5-DNT (Lent et al. 2012a; USAPHC 2011f), no effects on kidney weight, kidney histopathology, or serum parameters indicative of renal toxicity were seen in male rats exposed by gavage to doses up to 39 mg/kg/day for 14 days.

The kidney does not appear to be a sensitive target of DNT toxicity for all species tested. Severe renal effects were observed only in CD-1 mice fed 14 mg/kg/day 2,4-DNT for 24 months, and less severe renal effects were observed in dogs administered 20 mg/kg/day 2,6-DNT for 13 weeks.

In the 14-day gavage studies that compared the effects of the various DNT isomers on the kidneys of male Sprague-Dawley rats (Lent et al. 2012a; USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f), only the

2,3-, 2,6-, and 3,4-DNT elicited adverse effects. Increased kidney weight accompanied by increased incidences of histopathologic lesions (trace tubular dilatation and lymphocytic infiltration) were observed for 2,3-DNT (LOAEL=275 mg/kg/day). Increased kidney weight (LOAEL=68 mg/kg/day) and increased incidences of proximal tubule degeneration and renal tubular basophilia were observed for 2,6-DNT (LOAEL=134 mg/kg/day). Increased incidences of proximal tubule degeneration, renal tubule basophilia, and lymphocytic infiltration were noted for 3,4-DNT (LOAEL=227 mg/kg/day).

Endocrine Effects. No studies were located regarding endocrine effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Administration of 2,4-DNT in the diet for 14 days, at 78 mg/kg/day for males or 82 mg/kg/day for females did not cause any histopathological changes in adrenal, pituitary, or thyroid glands of Sprague-Dawley rats (McGown et al. 1983).

No histopathological effects on adrenal, pituitary, or thyroid glands were found in rats treated with 14 mg/kg/day Tg-DNT for up to 2 years or 35 mg/kg/day for 1 year (Hazleton Laboratories 1982). Increases in the incidence and severity of parathyroid hyperplasia (males) and increases in the incidence and severity of fatty metamorphosis and vascular ectasia (males and females) were found in rats fed 14 mg/kg/day Tg-DNT in the diet in a chronic study (Hazleton Laboratories 1982).

No studies were located regarding endocrine effects in animals after oral exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Dermal Effects. No studies were located regarding dermal effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Concentrations of up to 261 mg/kg/day 2,4-DNT for males or 273 mg/kg/day 2,4-DNT for females administered in the diet for 14 days to Sprague-Dawley rats caused no histopathological changes in their skin (McGown et al. 1983).

No effects were found on the skin of rats treated for up to 2 years with 14 mg/kg/day Tg-DNT or up to 1 year with 35 mg/kg/day Tg-DNT (Hazleton Laboratories 1982).

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

Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

The eyes of male and female Sprague-Dawley rats administered up to 261 or 273 mg/kg/day 2,4-DNT, respectively, in the diet for 14 days did not exhibit any alterations upon histopathological examination (McGown et al. 1983).

No effects were found on the eyes of rats treated for up to 2 years with 14 mg/kg/day Tg-DNT in feed or up to 1 year with 35 mg/kg/day Tg-DNT in feed (Hazleton Laboratories 1982).

No studies were located regarding ocular effects in animals after oral exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Body Weight Effects. No studies were located regarding body weight effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Adverse effects on body weight and body weight gain in rats, mice, and dogs were observed after oral administration of 2,4-DNT, 2,6-DNT, and Tg-DNT, and in rats exposed to 3,4-DNT. In most of these studies, a concurrent decrease in food consumption was also observed. Because exposure resulted from intake of the test article in feed in most of these studies, it is possible that some of the body weight changes resulted from unpalatability.

Body weight was not altered in male rats receiving doses up to 275 mg/kg/day 2,3-DNT by gavage for 14 days (Lent et al. 2012a; USAPHC 2011a).

Adverse effects on body weight, including body weight loss, have been reported after almost all acute-, intermediate-, and chronic-duration oral administration of 2,4-DNT (Bloch et al. 1988; Deng et al. 2011; Ellis et al. 1985; Hazleton Laboratories 1982; Hong et al. 1985; Kozuka et al. 1979; Lane et al. 1985; Lee et al. 1985; Lent et al. 2012a; USAPHC 2011b; Leonard et al. 1987; McGown et al. 1983; NCI 1978; U.S. Army 1978b, 1979). Female Sprague-Dawley rats (5/group) administered 2,4-DNT (in 5% DMSO in corn oil) via gavage at 398 mg/kg showed decreased body weight gain (1–4 g compared to 17 g for

controls) 48 hours after dosing (Deng et al. 2011). In another acute study, rats dosed by gavage with 240 mg/kg 2,4-DNT for 5 days lost weight (Lane et al. 1985). Lent et al. (2012a; USAPHC 2011b) reported a 12% decrease in body weight (relative to controls) in male rats given 2,4-DNT by gavage for 14 days at a dose of 142 mg/kg/day. Wilbanks et al. (2014) observed greater body weight loss (0.96 versus 0.35 g), compared with untreated controls, in wild type C57Bl/6N mice exposed to 134 mg/kg/day 2,4-DNT via gavage for 14 days. In general, there were losses of 10–40% in body weight in acute-, intermediate-, and chronic-duration studies in rats. After 6 months, decreases in body weight gain were noted in rats fed 27 mg/kg/day 2,4-DNT (Leonard et al. 1987), and a 25% decrease in body weight was seen in rats fed 34.5 mg/kg/day (Lee et al. 1985; U.S. Army 1978b, 1979). This reduction in body weight gain tended to become more pronounced when 2,4-DNT was continued for periods of 1–2 years (Leonard et al. 1987). Body weight was decreased 25% in rats that received 20 mg/kg/day 2,4-DNT in the diet for 78 weeks; the NOAEL in this study was 8 mg/kg/day (NCI 1978). Mice showed similar decreases in body weight after intermediate- (13 weeks) and chronic-duration (78–104 weeks) exposure, but the doses of the test article needed to evoke this effect were considerably higher than in rats (Hong et al. 1985; NCI 1978; U.S. Army 1978b). An 18–24% decrease in body weight was seen in rats receiving 72–76 mg/kg/day 2,4-DNT in the diet for 78 weeks (NCI 1978).

In a 14-day study of 2,5-DNT, body weight was not altered in male rats receiving doses up to 308 mg/kg/day (Lent et al. 2012a; USAPHC 2011c).

Female Sprague-Dawley rats (5/group) administered 2,6-DNT (in 5% DMSO in corn oil) via gavage at 50 or 99 mg/kg gained less weight than control animals over the 48-hour observation period (1–4 g compared to 17 g for controls); rats from the same study administered 2,6-DNT at 199 mg/kg lost weight over the course of 48 hours (Deng et al. 2011). Body weight reductions of at least 10% were observed in male rats receiving gavage doses ≥35 mg/kg/day for 14 days, although the reductions were statistically significant only at the highest dose of 134 mg/kg/day (Lent et al. 2012a; USAPHC 2011d). Reduced body weight was reported in male Sprague-Dawley rats exposed to 29 consecutive daily oral doses of 33 mg/kg/day 2,6-DNT (Rothfuss et al. 2010). Administration of 2,6-DNT also caused decreased body weight gain or body weight loss in rats, mice, and dogs at doses ranging from 14 to 145 mg/kg/day in intermediate-duration studies (U.S. Army 1976). Treatment with 7 mg/kg/day 2,6-DNT for 52 weeks decreased body weight in rats by 18% (Leonard et al. 1987).

A 10% reduction in terminal body weight was seen in male rats exposed to 227 mg/kg/day 3,4-DNT by gavage for 14 days; however, the difference from control weight was not statistically significant (Lent et al. 2012a; USAPHC 2011e).

Body weight was not altered in male rats receiving gavage doses up to 39 mg/kg/day 3,5-DNT for 14 days (Lent et al. 2012a; USAPHC 2011f).

A 29% decrease in absolute maternal weight gain was observed in dams fed 14 mg/kg/day Tg-DNT for 14 days during gestation (Jones-Price et al. 1982). Decreased body weight or decreased body weight gain was reported in rats at levels as low as 14 mg/kg/day Tg-DNT in intermediate- or chronic-duration studies (Hazleton Laboratories 1982). Other intermediate- and chronic-duration studies also confirmed these body weight effects (Hazleton Laboratories 1977; Leonard et al. 1987; NCI 1978).

Metabolic Effects. No studies were located regarding metabolic effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Female Sprague-Dawley rats (5/group) administered 2,4-DNT (at 198 or 398 mg/kg) or 2,6-DNT (at 199 mg/kg) via gavage (in 5% DMSO in corn oil) showed small but statistically significant reductions in sodium levels in the serum (4–11% lower than controls) 24 and/or 48 hours after dosing (Deng et al. 2011). Rats treated with 2,4-DNT at 398 mg/kg also had elevated levels of blood glucose (increased 2.6-fold) 48 hours after dosing.

No studies were located regarding metabolic effects in animals after oral exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNT.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Testing for immunological effects of DNTs is limited. Acute (14-day) exposure of male rats to 2,3-DNT resulted in lymphoid hyperplasia of the spleen and lymphoid depletion at gavage doses of 275 mg/kg/day (Lent et al. 2012a; USAPHC 2011a). In the corresponding study of 2,4-DNT, lymphoid hyperplasia was seen at doses ≥35 mg/kg/day, and lymphoid depletion was seen at 134 mg/kg/day (Lent et al. 2012a;

USAPHC 2011b). No changes in serum concentrations of IgE were observed in rats and dogs administered 2,4-DNT at levels up to 206 and 25 mg/kg/day, respectively, for 13 weeks (Ellis et al. 1985; Lee et al. 1985; U.S. Army 1978b). In these studies, the rats received the test article in feed, while the dogs received it in capsules. No histopathological changes were found in the spleen or thymus of Sprague-Dawley male rats fed 78 mg/kg/day 2,4-DNT or female rats fed 82 mg/kg/day 2,4-DNT in the diet for 14 days (McGown et al. 1983).

Administration of 2,6-DNT to dogs (up to 100 mg/kg) and rats (up to 145 mg/kg/day) for 13 weeks resulted in no observable changes in IgE serum concentrations (U.S. Army 1976). IgE is the antibody associated with allergic or hypersensitive reactions, and so it may be expected that the human sensitizing potential of 2,4- and 2,6-DNT would be low. Involution of the thymus was noted when dogs were administered 100 mg/kg 2,6-DNT, but was not noted when they were administered 20 mg/kg 2,6-DNT by capsule for 13 weeks (U.S. Army 1976).

Exposure to 3,4-DNT by gavage for 14 days induced lymphoid hyperplasia of the spleen in male rats exposed to doses ≥57 mg/kg/day (Lent et al. 2012a; USAPHC 2011e).

All LOAEL values from each reliable study for immunological/lymphoreticular effects in each species and duration category for 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT are recorded in Tables 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively, and plotted in Figures 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively.

No studies were located regarding immunological and lymphoreticular effects in animals after oral exposure to 2,5- or 3,5-DNT, although Lent et al. (2012a; USAPHC 2011c, 2011f) did not observe splenic lymphoid lesions in rats exposed to these two compounds for 14 days.

3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Neurotoxicity appears to be a characteristic syndrome of subchronic and chronic DNT poisoning of animals. Neurotoxic symptoms, of decreased severity compared to dogs, were observed in mice and rats at doses higher than neurotoxic doses in dogs; however, the test article was administered in feed to rodents and in capsules to dogs.

Acute (14-day) oral exposure of male Sprague-Dawley rats to 2,3-DNT at doses up to 275 mg/kg/day or 2,4-DNT at doses up to 142 mg/kg/day did not lead to any clinical signs of neurotoxicity, nor was brain weight affected (Lent et al. 2012a; USAPHC 2011a).

In a subchronic study, beagle dogs (4/sex/group) administered 2,4-DNT at 1 or 5 mg/kg/day showed no signs of behavioral changes or clinical signs of neurotoxicity. Neurotoxicity was observed at 25 mg/kg/day, with signs of neurotoxicity (loss of hind leg control) first observed in a female dog on day 12 of treatment. Three additional male dogs showed similar signs (signs not specified) on day 14 of treatment. All dogs administered 25 mg/kg/day showed signs of neurotoxicity after treatment for 12–22 days. The onset and severity of toxic signs reportedly varied among dogs within the same treatment group; some dogs were moribund at the same time that others began experiencing symptoms. In individual dogs, symptom severity varied over time, with no duration-related pattern of severity. The specific neurotoxic effects in dogs affected within the first 14 days of treatment were not specified. The NOAEL for the neurotoxicity observed after 12 days was 5 mg/kg/day. No histopathological changes were found in the brain or spinal cord of male and female Sprague-Dawley rats fed 2,4-DNT for 14 days in the diet at doses of 78 and 82 mg/kg/day, respectively (McGown et al. 1983).

Neurotoxicity has been reported in laboratory animals after intermediate- or chronic-duration exposure to 2,4-DNT with symptoms ranging from tremors, convulsions, and ataxia to paralysis. These effects were observed in 13-week studies of rats and dogs. Administration of 93 mg/kg/day 2,4-DNT in the diet for 13 weeks caused demyelinization in the cerebellum and brain stem of 1 male rat, while at 266 mg/kg/day, some rats exhibited a widespread or stiff-legged gait that did not progress to the rigid paralysis observed in dogs (Lee et al. 1985; U.S. Army 1978b). After 3 months of being fed 2,4-DNT at 0.5% in the diet (an estimated dose of 350 mg/kg/day based on body weight and feed consumption data provided by the study authors), Wistar rats exhibited humpback and jerky incoordination (Kozuka et al. 1979). Dogs that were administered 25 mg/kg/day 2,4-DNT in capsules for 13 weeks began to show neurotoxic effects within 2 months; these effects included incoordination, abnormal gait, rigid paralysis of the hind legs, eventually progressing to paralysis up to the neck (Ellis et al. 1985; U.S. Army 1978b). No neurological signs were observed in mice fed 413 mg/kg/day in males or 468 mg/kg/day in females 2,4-DNT in the diet for 13 weeks (Hong et al. 1985; U.S. Army 1978b).

An abnormal gait was also observed in chronic studies of laboratory animals fed 2,4-DNT. The characteristic widespread and stiff-legged gait was observed after feeding 34.5 or 45.3 mg/kg/day

2,4-DNT to male and female rats, respectively, for up to 2 years (Lee et al. 1985; U.S. Army 1978b, 1979). This stiff-legged gait and hyperactive behavior were also noted in mice fed 898 mg/kg/day in the diet for 24 months but were not observed in mice at 95 mg/kg/day (Ellis et al. 1985; U.S. Army 1979). In dogs dosed at 1.5 mg/kg/day 2,4-DNT in a 2-year study, one of six dogs showed intermittent loss of hindquarter control (Ellis et al. 1985; U.S. Army 1979). Central nervous system lesions were identified in high-dose (10 mg/kg/day) dogs in this study and included vacuolization, hypertrophy, endothelial mitosis, and focal gliosis in the cerebellum, as well as some perivascular hemorrhage in the cerebellum and brain stem (Ellis et al. 1985; U.S. Army 1979).

Male rats exposed for 14 days by gavage to 2,5-DNT at doses up to 308 mg/kg/day or 2,6-DNT at doses up to 134 mg/kg/day did not display clinical signs of neurotoxicity or alterations in brain weight (Lent et al. 2012a; USAPHC 2011c).

Dogs dosed with 20 or 100 mg/kg/day of 2,6-DNT for 13 weeks exhibited dose-related neurotoxic symptoms that included muscular incoordination, weakness, tremors, and paralysis (U.S. Army 1976). Rats and mice dosed at 145 and 289 mg/kg/day of 2,6-DNT, respectively, for 13 weeks did not display neurotoxic symptoms (U.S. Army 1976).

Clinical signs of neurotoxicity consisting of facial twitching, hypoactivity, and staring were noted in male rats exposed by gavage to 227 mg/kg/day 3,4-DNT for 14 days (Lent et al. 2012a; USAPHC 2011e).

Male rats exposed to ≥39 mg/kg/day 3,5-DNT by gavage for 14 days were reported to exhibit neurological signs progressing from facial twitching to paralysis of the forelimbs (Lent et al. 2012a; USAPHC 2011f). However, all rats exposed to higher doses died or were sacrificed moribund, as did one rat exposed to 39 mg/kg/day. It is not clear whether these signs were seen only in the animals that were moribund or if some neurological signs were seen in animals that survived treatment. Animals in the 39 mg/kg/day group also showed a 30% increase (compared with controls) in brain weight relative to body weight, as well as mild or moderate inflammatory infiltrates in the brain (Lent et al. 2012a; USAPHC 2011f).

Administration of 150 mg/kg/day Tg-DNT to F344 dams during gestation days 7–20 caused hind limb weakness in 7 of 13 animals (Jones-Price et al. 1982). No clinical signs of neurotoxicity or histopathological changes were found in rats fed up to 35 mg/kg/day Tg-DNT in the diet for 26 or 52 weeks (Hazleton Laboratories 1982).

In the 14-day gavage studies that compared the effects of the various DNT isomers on neurological end points in male Sprague-Dawley rats (Lent et al. 2012a; USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f), adverse effects were elicited only by 3,4-DNT (facial twitching, hypoactivity, staring; LOAEL=227 mg/kg/day) and 3,5-DNT (facial twitching and paralysis, inflammatory infiltrates in the brain; LOAEL=39 mg/kg/day).

All LOAEL values from each reliable study for neurological effects in each species and duration category for 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT are recorded in Tables 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively, and plotted in Figures 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Studies in laboratory animals have shown that oral exposure to 2,4-DNT can result in adverse effects on reproduction, as shown by decreased fertility and the development of lesions of the male and female reproductive tracts. The male reproductive system seems to be particularly sensitive; observed effects include decreased sperm production, testicular atrophy, changes in Sertoli cell morphology, and degenerated seminiferous tubules (Bloch et al. 1988; Kozuka et al. 1979; Lane et al. 1985; McGown et al. 1983; U.S. Army 1976, 1978b, 1979). In the female reproductive system, ovarian atrophy and dysfunction were observed (U.S. Army 1979).

Fourteen-day exposure to 2,3-DNT at doses up to 275 mg/kg/day did not result in alterations in testes or epididymides weights in male Sprague-Dawley rats exposed by gavage (Lent et al. 2012a; USAPHC 2011a).

The effects on the male reproductive system have been reported in studies of brief durations. Decreased fertility was noted in male rats dosed with 180 mg/kg 2,4-DNT for 5 days; no dominant lethal effect was observed at this dose (Lane et al. 1985). Decreased absolute and relative testes weight, seminiferous tubular degeneration, multinucleated giant cell formation, and interstitial atrophy of the testes were noted in male rats after 14 days of gavage doses of 142 mg/kg/day 2,4-DNT (Lent et al. 2012a; USAPHC 2011b). Sprague-Dawley rats administered 104, 165, or 261 mg/kg/day 2,4-DNT in the diet for 14 days

exhibited oligospermia with degenerative changes, such as syncytial cell formation and focal spermatic granuloma, in a dose-related manner (McGown et al. 1983). A dose of 78 mg/kg/day 2,4-DNT caused a decrease in the thickness of spermatogenic cell layers. No histopathological changes were found in the reproductive organs of females in this study (McGown et al. 1983). Although no changes were found in sperm morphology of male mice that were administered 250 mg/kg/day 2,4-DNT for 2 days, significant decreases in fertile matings of these animals were observed during weeks 2, 3, and 6 post-treatment (Soares and Lock 1980). However, sperm morphology was examined at 8 weeks post-treatment, so it is possible that a toxic effect was selective for specific types of sperm cells.

In intermediate studies of 2,4-DNT, serious effects on the male reproductive system have been observed in numerous animal studies. In a series of three dominant lethal studies using male rats for 13 weeks, 45 mg/kg/day 2,4-DNT in the diet caused severe atrophy and degeneration of the seminiferous tubules, resulting in decreased fertility, although no dominant lethal effect was observed (U.S. Army 1979). Another study using CD rats found that spermatogenesis was impaired after 4 weeks of feeding 93 mg/kg/day 2,4-DNT in the diet and had completely ceased after 13 weeks (Lee et al. 1985; U.S. Army 1978b). This effect was not reversible after a 4-week post-treatment period. Higher concentrations of 2,4-DNT were needed to cause these effects in mice. Testicular atrophy and aspermatogenesis occurred in CD-1 mice fed 413 mg/kg/day 2,4-DNT for 13 weeks (Hong et al. 1985; U.S. Army 1978b) and rats fed a TWA dose of 371 mg/kg/day 2,4-DNT for 6 months in feed (Kozuka et al. 1979). Decreased fertility was observed after male mice were treated with 1,032 mg/kg/day, but not 295 mg/kg/day, 2,4-DNT in the feed for 4 weeks in a dominant lethal study (U.S. Army 1978b). The decreased fertility was not observed in mice fed 295 mg/kg/day (U.S. Army 1978b). The testicular atrophy was considered to be due to a direct toxic effect on spermatogenic cells. Mild-to-severe testicular degeneration with decreased spermatogenesis has also been observed in dogs administered 25 mg/kg 2,4-DNT in capsules for 13 weeks (Ellis et al. 1985; U.S. Army 1978b). No testicular effects were found at 5 mg/kg in the study.

Histopathological examination of the testes after treatment with 2,4-DNT revealed changes, which suggest specific causes for the male infertility observed in animal studies. Dose-related changes in sperm cell morphology were found in Sprague-Dawley rats fed 76.7 or 153.4 mg/kg/day 2,4-DNT in the diet for 3 weeks (Bloch et al. 1988). At the low dose, vacuolation and lipid accumulation were noted in Sertoli cells; multinucleated spermatid and irregularities of the basal lamina were also found. These changes were limited and variable with most samples, demonstrating patchy damage. More extensive degenerative changes in both spermatocytes and spermatids were found at the high dose as well as

ultrastructural changes in Sertoli cells; epididymal sperm counts were decreased 63%. The high-dose animals also had increased levels of serum luteinizing hormone (LH) and FSH but not testosterone (Bloch et al. 1988).

Chronic-duration studies in laboratory animals have also demonstrated both male and female reproductive effects. Male CD-1 mice fed 14 mg/kg/day 2,4-DNT for 12 months showed atrophy of the testes and decreased spermatogenesis (Hong et al. 1985). Female mice fed 898 mg/kg/day 2,4-DNT in this study had ovarian atrophy with non-functioning follicles, with a NOAEL of 95 mg/kg/day (Hong et al. 1985). Male CD rats that received 34 mg/kg/day 2,4-DNT in the diet for 12 months showed an increased incidence of seminiferous tubule atrophy compared to controls (100% affected at the high dose versus 0% of controls), with a NOAEL of 3.9 mg/kg/day (Lee et al. 1985; U.S. Army 1978b, 1979). No adverse reproductive effects were found in dogs fed 10 mg/kg/day 2,4-DNT for 24 months (Ellis et al. 1985; U.S. Army 1979).

A three-generation reproductive toxicity study was performed in rats fed 2,4-DNT for up to 6 months before mating of original prenatal animals (U.S. Army 1979). Effects on neonatal viability were observed at the highest concentration of 2,4-DNT used, 40 mg/kg/day. Reductions in neonatal viability became more severe with successive litters within each generation, such that no second litters were produced by the second generation of high-dose animals, which were fed 34.5 mg/kg/day (male) or 45.3 mg/kg/day (female) 2,4-DNT. Decreased fetal viability was attributed to maternal neglect and maternal death during parturition. Decreases in the number of fetal implants were attributed to the adverse impact of 2,4-DNT on sperm production.

No effects on testes or epididymides weights were observed in male rats receiving 308 mg/kg/day 2,5-DNT by gavage for 14 days (Lent et al. 2012a; USAPHC 2011c).

In contrast, seminiferous tubular degeneration, multinucleated giant cell formation, and interstitial atrophy of the testes were reported in rats after gavage doses ≥68 mg/kg/day 2,6-DNT for 14 days (Lent et al. 2012a; USAPHC 2011d). At the highest dose (134 mg/kg/day 2,6-DNT), these effects were accompanied by marked decreases (49–57% compared with controls) in absolute and relative testes weight (Lent et al. 2012a; USAPHC 2011d).

In studies of rats, mice, and dogs dosed with 2,6-DNT for 13 weeks (U.S. Army 1976), decreased spermatogenesis was observed in male mice administered 51 mg/kg/day, but normal spermatogenesis was

observed in animals dosed with 11 mg/kg/day. Testicular atrophy was reported in rats administered 35 mg/kg/day 2,6-DNT, and no effects were observed in rats dosed with 7 mg/kg/day (U.S. Army 1976). Dogs dosed with 20 and 100 mg/kg/day had testicular degeneration, but no effects were observed in dogs dosed with 4 mg/kg/day (U.S. Army 1976).

3,4-DNT doses up to 227 mg/kg/day administered by gavage for 14 days to male Sprague-Dawley rats did not result in any changes in testicular or epididymides weights (Lent et al. 2012a; USAPHC 2011e).

Exposure to 3,5-DNT, in contrast, resulted in significant reductions in absolute and relative testes weights (≥40% lower than controls), as well as seminiferous tubular degeneration and multinucleated giant cell formation in the testes of male rats after exposure to doses of 19 and 39 mg/kg/day for 14 days (Lent et al. 2012a; USAPHC 2011f).

Based upon the testicular effects observed after administration of 2,4-, 2,6-, and 3,5-DNT it is not surprising that these effects are found after treatment with Tg-DNT. Testicular degeneration was found in male rats fed 35 mg/kg/day Tg-DNT for 26 weeks, but since the finding was unilateral, the relationship to treatment may be considered equivocal (Hazleton Laboratories 1982). When treatment with this concentration was carried through 52 weeks, however, bilateral mild-to-severe testicular degeneration and hypospermatogenesis were observed (Hazleton Laboratories 1982). No changes were found in the fertility or sperm morphology of male mice that received 250 mg/kg Tg-DNT by gavage for 2 days in a dominant lethal study (Soares and Lock 1980).

In the 14-day gavage studies that compared the effects of the various DNT isomers on reproductive end points in male Sprague-Dawley rats (Lent et al. 2012a; USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f), adverse effects were elicited only by 2,4-DNT (decreased weights of testis and epididymis, tubular degeneration, multinucleated giant cell formation, and interstitial atrophy of the testes; LOAEL=142 mg/kg/day), 2,6-DNT (decreased testis weight at a LOAEL of 134 mg/kg/day; tubular degeneration, multinucleated giant cell formation, and interstitial atrophy of the testes at a LOAEL of 68 mg/kg/day), and 3,5-DNT (small testes, significantly reduced testes weight, tubular degeneration and multinucleated giant cell formation in the testes; LOAEL=19 mg/kg/day).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category for 2,3-, 2,4-, 2,5-, 2,6, 3,4-, and 3,5-DNT are recorded in Tables 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively, and plotted in Figures 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, respectively.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT. However, developmental toxicity from DNTs could potentially occur because exposure to any substance that depletes the amount of oxygen available to developing fetal tissues may have adverse consequences. U.S. Army (1979) conducted a 3-generation reproductive study in which 2,4-DNT was administered to male and female rats at doses up to 34.5 and 45.3 mg/kg/day, respectively. Normal birth weights, liveborn index, and weight at weaning were observed. Decreases in pup viability at 45.3 mg/kg/day in this study resulted from maternal neglect and a high incidence of maternal death during parturition; these decreases did not appear to result from pup defects since no anomalies were detected in offspring from any generation. These effects were not observed in animals fed 5.1 mg/kg/day 2,4-DNT (U.S. Army 1979).

Tg-DNT was administered by gavage to pregnant rats for 14 days during gestation, and pups were evaluated for developmental toxicity either at gestation day 20 or postpartum day 60 (Jones-Price et al. 1982). Adverse effects on hematologic parameters and altered organ weights were observed in both dams and fetuses when dams were administered 100 or 150 mg/kg/day. However, the fetal toxicity was not dose related. A decrease in relative liver weight was observed, however, in the postpartum pups at the low dose of 14 mg/kg/day; this dose is considered to be a LOAEL. Dose-related effects on postnatal development were not observed in pups when dams were administered 35 or 75 mg/kg/day. Transient and statistically significant signs of neurotoxicity, which were not dose-related, included delayed eye opening and cliff avoidance when dams were treated with 35 or 75 mg/kg/day. No evidence of toxicity was found in pups at postpartum day 60 of the postnatal study.

No studies were located regarding developmental effects in animals after oral exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category for 2,4-DNT are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2.7 Cancer

No studies were located regarding cancer in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

The carcinogenic activity of DNTs has been extensively studied in typical chronic bioassays and in some less-than-lifetime studies. 2,4-DNT produced renal tumors in male mice and was hepatocarcinogenic in rats. 2,6-DNT and Tg-DNT are potent hepatocarcinogens in rats (Lee et al. 1985; U.S. Army 1978b, 1979).

2,4-DNT (98% 2,4-DNT, 2% 2,6-DNT) produced renal tumors (76%) in male CD-1 mice fed 95 mg/kg/day for 2 years (U.S. Army 1979). A statistically significant increase in renal tumors in female mice was not observed. A National Cancer Institute (NCI) bioassay (NCI 1978) of 2,4-DNT (95% 2,4-DNT, the other components not specified) did not detect a carcinogenic effect in mice dosed with 72 mg/kg/day for 78 weeks. The NCI bioassay used the C57BL/6N strain of mouse, lower doses, and a shorter treatment schedule than did U.S. Army (1979).

Hepatocellular carcinoma were significantly increased in male CD rats fed 34.5 mg/kg/day 2,4-DNT and in females fed 45.3 mg/kg/day 2,4-DNT for 2 years (U.S. Army 1979). The tumor response in females was higher than in the males. Two other studies of rats in which malignancies were not observed used the F344 strain, lower doses, and shorter exposure durations than did U.S. Army (1979): 10 mg/kg/day for 78 weeks (NCI 1978) and 27 mg/kg/day for 52 weeks (Leonard et al. 1987). NCI (1978) reported significant increases in subcutaneous tissue fibroma in male rats at 7.5–8 mg/kg/day and mammary gland fibroadenomas in female rats at 22 mg/kg/day. U.S. Army (1979) found significant increases in subcutaneous tissue fibromas in male rats at 34.5 mg/kg/day and mammary gland fibroadenomas in female rats at 45.3 mg/kg/day; these were benign tumors.

2,4-DNT was not found to be carcinogenic in the Strain A/J mouse pulmonary tumor bioassay when 250 mg/kg was administered by gavage twice a week for 12 weeks (Stoner et al. 1984). 2,4-DNT was a hepatic tumor promoter, but not a tumor initiator, using *in vivo* hepatic initiation-promotion protocols (Leonard et al. 1986).

2,6-DNT administered for 1 year at 7 and 14 mg/kg/day produced hepatocellular carcinomas in 85% and 100%, respectively, of male F344 rats (Leonard et al. 1987). Pulmonary metastases of hepatocytic origin were also observed. Both tumor-initiating and tumor-promoting activities of 2,6-DNT in rat liver were reported (Leonard et al. 1983, 1986; Mirsalis and Butterworth 1982). 2,6-DNT was not found to be a lung carcinogen in the Strain A/J mouse pulmonary tumor bioassay when 250 mg/kg was administered by gavage twice a week for 12 weeks (Schut et al. 1983; Stoner et al. 1984).

The effect of diet-induced changes in gut microflora on the hepatocarcinogenicity of 2,6-DNT was studied in male F344 rats (Goldsworthy et al. 1986). Groups of the rats were placed on one of three diets containing 2,6-DNT at doses of 0, 0.6–0.7, or 3–3.5 mg/kg/day. Ten animals from each group were sacrificed at 3, 6, and 12 months, and their livers were evaluated histopathologically. The diets used were NIH-07, an open formula cereal-based diet high in pectin content; AIN-76A, a purified pectin-free diet; or AP, which is AIN-76A supplemented with 5% pectin. The number and size of γ-glutamyl transpeptidase-staining foci in the liver increased in a dose- and time-related manner in animals given 2,6-DNT in the NIH-07 diet. Hepatocellular carcinomas and neoplastic nodules were observed only in rats fed NIH-07 containing 2,6-DNT. No tumor was observed in rats receiving the control diets or 2,6-DNT in the AIN-76 diet with or without pectin. This finding suggested that pectin did not influence the tumor outcome of the experiment. Unidentified contaminants in cereal-based diets may influence liver foci and tumor production in the rat liver during carcinogen treatment.

Tg-DNT provided positive hepatocarcinogenic results in two bioassays of less-than-lifetime duration. In a 52-week study of male rats dosed with 35 mg/kg/day of Tg-DNT, Leonard et al. (1987) observed a 47% increase in hepatocellular carcinoma; cholangiocarcinomas were also found in 10% of rats treated with 35 mg/kg/day Tg-DNT in the Leonard et al. (1987) study. Hazleton Laboratories (1982) reported that dietary administration of 35 mg/kg/day Tg-DNT to rats for 55 weeks resulted in an increased incidence (100% in males and 55% in females) of hepatocellular carcinoma; this lesion was found in some animals treated at this level for 26 weeks. The administration of 3.5 mg/kg/day Tg-DNT for 104 weeks caused hepatocellular carcinoma in 9 of 70 males compared to 1 of 61 controls. Mammary fibroadenoma and subcutaneous fibroma were also found in both sexes at 3.5 mg/kg/day after 104 weeks (Hazleton Laboratories 1982). Administration of 14 mg/kg/day Tg-DNT for 104 weeks caused cholangio-carcinomas and parathyroid adenomas in males and hepatocellular carcinomas and hepatocholangio-carcinomas in females (Hazleton Laboratories 1982).

Tg-DNT contains about 76% 2,4-DNT and 19% 2,6-DNT, as well as small amounts of other isomers. Rats that received 35 mg/kg/day in the Leonard et al. (1987) and Hazleton Laboratories (1982) studies were provided approximately 28 and 7 mg/kg/day of 2,4- and 2,6-DNT, respectively. This dose of 2,6-DNT in Tg-DNT bioassays is equivalent to the low dose of 2,6-DNT administered to rats by Leonard et al. (1987) that produced hepatocellular carcinomas.

In hepatic tumor initiation-promotion protocols, Tg-DNT was reported to have tumor-promoting and tumor-initiating activity (Leonard et al. 1983, 1986; Mirsalis and Butterworth 1982). The results of the initiation-promotion protocols for 2,4-, 2,6-, and Tg-DNT indicate that 2,6-DNT is a complete hepatocarcinogen and is primarily responsible for the carcinogenic activity of Tg-DNT.

No studies were located regarding cancer in animals after oral exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNT.

3.2.3 Dermal Exposure

There are no data regarding adverse health effects associated with dermal exposure of humans or animals to 2,3-, 2,5-, 3,4-, or 3,5-DNT. There are data on occupational exposure of humans to 2,4-DNT and Tg-DNT (see Section 3.2.1) in which dermal exposure probably occurred, but the primary route of exposure in these studies is believed to be inhalation. The relative contribution of dermal exposure to total occupational exposure cannot be determined from these studies. Levine et al. (1985b) reported that small amounts of 2,4-DNT were detected on the hands, face, and forehead when a wipe-sample survey was conducted on workers in a DNT manufacturing plant. The highest quantity found on a worker's skin was 180 μg (obtained from wipe sampling of hands and face; total surface area wiped was not reported) and may account for the quantity of excreted urinary metabolites that exceeded the amount of inhaled DNT in the operators and loaders. Animal data associated with dermal exposure of animals to DNTs are limited to studies of dermal irritation, eye irritation, and dermal sensitization of 2,4-, 2,6-, or Tg-DNT in rabbits.

3.2.3.1 Death

No studies were located regarding mortality in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

One study was located that examined death among humans exposed to DNTs. A retrospective mortality study of munitions workers exposed to either 2,4-DNT or Tg-DNT revealed an increased death rate due to ischemic heart disease and residual diseases of the circulatory system in the exposed cohort (Levine et al. 1986a, 1986b). Exposure concentrations to 2,4-DNT or Tg-DNT were not reported. The residual diseases included cardiac arrest and arteriosclerosis. Exposure levels were not reported, and the study is further limited by the small cohort size and concurrent inhalation exposure of the workers.

No studies were located regarding death in animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, renal, body weight, or endocrine effects in humans or animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Gastrointestinal complaints of munitions workers exposed to either 2,4-DNT or Tg-DNT included nausea and vomiting (McGee et al. 1947). These workers also presumably inhaled DNT in the occupational setting. Exposure concentrations to 2,4-DNT or Tg-DNT were not reported.

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

Cardiovascular Effects. No studies were identified with respect to cardiovascular effects after dermal exposure of humans to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Levine et al. (1986a) reported a significant increase in heart disease mortality in workers involved in the manufacture and processing of 2,4-DNT and/or Tg-DNT.

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

Hematological Effects. No studies were located regarding hematological effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Hematological effects, such as anemia and cyanosis (which can be indicative of anemia), have been found in men employed at munitions factories (McGee et al. 1947; Perkins 1919). These workers were exposed to either 2,4-DNT or Tg-DNT. Because these studies lacked worker histories, exposure data, and

reported on small cohorts, the results are equivocal and are best used to qualitatively describe symptoms. In addition, the workers probably received their primary exposure via the inhalation pathway.

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

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Muscle weakness and joint pain have been reported by munitions workers after occupational exposure to unspecified concentrations of 2,4-DNT or Tg-DNT (McGee et al. 1947; Perkins 1919).

In the Perkins (1919) study, joint pain and other incapacitating symptoms were noted following exposure to what were presumed to be very high concentrations of Tg-DNT since the processes described required direct handling without protective equipment. In both of these studies, however, no exposure data were available; exposure to other compounds may have occurred, and concomitant exposure via inhalation was also likely.

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

Hepatic Effects. No studies were located regarding hepatic effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

In a study of male munitions workers exposed to unspecified concentrations of 2,4-DNT, 29 of 714 workers displayed tenderness of the liver (McGee et al. 1947). No other clinical evaluation was performed that might provide further insight into the significance of this finding. These workers were also exposed to DNTs via inhalation.

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

Dermal Effects. No studies were located regarding dermal effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

A study of dermal effects of topical exposure to 2,4-DNT in workers employed by a munitions factory during World War II reported that 32 of 714 workers complained of dermatitis (McGee et al. 1947). Exposure levels were not quantified in this study.

Both 2,4- and 2,6-DNT were shown to be mild primary dermal irritants in rabbits (U.S. Army 1975, 1978a).

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

Ocular Effects. No studies were located regarding ocular effects in humans after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

No ocular irritation was found in rabbits in a primary eye irritation test using unspecified concentrations of 2,4- or 2,6-DNT (U.S. Army 1975, 1978a). However, mild eye irritations were reported in rabbits treated with 2,4-DNT (Ford 1981).

No studies were located regarding ocular effects in animals after dermal exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNT.

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

In dermal sensitization tests, 2 of 10 guinea pigs exhibited mild sensitization to 2,6-DNT, but no sensitization was evident when 2,4-DNT was tested (U.S. Army 1975, 1978a).

No studies were located regarding immunological and lymphoreticular effects in animals after dermal exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNT.

3.2.3.4 Neurological Effects

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

Various neurological symptoms, including headache, vertigo, and pain or numbness in the extremities, have been reported in surveys of munitions workers exposed to unspecified concentrations of 2,4-DNT (McGee et al. 1947). Although it is assumed that some dermal exposure to 2,4-DNT occurred in these workers, inhalation was the probable primary route of exposure.

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

3.2.3.5 Reproductive Effects

No significant effects on fertility were observed in workers occupationally exposed to Tg-DNT (Levine et al. 1985a). However, Levine et al. estimated that only a 50–70% reduction in fertility could have been detected in the worker population that they studied.

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

3.2.3.6 Developmental Effects

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

3.2.3.7 Cancer

In a case-cohort study of German copper miners with exposure to Tg-DNT (see Section 3.2.1 for additional detail), Seidler et al. (2014b) observed elevated Cox proportional HRs for renal cancer among workers with medium or high dermal exposure to Tg-DNT (HR 2.73, 95% CI 1.0–7.42 for medium exposure and HR 1.81, 95% CI 0.75–4.33 for high exposure). When combined across medium and high exposure categories for both dermal and inhalation exposure, the HR for renal cancer was significantly increased (HR 2.12, 95% CI 1.03–4.37).

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

3.3 GENOTOXICITY

Data are available for 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, 3,5-, and Tg-DNT from *in vitro* tests in prokaryotic organisms (gene mutation in *Salmonella typhimurium*) and in mammalian cell systems (gene mutation, chromosomal aberrations, DNA damage, and morphological transformation). Results of *in vitro* genotoxicity assays are presented in Table 3-9. *In vivo* data for 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, 3,5-, and Tg-DNT, including evaluations of unscheduled DNA synthesis (UDS), DNA damage and DNA binding, gene mutations, and/or chromosomal aberrations, are presented in Table 3-10. Some isomers of DNT (2,4-DNT and especially 2,6-DNT) have tested positive *in vitro* and/or *in vivo* in other genotoxicity assays, with evidence of chromosomal aberrations, DNA damage and adduct formation, and morphological transformation. 2,3-, 2,5-, and 3,5-DNT generally tested negative in genotoxicity assays. Taken together, results of *in vitro* studies do not provide strong evidence that DNT directly induces gene mutations; however, there is some evidence that 2,4-DNT induces mutations *in vivo*.

In vitro studies assessing genotoxicity of DNT have yielded mixed results in *S. typhimurium*, presumably due to differences in the need for metabolic activation and the sensitivity of the tester strains. 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT have been shown to induce gene mutations in *S. typhimurium* in the presence or absence of metabolic activation in some assays, but produced negative or equivocal results in several others (Couch et al. 1981; Dellarco and Prival 1989; Einistö et al. 1991; Kawai et al. 1987; Mori et al. 1982; Neuwoehner et al. 2007; Padda et al. 2003; Sayama et al. 1989; Simmon et al. 1977; Spanggord et al. 1982b; Suzuki et al. 2011; Tokiwa et al. 1981; U.S. Army 1978a). Sayama et al. (1998) showed that 2,4-DNT was mutagenic in *S. typhimurium* strains possessing high levels of nitroreductase and *O*-acteyltransferase and 2,6-DNT was mutagenic in strains possessing high levels of *O*-acetyltransferase. 2,4-DNT was nonmutagenic in *Escherichia coli* (Dunkel et al. 1985). Mixed results were found for 2,4-DNT in tests of DNA damage in bacteria; positive results were found in the umu test using *S. typhimurium* and negative results were found in the SOS chromotest using *E. coli* (Öztürk and Durusoy 1999). In general, 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT did not induce gene mutations in mammalian cells (Abernathy and Couch 1982; Styles and Cross 1983); however, positive results were obtained for 2,4- and/or 2,6-DNT in assays that evaluated chromosomal aberrations in Chinese hamster lung fibroblasts or human peripheral lymphocytes (Huang et al. 1996; Suzuki et al. 2011), morphological

Table 3-9. Genotoxicity of Dinitrotoluene Isomers *In Vitro*

Species (test system)	Isomer	End point	Results		Reference
			With activation	Without activation	
Prokaryotic organisms:					
Salmonella typhimurium, TA98	2,3-DNT	Gene mutation	+	+	Couch et al. 1981
S. typhimurium, TM677	2,3-DNT	Gene mutation	–	–	Couch et al. 1981
S. typhimurium, TA98, TA100	2,3-DNT	Gene mutation	–	–	Dellarco and Prival 1989
S. typhimurium, TA98; with flavin mononucleotide (FMN)	2,3-DNT	Gene mutation (modified assay)	+	NT	Dellarco and Prival 1989
S. typhimurium, TA100; with FMN	2,3-DNT	Gene mutation (modified assay)	–	NT	Dellarco and Prival 1989
S. typhimurium, TA98	2,3-DNT	Gene mutation	–	+	Spanggord et al. 1982b
S. typhimurium, TA100	2,3-DNT	Gene mutation	+	+	Spanggord et al. 1982b
S. typhimurium, TA1538	2,3-DNT	Gene mutation	–	+	Spanggord et al. 1982b
S. typhimurium, TA1535, TA1537, TA100 NR3	2,3-DNT	Gene mutation	–	–	Spanggord et al. 1982b
S. typhimurium, TA100	2,3-DNT	Gene mutation	±	–	Kawai et al. 1987
S. typhimurium, TA98	2,3-DNT	Gene mutation	–	±	Kawai et al. 1987
S. typhimurium, TA98, TM677	2,4-DNT	Gene mutation	+	+	Couch et al. 1981
S. typhimurium	2,4-DNT	Gene mutation	NT	–	Chiu et al. 1978
S. typhimurium, TA98, TA100	2,4-DNT	Gene mutation	–	+	Tokiwa et al. 1981
S. typhimurium, TA98, TA100	2,4-DNT	Gene mutation	–	–	Dellarco and Prival 1989
S. typhimurium, TA98; with FMN	2,4-DNT	Gene mutation (modified assay)	+	NT	Dellarco and Prival 1989
S. typhimurium, TA100; with FMN	2,4-DNT	Gene mutation (modified assay)	–	NT	Dellarco and Prival 1989
S. typhimurium	2,4-DNT	Gene mutation	NT	+	Mori et al. 1982
S. typhimurium, TA100	2,4-DNT	Gene mutation	–	+	Spanggord et al. 1982b
S. typhimurium, TA100 NR3	2,4-DNT	Gene mutation	+	–	Spanggord et al. 1982b
S. typhimurium, TA1535, TA1537, TA1538, TA98	2,4-DNT	Gene mutation	–	–	Spanggord et al. 1982b
S. typhimurium, TA100	2,4-DNT	Gene mutation	+	–	U.S. Army 1978a
S. typhimurium, TA1535	2,4-DNT	Gene mutation	–	+	U.S. Army 1978a
S. typhimurium, TA98, 1537	2,4-DNT	Gene mutation	–	–	U.S. Army 1978a

Table 3-9. Genotoxicity of Dinitrotoluene Isomers *In Vitro*

Species (test system)	Isomer	End point	Results		Reference
			With activation	Without activation	
S. typhimurium, TA1538	2,4-DNT	Gene mutation	+	+	U.S. Army 1978a
S. typhimurium	2,4-DNT	Gene mutation	+	NT	Pearson et al. 1978
S. typhimurium, TA98, TA98 NR, TA98/,8-DNP$_6$, YG1021, YG1024	2,4-DNT	Gene mutation	NT	+	Einistö et al. 1991
S. typhimurium, TA100	2,4-DNT	Gene mutation	±	±	Kawai et al. 1987
S. typhimurium, TA98	2,4-DNT	Gene mutation	–	–	Kawai et al. 1987
S. typhimurium, TA100	2,4-DNT	Gene mutation	NT	–	Padda et al. 2003
S. typhimurium, TA98,	2,4-DNT	Gene mutation	NT	–	Sayama et al. 1998
S. typhimurium, TA100 YG1021, YG1024, YG1041, YG1026, YG1029, YG1042	2,4-DNT	Gene mutation	NT	+	Sayama et al. 1998
Escherichia coli	2,4-DNT	Gene mutation	–	–	Dunkel et al. 1985
S. typhimurium, NM2009, NM3009	2,4-DNT	DNA damage (umu test)	NT	+	Öztürk and Durusoy 1999
E. coli, PQ37	2,4-DNT	DNA damage (SOS chromotest)	–	–	Öztürk and Durusoy 1999
S. typhimurium, TA98, TM677	2,5-DNT	Gene mutation	+	+	Couch et al. 1981
S. typhimurium, TA98	2,5-DNT	Gene mutation	±	No data	Dellarco and Prival 1989
S. typhimurium, TA100	2,5-DNT	Gene mutation	–	–	Dellarco and Prival 1989
S. typhimurium, TA98; with FMN	2,5-DNT	Gene mutation (modified assay)	+	NT	Dellarco and Prival 1989
S. typhimurium, TA100; with FMN	2,5-DNT	Gene mutation (modified assay)	–	NT	Dellarco and Prival 1989
S. typhimurium, TA1535, TA1538, TA98, TA100	2,5-DNT	Gene mutation	+	+	Spanggord et al. 1982b
S. typhimurium, TA1537, TA100 NR3	2,5-DNT	Gene mutation	–	–	Spanggord et al. 1982b
S. typhimurium, TA98, TM677	2,6-DNT	Gene mutation	+	+	Couch et al. 1981
S. typhimurium, TA100, TA1535, TA1537, TA98	2,6-DNT	Gene mutation	–	–	U.S. Army 1978a
S. typhimurium, TA1538	2,6-DNT	Gene mutation	–	+	U.S. Army 1978a
S. typhimurium	2,6-DNT	Gene mutation	NT	+	Simmon et al. 1977
S. typhimurium, TA98, TA100	2,6-DNT	Gene mutation	–	–	Sayama et al. 1989
S. typhimurium, TA98, TA100	2,6-DNT	Gene mutation	–	+	Tokiwa et al. 1981

Table 3-9. Genotoxicity of Dinitrotoluene Isomers *In Vitro*

Species (test system)	Isomer	End point	Results		Reference
			With activation	Without activation	
S. typhimurium, TA100	2,6-DNT	Gene mutation	+	+	Spanggord et al. 1982b
S. typhimurium, TA1535, TA1537, TA1538, TA98, TA100 NR3	2,6-DNT	Gene mutation	–	–	Spanggord et al. 1982b
S. typhimurium, TA98, TA100	2,6-DNT	Gene mutation	–	–	Dellarco and Prival 1989
S. typhimurium, TA98; with FMN	2,6-DNT	Gene mutation (modified assay)	+	NT	Dellarco and Prival 1989
S. typhimurium, TA100; with FMN	2,6-DNT	Gene mutation (modified assay)	–	NT	Dellarco and Prival 1989
S. typhimurium, TA98, TA100, TA1537	2,6-DNT	Gene mutation	+	+	Suzuki et al. 2011
S. typhimurium, TA98, TA98/1,8-DNP, YG1021,YG1024	2,6-DNT	Gene mutation	NT	+	Einistö et al. 1991
S. typhimurium, TA98 NR	2,6-DNT	Gene mutation	+	–	Einistö et al. 1991
S. typhimurium	2,6-DNT	Gene mutation	+	NT	Pearson et al. 1979
S. typhimurium, TA98, TA100	2,6-DNT	Gene mutation	±	±	Kawai et al. 1987
S. typhimurium, TA100	2,6-DNT	Gene mutation	NT	–	Padda et al. 2003
S. typhimurium, TA98, TA100 YG1021,	2,6-DNT	Gene mutation	NT	–	Sayama et al. 1998
S. typhimurium, YG1024, YG1041, YG1026, YG1029, YG1042	2,6-DNT	Gene mutation	NT	+	Sayama et al. 1998
S. typhimurium, TA100	3,4-DNT	Gene mutation	+	+	Kawai et al. 1987
S. typhimurium, TA98	3,4-DNT	Gene mutation	–	–	Kawai et al. 1987
S. typhimurium, TA100	3,4-DNT	Gene mutation	+	+	Spanggord et al. 1982b
S. typhimurium, TA1535, TA1537, TA1538, TA98, TA100 NR3	3,4-DNT	Gene mutation	–	–	Spanggord et al. 1982b
S. typhimurium, TA98	3,4-DNT	Gene mutation	–	–	Tokiwa et al. 1981
S. typhimurium, TA100	3,4-DNT	Gene mutation	–	+	Tokiwa et al. 1981
S. typhimurium, TA98, TM677	3,5-DNT	Gene mutation	+	+	Couch et al. 1981
S. typhimurium, TA1537, TA1538, TA98, TA100	3,5-DNT	Gene mutation	+	+	Spanggord et al. 1982b
S. typhimurium, TA1535, TA100 NR3	3,5-DNT	Gene mutation	–	–	Spanggord et al. 1982b

Table 3-9. Genotoxicity of Dinitrotoluene Isomers *In Vitro*

Species (test system)	Isomer	End point	Results		Reference
			With activation	Without activation	
S. typhimurium, TA98, TM677	Tg-DNT	Gene mutation	+	+	Couch et al. 1981
S. typhimurium	Tg-DNT	Gene mutation	NT	+	Chadwick et al. 1990
Mammalian cells:					
CHO cells	2,4-DNT	Sister chromatid exchange	+	–	Loveday et al. 1989
CHO cells	2,4-DNT	Chromosomal aberrations	–	–	Loveday et al. 1989
CHO/HGPRT	2,3-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO/HGPRT	2,4-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO/HGPRT	2,4-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO/HGPRT	2,5-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO-HGPRT	2,6-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO/HGPRT	3,5-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO-HGPRT	Tg-DNT	Gene mutation	–	–	Abernethy and Couch 1982
P388 mouse lymphoma TK	2,4-DNT	Gene mutation	–	+	Styles and Cross 1983
P388 mouse lymphoma TK	2,6-DNT	Gene mutation	–	–	Styles and Cross 1983
P388 mouse lymphoma TK	Tg-DNT	Gene mutation	–	–	Styles and Cross 1983
Chinese hamster lung fibroblasts	2,6-DNT	Chromosomal aberrations	+	+	Suzuki et al. 2011
SHE cells	2,4-DNT	Morphological transformation	NT	–	Holen et al. 1990
SHE cells	2,4-DNT	Morphological transformation	NT	+	Engelhardt et al. 2004
SHE cells	2,6-DNT	Morphological transformation	NT	+	Engelhardt et al. 2004
SHE cells	2,6-DNT	Morphological transformation	NT	–	Holen et al. 1990
Kumming rat Sertoli cells	2,4-DNT	DNA damage (comet assay)	NT	+	Yang et al. 2005

Table 3-9. Genotoxicity of Dinitrotoluene Isomers *In Vitro*

Species (test system)	Isomer	End point	Results		Reference
			With activation	Without activation	
Kumming rat Sertoli cells	2,6-DNT	DNA damage (comet assay)	NT	+	Yang et al. 2005
Human peripheral lymphocytes	2,4-DNT	Chromosomal aberrations	NT	+	Huang et al. 1996

+ = positive; – = negative; ± = weakly positive or equivocal; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; DNT = dinitrotoluene; FMN = flavin mononucleotide; NT = not tested; SHE = Syrian hamster embryo; Tg = technical grade

Table 3-10. Genotoxicity of Dinitrotoluene Isomers *In Vivo*

Species (test system)	Isomer	End point	Results	Reference
DNT and TNT workers	Tg-DNT	Chromatid-type chromosomal aberrations	+	Sabbioni et al. 2006
Rat hepatocyte	2,4-DNT	UDS	+	Mirsalis et al. 1989
Rat hepatocyte	2,4-DNT	UDS	+	Mirsalis and Butterworth 1982
Rat hepatocyte (male; AP or F344)	2,4-DNT[a]	UDS	+	Ashby et al. 1985
Rat hepatocyte	2,4-DNT	S-phase synthesis	+	Mirsalis et al. 1989
Rat hepatocyte (male; Sprague-Dawley)	2,3-DNT	DNA damage; comet assay	–	Lent et al. 2012b
Rat hepatocyte (male; Sprague-Dawley)	2,4-DNT	DNA damage; comet assay	–	Lent et al. 2012b
Rat hepatocyte (male; Sprague-Dawley)	2,5-DNT	DNA damage; comet assay	–	Lent et al. 2012b
Rat hepatocyte (male; Sprague-Dawley)	2,6-DNT	DNA damage; comet assay	+	Lent et al. 2012b
Rat hepatocyte (male; Crl:CD(SD)	2,6-DNT	DNA damage; comet assay	+	Suzuki et al. 2011
Rat hepatocyte (male; Sprague-Dawley)	2,6-DNT	DNA damage; comet assay	+	Rothfuss et al. 2010
Rat hepatocyte (male; Sprague-Dawley)	3,5-DNT	DNA damage; comet assay	–	Lent et al. 2012b
Rat peripheral blood (male; Sprague-Dawley)	2,6-DNT	DNA damage; comet assay	+	Rothfuss et al. 2010
Rat hepatocyte	2,4-DNT	DNA binding (adduct formation)	+	La and Froines 1993
Rat hepatocyte (male; F344)	2,4-DNT	DNA binding (adduct formation)	+	Chadwick et al. 1993
Rat hepatocyte (female; Wistar)	2,4-DNT	DNA binding (adduct formation)	+	Jones et al. 2005a
Rat hepatocyte (female; Wistar)	2,6-DNT	DNA binding (adduct formation)	+	Jones et al. 2005a
Rat (male; F344) and mouse (male; B6C3F1) hepatocytes	2,6-DNT	DNA binding (adduct formation)	+	George et al. 1996
Rat peripheral blood (male; Sprague-Dawley)	2,3-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012b
Rat hepatocyte (male; F344)	2,4-DNT	Chromosomal aberrations; micronucleus assay	+	Suzuki et al. 2009
Rat hepatocyte (male; F344)	2,4-DNT	Chromosomal aberrations; micronucleus assay	+	Takasawa et al. 2010
Rat peripheral blood (male; Sprague-Dawley)	2,4-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012b
Rat peripheral blood (male; Sprague-Dawley)	2,5-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012b

Table 3-10. Genotoxicity of Dinitrotoluene Isomers *In Vivo*

Species (test system)	Isomer	End point	Results	Reference
Rat peripheral blood (male; Sprague-Dawley)	2,6-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012b
Rat hepatocyte (male; F344)	2,6-DNT	Chromosomal aberrations; micronucleus assay	±	Takasawa et al. 2010
Rat peripheral blood (male; Sprague-Dawley)	3,5-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012b
Rat bone marrow (male; Sprague-Dawley)	2,6-DNT	Chromosomal aberrations; micronucleus assay	–	Rothfuss et al. 2010
Mouse bone marrow (male; CBA x BALBc)F1)	2,4-DNT[a]	Chromosomal aberrations; micronucleus assay	–	Ashby et al. 1985
Mouse (female; C57BL/6J or T stock)	2,4-DNT[a]	Gene mutation, spot test	–	Soares and Lock 1980

[a]The test substance is referred to in the publication as technical-grade 2,4-DNT.

+ = positive result; – = negative result; ± = equivocal result; DNA = deoxyribonucleic acid; DNT = dinitrotoluene; Tg = technical grade; TNT = trinitrotoluene; UDS = unscheduled DNA synthesis

transformation in Syrian hamster embryo cells (Engelhardt et al. 2004; Holen et al. 1990), or DNA damage in rat Sertoli cells (Yang et al. 2005).

UDS and/or *S*-phase synthesis (SPS) were induced *in vitro* in the hepatocytes of F344 rats treated with 2,4-DNT or Tg-DNT *in vivo* (Ashby et al. 1985; Mirsalis et al. 1989). The mutagenicity of several of the metabolites of 2,6-DNT have been tested in *S. typhimurium*. Although neither 2,6-DNT nor its metabolites, 2-amino-6-nitrotoluene, 2,6-dinitrobenzylalcohol, 2-acetylamino-6-nitrobenzoic acid, and 2-amino-6-nitrobenzoic acid, were mutagenic in this assay with or without S9 activation, other metabolites of 2,6-DNT were found to possess mutagenic activity (Sayama et al. 1989). The putative metabolite 2,6-dinitrobenzaldehyde was a direct acting mutagen (i.e., it did not require activation) (Sayama et al. 1989). Urine from F344 rats administered 75 mg/kg 2,6-DNT by gavage tested positive for mutagenicity using *S. typhimurium* TA98 without S9 activation (Chadwick et al. 1993).

2,4-DNT induced lethal mutations but not reciprocal translocations in mutagenicity testing using *Drosophilia melanogaster* (Woodruff et al. 1985). Color coat mutations were also observed in the offspring of pregnant C57BL/6J or T stock mice administered 1,000 mg/kg technical-grade 2,4-DNT via gavage or intraperitoneal injection on gestation day 10 (Soares and Lock 1980).

Micronuclei formation was observed in two assays in liver cells of 2,4-DNT-treated F344 rats (Suzuki et al. 2009; Takasawa et al. 2010), but were not observed in the peripheral blood of 2,4-DNT-treated Sprague-Dawley rats (Lent et al. 2012b). Similarly, no micronuclei were observed in the peripheral blood of Sprague-Dawley rats administered 2,6-DNT via gavage for 2 or 14 days (Lent et al. 2012b; Takasawa et al. 2010) or in the bone marrow of Sprague-Dawley rats exposed for 3 or 29 days (Rothfuss et al. 2010); however, micronuclei were induced in the hepatocytes of 2,6-DNT-treated rats. Other isomers of DNT (2,3-, 2,5-, or 3,5-DNT) tested negative in micronucleus assays in rats (Lent et al. 2012b). In workers at a DNT and TNT manufacturing facility, significant increases in chromatid-type chromosomal aberrations were observed, as compared to factory controls (Sabbioni et al. 2006). When workers were divided into three age categories, a significant association between 2,4-DNT hemoglobin adducts and chromosomal frequency was observed in the youngest group, but not in the two older groups.

The formation of DNA adducts is generally thought to indicate carcinogenic risk (La and Froines 1993). Both 2,4- and 2,6-DNT have induced DNA adducts in rat liver. Following treatment with 2,4-DNT at up to 375 mg/kg via intraperitoneal injection, three DNA adducts were found in the liver of F344 rats (La and Froines 1992). Four adducts, identified as 4-amino-2-nitrotoluene (4A2NT), 24TDA, and

4-acetylamino-2-aminotoluene (4AA2AT) (with 4A2NT being the predominant form) were found in the liver of Wistar rats administered 0.5 mmol/kg (approximately 91 mg/kg) 2,4-DNT as a single gavage dose (Jones et al. 2005a). Following treatment with 2,6-DNT, four DNA adducts (not identified) were found in the liver of rats treated with 75 mg/kg 2,6-DNT by gavage (Chadwick et al. 1993). In Wistar rats treated with 2,6-DNT at 0.5 mmol/kg (about 91 mg/kg) via gavage as a single dose, three adducts, identified as 2-amino-6-nitrotoluene (2A6NT), 26TDA, and 2-acetylamino-6-aminotoluene (2AA6AT) (with 2A6NT being the predominant form) were detected (Jones et al. 2005a). Two types of DNA adducts were detected in liver DNA of B6C3F1 mice administered 2,6-DNT at 50 mg/kg via gavage; these adducts differed from the four types of adducts identified in F344 rats administered 2,6-DNT via gavage (George et al. 1996). The formation of 4 DNA adducts was also observed after intraperitoneal administration of 219 mg/kg 2,6-DNT to F344 rats (La and Froines 1992, 1993). One adduct accounts for the majority of the radioactivity measured; about 85% of the total was in one adduct in the study using 2,4-DNT, while in the study with 2,6-DNT, about 60% of the total adducts measured were from a single adduct with the other adducts constituting 10–15% of the total (La and Froines 1992, 1993). No quantitative or qualitative differences in adduct formation were found when treatment occurred by gavage or intraperitoneal injection (La and Froines 1992). The proximate DNA binding species has been postulated to be 2-hydroxylamino-6-nitrobenzyl alcohol (La and Froines 1993; Rickert et al. 1984). The DNA adducts formed after exposure to 2,4- or 2,6-DNT were persistent over time; the persistence of these adducts was slightly >40% in the 2 weeks after exposure (La and Froines 1992).

Comet assays, which evaluated the ability of DNT to induce DNA damage in the hepatocytes of treated rats, were negative for 2,3-, 2,4-, 2,5-, and 3,5-DNT (administered via gavage at up to 275, 142, 308, or 39 mg/kg/day, respectively), but positive in Sprague-Dawley rats treated with 2,6-DNT via gavage at doses from 33 to 134 mg/kg/day (Lent et al. 2012b; Suzuki et al. 2011; Rothfuss et al. 2010). DNA damage (as measured by comet assay) was not detected in peripheral blood of Sprague-Dawley rats exposed to 100 mg/kg/day for 3 days or to 33 mg/kg/day for 29 days (Rothfuss et al. 2010)

Studies of the effects of various DNT isomers (2,4-, 2,6-, or 3,5-DNT) on sperm morphology (Soares and Lock 1980), spermatocyte DNA repair (Working and Butterworth 1984), and dominant lethal mutations (Soares and Lock 1980, U.S. Army 1979) were generally negative for these specific end points. Mice administered 2,4-DNT at 250 mg/kg on two consecutive days via gavage or intraperitoneal injection did not show evidence of an increase in morphologically aberrant sperm (Soares and Lock 1980), and UDS was not induced in the spermatocytes of rats treated with a single dose of 20 mg/kg 2,6-DNT via gavage (Working and Butterworth 1984). Dominant lethal effects were not induced in rats administered 2,4-DNT

at up to 0.07% (34 mg/kg) in the diet for 13 weeks (U.S. Army 1979) or in mice administered 250 mg/kg 2,4-DNT or 3,5-DNT on two consecutive days via gavage or intraperitoneal injection (Soares and Lock 1980). However, a significant increase in the number of dead implants/total implants was reported in females mated to males treated with 2,4-DNT at 0.2% in the diet for 13 weeks (Hodgson et al. 1976).

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

No studies were located regarding absorption of inhaled of 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT in humans or laboratory animals. However, detection of DNT metabolites in the urine of workers at DNT manufacturing plants provides evidence that DNT may be absorbed following presumed inhalation exposure (Levine et al. 1985b; Turner 1986; Woollen et al. 1985).

3.4.1.2 Oral Exposure

Rickert et al. (1983) suggested that the rapid disappearance of radioactivity from the first quarter of the small intestine of rats following the oral administration of uniformly [^{14}C]-ring-labeled 2,4- or 2,6-DNT indicates rapid and fairly complete absorption.

Excretion data and observed systemic effects indicate that DNTs are absorbed following oral administration to experimental animals. Several strains of rats, New Zealand rabbits, beagle dogs, and Rhesus monkeys excreted 55–90% of the radioactivity from orally-administered radiolabeled DNTs in the urine, primarily within the first 24 hours (Long and Rickert 1982; Rickert and Long 1981; U.S. Army 1978b). In mice, most of the radioactivity from ^{3}H-labeled 2,6-DNT was excreted in the urine (about 50% in 8 hours) (Schut et al. 1983), whereas most of the radioactivity from ^{14}C-labeled 2,4-DNT administered to mice was excreted in the feces, and only about 10% was excreted in the urine (U.S. Army 1978b). Increased fecal excretion could be due to reduced absorption or to greater excretion via the bile.

Studies on absorption of 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT following oral exposure of humans or animals were not identified.

3.4.1.3 Dermal Exposure

There were no *in vivo* data available specifically on the absorption of 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT via the dermal route of exposure. Two studies of occupational exposure to Tg-DNT have suggested that dermal absorption can be a significant route of entry for DNT in humans since the levels of urinary metabolites of 2,4- and 2,6-DNT in loaders and operators at a DNT manufacturing plant exceeded those that would have resulted from the inhaled concentrations (Levine et al. 1985b; Woollen et al. 1985). An *in vitro* study examined the dermal absorption of 2,4- and 2,6-DNT in soil or acetone using excised pigskin (Reifenrath et al. (2002). When in acetone, 36 and 24% of the 2,4- and 2,6-DNT, respectively, was absorbed. The absorption of radiolabel from soil was less; 15 or 16%, respectively, was absorbed from a low carbon soil and 5.4 and 3.8%, respectively, was absorbed from a high carbon soil.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution in humans or animals following inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

3.4.2.2 Oral Exposure

The tissue distribution of 2,4-DNT and its metabolites was studied by Rickert and Long (1980). 2,4-DNT was administered orally to male and female rats at doses of 10, 35, or 100 mg of ^{14}C-labeled 2,4-DNT/kg. When distribution is studied solely by detecting a radioisotope label, it is the labeled atom(s) that are being followed, and this label may be part of either the parent DNT molecule or a metabolite. Peak concentrations of radioactivity in plasma, red blood cells, liver, and kidney were proportional to dose. Levels in liver and kidney were 5–10 times higher than those in plasma or red blood cells. Levels of radioactivity in other tissues were lower than those in plasma. The only clear differences between males and females were the higher retention of radioactivity in red blood cells of females and the concentration of radioactivity in livers of females, which was only half that found in males. In addition, concentrations of 2,4-DNT in male kidneys peaked at 4–8 hours and were 3–10 times higher than the concentrations in female kidneys, which peaked 1 hour after the dose.

Rickert et al. (1983) observed that hepatic concentrations of radioactivity in male rats increased in two stages, with the first peak occurring 1–2 hours and a second peak occurring 8–12 hours after an oral

dose of 10 or 35 mg/kg of radiolabeled 2,4- or 2,6-DNT. The second peak was followed by a gradual decline up to 16 days and was thought to be the result of enterohepatic cycling.

In mice administered ^{3}H-labeled 2,6-DNT, the distribution of the label was similar in the blood, liver, kidneys, lungs, and small and large intestines at 8 hours after administration, with very low levels detected in the brain, lungs, heart, and spleen (Schut et al. 1983).

In a radioisotope labeling study in dogs and monkeys, total 2,4-DNT and its metabolites recovered in blood and other tissues were approximately 3.6% (dogs) and 2.2% (monkeys) of the administered dose (U.S. Army 1978b). Relative to blood concentrations, the liver had the highest levels of 2,4-DNT or metabolites. Detectable levels of 2,4-DNT or metabolites were also found in the kidney and in skeletal muscle (U.S. Army 1975, 1978b).

No studies were located regarding distribution in humans or animals following inhalation exposure to 2,3-, 2,5-, 3,4-, or 3,5-DNT.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals following dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

3.4.3 Metabolism

The metabolism of DNTs in humans has been studied in workers exposed to Tg-DNT by the analysis of urinary metabolites. The routes of exposure in these studies were multiple. Since the amounts of metabolites excreted could not be accounted for by the inhalation exposure route alone, dermal contact and ingestion routes of exposure may also be of importance (Levine et al. 1985b; Woollen et al. 1985). Woollen et al. (1985) found that the major metabolite excreted in the urine of workers exposed to Tg-DNT was 2,4-dinitrobenzoic acid (conjugates were hydrolyzed before analysis). There were wide variations in the excretion of the metabolites in different workers. Concentrations of 2,4-dinitrobenzoic acid in end-of-shift urine samples from 20 male and 8 female workers, however, did not suggest a difference in the excretion of this metabolite between males and females. The study authors stated that lesser amounts of the following metabolites were also found in the urine: 2-amino-4-nitro-, 4-amino-2-nitro-, and 2-amino-6-nitrobenzoic acids, and 4-(*N*-acetyl)amino-2-nitrobenzoic acid. Trace levels of

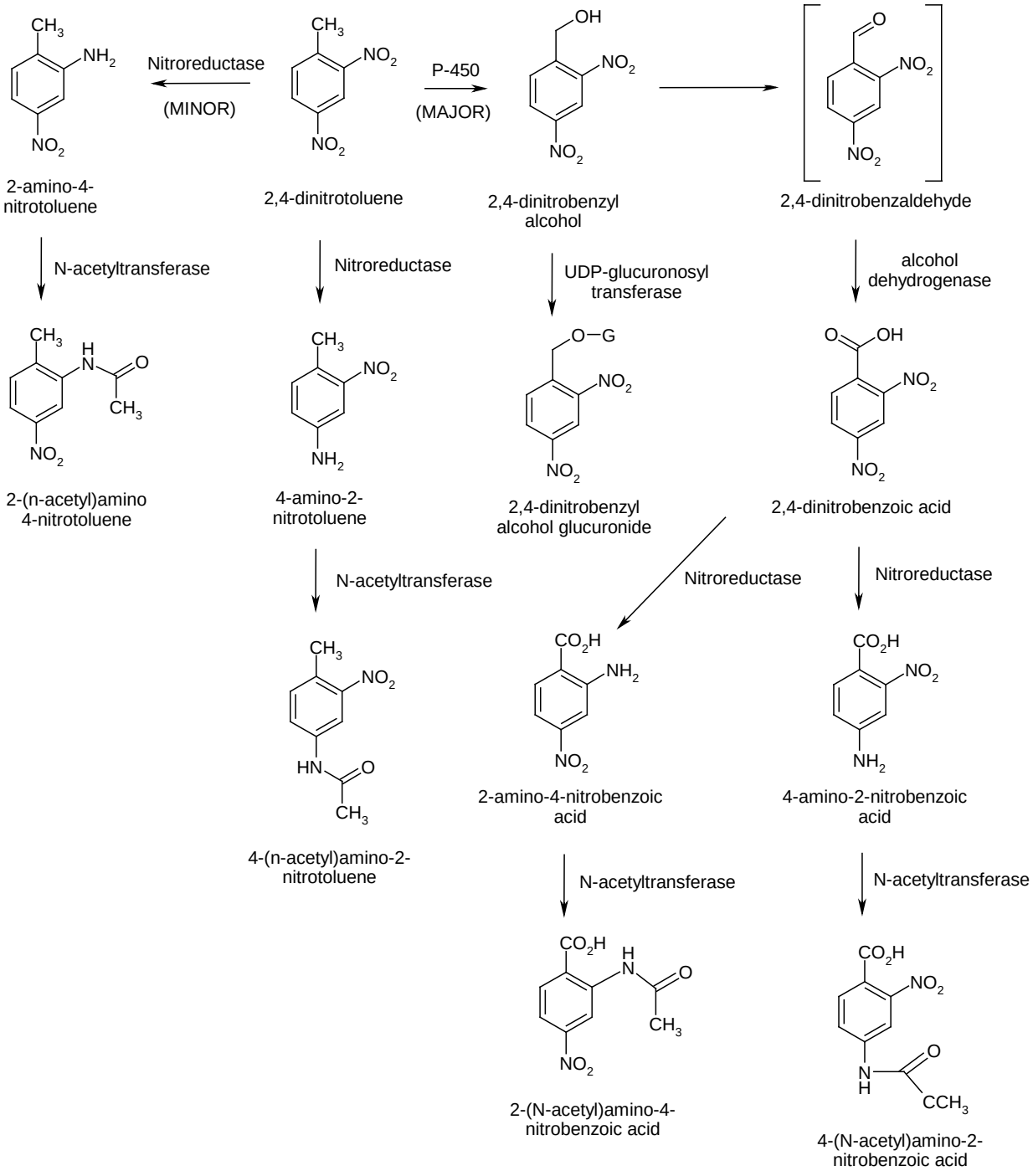
DNTs were also detected. Dinitrobenzyl alcohols were not detected. Neither amounts nor relative percentages of metabolites were reported.

Studies of workers at a Tg-DNT manufacturing plant (Levine et al. 1985b; Turner et al. 1985) provide more detailed information regarding the metabolism of Tg-DNT in occupationally exposed men and women. The principal metabolites detected in the urine of 14 men were dinitrobenzoic acids (2,4- and 2,6-) and 2-amino-4-nitrobenzoic acid. In the urine of three women, these metabolites were detected together with dinitrobenzyl alcohol glucuronides (2,4- and 2,6-). Expressed as percent of total urinary metabolites, the dinitrobenzoic acids, 2-amino-4-nitrobenzoic acid, and the dinitrobenzyl glucuronides constituted 52.5, 37.2, and 9.5%, respectively, of the total urinary DNT metabolites in men and 28.8, 37.6, and 33.3%, respectively, of the total urinary DNT metabolite in women. 2,4-DNT and 2,6-DNT metabolites were present in roughly the same proportions as in the Tg-DNT. Both men and women excreted relatively small amounts (<1% of urinary metabolites) of 2-(*N*-acetyl)amino-4-nitrobenzoic acid (Levine et al. 1985b).

In contrast to the findings in the Levine et al. (1985b) study, 2,4-dinitrobenzoic acid was not one of the primary urinary metabolites in Chinese workers exposed to Tg-DNT and mononitrotoluenes (Jones et al. 2005b). In these workers, 2,6-dinitrobenzoic acid, 4-amino-2-nitrobenzoic acid, 2-amino-4-nitrobenzoic acid, and 2,6-dinitrobenzl alcohol comprised 27.3, 26.0, 21.6, and 18.3%, respectively, of the total DNT metabolites.

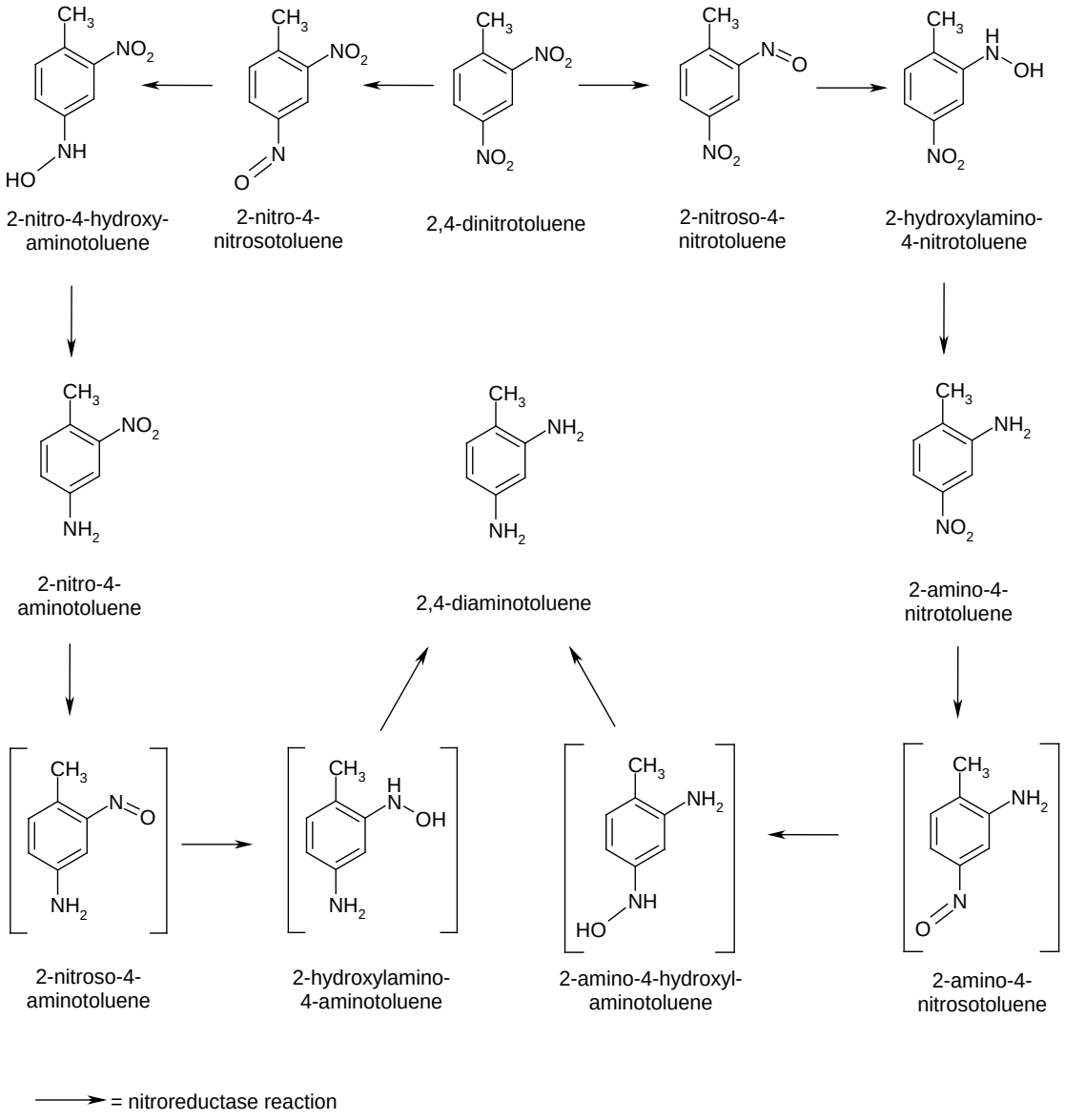
Studies in rats have identified a complex pathway for the metabolism of 2,4-DNT (Figures 3-7 and 3-8) and 2,6-DNT (Figure 3-9). Metabolism occurs in the liver and also in the intestine by microflora (Long and Rickert 1982; Rickert et al. 1981). Both oxidized and reduced metabolites are excreted in the urine after oral administration of the compounds. The main urinary metabolites of 2,4- and 2,6-DNT are the corresponding dinitrobenzyl alcohol glucuronide, dinitrobenzoic acid, and aminonitrobenzoic acid (Long and Rickert 1982). An additional urinary metabolite of 2,4-DNT is 4-(*N*-acetyl)amino-2-nitrobenzoic acid (Rickert et al. 1981). Although very little information on metabolism of 2,3-, 2,5-, or 3,5-DNT was identified, it is anticipated that metabolism of these isomers would follow similar pathways as those identified for 2,4- and 2,6-DNT. Results of an *in vitro* metabolism study using rat liver homogenate show that 2,3- and 2,5-DNT are reduced to monoaminonitrotoluenes and hydroxylaminonitrotoluenes (Kozuka et al. 1978).

Figure 3-7. Proposed Metabolic Pathways for the Hepatic Metabolism of 2,4-Dinitrotoluene



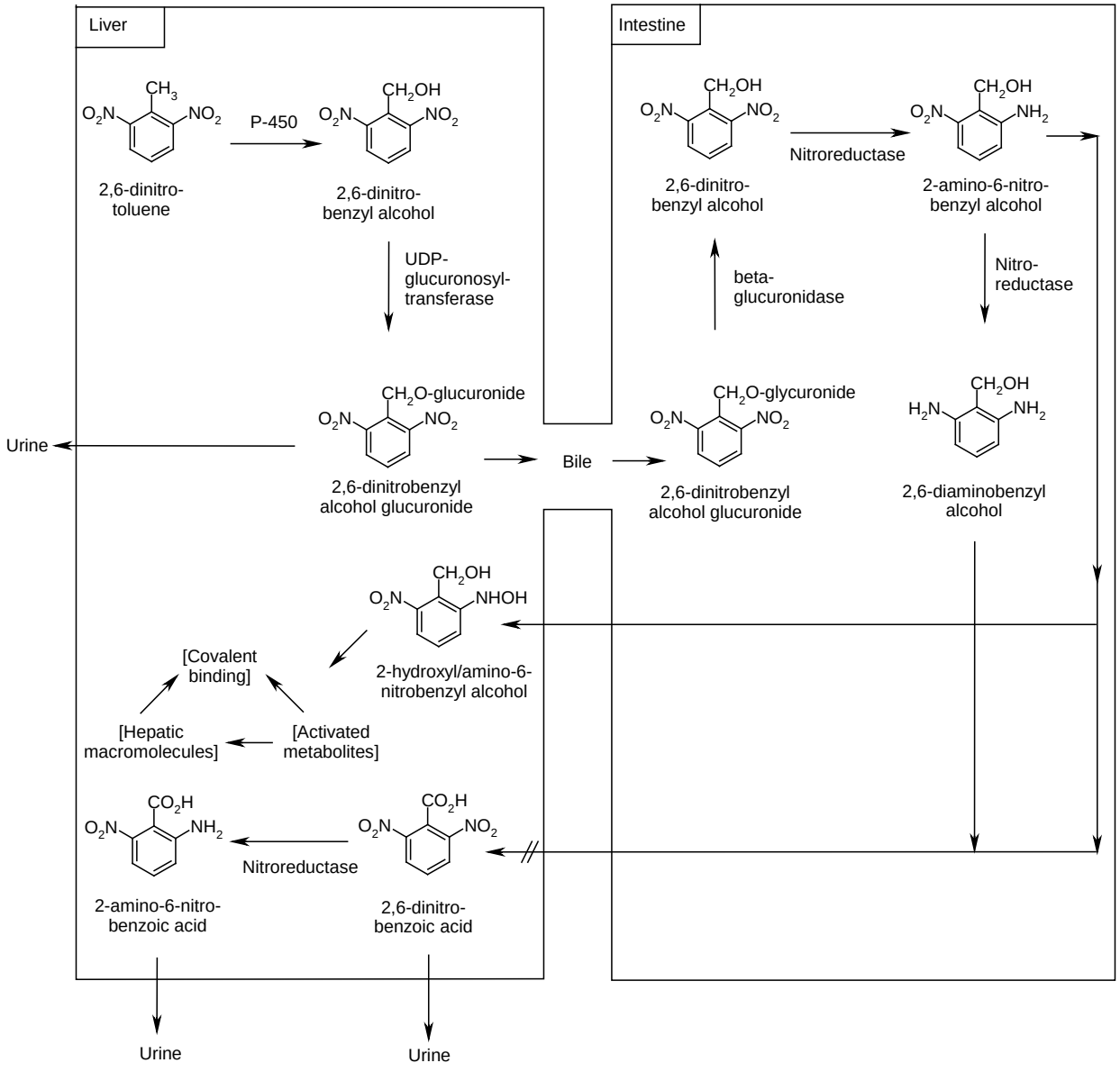
Sources: Bond and Rickert 1981; Bond et al. 1981; Smith et al. 1995

Figure 3-8. Proposed Pathways for the Anaerobic Metabolism of 2,4-Dinitrotoluene in Rat Intestinal Microflora



Sources: Guest et al. 1982; Mori et al. 1985

Figure 3-9. Proposed Pathways for Metabolism of 2,6-Dinitrotoluene



Sources: Chapman et al. 1993; La and Froines 1993; Rickert et al. 1984; Smith et al. 1995

Oxidative metabolism by cytochrome P450 predominates in the liver of experimental animals, leading to the formation of dinitrobenzyl alcohol, which is either converted to glucuronide conjugate or further oxidized to dinitrobenzoic acid. Dinitrobenzyl alcohol glucuronide is partially excreted into the bile, followed by metabolism by gut microflora and enterohepatic cycling (Long and Rickert 1982; Medinsky and Dent 1983; Mori et al. 1997; Rickert and Long 1981). Thus, DNTs appear to be first metabolized by the liver with the metabolites being excreted into the bile; the biliary metabolites are hydrolyzed and further metabolized in the intestine; after reabsorption and circulation back to the liver, the metabolites are activated and bound to macromolecules (Chadwick et al. 1993; Long and Rickert 1982).

2,4- and 2,6-dinitrobenzyl glucuronide have been detected directly in the bile following administration of 2,4- and 2,6-DNT to the male Wistar rat (Mori et al. 1997), accounting for about 35 and 51% of the dose respectively. Four other metabolites, 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,4-diamino-toluene, and 4-acetylamino-2-nitrobenzoic acid accounted for 0.02–0.12% of the dose; in addition to 2,4-dinitrobenzyl alcohol, 2,4-dinitrobenzaldehyde, and 2,4-dinitrobenzoic acid (0.09–0.14%) were detected in the bile of rats given 2,4-DNT. 2,6-Dinitrobenzyl alcohol, 2-amino-6-nitrotoluene, and 2,6-dinitrobenzaldehyde were detected in the bile of rats given 2,6-DNT.

Reductive metabolism of 2,3-, 2,4-, 2,5, and 2,6-DNT occurs by *Escherichia coli* isolated from human intestine (Mori et al. 1984). For all isomers, *E. coli* reduced DNTs to monoaminonitrotoluenes and hydroxylaminonitrotoluenes. Results are consistent with *E. coli* reduction of DNTs via hydroxylamino-nitrotoluenes to monoaminonitrotoluenes. Study authors suggest that reduced metabolites of DNT may play a role in the development of DNT-induced methemoglobinemia and anemia observed in humans and animals.

Studies of the metabolism of 2,4-DNT by intestinal microflora in rats and mice (Guest et al. 1982; Mori et al. 1985) and studies in germ-free rats (Rickert et al. 1981) have shown that intestinal microflora are responsible for reductive metabolism of DNTs. Intestinal microorganisms hydrolyze and reduce 2,4- and 2,6-dinitrobenzyl alcohol glucuronide to the corresponding aminonitrotoluenes, probably through nitroso derivatives and hydroxylamino derivatives (Mori et al. 1997). The deconjugated metabolites are reabsorbed and transported back to the liver by enterohepatic circulation (Medinsky and Dent 1983). In the liver, the newly formed amine group is N-hydroxylated by cytochrome P450 and conjugated with sulfate (Kedderis et al. 1984). The sulfate conjugate is unstable and can be decomposed to form a carbonium or nitrenium ion that can be bound to hepatic macromolecules; this ostensibly leads to mutations and the formation of liver tumors. Thus, sulfation may be involved in the initiation stage of

hepatocarcinogenesis by 2,6-DNT. Metabolism by intestinal microflora appears to be essential for the production of metabolites that bind covalently to liver macromolecules.

The intestinal biotransformation of 2,6-DNT was investigated *in vitro* using suspended microflora preparation from the intestinal contents of male Wistar rats (Sayama et al. 1993). It was determined that the metabolites formed with the incubation of 2,6-DNT were 2-nitroso-, 2-hydroxyl amino-, and 2-amino-6-nitrotoluene and 2,6-diaminotoluene. Since no metabolites were detected when 2,6-diaminotoluene was incubated and the recovery of 2,6-diaminotoluene was about 95%, it appears that 2,6-diaminotoluene is the terminal intestinal metabolite of 2,6-DNT (Sayama et al. 1993). When 2,4-DNT was examined in this system, two nitroazoxy compounds (2,2'-dimethyl-5-5'-dinitroazoxybenzene and 4,4'-dimethyl-3,3'-dinitro azoxybenzene) were detected in addition to other known metabolites, such as nitrosonitrotoluenes, hydroxyl aminonitrotoluenes, aminonitrotoluenes, and diaminotoluene (Sayama et al. 1993). The nitroazoxy compounds were believed to be non-enzymatic products (Sayama et al. 1993).

The metabolites formed by the anaerobic incubation of potassium 2,4-dinitrobenzyl glucuronide or potassium 2,6-dinitrobenzyl glucuronide with rat intestinal microflora have been examined (Mori et al. 1997). Metabolites transformed from 2,4-dinitrobenzyl glucuronide were 2,4-dinitrobenzyl alcohol, 4-amino-2-nitrobenzyl alcohol, and 2-amino-4-nitrobenzyl alcohol, which peaked at 30, 75, and 120 minutes of the incubation. 2,6-Dinitrobenzyl alcohol and 2-amino-6-nitrobenzyl alcohol were detected from potassium 2,6-dinitrobenzyl glucuronide incubation. Thus, intestinal metabolism includes the deconjugation of the glucuronide and the reduction of the nitro compound.

In rats, sex differences in the metabolism of 2,4-DNT have been observed. A larger percentage of the administered dose is excreted in the bile of male rats than is excreted in the bile of females. In females, a greater percentage of the dose is excreted in urine as the dinitrobenzyl alcohol glucuronide (Medinsky and Dent 1983; Rickert and Long 1981). The quantitative differences in urinary versus biliary excretion of the glucuronide conjugates by females may account for the sex differences in the susceptibility of the rat to the hepatocarcinogenic effects of 2,4-DNT (U.S. Army 1979). Greater urinary excretion may decrease the amount of the glucuronide available to the intestinal microflora for metabolism to a carcinogenic metabolite.

Metabolism studies in rats, rabbits, dogs, and monkeys with 2,4-DNT revealed the major urinary metabolites as glucuronide conjugates of 2,4-dinitrobenzyl alcohol (20–33% of the dose) and 2,4-aminonitro-benzyl alcohols (8–19% of the dose). Lesser amounts of aminonitrotoluene, 2,4-diamino-

toluene, 2,4-aminobenzyl alcohol, and 2,4-dinitrobenzoic acid were also identified in all four species. Mice were also evaluated in this same group of studies. In mice, approximately 3% of the administered dose was excreted in the urine as the glucuronide conjugate of the 2,4-dinitrobenzyl alcohol and approximately 3% as the glucuronide conjugates of 2,4-aminonitrobenzyl alcohol (U.S. Army 1978b).

Another study using rats dosed with either 2,4- or 2,6-DNT also demonstrated that the primary urinary conjugate was the respective dinitrobenzyl alcohol glucuronide (11–17% of administered dose) (Mori et al. 1996). Other metabolites in rats administered 2,4-DNT included 2-amino-4-nitrobenzoic acid (0.71%), 4-amino-2-nitrobenzoic acid (0.52%), 4-acetylamino-2-nitrobenzoic acid (3.9%), 4-amino-2-nitrotoluene (0.04%), 2,4-dinitrobenzyl alcohol (0.25%), 2,4-dinitrobenzoic acid (6.9%), and 4-acetylamino-2-aminobenzoic acid (3.4%). After administration of 2,6-DNT, other metabolites in urine included 2,6-dinitrobenzoic acid (0.17%), 2-amino-6-nitrotoluene (0.44%), and 2,6-dinitrobenzyl alcohol (0.53%) (Mori et al. 1996).

The urinary metabolites of DNTs, and probably the glucuronides resulting from occupational exposure of humans, are qualitatively the same as those resulting from oral administration to rats, but the proportions of nitro-reduced metabolites were lower relative to oxidized metabolites in the urine from humans (Turner et al. 1985). These differences may be due more to the particular routes of exposure (inhalation and dermal for humans; oral for rats) than differences in species. As seen in experimental animals, female subjects excreted a higher proportion of urinary metabolites as dinitrobenzyl alcohol glucuronides than did males.

Metabolism of DNTs has not been studied in children. However, fetuses and neonates have been shown to be limited in their ability to biotransform xenobiotics. Although the cytochrome P450 isoforms responsible for DNT metabolism have not been identified, cytochromes CYP2E1, CYP2B1/2, and CYP2C11/6 are known to contribute to the side-chain oxidation of toluene by the rat liver, and multiple cytochrome P450 isoforms may contribute to the side-chain oxidation of DNTs (Chapman et al. 1993). In humans, CYP2E1 protein is absent from fetal and neonatal livers, but steadily increases during the first year of life (Vieira et al. 1996). Other isoforms' expression in fetuses and neonates is also qualitatively and quantitatively different from the expression observed in adults (Komori et al. 1990; Leeder and Kearns 1997). In rats, while sulfotransferase (the enzyme that catalyzes sulfation) activity is almost at adult levels at birth, UDP-glucuronosyltransferase (the enzyme that produces glucuronide conjugates) activity towards different xenobiotics varies with maturation (Young and Lietman 1978). Similarly, in humans, sulfation capabilities develop faster than glucuronidation capabilities (Leeder and Kearns 1997).

While the activity of some isoforms of sulfotransferase may exceed those seen in adults during infancy and early childhood, the activity of UDP-glucuronosyltransferase depends on the specific isoforms of the enzyme, and adult levels are generally attained by 6–18 months (Leeder and Kearns 1997). Since DNTs undergo bioactivation in the liver and by the intestinal microflora, the toxicity of DNTs may be different in children. Newborns have a transient deficiency in methemoglobin reductase (Gruener 1976) and have a high concentration of fetal hemoglobin in their erythrocytes. Consequently, they are highly sensitive to methemoglobin-generating chemicals and to methemoglobinemia generated by DNTs.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

In occupational settings, in addition to inhalation, some oral and dermal exposure can occur. The elimination of DNTs in the urine of workers exposed to Tg-DNT has been studied by several investigators (Levine et al. 1985b; Turner et al. 1985; Woollen et al. 1985).

Woollen et al. (1985) observed that the highest rates of excretion of 2,4-dinitrobenzoic acid occurred near the end of the work shift. The half-life for urinary excretion of 2,4-dinitrobenzoic acid was calculated to be 2–5 hours. This estimate appears to be the initial phase of a biphasic elimination profile since even 3 days after the last exposure, detectable levels of 2,4-dinitrobenzoic acid were present in urine.

Turner et al. (1985) determined the metabolic profiles in workers exposed to DNTs. The half-life for excretion of DNT metabolites in urine ranged from 0.8 to 4.5 hours. The half-lives for 2,4-dinitrobenzoic acid and 2,4-dinitrobenzyl alcohol glucuronide tended to be shorter than those for the metabolites that resulted from both oxidative and reductive metabolism.

No studies investigating elimination and excretion of 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT following inhalation exposure of humans or animals were located.

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans following oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

Schut et al. (1983) reported that in mice, urine was the main route of elimination of ^{3}H-labeled 2,6-DNT, with about 50% excreted after 8 hours. U.S. Army (1978b) observed that most of the radioactivity from ^{14}C-labeled 2,4-DNT administered to mice was excreted in the feces and only about 10% in the urine. Differences between these two studies could be due, in part, to the use of different species of mice.

Male and female rats excreted 55–90% of the radioactivity from ^{14}C-2,4-DNT or ^{14}C-2,6-DNT in the urine, and 15–30% in the feces, within 72 hours after dosing (Long and Rickert 1982; Rickert and Long 1981). With 2,4-DNT, the females excreted a greater percentage of the dose in the urine as 2,4-dinitrobenzylalcohol glucuronide than did the males (except at the highest dose), but with 2,6-DNT, no sex-related difference in urinary excretion was seen.

In experiments with bile duct-cannulated rats, male rats excreted 25% of the radioactivity from ^{14}C-2,4-DNT into the bile over a 36-hour period, whereas female rats excreted 18% (Medinsky and Dent 1983). Biliary excretion of radioactivity was linearly related to dose in males; females were evaluated only at one dose. Biliary excretion of radioactivity was virtually complete within 24 hours for males and 12 hours for females. Mean half-times of biliary excretion ranged from 3.3 to 5.3 hours. Urinary excretion was also significant, with greater amounts of radioactivity excreted in the urine of rats from which bile was not collected (60–90% of the dose) than in the urine of rats from which bile was collected (20–60% of the dose). This finding indicates that biliary metabolites were absorbed from the intestines (enterohepatic cycling). Whether or not bile was collected, female rats excreted more radioactivity in urine than did male rats. Greater than 90% of the urinary excretion of labeled metabolites appeared in urine collected during the first 24 hours. At the end of 36 hours, only 0.02–0.05% of the radioactivity was detectable in the livers; 20–60% of this was covalently bound.

No studies investigating elimination and excretion of 2,3-, 2,5-, 3,4-, or 3,5-DNT following oral exposure of humans or animals were located.

3.4.4.3 Dermal Exposure

There are no kinetic data in humans in which the route of exposure was specifically dermal. Occupational exposure studies available for Tg-DNT involved multiple routes of exposure (Levine et al. 1985b; Turner et al. 1985; Woollen et al. 1985). The major routes of exposure in these studies were considered to be inhalation and dermal. The results were discussed previously in Section 3.4.4.1.

No studies were located regarding excretion in animals following dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

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

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

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-10 shows a conceptualized representation of a PBPK model.

If PBPK models for 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

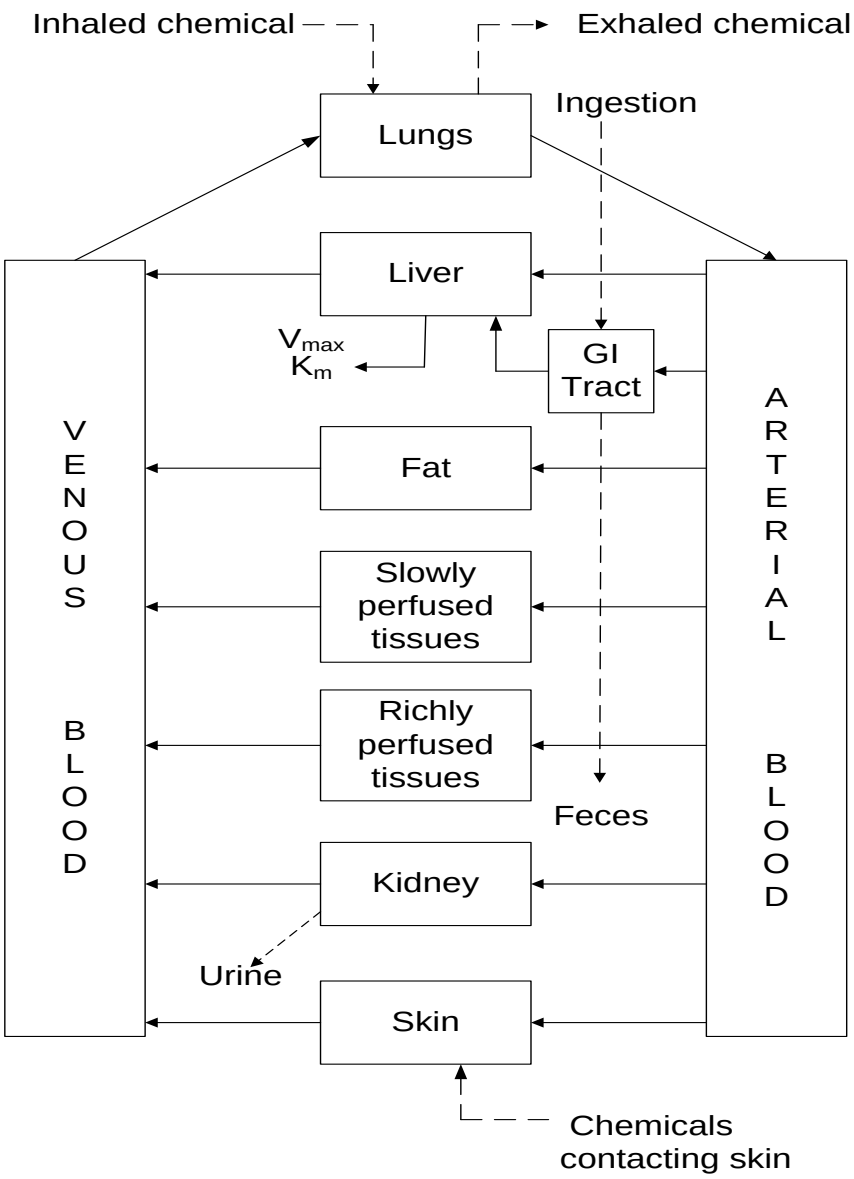
A PBPK model has not been developed for 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

No information was located regarding the mechanism of absorption of 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT. It is known that absorption occurs after inhalation exposure based on the metabolites found in the urine of workers at DNT manufacturing plants (Levine et al. 1985b; Turner 1986; Woollen et al. 1985). In studies of rats, rabbits, dogs, and monkeys, most orally administered 2,4- or 2,6-DNT has been shown to be absorbed (Long and Rickert 1982; Rickert and Long 1981; U.S. Army 1978b). There appears to be minimal accumulation of these compounds after a single exposure. After repeated oral exposure in rats, 2,4-DNT and its metabolites were preferentially distributed to the liver, kidney, brain, lung, and skeletal muscle. The primary metabolite of 2,4-DNT excreted by humans exposed via inhalation and dermal routes of exposure in occupational studies or animals exposed via the oral route is 2,4-dinitrobenzyl alcohol and/or its glucuronide (EPA 1992). In addition to this, humans also excrete

Figure 3-10. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: Krishnan and Andersen 1994

2-amino-4-nitrobenzyl alcohol in the urine. 2,4-Dinitrobenzoic acid is another major metabolite (EPA 1992). Both 2-nitroso-4-nitrotoluene and 2-amino-4-nitrotoluene, metabolites of 2,4-DNT in humans, have been shown to be mutagenic *in vitro* (EPA 1992). It has been suggested that these intermediates may bind covalently to hepatic macromolecules, such as DNA and RNA (EPA 1992).

3.5.2 Mechanisms of Toxicity

Effects of Metabolism on Toxicity. The primary mechanism of toxicity for DNTs involves bioactivation to form reactive intermediates (Kedderis et al. 1984; Sayama et al. 1989). Detailed information on the biotransformation of DNTs is presented in Section 3.4.3. Briefly, metabolism of DNTs begins in the liver, where it is oxidized by cytochrome P450 and conjugated with glucuronic acid to form the major metabolite, dinitrobenzyl alcohol glucuronide, and is excreted in bile or urine (Long and Rickert 1982; Medinsky and Dent 1983). The glucuronide excreted in bile undergoes biotransformation by intestinal microflora, where the conjugate is hydrolyzed and subsequently reduced by nitroreductase to the corresponding aminonitrobenzyl alcohol (Chadwick et al. 1993; Guest et al. 1982; Mori et al. 1985), probably through nitroso derivatives and hydroxylamino derivatives. The deconjugated metabolites are reabsorbed and transported back to the liver by enterohepatic circulation (Medinsky and Dent 1983). In the liver, the newly formed amine group is N-hydroxylated by cytochrome P450 and conjugated with sulfate (Kedderis et al. 1984). The sulfate conjugate is unstable and can be decomposed to form a carbonium or nitrenium ion that can be bound to hepatic macromolecules; this ostensibly leads to mutations and the formation of liver tumors. Thus, sulfation may be involved in the initiation stage of hepatocarcinogenesis by 2,6-DNT.

Target Organ Toxicity. The mechanism of toxicity of the hematological effects of DNTs is described by U.S. Army (1979). The effect of DNTs on the blood is also produced by aromatic amines and most organic and inorganic nitrates. These compounds or their metabolites oxidize the ferrous ion in hemoglobin and produce methemoglobin. Hydroxylamine is probably the oxidizing species, because it is an intermediate in the reduction of nitro to amines. Within limits, the body can correct methemoglobinemia, but the corrective measures can be overwhelmed, producing numerous secondary effects including anoxia. The presence of methemoglobin leads to the formation of aggregates of hemoglobin degradation products called Heinz bodies. The presence of Heinz bodies is a sensitive indicator of blood toxicity as it indicates that some hemoglobin has been destroyed. High levels of methemoglobin are removed by catabolism, leading to the development of anemia. The body compensates for the destruction of red blood cells by increasing erythrocyte production, resulting in large numbers of immature erythrocytes, called

reticulocytes, in the blood. If the toxic dose is not too severe, these compensatory mechanisms suffice. Thus, "compensated anemia," normal erythrocyte levels with reticulocytosis, may exist in exposed individuals. When the production of red blood cells can no longer keep pace with the hemolysis, frank anemia may be present (U.S. Army 1979).

As discussed in Section 3.2.2.7 (Oral Exposure; Cancer), 2,4-, 2,6-, or Tg-DNT have been shown to induce hepatocellular carcinoma following chronic-duration oral exposure. Thus, hepatic effects observed at less than chronic exposure durations or at lower doses may represent early stages of progressive development to hepatic cancer or threshold level effects for development of cancer; however, the mechanism of toxicity for hepatic effects has not been elucidated. A study examining changes in gene expression in the livers of female rats administered 2,4-DNT at 5–398 mg/kg/day or 2,6-DNT at 5–199 mg/kg/day via gavage as a single dose showed that treatment with these compounds perturbed pathways involved in DNA damage response, cell death signaling, detoxification and lipid metabolism, the oxidative stress response, and the immune response 24 and 48 hours after dosing (Deng et al. 2011). There are limited data on the mechanisms of hepatotoxicity for the six isomers. Spanggord et al. (1990) exposed rat hepatocytes to each of the six isomers of DNT *in vitro* and observed greater toxicity to hepatocytes (based on dose-response relationships for protein synthesis and LDH release) from 2,3-, 2,5-, and 3,4-DNT than from 2,4-, 2,6-, or 3,5-DNT.

The mechanisms of toxicity for reproductive effects (testicular atrophy, degeneration of the seminal vesicles, and decreased sperm production) observed in animal studies (Bloch et al. 1988; U.S. Army 1976, 1978b, 1979) have not been elucidated. However, data from i*n vitro* studies indicate that testicular degeneration may be associated with structural changes in Sertoli cells (Reader and Foster 1990). Bloch et al. (1988) reported that diminished sperm counts in rats acutely exposed to 2,4-DNT were accompanied by fine structural alterations of Sertoli cells. Increased serum levels of FSH, also indicative of Sertoli cell malfunction, were also reported (Bloch et al. 1988). Reader and Foster (1990) reported that Sertoli cell cultures prepared from the testes of Wistar rats and treated with up to 100 µM 2,3-, 2,4- or 2,6-DNT remained intact; however, cells treated with 2,3-, 2,4-, or 2,6-DNT showed some evidence of damage (absence of germ cells and some Sertoli cells containing cytoplasmic vacuoles). Treatment of the Sertoli cell cultures with 100 µM 3,4-DNT, in contrast, resulted in subconfluent monolayers with a significant decrease in total cellular protein, as well as stellate appearance and prominent cytoplasmic vacuolation. Germ cell detachment from Sertoli germ cell cocultures was significantly increased ($p<0.05$) compared to controls at 10 µM 2,4- or 2,6-DNT and at 0.01 µM 2,3- or 3,4-DNT (Reader and Foster 1990). Further evidence of disruption of Sertoli cell function was observed as increased production of lactate and

pyruvate (including marked increases in pyruvate production with exposure to 3,4-DNT), although increases in pyruvate production were minimal with 2,6-DNT (Reader and Foster 1990). Another study showed that adult immortalized rat Sertoli cells exposed to 2,4-DNT at 25–200 µmol/L showed increased numbers of autophagosomes and autophagic lysosomes, which may signal the removal of cellular components damaged by exposure or impending apoptosis (Sorenson and Brabec 2003). Thus, it appears that structural changes in the Sertoli cells may be precipitating events responsible for reproductive effects observed in animals exposed to DNTs.

The effects of 2,4-DNT on body weights of animals may be mediated through PPARα signaling. Wilbanks et al. (2014) observed decreased body weight and lower swim-to-exhaustion times in wild type C57Bl/6N mice exposed to 134 mg/kg/day 2,4-DNT via gavage for 14 days, while these effects were much less severe in exposed PPARα-null mice. Additional support for the role of PPARα signaling was shown by the decreases in expression of genes downstream of this signaling pathway (especially those involved in fatty acid transport) in wild type mice. Wilbanks et al. (2014) proposed that impairment of the PPARα signaling pathway was a key event in the mechanism of 2,4-DNT-induced effects on energy metabolism and weight loss.

Carcinogenesis. The mechanisms of DNT-induced carcinogenicity have not been described. However, genotoxicity assays *in vitro* and *in vivo* indicate that DNT isomers have the potential to induce gene mutations and other forms of DNA damage (DNA adduct formation, chromosomal aberrations, and UDS). The mechanism underlying the development of renal tumors in CD-1 male mice exposed to oral 2,4-DNT at 95 mg/kg/day for 2 years (U.S. Army 1979) has not been determined. However, workers exposed occupationally to explosives containing 30% Tg-DNT with renal cancer typically showed pathological patterns of urinary excretion, indicated by the molecular weight of excreted proteins (i.e., increased excretion of tubular proteins of low molecular weight and glomerular leakage with excretion of high molecular weight proteins), and/or specific biomarkers of damage to the proximal tubule of the nephron (α_1-microglobulin and glutathione-S-transferase α). These data are consistent with the hypothesis that renal tumors are initiated by DNT isomers and that promotion occurs via damage to the proximal tubule of the nephron (Brüning et al. 2001).

In hepatic tumor initiation-promotion experiments, Tg-DNT and 2,6-DNT were found to have tumor-promoting and tumor-initiating activity; 2,4-DNT showed only tumor-promoting activity (Leonard et al. 1983, 1986; Mirsalis and Butterworth 1982; Popp and Leonard 1982). 2,6-DNT was indicated to be a complete hepatocarcinogen and is primarily responsible for the carcinogenic activity of Tg-DNT. Hepatic

DNA adducts have been detected by ^{32}P-postlabeling technique in 2,6-DNT-treated B6C3F$_1$ mice and F344 rats (George et al. 1996). In male Wistar rats administered 2,4- or 2,6-dinitrobenzyl glucuronide, the major compounds excreted in the bile after treatment with 2,4- or 2,6-DNT, secondary metabolism led to the production of genotoxic agents in the urine and bile (Mori et al. 2000). 2,4-Dinitrobenzaldehyde (mutagenic in the Ames assay using *S. typhimurium* strains TA98 and TA100) and 2,4-diaminotoluene (carcinogenic) were detected in the urine and bile of 2,4-dintrobenzyl glucuronide-treated rats; 2-amino-6-nitrobenzyl alcohol (genotoxic) was detected in 2,6-dintrobenzyl glucuronide-treated rats. The formation of these metabolites may contribute to the hepatocarcinogenesis induced by 2,4- and 2,6-DNT. 2,6-Dinitrobenzaldehyde, another metabolite of 2,6-DNT, was found to be a direct-acting mutagen in the *S. typhimurium* strain TA98 and TA100 systems, not requiring metabolic activation by the S9 mix. 4-Amino-2-nitrobenzyl alcohol, 2-amino 4 nitrobenzyl alcohol, and 2-amino-6-nitrobenzyl alcohol are also mutagenic metabolites of 2,4- and 2,6-DNT, with their mutagenicity requiring metabolic activation (Mori et al. 1982; Sayama et al. 1989). Kedderis et al. (1984) proposed a bioactivation mechanism relating to the genotoxicity of 2,6-DNT in male F344 rats. They showed that the active metabolite of 2,6-DNT in the male F344 rat is the hydroxylamino sulfate of aminonitrobenzyl alcohol formed by the intestinal metabolism of benzyl glucuronide of 2,6-dinitrobenzyl alcohol excreted in bile. The sulfate conjugate is unstable, and the formation of electrophilic carbonium or nitrenium ions from these conjugates leads to subsequent binding to DNA.

3.5.3 Animal-to-Human Extrapolations

Correlation of toxic effects between humans and animals for 2,4- and 2,6-DNT with regard to hematologic and neurological effects has been noted (Ellis et al. 1985; Hong et al. 1985; Lane et al. 1985; Lee et al. 1985; McGee et al. 1947; U.S. Army 1978b, 1979). Other effects for 2,4- and 2,6-DNT, such as reproductive, hepatic, renal, and cancer have been noted in animals (Ellis et al. 1985; Hong et al. 1985; Lee et al. 1985; Leonard et al. 1983, 1986; McGown et al. 1983; Stoner et al. 1984; U.S. Army 1976, 1978b, 1979), but insufficient data are available to state definitively whether they are effects in humans. Two mutagenic metabolites of 2,4-DNT have been found in humans, mice, and rats (EPA 1992). Although rats appear to be more sensitive to the effects of 2,4-, 2,5-, 2,6-, and 3,4-DNT than mice (Hong et al. 1985; Lane et al. 1985; U.S. Army 1975, 1978a, 1978b; Vernot et al. 1977), dogs appear to be the most sensitive of the three species (Ellis et al. 1985; U.S. Army 1976, 1978b, 1979). However, limited intermediate-duration data using 2,6-DNT have shown mice to be more sensitive than rats (U.S. Army 1976). It should be noted that dogs were fed DNTs by capsule in experimental studies, whereas the

rodents received the test chemical in feed (Ellis et al. 1985; Hong et al. 1985; Lee et al. 1985; U.S. Army 1975, 1976, 1978b, 1979).

Extrapolating animal toxicity data to predict human risk from exposure to 2,4- and 2,6-DNT appears to be reasonable because of qualitative similarities in metabolism and known toxic effects. Based on what is known about these two isomers, extrapolation from animal toxicity data to predict human risk from exposure to the remaining isomers (2,3-, 2,5-, 3,4-, and 3,5-DNT) is also assumed to be reasonable.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought

to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding endocrine disruption in humans and/or animals after exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT.

No *in vitro* studies were located regarding endocrine disruption of 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek

1966; Widdowson and Dickerson 1964). The fetus/infant has an immature (developing) blood-brain barrier that past literature has often described as being leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the blood-brain barrier, there are differences between fetuses/infants and adults which are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; as it raises a very important toxicological question as to whether these mechanisms provide protection for the developing brain or do they render it more vulnerable to toxic injury. Each case of chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also

have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

No specific health effects resulting from DNT exposure have been observed in children. Generally, health effects observed in adults should also be of potential concern in children.

No direct information is available regarding the effects of DNTs on the developmental process in humans, and there are few developmental studies on animals. When Tg-DNT was administered by gavage to pregnant rats for 14 days during gestation, and pups were evaluated for developmental toxicity either at gestation day 20 or postpartum day 60 (Jones-Price et al. 1982), adverse effects on hematologic parameters and altered organ weights were observed in both dams and fetuses when dams were administered 100 or 150 mg/kg/day. However, the fetal toxicity was not dose-related. A decrease in relative liver weight was observed in the postpartum pups at the low dose of 14 mg/kg/day. Dose-related effects on postnatal development were not observed in pups when dams were administered 35 or 75 mg/kg/day.

No consistent changes were observed in the number of preimplantation losses, implantation sites, or living or non-living fetuses in male Sprague-Dawley rats gavaged with 2,4 DNT at 0, 60, 180, or 240 mg/kg/day for 5 days (Lane et al. 1985). Exposure of male animals to DNTs does not cause dominant lethal mutation or increases in the proportion of nonviable conception (U.S. Army 1979).

DNTs have been found to be genotoxic using *in vivo* test systems (Ashby et al. 1985; Huang et al. 1995; Mirsalis et al. 1989). Although 2,6-DNT itself showed no mutagenicity towards *S. typhimurium* strains TA98 and TA100 with or without activation by S9 mix, 2,6-dinitrobenzaldehyde, a metabolite of 2,6-DNT, was found to be a direct-acting mutagen, not requiring metabolic activation (Sayama et al. 1989). The reason that DNTs are not shown to bind to DNA and cause mutations in most of the short-term *in vitro* assays for genotoxicity is that the formation of DNA-reactive DNT metabolites involved several different biotransforming enzymes in the intestinal microflora and in the liver. However, DNTs

did not cause dominant lethal mutation or increases in the proportion of nonviable conceptions following exposure of male animals (U.S. Army 1979), so it is not clear if the genotoxic form of DNT might potentially reach the germ cells following oral, inhalation, or dermal exposure.

It is unlikely that DNTs and its metabolites will accumulate in maternal tissues because of its low octanol-water partition coefficient. No studies are available that demonstrate DNTs or their metabolites cross the placenta or get into breast milk. Thus, it is unlikely that the developing fetus or nursing infant would be exposed to DNTs as a consequence of maternal exposure prior to gestation. However, developmental toxicity from DNTs could potentially occur because of its ability to deplete the amount of oxygen available to the developing fetus. Pregnant women and their fetuses may be susceptible to the oxygen depletion implied by the hematotoxicity of DNTs based on a study of rats (Jones-Price et al. 1982). Newborns have a transient deficiency in methemoglobin reductase which reduces methemoglobin back to hemoglobin (Gruener 1976). They also have a high concentration of fetal hemoglobin in their erythrocytes (Smith 1996). Thus, newborns are unusually sensitive to methemoglobin-generating chemicals such as DNT. The metabolism of DNTs has not been studied in children or appropriate animal models. However, while some of the enzymes involved in DNT metabolism reach or exceed adult levels during infancy and early childhood, other enzymes such as UDP-glucuronosyltransferase may attain adult levels by 6–18 months of age (Leeder and Kearns 1997). Thus, the toxicity of DNTs may be different in children.

3.8 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 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. 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 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT are discussed in Section 3.8.1.

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 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by DNTs are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

There are no biomarkers of exposure or effects that have been validated in children or in adults exposed as children.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to DNTs

Workers exposed to DNTs in a manufacturing plant excreted 2,4-DNT, 2,6-DNT, and their metabolites in the urine (Levine et al. 1985b). The concentrations of DNT in air ranged from 0.1 to 5.9 mg/m^3. Concentrations of DNTs and metabolites ranged from 1.68 to 16.74 mg/day (or 1.74–17.31 mg/L, based on an average daily urine volume of 967 mL), with widespread daily variations. Estimates of inhaled DNTs ranged from 0.5 to 4.9 mg/day, less than the total excreted suggesting that dermal exposure, and possibly oral exposure, contributed to the body burden of DNTs. Jones et al. (2005b) did not find a significant correlation between the levels of air concentrations of 2,4- and 2,6-DNT and the sum of urine

metabolites among workers at a DNT and mononitrotoluene manufacturing facility. The 8-hour TWA air concentrations were 0.043 and 0.014 mg/m^3 for 2,4- and 2,6-DNT, respectively.

Woollen et al. (1985) determined that the urinary concentration of a Tg-DNT metabolite, 2,4-dinitro-benzoic acid, was <1 mg/L at the beginning of the work week and ranged from 3.4 to 41 mg/L at the end of the shift. Atmospheric DNT levels of undetectable to 0.03 mg/m^3 were monitored with personal air samples. Static samples near dusty process areas monitored were 0.02–2.68 mg/m^3. The study authors estimate that inhalation exposures ranged from 1 to 14 mg/day. As in Levine et al. (1985b), inhalation exposure does not account for the entire amount of DNT metabolites excreted in urine. Dermal and ingestion exposures are, therefore, likely to have occurred.

Significant correlations between urinary levels of hemoglobin adducts and urinary metabolites were found for 2,4- and 2,6-DNT in workers exposed to Tg-DNT (Sabbioni et al. 2006). The hemoglobin adducts resulting in exposure to 2,4-DNT were 4-amino-2-nitrotoluene and 2,4-toluenediamine; the hemoglobin adducts from 2,6-DNT exposure were 2-amino-6-nitrotoluene and 2,6-toluenediamine. The best correlations between urinary metabolites and hemoglobin adducts were 2,4-dinitrobenzyl alcohol and 2,6-dinitrobenzyl alcohol with hemoglobin 2-4-nitrotoluene and hemoglobin-2-amino-6-nitrotoluene, respectively.

3.8.2 Biomarkers Used to Characterize Effects Caused by DNTs

After exposure to DNTs, methemoglobin levels in the blood may be elevated (Bentur and Keyes 2004). The methemoglobinemia present may be quite profound and its onset is often delayed by up to 4 hours (Bentur and Keyes 2004). Another hematological change that might be present in individuals who have undergone repeated or prolonged exposure to DNTs is that which is consistent with Heinz bodies and hemolytic anemia.

DNA adducts have been found in the livers of rats treated orally with either 2,4- or 2,6-DNT (La and Froines 1992, 1993). The formation of DNA adducts is believed to be indicative of carcinogenic risk.

Decreased spermatogenesis has been reported in treated rats, mice, and dogs (Bloch et al. 1988; U.S. Army 1979). However, decrease in sperm counts in workers exposed to DNTs has been reported in only one study (CDC 1981).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Perkins (1919) reported that "alcoholic subjects have very little resistance to DNT."

Exposure of male rats to 2,6-DNT for 5 days reduced the rate of metabolism of phenobarbital; exposure to 2,6-DNT for 4 weeks increased phenobarbital metabolism (Short and Lee 1980). Exposure of rats to 2,4-DNT did not affect the rate of phenobarbital metabolism.

The effects of the herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), on 2,6-DNT genotoxicity were examined in male weanling F344 rats (George et al. 1992). The rats were treated orally with 54.4 mg/kg 2,4,5-T for 4 weeks, then with 75 mg/kg 2,6-DNT, 1, 2, or 4 weeks after the first dose of 2,4,5-T; urine was then collected for 24 hours. In animals treated for 1 week with 2,4,5-T, there was a decrease in transformation of 2,6-DNT to mutagenic metabolites in the urine, but there were no changes in intestinal enzyme activities (George et al. 1992). Longer treatments with 2,4,5-T did not alter urine genotoxicity compared to controls, and there was a transient increase in cecal azo reductase and nitroreductase after 2 weeks with a decrease in intestinal β-glucuronidase activity, but all levels were normal after 4 weeks.

Pretreatment of rats with alachlor for 3 weeks prior to a single oral administration of 75 mg/kg 2,6-DNT resulted in the production of genotoxic metabolites, as evaluated in a *S. typhimurium* histidine reversion bioassay in the absence of S9 activation (George et al. 1998). Significant increases, compared to 2,6-DNT-only exposed rats, were not observed in rats exposed to alachlor for 1 or 5 weeks.

Interaction of DNTs with other chemicals has not been observed in children.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to DNTs than will most persons exposed to the same level of DNTs in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of DNTs, or compromised function of organs affected by DNTs. Populations who are at greater risk due to their unusually high exposure to DNTs are discussed in Section 6.7, Populations with Potentially High Exposures.

Several studies have examined the possible association between DNT toxicity and genetic polymorphisms. Sabbioni et al. (2006) found that workers exposed to DNTs and TNT with the

glutathione S-transferase T1 null genotype or *N*-acetyltransferase 1 fast acetylators genotype had significantly more chromatid-type chromosomal aberrations than controls of the same genotypes. Workers with the sulfotransferase 1A1 Arg/Arg or sulfotransferase 1A2 Asn/Asn genotypes or *N*-acetyltransferase 1 fast acetylators had significantly more total aberrations than controls with the same genotypes. These results suggest differences in susceptibility to DNT toxicity. In contrast, Brüning et al. (1999) found that all of the workers at a copper mine using Tg-DNT explosives with urothelial cancer were slow acetylators.

Humans sensitive to DNTs may include individuals with cardiovascular problems. Hematological effects associated with exposure to 2,4-DNT may place persons with anemia, including sickle cell anemia or other diseases of the blood, at an increased risk.

Persons with chronic neurological disorders may also have an increased sensitivity to DNT exposure. Although there are insufficient data available to draw firm conclusions, it appears that pregnant women and their fetuses may be susceptible to the oxygen depletion implied by the hematotoxicity of DNTs based on a study on rats (Jones-Price et al. 1982). Although it has been reported that alcoholics may have a decreased resistance to the effects of Tg-DNT (Perkins 1919), the extent of this compromise has not been determined.

The susceptibility of children to the health effects of DNTs may be different from that of adults, as discussed in Section 3.7.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

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

Bentur Y, Keyes DC. 2004. Explosives. In: Dart RC, ed. Medical Toxicology. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 1258-1279.

Currance PL, Clements B, Bronstein AC. 2007. Dinitrophenol and related compounds. In: Emergency care for hazardous materials exposure. St. Louis, MO: Mosby Jems, 335-338.

There are no known pediatric-specific methods for reducing peak absorption following exposure, reducing burden, or interfering with the mechanism of action for toxic effects.

3.11.1 Reducing Peak Absorption Following Exposure

Limited information from humans indicates that DNTs is absorbed after inhalation exposure, while animal data suggest that DNTs are rapidly and completely absorbed after oral exposure. Efforts to reduce absorption following acute exposure to DNTs should focus on removing the individual from the site of exposure and decontaminating exposed areas of the body. Contaminated clothing and jewelry should be removed and skin should be washed with soap and water; eyes exposed to DNTs should be immediately irrigated with water and normal saline (Bentur and Keyes 2004; Currance et al. 2007). If ingestion of DNTs occurs, emetics are not recommended due to the potential for central nervous system depression; the mouth should be rinsed, and water can be administered for dilution if the patient can swallow, has a good gag reflex, and is not drooling (Bentur and Keyes 2004; Currance et al. 2007). Gastric lavage and administration of activated charcoal may be considered shortly following ingestion provided airways are protected (Bentur and Keyes 2004; Currance et al. 2007).

3.11.2 Reducing Body Burden

There are no data to support the use of hemodialysis, forced diuresis, hyperbaric oxygen, or hemoperfusion for treatment of methemoglobinemia alone, but these treatments may provide adjunctive care after DNT ingestion when supportive care is inadequate (Bentur and Keyes 2004).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Methods for reducing DNT-induced methemoglobinemia (Bentur and Keyes 2004) are the same as those recommended for treating methemoglobinemia caused by overexposure to nitrate and/or nitrite (Agency for Toxic Substances and Disease Registry 2013; Leikin and Paloucek 2008; Seifert 2004). Severe methemoglobinemia (methemoglobin levels generally >30% of total hemoglobin) can be reduced by intravenous administration of methylene blue (1–2 mg/kg). Exchange transfusions may be considered for patients who do not respond to methylene blue (particularly patients with glucose-6-phosphate dehydrogenase deficiency or hemoglobin M), and patients where methylene blue is contraindicated (e.g., patients on serotonin uptake inhibitors). In symptomatic patients, 100% oxygen and assisted ventilation should be considered; seizures can be treated with oxygen and benzodiazepines, followed by

phenobarbital (Seifert 2004). Hyperbaric oxygen therapy may be of some benefit, but has not been demonstrated in controlled studies (Leikin and Paloucek 2008; Seifert 2004).

3.12 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 DNTs 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 DNTs.

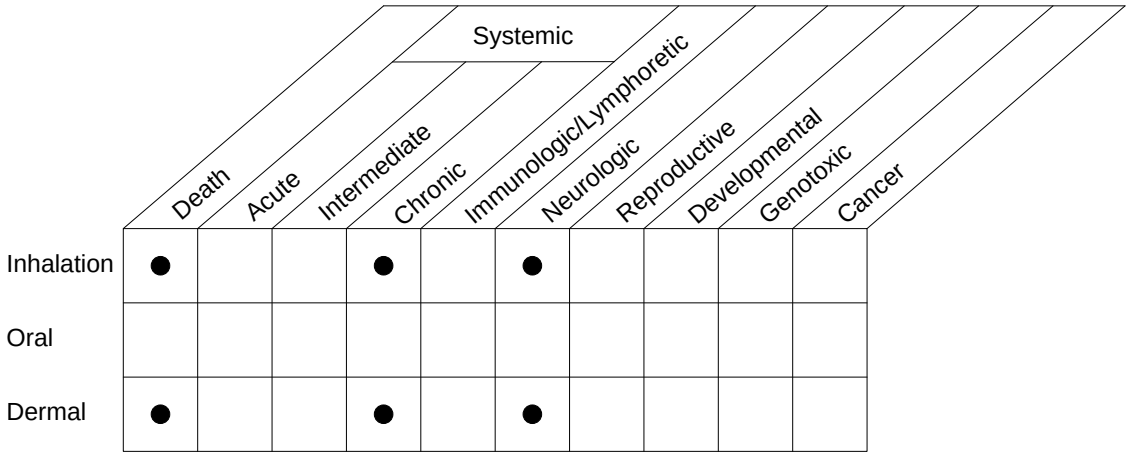
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean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

3.12.1 Existing Information on Health Effects of DNTs

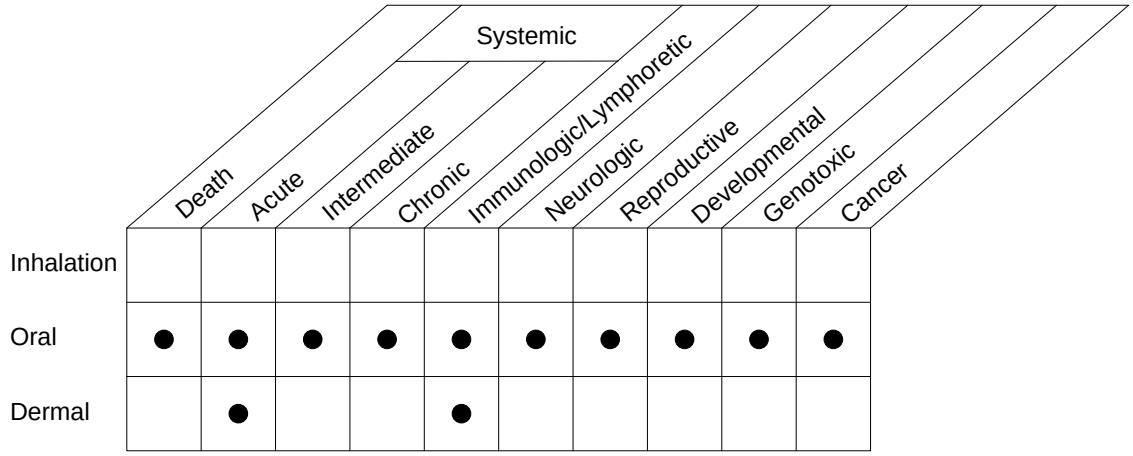
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 2,4-DNT; 2,6-DNT; 2,3-, 2,5-, 3,4-, and 3,5-DNT; and Tg-DNT are summarized in Figures 3-11, 3-12, 3-13, and 3-14, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of 2,4-, 2,6-, 2,3-, 2,5-, 3,4-, 3,5-, and Tg-DNT. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments.

Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 3-11. Existing Information on Health Effects of 2,4-Dinitrotoluene



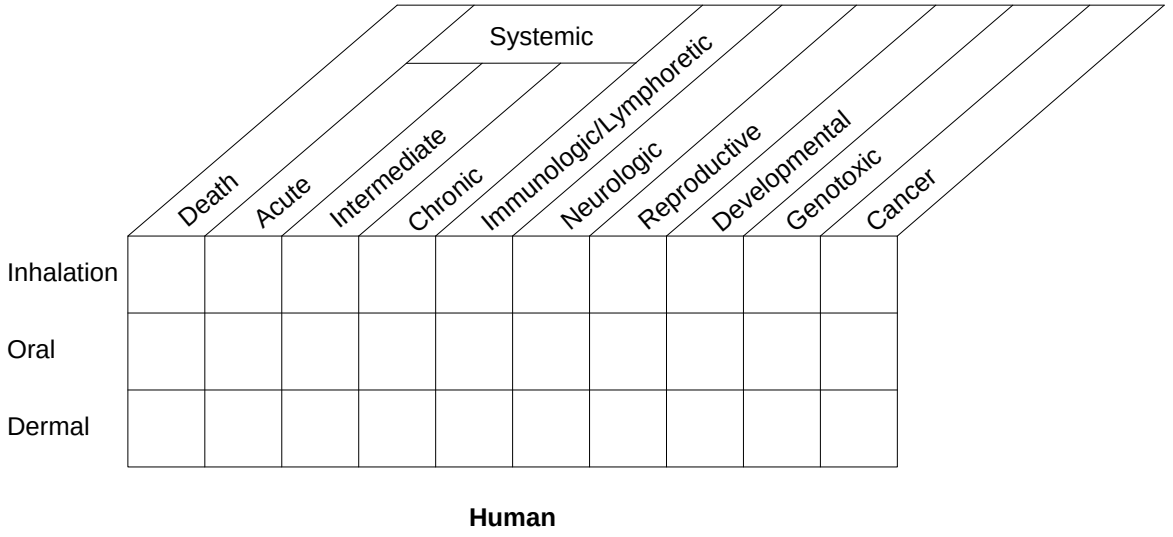
Human



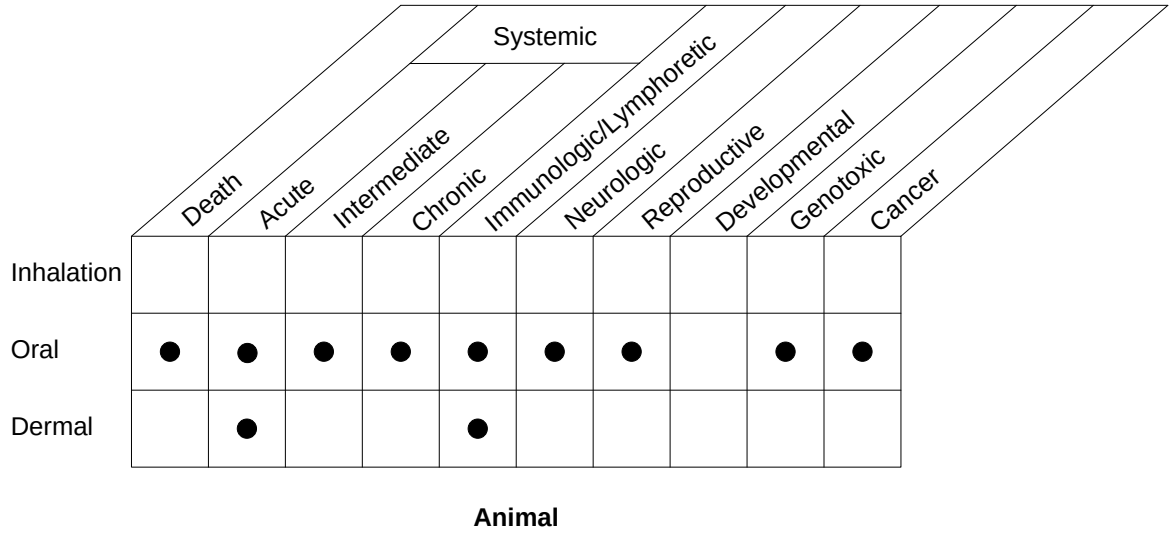
Animal

● Existing Studies

Figure 3-12. Existing Information on Health Effects of 2,6-Dinitrotoluene



Human



Animal

● Existing Studies

Figure 3-13. Existing Information on Health Effects of 2,3-, 2,5-, 3,4-, and 3,5-Dinitrotoluene

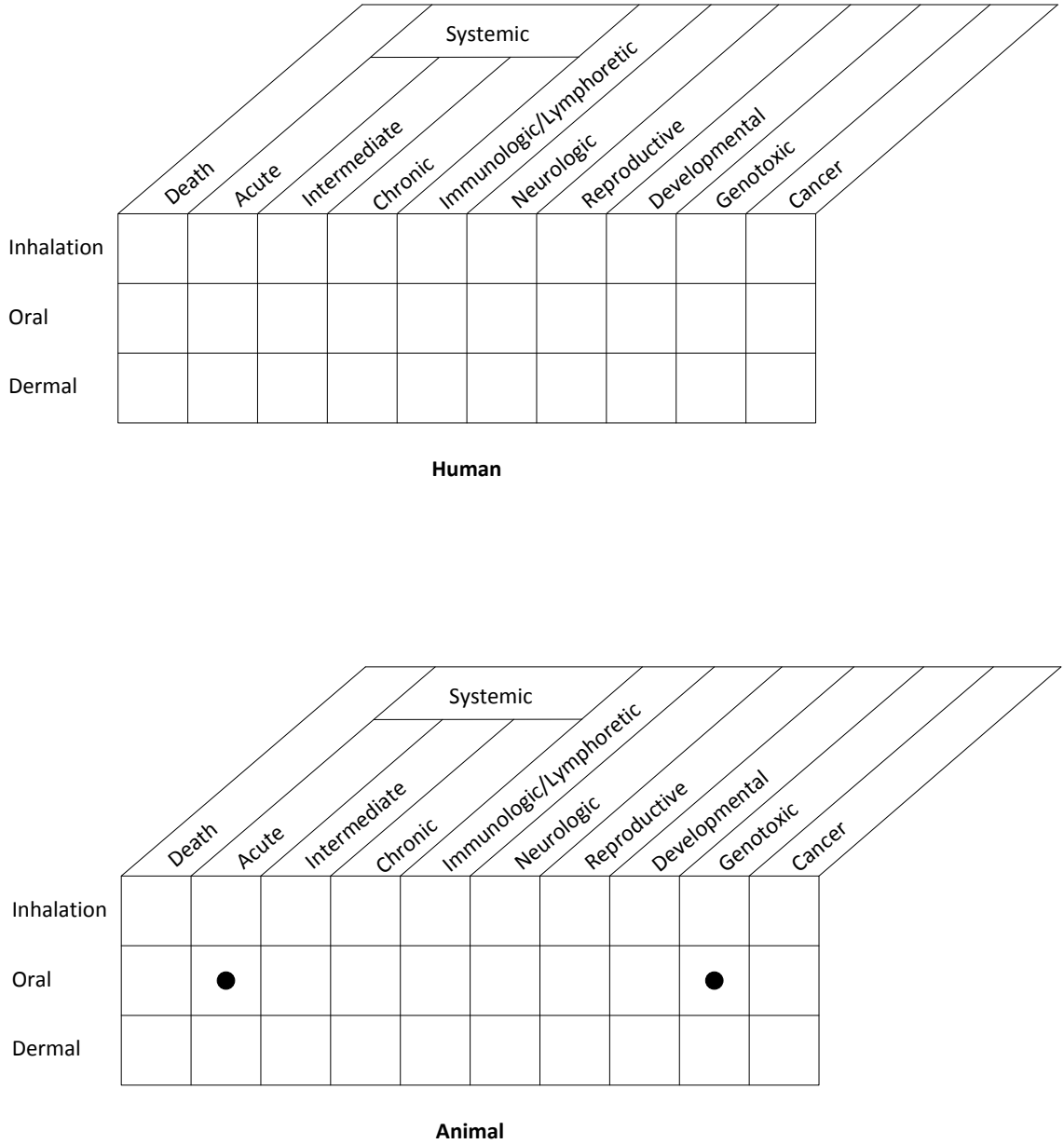
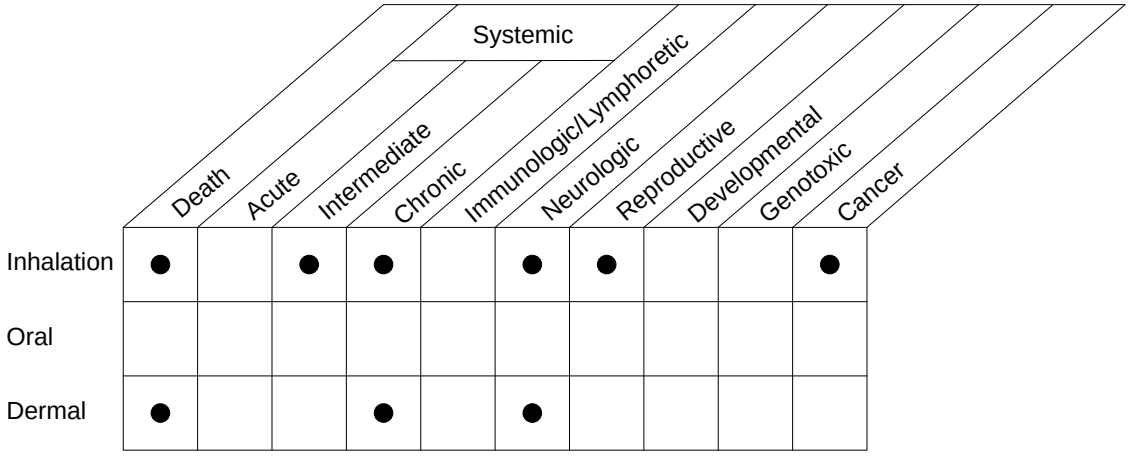
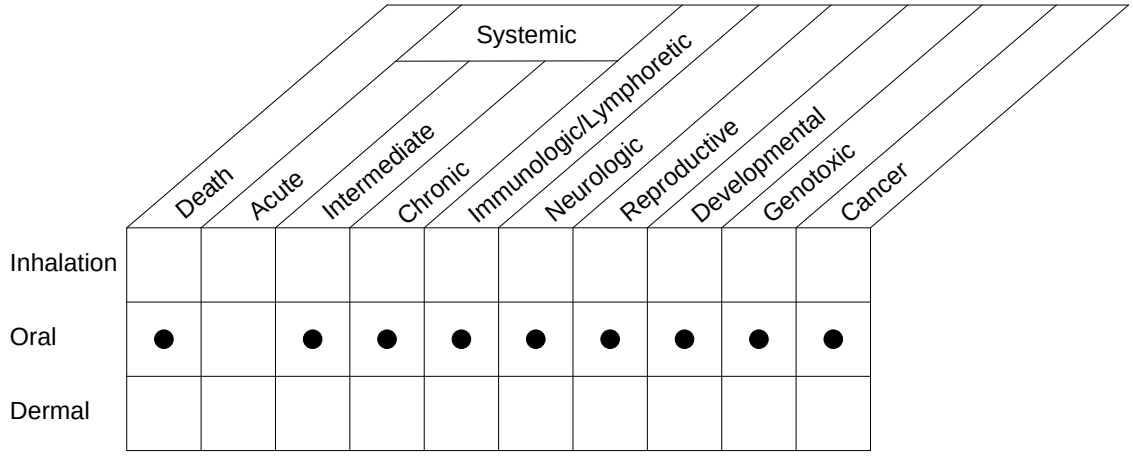


Figure 3-14. Existing Information on Health Effects of Technical-Grade Dinitrotoluene



Human



Animal

● Existing Studies

3.12.2 Identification of Data Needs

There are no data on the health effects of 2,3-, 2,5-, 3,4-, or 3,5-DNT in humans by any route of exposure. As shown in Figures 3-11 (2,4-DNT), 3-9 (2,6-DNT), and 3-11 (Tg-DNT), there are limited data on health effects in humans, primarily for Tg-DNT, following inhalation exposure. With the exception of animal oral LD_{50} values, the animal data for 2,3-, 2,5-, 3,4-, and 3,5-DNT are limited to a single 14-day gavage study in rats (Figure 3-13).

The available reports generally lack quantitative information on exposure levels. Human data are particularly sparse. Most toxicity studies have focused on the main systemic effects of obvious clinical significance, as described in the previous sections.

The toxicity of these chemicals has been extensively investigated in animals after oral exposure, but not after inhalation exposure, and only in a very limited way after dermal exposure. The potential carcinogenicity of these chemicals has been investigated following oral exposure in typical chronic bioassays as well as in less-than-lifetime studies.

Acute-Duration Exposure. Although there are no human data available from acute-duration oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-DNT, data currently available from animal studies of 2,3-, 2,4-, 2,5-, 2,6-, and 3,4-DNT are appropriate for evaluation of oral toxicity (Lent et al. 2012a; USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f; U.S. Army 1975, 1978a; Vernot et al. 1977). In gavage studies in rats and mice, LD_{50} values of 1,120 and 1,070 mg/kg, respectively, were identified for 2,3-DNT (Vernot et al. 1977). The LD_{50} values for 2,4-DNT determined after gavage dosing ranged from 270 to 650 mg/kg in rats and from 1,340 to 1,954 mg/kg in mice (U.S. Army 1975, 1978a; Vernot et al. 1977). Gavage dosing of 2,5-DNT identified LD_{50} values of 710 mg/kg in rats and 1,230 mg/kg in mice (Vernot et al. 1977). After oral administration of 2,6-DNT, LD_{50} values ranged from 180 to 795 mg/kg in rats and from 621 to 807 mg/kg in mice (U.S. Army 1975, 1978a; Vernot et al. 1977). Ataxia was observed in these animals before death. Oral LD_{50} values of 1,070 and 1,410 mg/kg 3,4-DNT in rats and mice were determined after gavage dosing (Vernot et al. 1977). Decreased body weight gain was noted in rats administered 2,4-DNT via gavage at 398 mg/kg and observed for 48 hours and in rats administered 2,6-DNT at ≥50 mg/kg and observed for 48 hours; body weight loss occurred at 199 mg/kg 2,6-DNT (Deng et al. 2011). After 14 days of gavage administration of 2,4-, 2,6-, or 3,4-DNT, decreased body weights were observed at doses of 142, 35, and 227 mg/kg/day, respectively (Lent et al. 2012a USAPHC

2011b, 2011d, 2011e). Slight cyanosis was observed in rats administered 60 mg/kg 2,4-DNT by gavage for 5 days (Lane et al. 1985), but no changes in hematological parameters were found in rats fed up to 273 mg/kg/day in the diet for 14 days (McGown et al. 1983). Evidence of erythrocytosis (increased red blood cells, hemoglobin, and hematocrit) was observed in rats administered 2,4-DNT at ≥99 mg/kg and in rats administered 2,6-DNT at 199 mg/kg at 24 and/or 48 hours after dosing (Deng et al. 2011). Increased incidences of splenic extramedullary hematopoiesis were seen in male Sprague-Dawley rats exposed by gavage for 14 days to DNT isomers; the LOAELs for this effect were 275, 71, 39, 68, and 57 mg/kg/day for 2,3-, 2,4-, 2,5-, 2,6-, and 3,4-DNT; 3,4-DNT did not induce this effect at doses up to 39 mg/kg/day (Lent et al. 2012a USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f). Hepatic effects, including increased blood cholesterol and alanine aminotransferase levels, and renal effects, such as hyaline droplet accumulation, were observed in rats fed 2,4-DNT in the diet for 14 days (McGown et al. 1983). Sinusoid congestion was noted in the livers of rats treated with 2,4-DNT at 398 mg/kg and in rats treated with 2,6-DNT at 199 mg/kg and evaluated at 24 or 48 hours; levels of albumin in the serum were also increased in rats treated with 2,4-DNT at 99 mg/kg (Deng et al. 2011). In studies by Lent et al. (2012a; USAPHC 2011a, 2011b, 2011c, 2011d, 2011e, 2011f) of male rats exposed by gavage to DNT isomers for 14 days, single cell necrosis and increased glycogen deposition were observed at 36 mg/kg/day 2,4-DNT; hepatocellular and oval cell hyperplasia, along with hepatocellular hypertrophy were observed at 35 mg/kg/day 2,6-DNT. The other isomers did not induce hepatic effects at the doses up to those causing lethality. Effects on the male reproductive tract, including decreased organ weights and testicular lesions, were seen in rats exposed for 14 days by gavage to doses of 142, 68, and 19 mg/kg/day of 2,4-, 2,6-, and 3,5-DNT, respectively (Lent et al. 2012a; USAPHC 2011b, 2011d, 2011f).

There were no acute-duration inhalation or dermal studies in humans available for evaluation. The LD_{50} value determined after exposure to 2,6-DNT as an aerosol in rats (combined males and females) was 0.43 mg/L (0.24 mg/L for males and 0.66 mg/L for females) (CMA 1991). Treated rats showed evidence of respiratory distress (exaggerated breathing, ataxia, and lethargy); rats that died experienced lung congestion and increased relative lung weights. Both 2,4- and 2,6-DNT were shown to be mild primary dermal irritants in rabbits (U.S. Army 1975, 1978a). Additional acute inhalation studies and dermal studies would be useful for determining route-specific toxicity.

Intermediate-Duration Exposure. Currently available animal studies using repeated-dose exposure are appropriate for evaluation of oral toxicity for both 2,4- and 2,6-DNT (Ellis et al. 1985; Hazleton Laboratories 1977, 1982; Hong et al. 1985; Jones-Price et al. 1982; Lee et al. 1985; McGown et al. 1983; U.S. Army 1976, 1978b, 1979); no data were located regarding repeated-dose toxicity for 2,3-, 2,5-, 3,4-,

or 3,5-DNT. Methemoglobinemia and its sequelae (Heinz bodies, anemia, reticulocytosis), hemosiderosis, extramedullary hematopoiesis, and cyanosis have been observed in animals after oral treatment with 2,4-, 2,6-, or Tg-DNT (Hazleton Laboratories 1977, 1982; U.S. Army 1976, 1978b). Mild hepatocellular dysplasia was observed in mice fed 2,4-DNT in the diet for 13 weeks (Hong et al. 1985; U.S. Army 1978b), but no hepatotoxicity was observed after 2,4-DNT administration to rats or dogs for the same duration (U.S. Army 1978a, 1978b). However, treatment with 2,6-DNT did cause bile duct hyperplasia in rats and mice (U.S. Army 1976). This lesion, as well as hepatic degeneration, was observed in dogs dosed with 2,6-DNT (U.S. Army 1976).

Oral administration of 2,4-DNT to rats, mice, or dogs for 13 weeks did not cause any significant adverse renal effects (Hong et al. 1985; U.S. Army 1978b). Administration of 2,6-DNT to dogs for 13 weeks caused renal inflammation and degeneration, which were not observed in rats or mice (U.S. Army 1976). Decreased body weight gain or weight loss was observed in rats and mice after administration of 2,4-DNT (Hong et al. 1985; Lee et al. 1985; Leonard et al. 1987; NCI 1978; U.S. Army 1978b, 1979) and in rats, mice, and dogs after administration of 2,6-DNT (U.S. Army 1976). Subchronic inhalation would be useful for determination of toxic effects in order to derive an MRL for 2,4-DNT and to determine a mechanism of action from routes of exposure that are more characteristic of occupational exposure.

Chronic-Duration Exposure and Cancer. There are no data available in humans regarding the carcinogenicity of 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNT. A retrospective cohort mortality study performed using data from workers at ammunition plants that used 2,4-DNT or Tg-DNT found no increases in mortality due to either malignant neoplasms as a whole or from particular cancers (Levine et al. 1986b). However, the small cohort examined in this study limited its statistical power. Both 2,4- and 2,6-DNT have been found to cause hepatocellular carcinoma in rats (Leonard et al. 1987; U.S. Army 1979). Renal cancer was observed in mice after administration of 2,4-DNT in the diet (U.S. Army 1979). EPA has derived a cancer slope factor for oral exposure to 2,4-DNT/2,6-DNT mixture (IRIS 2003). No animal data pertaining to the carcinogenicity of 2,3-, 2,5-, 3,4-, or 3,5-DNT were located.

Excessive mortality rates from ischemic heart disease and residual diseases of the circulatory system were observed in ammunition plant workers (Levine et al. 1986a). Because it is expected that these workers would have a lower incidence of cardiovascular disease due to the healthy worker effect, this finding is unusual. Further epidemiological studies to verify these findings are needed; newer analyses should control for risk factors, particularly cigarette smoking, which was not done by the Levine et al. (1986a) study.

No data were located regarding the chronic toxicity of 2,3-, 2,5-, 3,4-, or 3,5-DNT. The currently available studies in laboratory animals on the effects of 2,4- and 2,6-DNT after chronic exposure are appropriate for evaluation of chronic oral toxicity (Ellis et al. 1985; Hazleton Laboratories 1982; Lee et al. 1985; Leonard et al. 1978; NCI 1978; U.S. Army 1978b, 1979). Hematological effects, including anemia, compensatory anemia, methemoglobinemia, and Heinz bodies, have been observed after chronic administration of 2,4-DNT to dogs, mice, and rats (Ellis et al. 1985; Hong et al. 1985; U.S. Army 1978b, 1979). Data were insufficient for the derivation of a chronic-duration oral MRL for 2,6-DNT. Severe hepatocellular changes, such as degeneration and vacuolation and dysplasia, were found in rats, mice, and dogs administered 2,4- or 2,6-DNT for chronic durations in oral exposure studies (Ellis et al. 1985; Hong et al. 1985; Leonard et al. 1987; U.S. Army 1979). Renal cystic dysplasia was observed in mice, but not rats or dogs, treated orally with 2,4-DNT for chronic-duration periods (Hong et al. 1985; U.S. Army 1979). Chronic-duration studies have not been performed in mice using 2,6-DNT to determine whether these findings would also result after administration of this isomer. Although no histopathological effects were found in adrenal, pituitary, or thyroid glands of rats after chronic oral administration of Tg-DNT, increases in parathyroid hyperplasia, fatty metamorphosis, and vascular ectasia were found (Hazleton Laboratories 1982). Further studies may be useful to verify these findings. Effects on body weight, including body weight loss, were reported in almost all chronic-duration oral studies (Ellis et al. 1985; Hazleton Laboratories 1982; Hong et al. 1985; Leonard et al. 1987; NCI 1978; U.S. Army 1979).

A well-controlled chronic inhalation study and dermal studies would be useful for determination of the potential for route-specific toxicity. In addition, for both 2,4- and 2,6-DNT, well-controlled epidemiological evaluations of larger occupationally exposed populations would contribute valuable insights regarding the human relevancy of chronic health effects observed in animal studies.

Genotoxicity. All isomers of DNT (2,3-, 2,4-, 2,6-, 3,4-, and 3,5-DNT) have caused gene mutations in one or more reverse mutation assays using *S. typhimurium* (Couch et al. 1981; Dellarco and Prival 1989; Spanggord et al. 1982b; Tokiwa et al. 1981; U.S. Army 1978a). However, the test system has given variable results because of the need for metabolic activation and the sensitivity of the tester strains. *In vivo* assays using 2,4-DNT have shown UDS and *S*-phase synthesis using rat hepatocytes (Ashby et al. 1985; Mirsalis and Butterworth 1982; Mirsalis et al. 1989), chromosomal aberrations using human lymphocytes (Huang et al. 1995), and DNA binding in rat hepatocytes (Chadwick et al. 1993; La and Froines 1993). The genotoxicity of Tg-DNT is believed to be due to the potent genotoxicity of 2,6-DNT, as evidenced in an *in vivo-in vitro* hepatocyte UDS system (Mirsalis and Butterworth 1982). Both

2,4- and 2,6-DNT have induced DNA adducts in rat liver (La and Froines 1992, 1993). Studies currently available for 2,4- and 2,6-DNT are considered to be appropriate for evaluation of genotoxicity.

Reproductive Toxicity. The currently available laboratory data on reproductive toxicity are considered appropriate for evaluation of oral exposure of animals to both isomers. Several studies in rats, mice, and dogs with either isomer have shown impairment of the male reproductive system. The effects observed include testicular atrophy, degeneration of the seminal vesicles, and decreased sperm production (Bloch et al. 1988; U.S. Army 1976, 1978b, 1979). *In vitro* studies have shown that the testicular degeneration is due, at least in part, to structural changes in Sertoli cells (Reader and Foster 1990). Animal studies of reproductive toxicity using inhalation exposure would provide information relative to occupational exposure conditions.

Several assessments of reproductive function in exposed workers have been performed that did not detect differences in sperm production or fertility rates as a result of exposure (Hammill et al. 1982; Levine et al. 1985a; NIOSH 1982). However, an earlier study reported a significant reduction in the sperm counts of exposed workers, as well as an increase, of marginal statistical significance, in the number of spontaneous abortions in their wives (NIOSH 1980). These studies were all limited by the small exposure populations studied and the lack of historical individual exposure monitoring. Further epidemiological studies of larger exposed occupational populations with exposure data may be considered useful since questions of potential reproductive effects associated with these exposures have not yet been clearly resolved.

Developmental Toxicity. No data are available regarding developmental effects in humans after oral exposure to DNTs, but animal studies that have been performed show possible developmental effects. The only developmental effect observed in a three-generation reproductive study in rats using 2,4-DNT was a decrease in pup viability. This decrease was attributed to maternal neglect and a high incidence of maternal death during parturition. Tg-DNT administered to pregnant dams caused a decrease in relative liver weight in postpartum pups and possible transient neurotoxicity (Jones-Price et al. 1982). Further studies may be useful to elucidate these effects. Additional animal studies using 2,4- and 2,6-DNT by oral and inhalation routes should analyze fetal and maternal blood for hematological parameters. This is recommended because any factor that could reduce the amount of oxygen to developing tissue is expected to have adverse consequences in the offspring.

Immunotoxicity. Although no data are available regarding immunological or lymphoreticular effects in humans, some data on these end points are available in animals. The currently available information

on the potential immunotoxic effects of 2,4- and 2,6-DNT is sufficient to describe the sensitizing potential of DNTs. Mild sensitization has been reported in guinea pigs after dermal exposure to 2,6-DNT, but not 2,4-DNT (U.S. Army 1975, 1978a). No effects on IgE, the antibody associated with allergic or hypersensitive reactions, were reported in rats or dogs exposed to either the 2,4- or the 2,6-DNT isomer (Ellis et al. 1985; Lee et al. 1985; U.S. Army 1976, 1978b). Studies have not been performed that would describe effects on immunocompetence following exposure to DNTs. A battery of immunotoxicity tests would provide a better assessment of possible effects in humans.

Neurotoxicity. The nervous system has been shown to be a major target of 2,4- and 2,6-DNT toxicity in animals (Ellis et al. 1985; Kozuka et al. 1979; Lee et al. 1979, 1985; U.S. Army 1979). Clinical signs in dogs have included incoordination and stiffness of the hind legs leading to complete paralysis; cerebellar vacuolation, hypertrophy, and focal gliosis; and cerebellar and brain stem hemorrhage. In mice, depression and hyperexcitability were observed, while some rats administered 2,4-, 2,6-, 3,4-, and 3,5-DNT showed neuromuscular symptoms (including facial twitching, ataxia, and/or limb paralysis). Acute-duration exposure of rats to 3,5-DNT resulted in inflammatory infiltrates in the brain. More systematic examination of the neurological effects of these compounds in laboratory animals would be useful to fully assess behavioral abnormalities and morphological damage to the nervous system. Although results of an *in vitro* study in neuroblastoma cells show that 2,4-DNT is cytotoxic and produced cell death (Banerjee et al. 1999), the biochemical mechanisms of DNT neurotoxicity is not known.

Generalized symptoms of neurotoxicity, including headache, sleepiness, dizziness, and tingling pain in the extremities were reported in workers occupationally exposed to 2,4-DNT (McGee et al. 1947; Perkins 1919). However, the more recent occupational studies performed failed to examine workers for symptoms of neurotoxicity (Hammill et al. 1982; Levine et al. 1985a; NIOSH 1980, 1982). Because the early reports of potential neurotoxicity in exposed workers have not been followed-up in more recent studies, neurological examination of workers in occupational studies could provide additional information regarding the potential magnitude of neurotoxic effects.

Epidemiological and Human Dosimetry Studies. Epidemiology studies of workers exposed to DNTs suggest a potential for heart disease in exposed populations (Levine et al. 1986a). Doses of DNTs associated with heart disease in humans have not been determined. Further studies with historical cohort monitoring data and control for potentially confounding factors such as concomitant exposure to other chemicals and cigarette smoking would be useful to verify these findings.

Animal studies have indicated that the male reproductive system is a target of DNT toxicity. Epidemiological studies have provided only suggestive evidence of a reproductive effect in workers exposed to DNTs. Studies of larger worker populations may help to determine more conclusively the magnitude of the potential for reproductive toxicity in exposed humans.

Other effects that were observed in animal studies but not confirmed in human populations include liver and kidney toxicity, neurotoxicity, and cancer. Well-controlled epidemiological studies examining these end points in humans would be useful.

Biomarkers of Exposure and Effect

Exposure. A rapid, accurate method for determining exposure to DNTs has been developed using spectrophotometric analysis of complexes of primary arylamines, which result from the reduction of DNTs and its metabolites (Smith et al. 1995).

Effect. Epidemiological studies that correlate quantitative estimates of exposure with disease outcomes would be useful. Studies that identify subtle physiological changes, such as altered blood chemistry indices, associated with a particular disease state are not available.

A disease registry is not currently available. The development of a registry of exposures and diseases would provide a useful reference tool for assessing the variations in exposure concentrations and health effects from, for example, geography, season, regulatory actions, presence of hazardous waste landfills, or manufacturing and use facilities. These assessments, in turn, would provide a better understanding of the needs for some types of research or data acquisition based on the current exposure concentrations.

Absorption, Distribution, Metabolism, and Excretion. The toxicokinetics of 2,4- and 2,6-DNT in rats by the oral route have been extensively studied. That Tg-DNT is absorbed and excreted in the urine by humans in an occupational setting (where the main routes of absorption are considered to be inhalation and dermal) has also been documented. There are no data available in animals on the toxicokinetics of DNTs by the dermal or inhalation routes. Toxicokinetics studies in rats administered the test materials by the inhalation and dermal routes would be critical in understanding possible differences in the toxicity of DNTs by different routes of administration. The main routes of exposure of humans are dermal and inhalation. Understanding the possible differences in toxicity in animals by different routes

would be valuable in determining the significance of findings in humans who may be exposed by inhalation or dermal routes.

Comparative Toxicokinetics. Absorption and excretion studies in several species indicate that there are considerable differences between mice and the other species evaluated. More detailed study of the metabolism of DNTs by mice, including the role of biliary excretion and enterohepatic cycling, would assist in understanding why the metabolism in mice is different from other species and which species may be the most appropriate model for evaluating hazards and risks to humans.

Methods for Reducing Toxic Effects. The most important method for reducing the toxic effects of DNTs is removal of the person from the area of exposure. Skin and eyes should be rinsed copiously (Bronstein and Currance 1994), although absorption through the skin has not been adequately examined. Gastric lavage, with subsequent administration of activated charcoal, and cathartics may be of some benefit in reducing peak absorption after oral exposure to DNTs. Methylene blue treatment is used with patients presenting with serious methemoglobinemia (Bentur and Keyes 2004). No additional studies are considered necessary at this time to examine further methods for reducing body burden of DNTs. Further studies on supportive therapy after DNT exposure, such as the use of hemodialysis, forced diuresis, hyperbaric oxygen, or hemoperfusion might be useful.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

There is inadequate experimental evidence to evaluate if the pharmacokinetics of DNTs are different in children. There are no studies on whether DNTs or their active metabolites can cross the placenta or be excreted in breast milk, so it cannot be determined if fetuses may be exposed *in utero* or if infants may be exposed via breast milk ingestion. There are also no data to show if DNTs and their metabolites are stored in maternal tissues and thus might be later mobilized during gestation or lactation; however, DNTs and their metabolites are not likely to be stored because of their low octanol-water partition coefficient.

There is little experimental evidence to evaluate whether the metabolism of DNTs or their mechanisms of action are different in children. As discussed in Section 3.7, newborns are highly sensitive to the methemoglobin-generating effect of DNTs because of their deficiency in methemoglobin reductase (Gruener 1976), which reduces methemoglobin back to hemoglobin. In addition, newborns have a high

concentration of fetal hemoglobin in their erythrocytes. It will be useful to determine if fetal hemoglobin is more sensitive to the methemoglobin-generating effect of DNTs. It will also be helpful to have data on the metabolism and mechanism of action of DNTs on children to determine if children are more vulnerable than adults to health effects from exposure to DNTs, as some enzymes involved in DNT metabolism are known to have developmental regulation. There are no biomarkers of exposure or effect that have been validated in children or in adults exposed as children. There are no data to determine whether there are any interactions with other chemicals unique to children, or whether interactions observed in adults also occur in children. Although DNTs are shown to be genotoxic, it is not known if parental exposure to DNT may affect children via parental germ cells, or if DNTs may indirectly affect the fetus during maternal exposure.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

No ongoing studies were identified for DNTs.