2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of 2,4- and 2,6-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.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure-inhalation, oral, and dermal; and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in

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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 (LOAEL) 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.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of 2,4-DNT are indicated in Table 2-l. Because cancer effects could occur at lower exposure levels, Figure 2-l also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for 2,4- and 2,6-DNT. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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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.

Although the primary foci of this document are 2,4-DNT and 2,6-DNT, some information on technical grade DNT (Tg-DNT) is also provided. Tg-DNT contains approximately 76% 2,4-DNT, 19% 2,6-DNT, and other isomers. Unless specified otherwise, 2,4-DNT and 2,6-DNT are abbreviated generically as DNT in this document.

2.2.1 Inhalation Exposure

Most of the data on human health effects associated with exposure to 2,4-DNT or 2,6-DNT are derived from studies of workers in occupational settings. Exposure monitoring of workers in the past has generally been inadequate. Consequently, few dose-response data based on human exposure to 2,4- or 2,6-DNT are available.

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 DNT 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.

2.2.1.1 Death

In a retrospective cohort mortality study of 457 munitions workers who were exposed to either 2,4-DNT or Tg-DNT at 2 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 (Standard Mortality Rates [SMR] of 126 and 143; 95% confidence intervals [CI] 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 more than 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 more than 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 dinitrotoluenes. 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 DNT 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 located regarding death in animals after inhalation exposure to 2,4- or 2,6-DNT.

2.2.1.2 Systemic Effects

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

Cardiovascular Effects. As described in Section 2.2.1.1, 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 inhalation exposure to 2,4- or 2,6-DNT.

Gastrointestinal Effects. Vomiting and nausea were mentioned as health complaints in a survey of 154 male workers involved in the production of smokeless gunpowders during World War II (McGee et al. 1942). The exposure concentrations of 2,4-DNT were not specified but may have been relatively high because of the lack of modem industrial hygiene practices. These symptoms were still mentioned in a followup survey of workers that was undertaken at the same facility after the installation of safeguards to reduce

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DNT exposure (McGee et al. 1947). Since exposure to other compounds cannot be ruled out, attribution of these symptoms to DNT cannot be verified.

No studies were located regarding gastrointestinal effects in animals after inhalation exposure to 2,4- or 2,6-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. 1942, 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, the 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 located regarding hematological effects in animals after inhalation exposure to 2,4- or 2,6-DNT.

Musculoskeletal Effects. Men who worked in a munitions plant during World War II mentioned muscular weakness as a complaint in a survey of these workers (McGee et al. 1942). However, the lack of 2,4-DNT exposure data and lack of an adequate control population prevent these data from being useful for anything other than qualitative description. Joint pain, especially in the knees, and other incapacitating symptoms were found in unspecified numbers of French workers in a plant that produced DNT 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.

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No studies were located regarding musculoskeletal effects in animals after inhalation exposure to 2,4- or 2.6-DNT.

Hepatic Effects. Two of 154 workers at a munitions plant suffered from acute hepatitis with jaundice but recovered soon after they were no longer exposed to 2,4-DNT (McGee et al. 1942). Although the exposure concentrations were not known, they were assumed to be high based upon the industrial hygiene practices prevalent at that time. Furthermore, since an adequate control population was not used, this study provides only a qualitative description of symptoms, at best. A later study of 714 workers at this same plant found that 29 experienced liver tenderness (McGee et al. 1947). This incidence would indicate an increased effect on the liver from the 1942 study, despite improvements in industrial hygiene and engineering practices designed to decrease worker exposure in the interval between the two studies. 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 (Ahrenholz and Meyer 1982). Air samples contained concentrations ranging from 0.026 to 0.890 mg/m^3 Tg-DNT (mean 0.207 mg/m^3 Tg-DNT).

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

Renal Effects. No effects were observed on either of the renal parameters (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 (Ahrenholz and Meyer 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/m3 Tg-DNT). The study was limited by a small exposure population and lack of historical individual exposure monitoring.

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

2.2.1.3 Immunological and Lymphoreticular Effects

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

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2.2.1.4 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. (1942,1947). Neurological signs reported by these workers included headache, dizziness, insomnia, unpleasant taste in the mouth, 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 animals after inhalation exposure to 2,4- or 2,6-DNT.

2.2.1.5 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 in the incidence of miscarriage in their wives compared to controls (Ahrenholz and Meyer 1982; Hamill et al. 1982). In the Ahrenholz and Meyer (1982) study, DNT concentrations ranged from 0.026 to 0.890 mg/m^3 (mean 0.207 mg/m^3 Tg-DNT). Interpretation of these studies is somewhat confounded by the lack of distinction between DNT and TDA exposure and by 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 (Ahrenholz and Meyer 1982; Hamill et al. 1982; Levine et al. 1985a). 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.

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One study by the CDC (1981) noted that sperm counts were decreased by more than 50% in workers in a Kentucky chemical plant exposed to DNT and TDA compared to workers unexposed 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 DNT/TDA.

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

2.2.1.6 Developmental Effects

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

2.2.1.7 Genotoxic Effects

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

Genotoxicity studies are discussed in Section 2.5.

2.2.1.8 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 unexposed 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 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 again 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.

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

2.2.2 Oral Exposure

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

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to 2,4- or 2,6-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,,s that have been determined for rats after gavage dosing with 2,4-DNT range from 270 to 650 mgkg (Ellis et al. 1978; Lee et al. 1975; Vernot et al. 1977); in mice, LD_{50}s were reported to be between 1,340 and 1,954 mg/kg after 2,4-DNT administration (Ellis et al. 1978; Lee et al. 1975; Vernot et al. 1977). In a dominant lethal study by Lane et al. (1985), 8 of 15 male Sprague-Dawley rats died after receiving 5 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).

Death has been reported after intermediate- and chronic-duration exposure to 2,4-DNT in numerous studies. One of 8 male and 8 of 8 female CD rats died after 3-13 weeks of ingesting 2,4-DNT in the diet (Lee et al. 1978,1985). 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 347-413 mg/kg 2,4-DNT in the diet for up to 6 months (Hong et al. 1985; Kozuka et al. 1979; Lee et al. 1978). 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

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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,, dams during parturition after receiving 45.3 mg/kg/day 2,4-DNT in the diet for 6 months (Ellis et al. 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 (Ellis et al. 1979).

In a 13-week study, some dogs fed 25 mg/kg/day became moribund after 22 or more days and had to be terminated, whereas no treatment-related deaths were reported in dogs fed 5 mg/kg/day (Ellis et al. 1985; Lee et al. 1978). 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. 1979, 1985). 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-l mice at 898 mg/kg/day (Ellis et al. 1979; Hong et al. 1985; Lee et al. 1978,1985).

The experimental data are more limited for 2,6-DNT than for 2,4-DNT. After administration of 2,6-DNT, LD_{50}s have been reported to range from 180 to 795 mg/kg in rats and from 621 to 807 mg/kg in mice (Ellis et al. 1978; Lee et al. 1975; Vernot et al. 1977). 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).

Intermediate-duration studies have also 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 (Lee et al. 1976). No treatment-related deaths were reported when rats were fed up to 155 mg/kg/day 2,6-DNT for the same duration (Lee et al. 1976). Two of 8 dogs treated with 20 mg/kg 2,6-DNT by capsule died in a 13-week study (Lee et al. 1976). Thus, dogs seem to be the most sensitive of the three species to intermediateduration oral 2,6-DNT exposure.

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Administration of up to 150 mg/kg/day Tg-DNT for 14 days was lethal to 6 of 13 pregnant Fischer-344 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).

For 2,4-DNT, all LOAEL values from each reliable study for death in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l. For 2,6-DNT, all LOAEL values from each reliable study for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies were located regarding musculoskeletal effects in humans or animals after oral exposure to 2,4-DNT or 2,6-DNT. The systemic effects observed after oral exposure are discussed below.
For 2,4-DNT, the highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l. For 2,6-DNT, all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

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

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
	ACUTE EXPOSURE						
	Death						
1	Rat (Sprague-Dawley)	5 d 1x/d (GO)				240 M (8/15 died)	Lane et al. 1985
2	Rat (CD)	once (GO)				568M (LD_{50}) 650 F (LD_{50})	Lee et al. 1975; Ellis et al. 1978
3	Rat (Sprague-Dawley)	once (G)				270M (LD_{50})	Vernot et al. 1977
4	Mouse (Swiss albino)	once (GO)				1,954 M (LD_{50}) 1,340 F (LD_{50})	Lee et al. 1975
5	Mouse (CF-1)	once (G)				1,630 M (LD_{50})	Vernot et al. 1977
	Systemic						
6	Rat (Sprague-Dawley)	5 d 1x/d (GO)	Hemato		60M (slight cyanosis)		Lane et al. 1985
			Bd Wt	180M	240M (weight loss)		

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less serious (mg/kg/day)		Serious (mg/kg/day)	
7	Rat (Sprague-Dawley)	14d ad lib	Resp	260.9 M 272.7 F				McGown et al. 1983
			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				
	Immunological/Lymphoreticular							
8	Rat (Sprague-Dawley)	14d ad lib		260.9 M 272.7 F				McGown et al. 1983
	Neurological							
9	Dog (Beagle)	12d 1x/d (C)		5[b]			25 (incoordination, stiffness, abnormal gait)	Ellis et al. 1985; Lee et al. 1978

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)		
Reproductive								
10	Rat (Sprague-Dawley)	5 d 1x/d (GO)		60M		180M	(decreased fertility)	Lane et al. 1985
11	Rat (Sprague-Dawley)	14d ad lib				78.3M	(decreased thickness of spermatogenic sperm layers)	McGown et al. 1983
12	Mouse DBA/2J	2 d 1x/d (G)				250M	(decreased fertility)	Soares and Lock 1980

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)		
	INTERMEDIATE EXPOSURE							
	Death							
13	Rat (CD)	3 or 6 mo ad lib (F)				45.3 F	(increased incidence of death during parturition)	Ellis et al. 1979
14	Rat (Wistar)	6 mo ad lib (F)				347M	(71% died)	Kozuka et al. 1979
15	Rat (CD)	4 or 13 wk ad lib (F)				93M 145 F	(1/8 died) (8/8 died)	Lee et al. 1985
16	Mouse (CD-1)	4 or 13 wk ad lib (F)				413	(2/16M, 2/16F died))	Hong et al. 1985; Lee et al. 1978
17	Dog (Beagle)	6 mo 1x/d (C)				10M	(4/6 died)	Ellis et al. 1979, 1985
18	Dog (Beagle)	4 or 13 wk 1x/d (C)				25	(5/8 died)	Ellis et al. 1985; Lee et al. 1978

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Systemic						
19	Rat (Sprague-Dawley)	3 wk ad lib (F)	Bd Wt	76.7 M	156.4M (10% decrease body weight)		Bloch et al. 1988
20	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)	Ellis et al. 1979
21	Rat (Wistar)	6 mo ad lib (F)	Hemato		347M (increased methemoglobin)		Kozuka et al. 1979
			Hepatic		347M (increased relative liver weight; increased SGOT, LDH, alkaline phosphatase, acid phosphatase, triglycerides, glucose; formation of puruloid matter)		
			Renal	347M			
			Bd Wt			347M (41% decrease body weight)	

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
22	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)	Lee et al. 1978, 1985
			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)	
23	Rat (Fischer- 344)	6 or 26 wk ad lib (F)	Bd Wt		27M (11% decrease body weight)		Leonard et al. 1987
24	Mouse (CD-1)	4 or 13 wk ad lib (F)	Hemato	137 M 147 F	413 M (mild anemia, 468 F reticulocytosis)		Hong et al. 1985; Lee et al. 1978
			Hepatic	47 M 147 F		137 M (mild hepatocellular 468 F dysplasia)	
			Renal	413 M 468 F			
			Bd Wt			413 M (body weight loss with 468 F decreased food consumption)	

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
25	Dog (Beagle)	4 or 13 wk 1x/d (C)	Hemato	5		25 (anemia, Heinz bodies)	Ellis et al. 1985; Lee et al. 1978
			Hepatic	25			
			Renal	25			
Immunological/Lymphoreticular							
26	Rat (Wistar)	6 mo ad lib (F)			347M (increased relative spleen weight)		Kozuka et al. 1979
27	Dog (Beagle)	4 or 13 wk 1x/d (C)		25			Ellis et al. 1985; Lee et al. 1978
Neurological							
28	Rat (Wistar)	6 mo ad lib (F)				347M (humpback incoordination)	Kozuka et al. 1979
29	Rat (CD)	4 or 13 wk ad lib (F)		34 M 108 F		93M (demyelination of cerebellum and brain stem) (widespread and stiff-legged gait) 145 F	Lee et al. 1978, 1985
30	Mouse (CD-1)	4 or 13 wk ad lib (F)		413 M 468 F			Hong et al. 1985; Lee et al. 1978

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL				Reference
					Less serious (mg/kg/day)		Serious (mg/kg/day)		
31	Dog (Beagle)	4 or 13 wk 1x/d (C)		5			25	(incoordination, abnormal gait, paralysis)	Ellis et al. 1985; Lee et al. 1978
	Reproductive								
32	Rat (Sprague-Dawley)	3 wk ad lib (F)			76.7M	(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
33	Rat (CD)	3 or 6 mo ad lib (F)					34.5 M 45.3 F	(decreased fertility; difficult parturition)	Ellis et al. 1979
34	Rat (CD)	3 or 6 mo ad lib (F)		34.5 M			45 M	(decreased implantation index; severe atrophy/degeneration of seminiferous tubules)	Ellis et al. 1979
35	Rat (Wistar)	6 mo ad lib (F)					347 M	(testicular atrophy)	Kozuka et al. 1979
36	Rat (CD)	4 or 13 wk ad lib (F)		34 M			93 M	(severe decrease in spermatogenesis)	Lee et al. 1978, 1985

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)		
37	Rat (CD)	13 wk ad lib (F)		9.3 M		93 M	(decreased fertility)	Lee et al. 1978, 1985
38	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; Lee et al. 1978
39	Mouse (albino-Swiss	4 wk (C)		295 M		1032 M	(decreased fertility index)	Lee et al. 1978
40	Dog (Beagle)	4 or 13 wk 1x/d (C)		5 M 25 F		25 M	(testicular degeneration, decreased spermatogenesis)	Ellis et al. 1985; Lee et al. 1978
	Developmental							
41	Rat (CD)	3 or 6 mo ad lib (F)		5.1 F		45.3 F	(difficult parturition; decreased pup viability)	Ellis et al. 1979

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	CHRONIC EXPOSURE						
	Death						
42	Rat (CD)	1-2 yr ad lib (F)				3.9 M (decreased survival) 5.1 F (decreased survival)	Lee et al. 1978, 1985; Ellis et al. 1979
43	Mouse (CD-1)	24 mo ad lib (F)				898 (decreased survival)	Ellis et al. 1979; Hong et al. 1985
	Systemic						
44	Rat (CD)	1-2 yr ad lib (F)	Hemato	0.6 M 5.1 F	3.9M (decreased RBC count)	34.5 M (anemia) 45.3 F (anemia)	Ellis et al. 1979; Lee et al. 1978, 1985
			Hepatic	5.1 F		0.6 M (preneoplastic foci of 45.3 F altered or hyperplastic hepatocytes)	
			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)	
45	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
			Bd Wt			27 M (25% body weight decrease)	

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
46	Rat (Fischer- 344)	78 wk ad lib (F)	Bd Wt	8 M 8.8 F		20M (25% decrease body weight) (decrease body weight) 22 F	NCI 1978
47	Mouse (CD-1)	24 mo ad lib (F)	Hemato	95		898 (anemia; reticulocytosis; Heinz bodies)	Ellis et al. 1979; Hong et al. 1985
			Hepatic	95 F		14M (hepatocellular dysplasia) 898 F (hepatocellular dysplasia)	
			Renal			14M (cystic dysplasia; toxic nephropathy)	
			Bd Wt	14 M 95 F	95M (16% decrease in body weight)	898 F (20% decrease in body weight)	
48	Mouse (C57BL/6N)	78 wk ad lib (F)	Bd Wt	14.4 M	72M (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
49	Dog (Beagle)	24 mo 1x/d (C)	Hemato	0.2[c]	1.5 (methemoglobinemia, Heinz bodies)		Ellis et al. 1979, 1985
			Hepatic	0.2	1.5 (biliary hyperplasia)		
			Renal	10			
Neurological							
50	Rat (CD)	1-2 yr ad lib (F)				34.5M (wide-spread and stiff-legged gait) 45.3 F (wide-spread and stiff-legged gait)	Lee et al. 1978, 1985; Ellis et al. 1979

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)		
51	Mouse (CD-1)	24 mo ad lib		95		898	(stiff-legged gait, hyperactivity)	Ellis et al. 1979; Hong et al. 1985
52	Dog (Beagle)	24 mo 1x/d (C)		0.2		1.5	(loss of hindquarter control, convulsions)	Ellis et al. 1979, 1985
	Reproductive							
53	Rat (CD)	1-2 yr ad lib (F)				0.6 M	(atrophy of seminiferous tubules, aspermatogenesis)	Lee et al. 1978, 1985; Ellis et al. 1979
54	Mouse (CD-1)	24 mo ad lib				14 M	(decreased spermatogenesis and degenerative change; testicular atrophy)	Ellis et al. 1979; Hong et al. 1985
				95 F		898 F	(ovarian atrophy; nonfunctioning follicles)	
55	Dog (Beagle)	24 mo 1x/d (C)		10 M				Ellis et al. 1979, 1985
	Cancer							
56	Rat (CD)	1-2 yr ad lib (F)				34.5 M	(CEL: hepatocellular carcinoma, mammary and skin tumors)	Ellis et al. 1979; Lee et al. 1978, 1985
						45.3 F	(CEL: hepatocellular carcinoma; mammary and skin tumors)	

Table 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)		
57	Rat (Fischer- 344)	78 wk ad lib (F)				7.5-8 M	(CEL: skin and subcutaneous fibroma)	NCI 1978
						22 F	(CEL: mammary fibroadenoma)	
58	Mouse (CD-1)	24 mo ad lib				95 M	(CEL: renal solid carcinoma, cystic papillary carcinoma and adenoma, cystic adenoma)	Ellis et al. 1979; Hong et al. 1985

[a]The number corresponds to entries in Figure 2-1.
[b]Used to derive an acute-duration oral Minimal Risk Level (MRL) of 0.05 for 2,4-dinitrotoluene by dividing by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).
[c]Used to derive a chronic-duration oral MRL of 0.002 for 2,4-dinitrotoluene by dividing by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; d = day(s); (F) = feed; F = female; (G) = gavage; Gastro = gastrointestinal; (GO) = gavage, oil; Hemato = hematological; kg = kilogram; LD_{50} = lethal dose producing 50% death; M = male; mg = milligram; mo = month; Resp = respiratory; wk = week; x = times; yr = year;

Figure 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

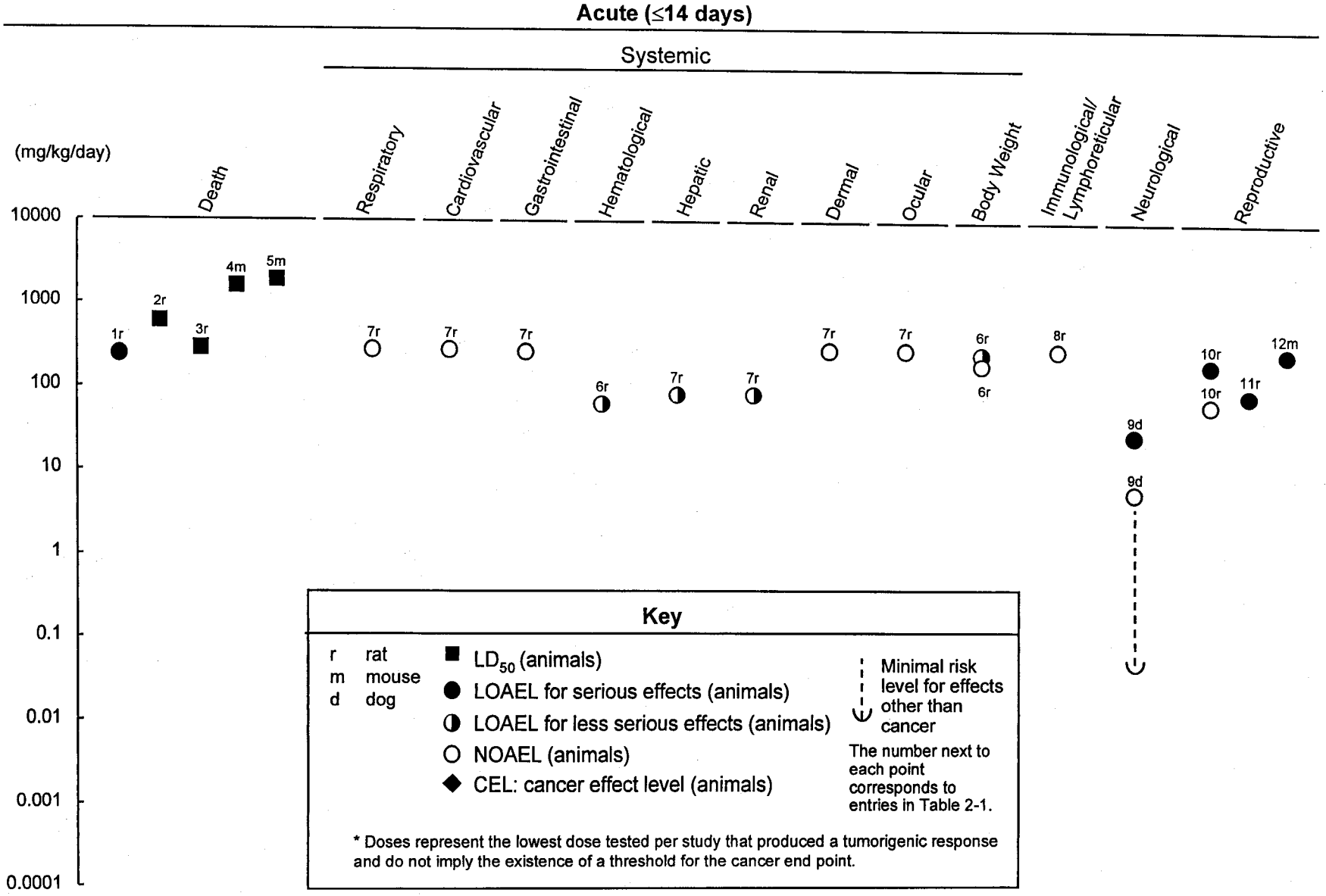


Figure 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Intermediate (15-364 days)

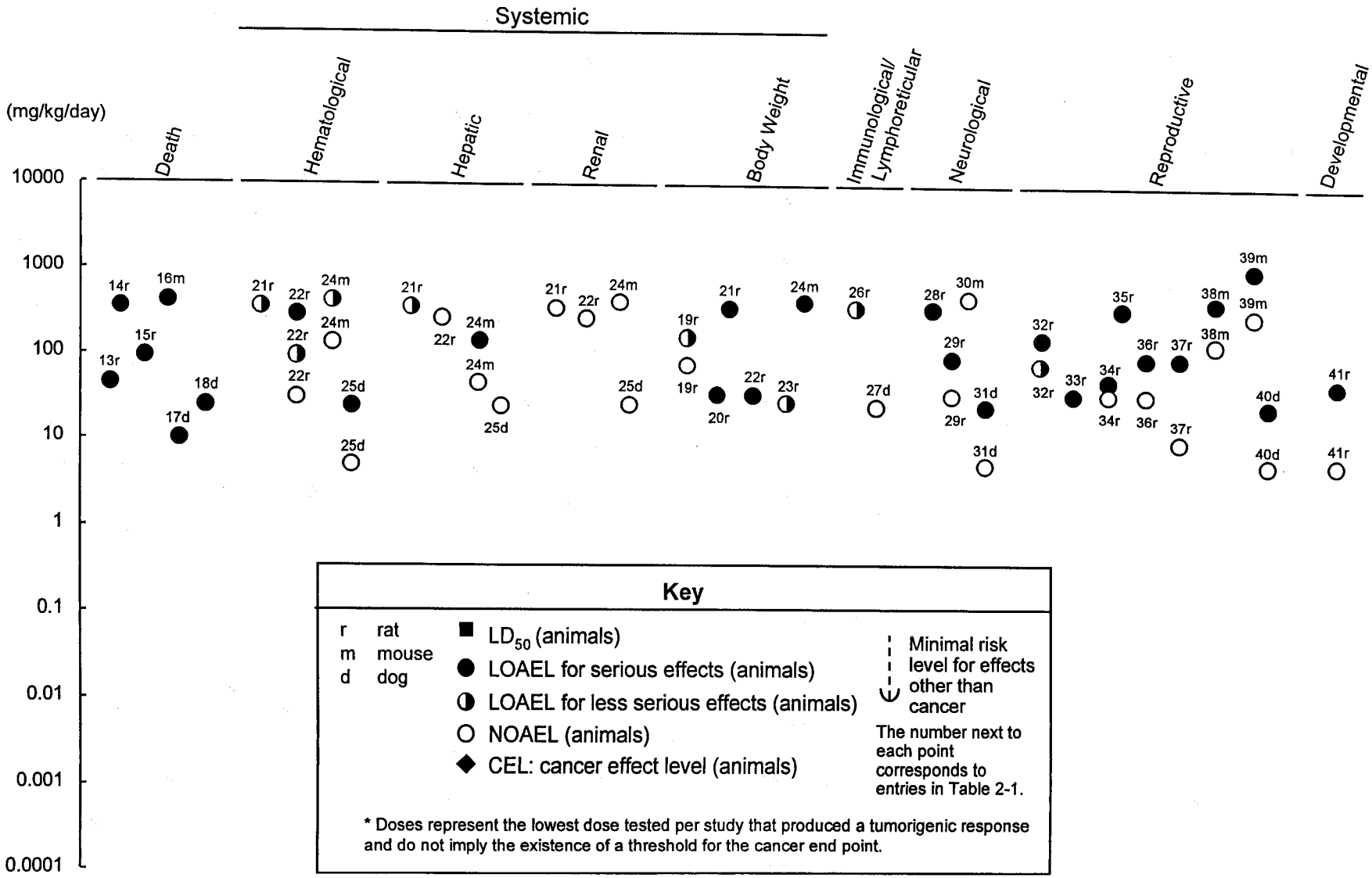


Figure 2-1. Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Chronic (≥365 days)

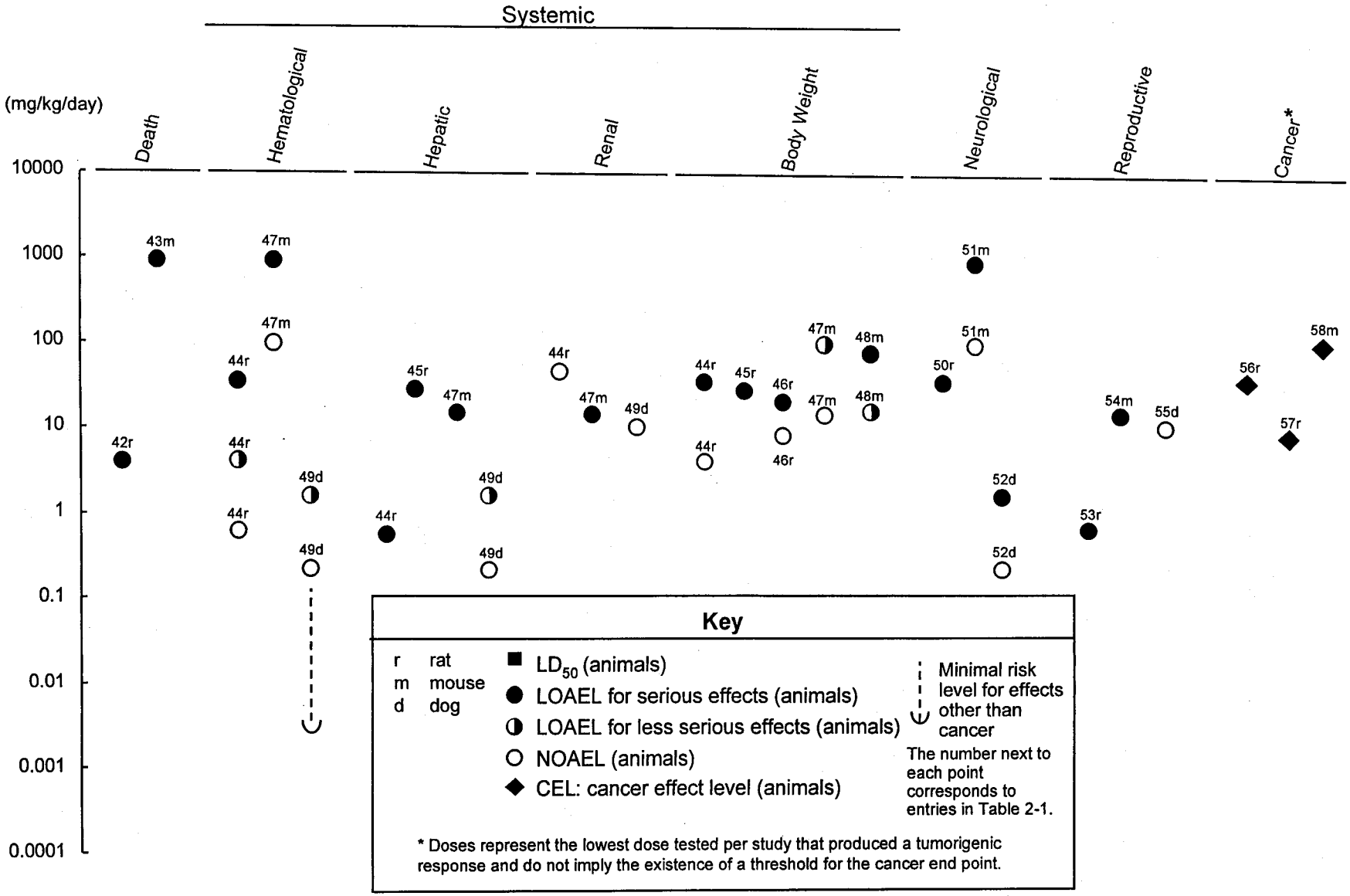


Table 2-2. Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
	ACUTE EXPOSURE						
	Death						
1	Rat (CD)	once (GO)				535 M (LD_{50}) 795 F (LD_{50})	Lee et al. 1975; Ellis et al. 1978
2	Rat (Sprague-Dawley)	once (G)				180 M (LD_{50})	Vernot et al. 1977
3	Mouse (CD)	once (GO)				621 M (LD_{50}) 807 F (LD_{50})	Lee et al. 1975
4	Mouse (CF-1)	once (G)				1,000 M (LD_{50})	Vernot et al. 1977

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

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
	INTERMEDIATE EXPOSURE						
	Death						
5	Mouse (Swiss-albino)	4 or 13 wk ad lib (F)				51 M (8/16 died) 55 F (6/16 died)	Lee et al. 1976
6	Dog (Beagle)	4 or 13 wk ad lib (C)				20 F (2/8 died)	Lee et al. 1976
	Systemic						
7	Rat (CD)	4 or 13 wk ad lib (F)	Hemato	7 M 7 F	35 M (splenic hemosiderosis; 37 F extramedullary hematopoiesis)		Lee et al. 1976
			Hepatic		35 M (bile duct hyperplasia; hemosiderosis) 37 F (bile duct hyperplasia; hemosiderosis)		
			Renal	145 M 155 F			
			Bd Wt	7	35 M (decreased body weight 37 F gain)	145 M (body weight loss) 155 F	
8	Rat (Fischer-344)	6 or 26 wk ad lib (F)	Bd Wt	7 M		14 M (20% decrease body weight)	Leonard et al. 1987

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

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
9	Mouse (Swiss-albino)	4 or 13 wk ad lib (F)	Hemato	11 M 11 F	51 M (extramedullary hematopoiesis) 55 F (extramedullary hematopoiesis)		Lee et al. 1976
			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)	
10	Dog (Beagle)	4 or 13 wk ad lib (C)	Hemato		4[b] (mild extramedullary erythropoiesis and lymphoid depletion)		Lee et al. 1976
			Hepatic	4		20 (bile duct hyperplasia; degenerative and inflammatory liver changes)	
			Renal	4	20 (dilated tubules, degenerative foci)		
			Bd Wt	4		20 (body wt. loss with decreased food consumption)	
	Immunological/Lymphoreticular						
11	Rat (CD)	4 or 13 wk ad lib (F)		145 M 155 F			Lee et al. 1976
12	Dog (Beagle)	4 or 13 wk ad lib (C)		20	100 (thymic involution)		Lee et al. 1976

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

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
	Neurological						
13	Rat (CD)	4 or 13 wk ad lib (F)		145 M 155 F			Lee et al. 1976
14	Mouse (Swiss-albino)	4 or 13 wk ad lib (F)		289 M 299 F			Lee et al. 1976
15	Dog (Beagle)	4 or 13 wk ad lib (C)		4		20 (incoordination, lack of balance)	Lee et al. 1976
	Reproductive						
16	Rat (CD)	4 or 13 wk ad lib (F)		7 M 155 F		35 M (decreased spermatogenesis; degeneration of testes)	Lee et al. 1976
17	Mouse (Swiss-albino)	4 or 13 wk ad lib (F)		11 M 299 F		51 M (decreased spermatogenesis)	Lee et al. 1976
18	Dog (Beagle)	4 or 13 wk ad lib (C)		4 M 100 F		20 M (testicular degeneration)	Lee et al. 1976

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

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
	CHRONIC EXPOSURE						
	Systemic						
19	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
			Bd Wt		7M (18% decrease body weight)		
	Cancer						
20	Rat (Fischer- 344)	52 wk ad lib (F)				7 M (CEL: cholangiocarcinoma, hepatocellular carcinoma)	Leonard et al. 1987

[a]The numbers correspond to entries in Figure 2-2.

[b]Used to derive an intermediate-duration oral Minimal Risk Level (MRL) of 0.004 for 2,6-dinitrotoluene by dividing by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for animal-to-human extrapolation, and 10 for human variability).

Bd Wt = body weight; (C) = capsule; F = female; (F) = feed; (G) = gavage; (GO) = gavage, oil; Hemato = hematological; kg = kilogram; LD_{50} = lethal dose producing 50% death; M = male; mg = milligram; wk = week;

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

Acute (≤14 days)

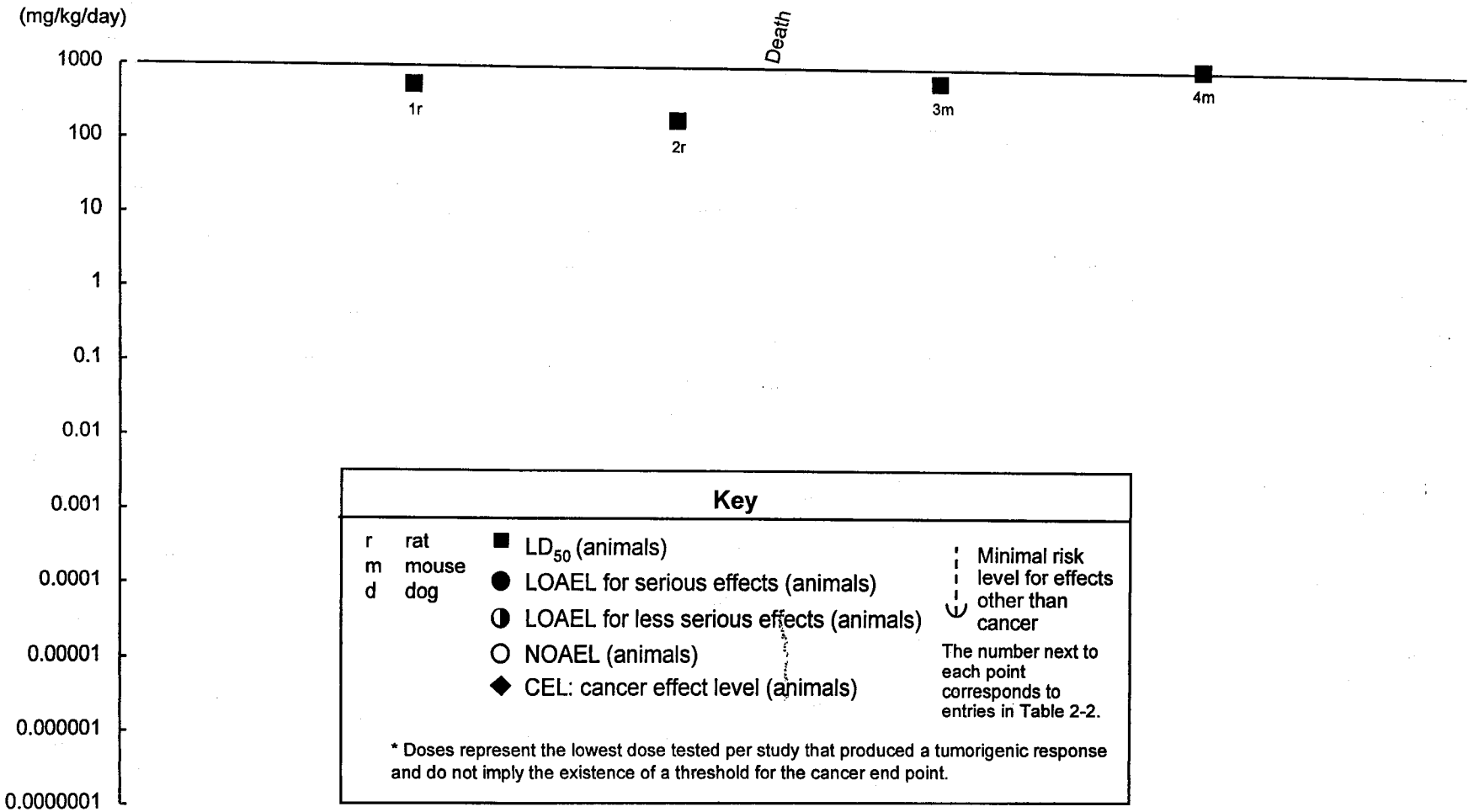


Figure 2-2. Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Intermediate (15-364 days)

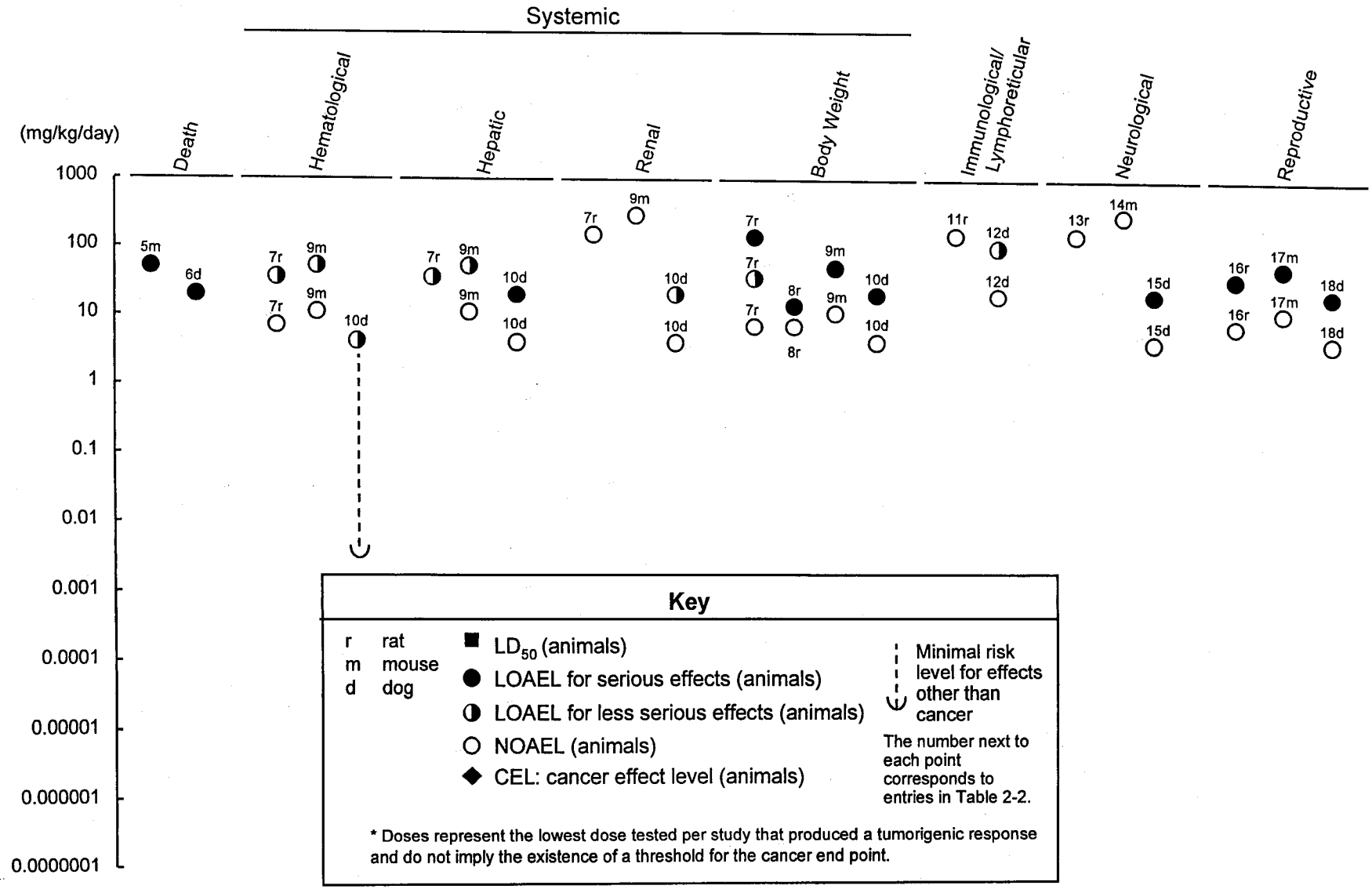
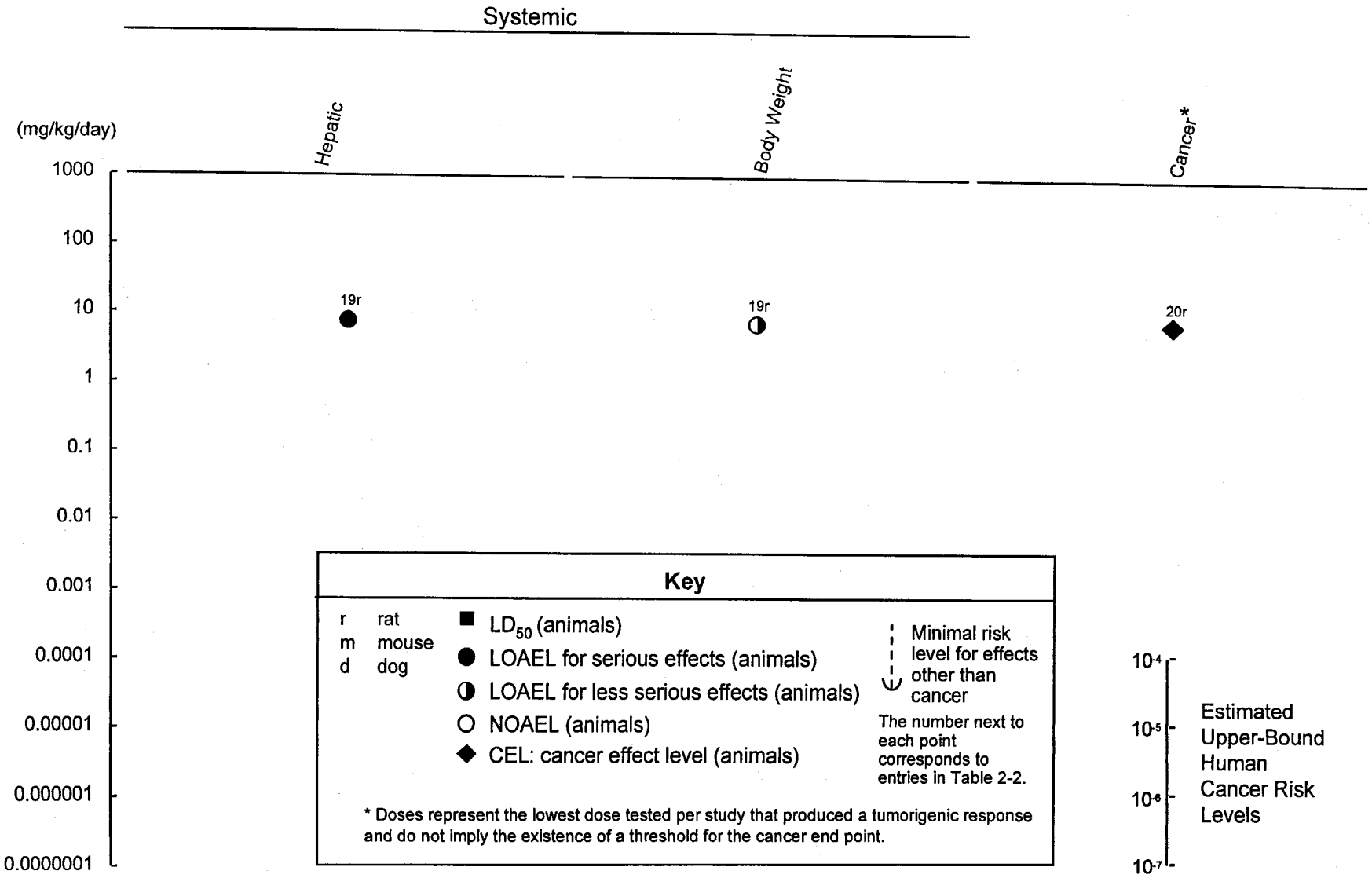


Figure 2-2. Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Chronic (≥365 days)



2. HEALTH EFFECTS

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

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).

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 2,4-DNT or 2,6-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) 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).

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

Hematological effects have been noted in virtually all animal studies of oral exposure to 2,4-DNT, 2,6-DNT, and Tg-DNT in which circulating blood has been examined. The most common findings are 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 [RBCs]) and is seen as a compensatory mechanism in anemia resulting from exposure to 2,4- and 2,6-DNT.

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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 (Ellis et al. 1979; Smith 1996).

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). Kozuka et al. (1979) found methemoglobin concentrations increased to 7 times those of controls in the blood of rats fed 347 mg/kg/day 2,4-DNT in the diet for 6 months. Anemia was observed in a 3-week feeding study in which male and female CD rats were fed 145 m/kg/day 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. 1978, 1985). 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-l mice administered 413 mg/kg/day and 468 mg/kg/day, 2,4-DNT, respectively in the diet for 13 weeks (Hong et al. 1985; Lee et al 1978). 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; Lee et al. 1978).

Subchronic administration of 2,6-DNT to dogs, rats, and mice resulted in hematological effects in dogs and rats at concentrations of 20 and 35 mg/kg/day, respectively (Lee et al. 1976). Dogs were found to be anemic and showed signs of compensatory reticulocytosis at this concentration, and rats showed hemosiderosis and extramedullary hematopoiesis. Statistically significant hematological effects were not observed in mice at levels up to 289 mg/kg/day 2,6-DNT but were observed in individual animals (Lee et al. 1976). Dogs administered 4 mg/kg/day 2,6-DNT by capsule for 13 weeks were found to have extramedullary erythropoiesis and lymphoid depletion of the spleen (Lee et al. 1976). An intermediate-duration oral MRL of 0.004 mg/kg/day for 2,6-DNT was derived from this LOAEL as described in the footnote in Table 2-2. The 2,6-DNT isomer was not tested for hematological endpoints in studies of chronic duration.

Chronic studies of animals administered 2,4-DNT fortify the weight-of-evidence supporting hematological effects. In 24-month studies, hematological effects were observed, but the animals often exhibited "compensated anemia," an adaptive response to 2,4-DNT exposure (Ellis et al. 1979). Methemoglobinemia and the presence of Heinz bodies were observed in dogs administered 1.5 mg/kg/day in capsules; no effect was observed at 0.2 mg/kg/day (Ellis et al. 1985; Lee et al. 1978). The NOAEL of 0.2 mg/kg/day in this dog study was used as the basis for a chronic oral MRL of 0.002 mg/kg/day for 2,4-DNT. In a 2-year study (with

2. HEALTH EFFECTS

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 REK count were found in mid-dose males compared to controls, and anemia was found in high-dose animals after 1 year (Ellis et al. 1979; Lee et al. 1978, 1985). No changes in methemoglobin or Heinz bodies were found, however. 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 at the high concentration, with compensatory increases in reticulocytes (Ellis et al. 1979; Hong et al. 1985).

Hematological changes consistent with those observed in anemia were found in pregnant Fischer-344 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 REXs along with hemosiderosis and extramedullary hematopoiesis in males, and increases in mean cell volume (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 DNT indicates that the blood is a primary target of DNT toxicity.

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Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans or animals after oral exposure to 2,4-DNT, 2,6-DNT, or Tg-DNT.

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

The hepatotoxic effects of DNT 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 DNT include liver discoloration and inflammation, alteration of hepatocytes, proliferation of bile duct epithelium, and hyperplastic foci.

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.

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; Lee et al. 1978). 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; Lee et al. 1978).

The most severe hepatotoxicity was found in rats. Concentrations of 2,4-DNT as low as 0.6 mg/kg/day caused foci of altered hepatocytes or hyperplastic nodules when fed to male rats for 1 year (Ellis et al. 1979; Lee et al. 1978, 1985). Hepatocellular degeneration and vacuolation accompanied by acidophilic foci and occasional basophilic foci of cellular alteration were found in Fischer-344 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 dietary concentrations of 347-395 mg/kg/day 2,4-DNT 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 (Ellis et al. 1979; Hong et al. 1985). Administration of 1.5 mg/kg/day 2,4-DNT for 24 months resulted in biliary hyperplasia in dogs; this effect was not seen in dogs administered 0.2 mg/kg/day (Ellis et al. 1979, 1985).

Six weeks of dietary consumption of 7 mg/kg/day 2,6-DNT caused a 380% increase in epoxide hydrolase (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 and dogs with 2,6-DNT for 13 weeks resulted in liver toxicity (Lee et al. 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 (Lee et al. 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 (Lee et al. 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 Fischer-344 rats (Leonard et al. 1987).

Irregular liver surfaces were found in male Fischer-344 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 Fischer-344 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).

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

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. Oral administration of 2,4-DNT to mice

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(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; Lee et al. 1978). 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 (Ellis et al. 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.

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 (Lee et al. 1976). No treatment-related effects on the kidney were found when rats were fed 2,6-DNT for 13 weeks (Lee et al. 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 (Lee et al. 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, blood urea nitrogen (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).

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-l mice fed 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.

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

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

Concentrations of up to 261 mgkg/day 2,4-DNT for males or 273 mgkg/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).

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

The eyes of male and female Sprague-Dawley rats administered up to 261 mg/kg/day 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).

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

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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; Ellis et al. 1979, 1985; Hazleton Laboratories 1982; Hong et al. 1985; Kozuka et al. 1979; Lane et al. 1985; Lee et al. 1978, 1985; Leonard et al. 1987; McGown et al. 1983; NCI 1978). In an acute study, rats dosed by gavage with 240 mg/kg 2,4-DNT for 5 days lost weight (Lane et al. 1985). 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 (Ellis et al. 1979; Lee et al. 1978, 1985). This reduction in body weight gain tended to become more pronounced when 2,4-DNT was continued for periods of l-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- and chronic-duration exposure, but the concentrations of the test article needed to evoke this effect were considerably higher than in rats (Hong et al. 1985; Lee et al. 1978; NCI 1978). 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).

Administration of 2,6-DNT also caused decreased body weight gain or body weight loss in rats, mice, and dogs at concentrations ranging from 14 to 145 mg/kg/day in intermediate-duration studies (Lee et al. 1976). Treatment with 7 mg/kg/day 2,6-DNT decreased body weight in rats at 52 weeks by 18% (Leonard et al. 1987).

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).

2.2.2.3 Immunological and Lymphoreticular Effects

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

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Testing for immunological effects of DNT is limited. 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. 1978, 1985). 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 (Lee et al. 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-DNT 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 (Lee et al. 1976).

For 2,4-DNT, the highest NOAEL values and all LOAEL values from each reliable study for immunological/ lymphoreticular effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2- 1. For 2,6-DNT, the highest NOAEL values and all LOAEL values from each reliable study for immunological/lymphoreticular effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to 2,4- or 2,6-DNT. Severe clinical signs of neurotoxicity, including incoordination and stiffness that led to an abnormal gait were observed in dogs given 25 mg/kg/day 2,4-DNT in capsules for 12 days (Ellis et al. 1985; Lee et al. 1978). These effects progressed with time and this dose was lethal after 22 days. The NOAEL for the neurotoxicity observed after 12 days was 5 mglkglday. An acute-duration oral MRL of 0.05 mg/kg/day was derived based on this NOAEL as described in the footnote in Table 2-l. 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.

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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. 1978, 1985). After 3 months of being fed 2,4-DNT in the diet, 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; Lee et al. 1978). No neurological signs were observed in mice fed 4 13 mgIkg/day in males or 468 mg/kg/day in females 2,4-DNT in the diet for 13 weeks (Hong et al. 1985; Lee et al. 1978).

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 mg/kg/day or 45.3 mg/kg/day 2,4-DNT to male and female rats, respectively, for up to 2 years (Ellis et al. 1979; Lee et al. 1978, 1985). This stifflegged 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. 1979,1985). Dogs dosed at 1.5 mg/kg/day 2,4-DNT in a 2-year study showed loss of hindquarter control (Ellis et al. 1979, 1985). 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. 1979, 1985).

Dogs dosed with 20 or 100 mg/kg/day of 2,6-DNT for 13 weeks exhibited dose-dependent neurotoxic symptoms that included muscular incoordination, weakness, tremors, and paralysis (Lee et al. 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 (Lee et al. 1976).

Administration of 150 mg/kg/day Tg-DNT to Fischer-344 dams during gestation days 7-20 caused hindlimb 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).

Neurotoxicity appears to be a characteristic syndrome of 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.

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For 2,4-DNT, the highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l. For 2,6-DNT, the highest NOAEL values and from each reliable study for neurological effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to 2,4- or 2,6-DNT. Studies in laboratory animals have shown that oral exposure to 2,4-DNT can result in adverse effects on reproduction. This is evidenced by decreased fertility as well as by 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; Ellis et al. 1979; Kozuka et al. 1979; Lane et al. 1985; Lee et al. 1976, 1978; McGown et al. 1983). In the female reproductive system, ovarian atrophy and dysfunction were observed (Ellis et al. 1979).

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). 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-dependent manner (McGown et al. 1983). A concentration 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 3 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 (Ellis et al. 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. 1978, 1985). 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 4 13 mg/kg/day 2,4-DNT for 13 weeks (Hong et al. 1985; Lee et al. 1978) and rats fed 347-395 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 (Lee et al. 1978). The decreased fertility was not observed in mice fed 295 mg/kg/day (Lee et al. 1978). 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; Lee et al. 1978). No testicular effects were found at 5 mg/kg in the study.

Chronic-duration studies in laboratory animals have also demonstrated both male and female reproductive effects. Rats that received 0.6 mg/kg/day 2,4-DNT in the diet for up to 2 years had an increased incidence of seminiferous tubule atrophy (29%) compared to controls (16%) (Ellis et al. 1979; Lee et al. 1978, 1985). This was the lowest concentration used in this study in which dose-related changes were found. At the highest dose, 35 mg/kg/day, an 8 1% increase in seminiferous tubule atrophy and spermatogenesis was reported. Similar changes were found in male CD-l mice fed 14 mg/kg/day 2,4-DNT for 24 months (Hong et al. 1985). Female mice fed 898 mg/kg/day 2,4-DNT in this study had ovarian atrophy with non-functioning follicles and, therefore, a lack of corpora lutea (Hong et al. 1985). The NOAEL for these effects was 95 mg/kg/day. No adverse reproductive effects were found in dogs fed 10 mg/kg/day 2,4-DNT for 24 months (Ellis et al. 1979,1985).

Histopathological examination of the testes after treatment with 2,4-DNT has revealed changes which suggest specific causes for the male infertility observed in animal studies. Dose-dependent 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).

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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 (Ellis et al. 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. Decreases in the number of fetal implants were attributed to the adverse impact of 2,4-DNT on sperm production.

In studies of rats, mice, and dogs dosed with 2,6-DNT for 13 weeks (Lee et al. 1976), decreased spermatogenesis was observed in male mice administered 5 1 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 (Lee et al. 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 (Lee et al. 1976).

Based upon the testicular effects observed after administration of 2,4-DNT or 2,6-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).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Tables 2-l and 2-2 and plotted in Figures 2-l and 2-2.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to 2,4- or 2,6-DNT. However, developmental toxicity from DNT could potentially occur because exposure to any substance that depletes the amount of oxygen available to developing fetal tissues can have adverse consequences. The hematological (and potential oxygen depleting) effects of DNT are reported in Section 2.2.1.2.

Ellis et al. (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 (Ellis et al. 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.

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are recorded in Tables 2-l and 2-3 and plotted in Figures 2-l and 2-3.

2.2.2.7 Genotoxic Effects

Studies of the effects of various DNT isomers on sperm morphology (Soares and Lock 1980), spermatocyte DNA repair (Working and Butterworth 1984), and dominant lethal mutations (Ellis et al. 1979; Hodgson et al. 1976; Soares and Locke 1980) were generally negative for these specific endpoints.

Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

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

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The carcinogenic activity of DNT 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 (Ellis et al. 1979; Lee et al. 1978, 1985).

2,4-DNT (98% 2,4-DNT, 2% 2,6-DNT) produced renal tumors (76%) in male CD-l mice fed 95 mg/kg/day for 2 years (Ellis et al. 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 Ellis et al. (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 (Ellis et al. 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 Fischer-344 strain, lower doses, and shorter exposure durations than did Ellis et al. (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. Ellis et al. (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 Fischer-344 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).

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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 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 the 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 AR, which is AlN-76A supplemented with 5% pectin. The number and size of γ-glutamyl transpeptidase-staining foci in the liver increased in a dose- and time-dependent manner in animals given 2,6-DNT in the NM-07 diet. Hepatocellular carcinomas and neoplastic nodules were observed only in rats fed NM-07 containing 2,6-DNT. No tumor was observed in rats receiving the control diets or 2,6-DNT in the AlN-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 cholangiocarcinomas and parathyroid adenomas in males and hepatocellular carcinomas and hepatocholangiocarcinomas 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 initiationpromotion 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.

2.2.3 Dermal Exposure

There are data on occupational exposure of humans to 2,4-DNT and Tg-DNT (see Section 2.2.1) 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 and may account for the quantity of excreted urinary metabolites that exceeded the amount of inhaled DNT in the operators and loaders.

2.2.3.1 Death

One study was located that examined death among humans exposed to DNT. 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). 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 2,4- or 2,6-DNT.

2.2.3.2 Systemic Effects

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

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Gastrointestinal Effects. Gastrointestinal complaints of munitions workers exposed to either 2,4-DNT or Tg-DNT included nausea and vomiting (McGee et al. 1942, 1947). These workers also presumably inhaled DNT in the occupational setting.

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

Hematological Effects. Hematological effects, such as anemia and cyanosis, have been found in men employed at munitions factories (McGee et al. 1942, 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 2,4-DNT or 2,6-DNT.

Musculoskeletal Effects. 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. 1942; 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 2,4-DNT or 2,6-DNT.

Hepatic Effects. In a follow-up 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 DNT via inhalation.

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

Dermal Effects. The only human studies that noted dermal effects upon topical exposure to 2,4-DNT were of workers employed by a munitions factory during World War II. The first study found 6 of 154 workers who complained of dermatitis, which the authors attributed to DNT exposure (McGee et al. 1942). A followup study, conducted after changes in the manufacturing process designed to reduce DNT exposure were implemented , reported that 32 of 714 workers complained of dermatitis (McGee et al. 1947). Exposure levels were not quantified in either of these studies.

Both 2,4- and 2,6-DNT were shown to be mild primary dermal irritants in rabbits (Ellis et al. 1978; Lee et al. 1975).

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

No ocular irritation was found in rabbits in a primary eye irritation test using unspecified concentrations of 2,4- or 2,6-DNT (Ellis et al. 1978; Lee et al. 1975). However mild eye irritations were reported in rabbits treated with 2,4- and Tg-DNT (Ford 1981; Henry 1982).

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after dermal exposure to 2,4-DNT or 2,6-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 (Ellis et al. 1978; Lee et al. 1975).

2.2.3.4 Neurological Effects

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. 1942, 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.

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

No studies were located regarding the following health effects in humans or animals after dermal exposure to 2,4- or 2,6-DNT:

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

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

2.3 TOXICOKINETICS

2.3.1 Absorption

No information regarding absorption of DNT in children has been located.

2.3.1.1 Inhalation Exposure

There are no available studies in which the absorption rates of inhaled 2,4- or 2,6-DNT in humans or laboratory animals have been evaluated. However, based on analyses of the urinary metabolites of workers in DNT manufacturing plants(Levine et al. 1985b; Turner 1986; Woollen et al. 1985), it is apparent that dermal absorption of these two compounds does occur under occupational settings.

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2.3.1.2 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 DNT is 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 DNT in the urine, primarily within the first 24 hours (Lee et al. 1978; Long and Rickert 1982; Rickert and Long 1981). 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% in the urine (Lee et al. 1978). Increased fecal excretion could be due to reduced absorption or to greater excretion via the bile.

2.3.1.3 Dermal Exposure

There were no data available specifically on the absorption of 2,4- or 2,6-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 these isomers 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).

2.3.2 Distribution

No information regarding distribution of DNT in children has been located.

2.3.2.1 Inhalation Exposure

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

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2.3.2.2 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 per kilogram. When distribution is studied solely by detecting a radioisotope label, it is the labeled atom(s) which 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 2 stages, with the first peak occurring l-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 (Lee et al. 1978). 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 (Lee et al. 1975, 1978).

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2.3.2.3 Dermal Exposure

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

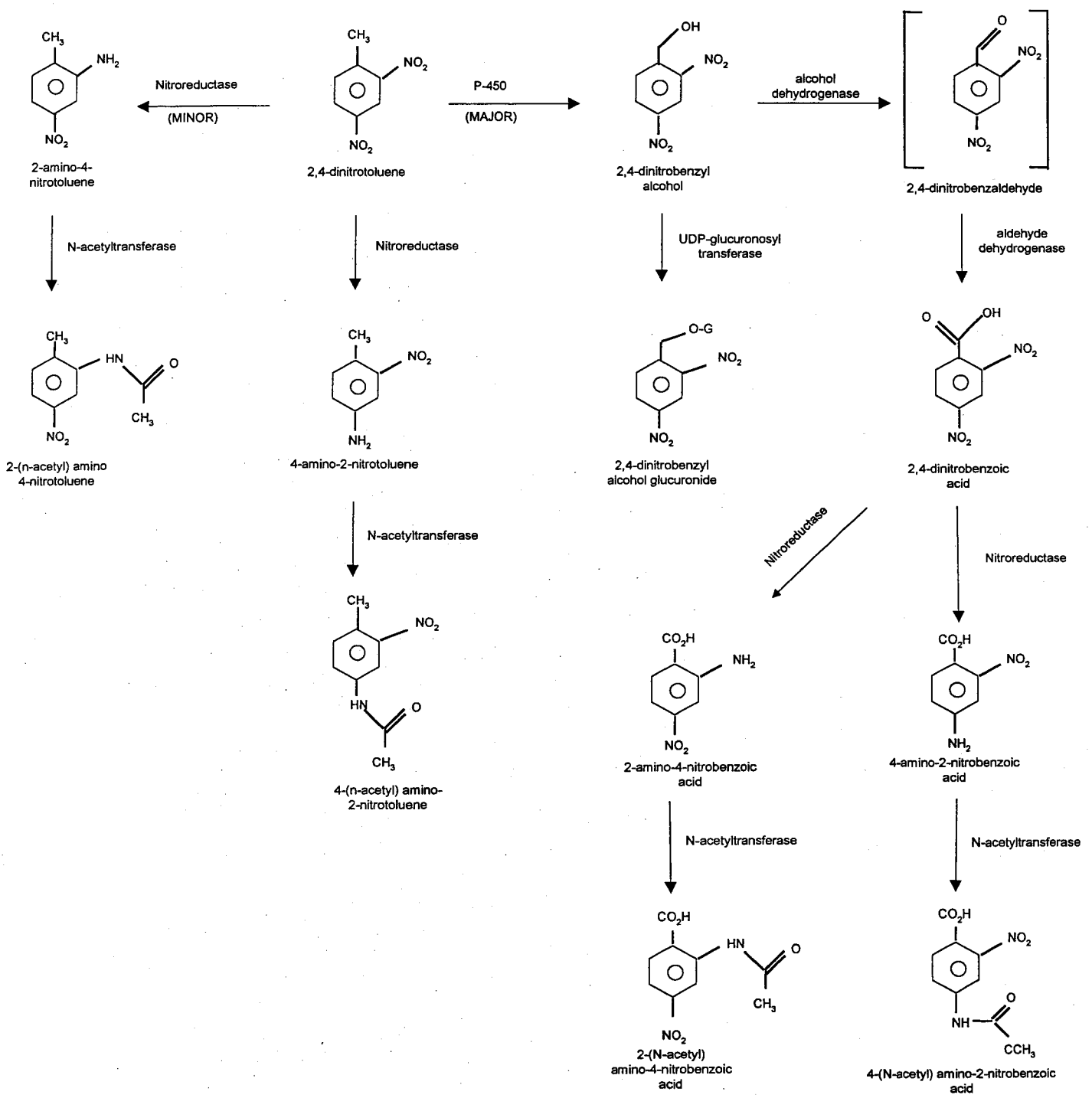
2.3.3 Metabolism

The metabolism of DNT 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-(Nacetyl) amino-2-nitrobenzoic acid. Trace levels of DNT 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- 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 (less than 1% of urinary metabolites) of 2-(N-acetyl)amino-4nitrobenzoic acid (Levine et al. 1985b).

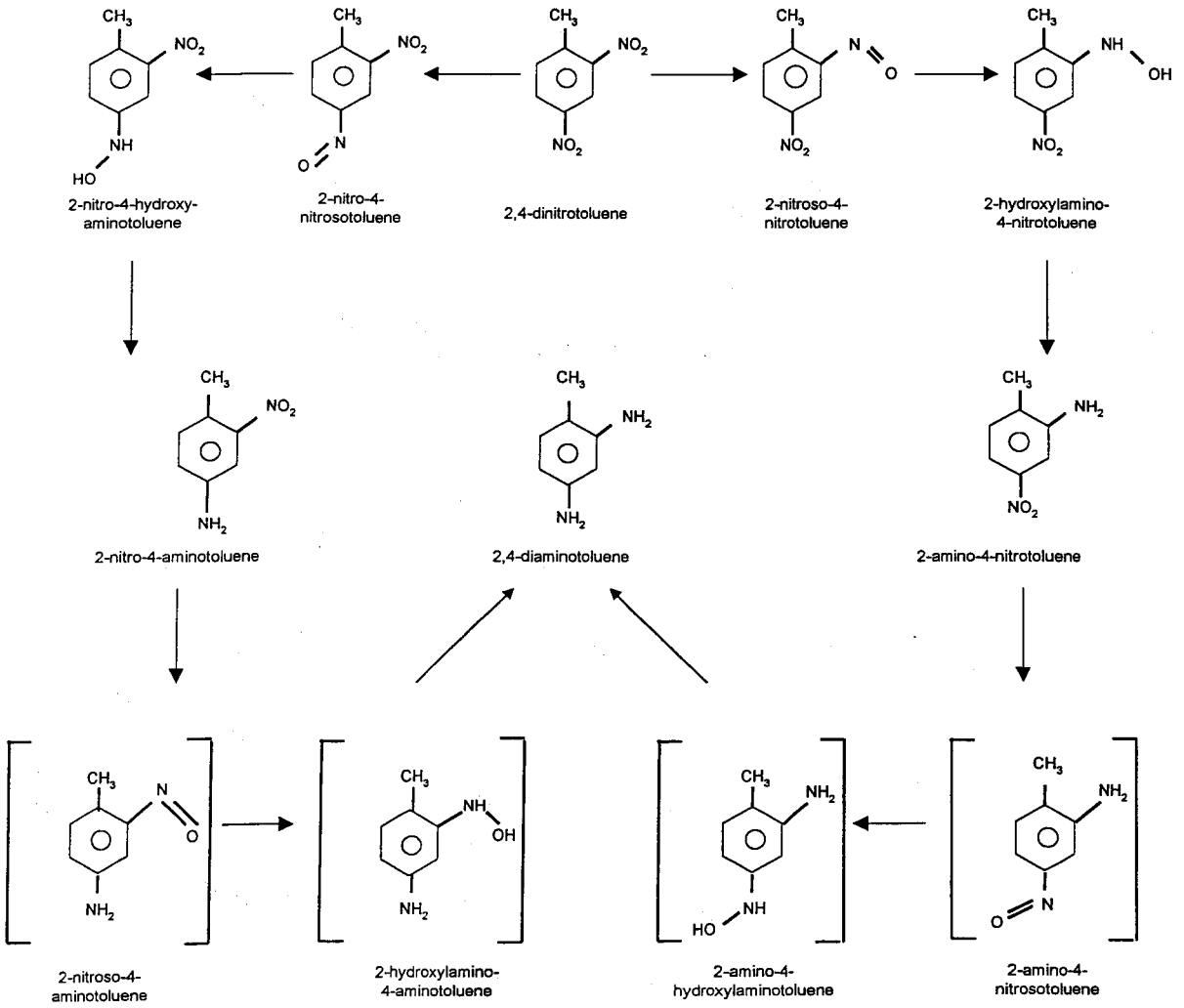
Studies in rats have identified a complex pathway for the metabolism of 2,4-DNT (Figures 2-3 and 2-4) and 2,6-DNT (Figure 2-5). Metabolism occurs in the liver and also in the intestine by microflora (Long and

Figure 2-3. Proposed Metabolic Pathways for the Hepatic Metabolism of 2,4-DNT*



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

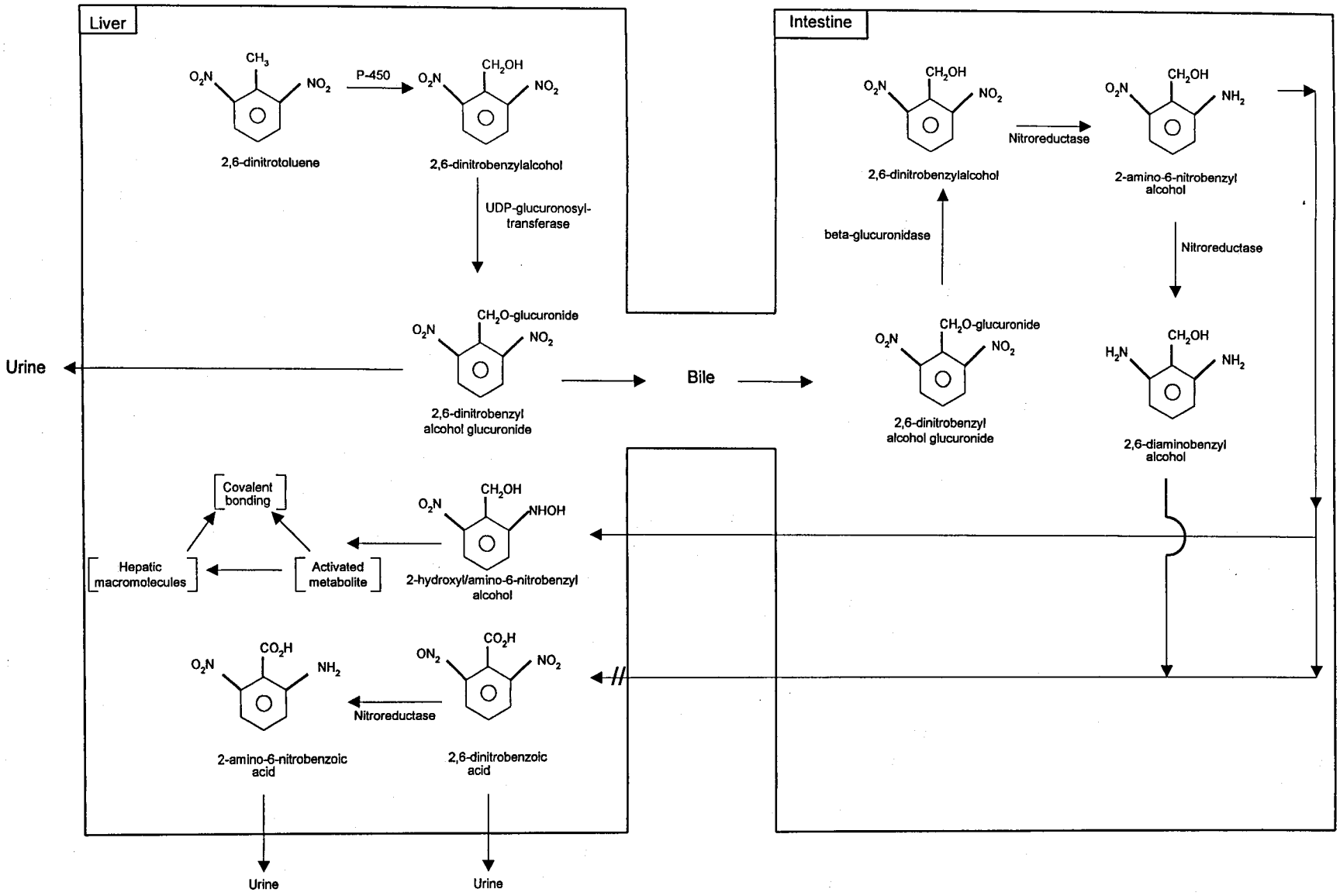
Figure 2-4. Proposed Pathways for the Anaerobic Metabolism of 2,4-DNT in Rat Intestinal Microflora*



⟶ = Nitroreductase reaction

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

Figure 2-5. Proposed Pathways for Metabolism of 2,6-DNT*



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

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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-2nitrobenzoic acid (Rickert et al. 1981).

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, DNT appears to be first metabolized by the liver with themetabolites 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-diaminotoluene, and 4-acetylamino-2-nitrobenzoic acid accounted for 0.02-0.12% of the dose; in addition to 2,4dinitrobenzyl alcohol, 2,4-dinitrobenzaldehyde and 2,4-dinitrobenzoic acid (0.09-o. 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.

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 DNT. 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-diarninotoluene 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 glucuromde 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 (Ellis et al. 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-diaminotoluene, 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 (Lee et al. 1978).

Another study using rats dosed with either 2,4- or 2,6-DNT also demonstrated that the primary urinary conjugate was the respective dinitrobenzyl 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 2,6dinitrobenzoic acid (0.17%), 2-amino-6-nitrotoluene (0.44%), and 2,6-dinitrobenzyl alcohol (0.53%) (Mori et al. 1996).

The urinary metabolites of DNT 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 DNT 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 P-450 isoforms responsible for DNT metabolism have not been identified, cytochromes CYP2E1, CYP2B l/2, and CYP2Cl l/6 are known to contribute to the side-chain oxidation of toluene by the rat liver, and multiple cytochrome P-450 isoforms may contribute to the side-chain oxidation of DNT (Chapman et al. 1993). In humans, CYP2El 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 quantitively different from the expression observed in adults (Komori et al. 1990; Leeder and Kearns 1997). In rats, while sulfotransferase (the enzyme which catalyzes sulfation) activity is almost at adult levels at birth, UDP-glucuronosyltransferase (the enzyme which 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 Keams 1997). While the activity of some isoforms of sulfotransferase may exceed those seen in adults during infancy and early childhood, the activity of UDP-lucuronosyltransferase

depends on the specific isoforms of the enzyme, and adult levels are generally attained by 6-18 months (Leeder and Kearns 1997). Since DNT undergoes bioactivation in the liver and by the intestinal microflora, the toxicity of DNT 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 DNT.

2.3.4 Elimination and Excretion

Information regarding excretion of DNT in children was not located.

2.3.4.1 Inhalation Exposure

In occupational settings, in addition to inhalation, some oral and dermal exposure can occur. The elimination of DNT 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 DNT. 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.

2.3.4.2 Oral Exposure

No studies were located regarding excretion in humans following oral exposure to 2,4- or 2,6-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. Lee et al. (1978) observed that most of the radioactivity from ^{14}C-labeled

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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.

2.3.4.3 Dermal Exposure

There are no kinetic data in humans in which the route of exposure was specifically dermal. Occupationalexposure 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 2.3.4.1.

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

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2.3.5 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 externaVexposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Ktishnan 1994). 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 parametrization, (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) is 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 2-6 shows a conceptualized representation of a PBPK model.

If PBPK models for 2,4- and 2,6-dinitrotoluene 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 2,4- or 2,6-DNT.

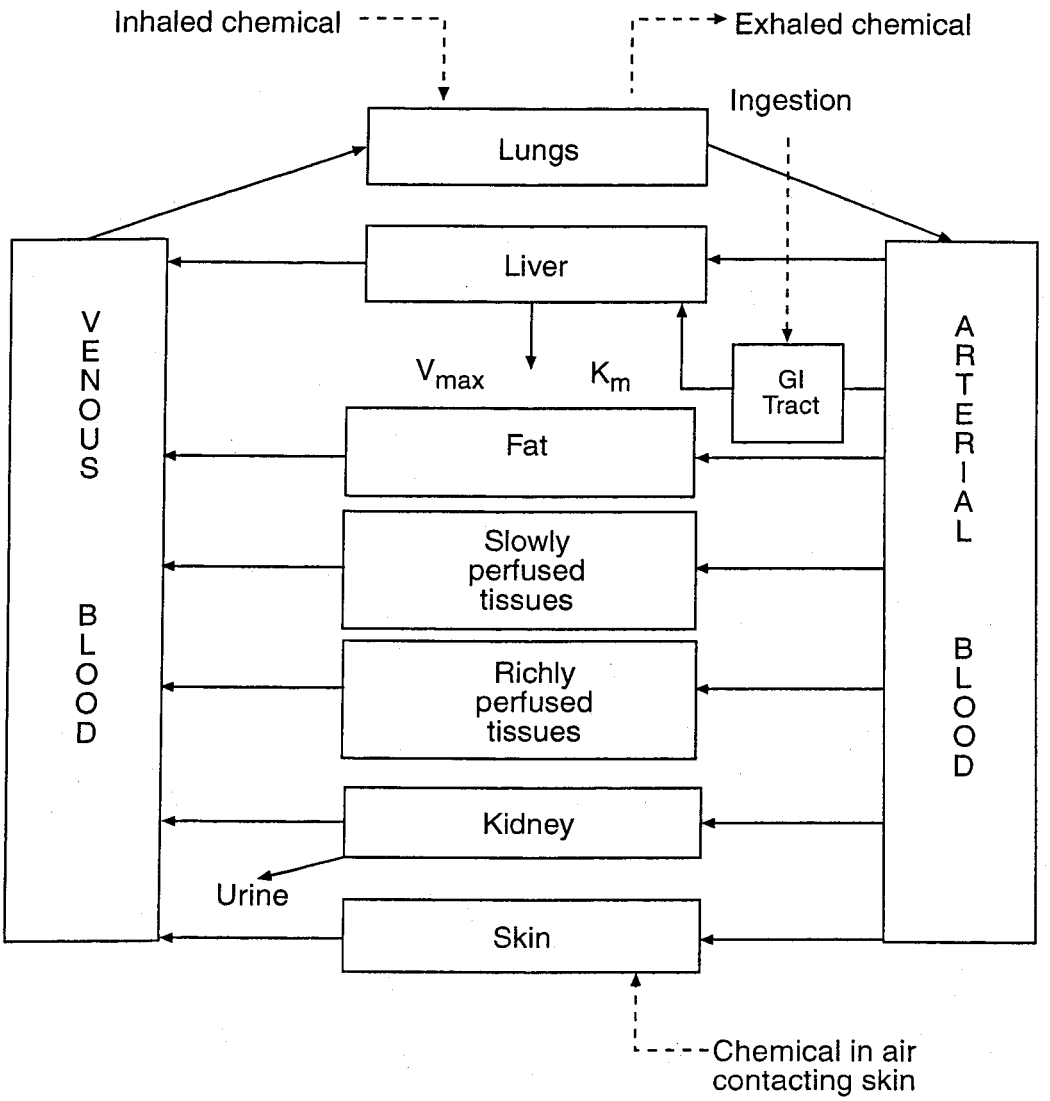
2.4 MECHANISMS OF ACTION

The mechanisms of action of DNT is not known to be different in children.

2.4.1 Pharmacokinetic Mechanisms

No information was located regarding the mechanism of absorption of 2,4- or 2,6-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 (Lee et al. 1978; Long and Rickert 1982; Rickert and Long 1981). 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 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,

Figure 2-6. 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.

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).

2.4.2 Mechanisms of Toxicity

Effects of Metabolism on Toxicity. The primary mechanism of toxicity for DNT involves bioactivation to form reactive intermediates (Kedderis et al. 1984; Sayama et al. 1989). Detailed information on the biotransformationof DNT is presented in Section 2.3.3. Briefly, metabolism of DNT 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 Nhydroxylated 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 DNT is described by Ellis et al. (1979). The effect of DNT 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 (Ellis et al. 1979).

Carcinogenesis. In hepatic tumor initiation-promotion experiments, Tg-DNT was found to have tumor promoting and tumor-initiating activity (Leonard et al. 1983, 1986; Mirsalis and Butterworth 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-DNTtreated B6C3F, mice and Fischer 344 rats (George et al. 1996). 2,6-Dinitrobenzaldehyde, one of the metabolites of 2,6-DNT, was found to be a direct-acting mutagen in the *Salmonella typhimurium* strain TA98 and Tal00 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. 1983; Sayama et al. 1989). Kedderis et al. (1984) proposed a bioactivation mechanism relating to the genotoxicity of 2,6-DNT in male Fischer 344 rats. They showed that the active metabolite of 2,6-DNT in the male Fischer 344 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.

2.4.3 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. 1979,1985; Hong et al. 1985; Lane et al. 1985; Lee et al. 1978, 1985; McGee et al. 1942,1947). Other effects for 2,4- and 2,6-DNT, such as reproductive, hepatic, renal, and cancer have been noted in animals (Ellis et al. 1979, 1985; Hong et al. 1985; Lee et al. 1976,1978, 1985; Leonard et al. 1983, 1986; McGown et al. 1983; Stoner et al. 1984), 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- and 2,6-DNT than are mice (Ellis et al. 1978; Hong et al. 1985; Lane et al. 1985; Lee et al. 1975, 1978; Vemot et al. 1977), dogs appear to be the most sensitive of the three species (Ellis et al. 1979, 1985; Lee et al. 1976, 1978). However, limited intermediate-duration data using 2,6-DNT have shown mice to be more sensitive than rats (Lee et al. 1976). It should be noted that dogs were fed DNT by capsule in experimental studies, whereas the rodents received the test chemical in feed (Ellis et al. 1979, 1985; Hong et al. 1985; Lee et al. 1975, 1976, 1978, 1985).

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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.

2.5 RELEVANCE TO PUBLIC HEALTH

Issues relevant to children are explicitly discussed in 2.6 Children's Susceptibility and 5.6 Exposures of Children.

Overview.

The major route of exposure to DNT for humans living near hazardous waste sites is via ingestion of contaminated water. Because of its low octanol-water partition coefficient, DNT is not expected to accumulate in homegrown fruits and vegetables. Dermal exposure to DNT could also occur when washing or bathing with contaminated water. The low vapor pressure of DNT makes inhalation unlikely, although it could possibly be present in particulate matter in ambient air from leaks in storage containers or from contaminated soil.

Data for humans exposed to DNT are derived from occupational studies in which exposure concentrations via the inhalation route were measured. Some dermal exposure may be expected to occur in the workplace although this should be minimized with modem industrial hygiene practices. No studies were located regarding health effects in animals following inhalation exposure to DNT. No studies were located regarding health effects in humans following oral exposure to DNT. Available animal data on the toxic effects of DNT were derived from oral exposure studies. Discussions of health effects observed in animals and humans are thus complicated by the need to compare toxicity across different exposure routes. Thus, the effects observed from inhalation exposure studies in humans may not accurately reflect the effects that might be predicted after oral exposure to DNT near hazardous waste sites. However, there does appear to be some suggestive evidence of common target organs following exposure of animals and humans to DNT by different routes.

Possible effects of exposure of humans to primarily Tg-DNT include heart disease, hematological effects, and neurological effects.

In animal species, 2,4-DNT caused adverse effects in a variety of organs and tissues including the blood, nervous system, liver, kidney, and gonads. Subcutaneous and mammary gland carcinomas were also

observed. 2,6-DNT affected the blood in all animal species tested. Effects on the liver or bile duct were generally observed, and males of all species exhibited adverse effects on the reproductive system. Two toxic effects, neurotoxicity and renal toxicity, were observed in dogs orally administered 2,6-DNT that were not observed in other animal species.

Minimal Risk Levels for 2,4- and 2,6-Dinitrotoluene

Inhalation MRLS.

Data were insufficient for the derivation of acute-, intermediate-, or chronic-duration inhalation MRLs for 2,4-DNT or 2,6-DNT. No suitable NOAEL or LOAEL was found for any duration category.

Oral MRLS.

Studies in humans did not provide sufficient data regarding exposure levels and their correlation with observed effects. Therefore, animal studies were used for the derivation of the oral MBLs for both 2,4- and 2,6-DNT.

2.4-DNT

- An acute-duration oral MRL of 0.05 mg/kg/day was derived for 2,4-DNT from a NOAEL value of 5 mg/kg/day for neurotoxicity observed in dogs (Ellis et al. 1985; Lee et al. 1978). Male and female beagle dogs were dosed by capsule with 0, 1,5, or 25 mg/kg/day 2,4-DNT in intermediate- and chronic-duration studies. However, after 12 days of treatment, the first clinical signs of neurotoxicity were evident. Minimal signs of neurotoxicity were incoordination and stiffness, giving the animals an abnormal gait. No clinical signs of neurotoxicity were observed at 5 mg/kg/day. The NOAEL of 5 mg/lcg/day was divided by an uncertainty factor (UF) of 100 (10 for animal-to-human extrapolation, and 10 for human variability).

- A chronic-duration oral MRL of 0.002 mg/kg/day was derived for 2,4-DNT from a NOAEL value of 0.2 mg/kg in dogs (Ellis et al. 1979, 1985). Beagle dogs were administered 0,0.2, 1.5, or 10 mgkg 2,4-DNT in capsules for up to 24 months. Methemoglobinemia and Heinz bodies were observed in dogs fed 1.5 mg/kg. Biliary hyperplasia and neurotoxicity (paralysis and cerebellar lesions) were also noted at this dose. No testicular degeneration was observed up to 10 mgkg 2,4-DNT. The NOAEL of 0.2 mgkg for hematological and neurological effects and biliary hyperplasia was divided by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

2. HEALTH EFFECTS

2,6-DNT

Data were insufficient for the derivation of acute-duration oral MRLs for 2,6-DNT. No suitable NOAEL or LOAEL was found.

- An intermediate-duration oral MRL of 0.004 mg/kg/day for 2,6-DNT was derived from a LOAEL value of 4 mg/kg, at which extramedullary erythropoiesis in the spleen and lymphoid depletion were observed in dogs (Lee et al. 1976). Beagle dogs were administered 0,4,20, or 100 mg/kg 2,6-DNT in capsules for up to 13 weeks. Treatment-related mortality occurred at 20 and 100 mg&g 2,6-DNT. No neurological effects were found in the 4-mg/kg group, but at 20 mg/kg, listlessness, incoordination, and lack of balance were found; effects became more severe at 100 mg/kg and progressed to paralysis, occasional tremors, and inability to eat. Body weight loss correlated with food consumption at 20 and 100 mg/kg. Anemia and compensatory reticulocytosis were also found at 20 and 100 mg!kg. Other treatment-related effects observed at mid and/or high dose were thymic involution, bile duct hyperplasia, testicular degeneration, hepatic inflammation, and dilated renal tubules. None of these effects were observed in animals treated with 4 mg/kg. However, after 13 weeks, mild extramedullary erythropoiesis in the spleen and lymphoid depletion were observed at 4 mg!kg. The LOAEL of 4 mgkg for hematological effects was divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for animal-to-human extrapolation, and 10 for human variability).

Data were insufficient for the derivation of chronic-duration oral MBLs for 2,6-DNT. No suitable NOAEL or LOAEL was found.

Death.

The limited data available on inhalation exposure to 2,4-DNT or Tg-DNT suggest that there may be an increase in death due to ischemic heart disease and diseases of the circulatory system (Levine et al. 1986a). There was no increase found in death due to cancer in this study. Although no data are available regarding death in animals after inhalation exposure to 2,4- or 2,6-DNT, and only one study reported an increased incidence of death after occupational exposure to 2,4- or Tg-DNT, it appears unlikely that death would occur in people living near hazardous waste sites and exposed to low levels of 2,4- or 2,6-DNT. No data are available on death in humans from oral exposure to 2,4- or 2,6-DNT, but from animal data it can be reasonably expected that consumption of these compounds may be fatal. Insufficient data are available to predict whether dermal exposure to 2,4- or 2,6-DNT would cause death in humans.

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Systemic Effects.

Respiratory Effects. Because no studies are available in humans or animals examining the respiratory endpoints after inhalation or dermal exposure to 2,4- or 2,6-DNT, it is not possible to predict whether exposure to these compounds via these routes would cause respiratory effects in humans living near hazardous waste sites. Human data are not available on respiratory effects after oral exposure to 2,4- or 2,6-DNT, but no effects on the respiratory system were found when rats were fed Tg-DNT for up to 2 years (Hazleton Laboratories 1982).

Cardiovascular Effects. Excessive rates of mortality from ischemic heart disease and residual diseases of the circulatory system were observed in workers exposed to Tg-DNT and 2,4-DNT at two separate facilities (Levine et al. 1986a). However, this finding was unusual. The heart disease mortality risk of workers is commonly lower than that of the general population because of the “healthy worker” effect. It is unlikely that a chance aggregation of risk factors independent of DNT exposure could explain the observed excess of heart disease mortality.

Although treatment-related cardiovascular lesions have not been reported in rat or mouse bioassays, these species are generally resistant to naturally-occurring or experimentally-induced atherosclerosis (Jokinen et al. 1985). Interim sacrifices in a 2-year bioassay in rats did show an exacerbation of spontaneous myocarditis, although it was not seen at study termination (Hazleton Laboratories 1982). Thus, insufficient data are available to determine whether cardiovascular effects might result from living near hazardous waste sites.

Gastrointestinal Effects. Some gastrointestinal symptoms, such as nausea and vomiting, have been reported after workers were exposed to 2,4-DNT, presumably via inhalation and dermal exposure (McGee et al. 1942, 1947). No animal studies are available regarding gastrointestinal effects after inhalation exposure to 2,4- and 2,6-DNT. The only data available after oral exposure are from a study in which no histopathological effects on the gastrointestinal tract were observed in rats fed Tg-DNT for up to 2 years (Hazleton Laboratories 1982). Thus, insufficient data are available to predict whether low-level exposure to 2,4- or 2,6-DNT might result in gastrointestinal effects in persons living near hazardous waste sites.

Hematological Effects. Early observations of workers exposed to 2,4-DNT (levels of exposure not described) report symptoms of cyanosis (McGee et al. 1942, 1947; Perkins 1919). McGee et al. (1942, 1947) also reported anemia. Occupational conditions at the time of these reports (pre-1950) undoubtedly

resulted in higher levels of human exposure than would occur in modem manufacturing facilities. Engineering, ventilation, and industrial hygiene improvements have reduced the levels of modem occupational exposure to DNT. Unfortunately, recent studies of exposed workers (Ahrenholz 1980; Ahrenholz and Meyer 1982; Hamill et al. 1982; Levine et al. 1985a) did not monitor hematological profiles.

In animal studies of intermediate duration, methemoglobinemia and its sequelae (Heinz bodies, anemia, reticulocytosis), hemosiderosis, extramedullary hematopoiesis, and slight cyanosis (Lane et al. 1985) were observed when 2,4-, 2,6-, and Tg-DNT were administered (Hazleton Laboratories 1977, 1982; Lee et al. 1976, 1978). Extramedullary erythropoiesis in the spleen and lymphoid depletion have been observed after intermediate-duration treatment of dogs with 2,6-DNT (Lee et al. 1976). In animal studies of chronic duration, anemia was observed (Ellis et al. 1979; Hazleton Laboratories 1982), but the animals often appeared to adapt, as indicated by a decrease in the hematological effects in the second year of exposure. Based on these findings in humans and animals, it appears that hematological effects may occur in persons exposed to 2,4-DNT at low levels near hazardous waste sites.

Musculoskeletal Effects. Workers at munitions plants that produced 2,4- or Tg-DNT have complained of muscle weakness, joint pain, and other incapacitating symptoms (McGee et al. 1942; Perkins 1919). Because these workers were most likely exposed to high levels of DNT without protective equipment, these effects may not necessarily be observed in persons exposed to low levels. No human or animal data are available on musculoskeletal effects of 2,4-, 2,6-, or Tg-DNT after oral exposure.

Hepatic Effects. Medical surveys of workers exposed to Tg-DNT performed during the 1970s and 1980s revealed no significant differences in hepatic blood chemistry profiles (Ahrenholz 1980; Ahrenholz and Meyer 1982). In earlier surveys of workers exposed presumably to 2,4-DNT, 2 of 154 workers (McGee et al. 1942) and 29 of 714 workers (McGee et al. 1947) indicated symptoms of liver tenderness. These reports are probably not significant. The incidence of liver tenderness reported by McGee et al. (1947) was increased over earlier reports by McGee et al. (1942), despite improvements in engineering, ventilation, and industrial hygiene in the intervening period that were likely to decrease the magnitude of worker exposures to DNT. In addition, alcohol consumption, which may also have been a cause of liver tenderness, was not taken into account.

A consistent finding in several studies of rats, mice, and dogs fed high doses of 2,4-, 2,6-, and Tg-DNT has been the occurrence of adverse effects on the liver or biliary tract (Ellis et al. 1979; Hazleton Laboratories

1982; Hong et al. 1985; Lee et al. 1976, 1978,1985). Lesions in rats were dose-dependent and may have progressed from foci of altered hepatocytes to proliferative nodules to hepatocellular carcinoma (Ellis et al. 1979; Hazleton Laboratories 1982; Leonard et al. 1987).

The significance of hepatotoxicity observed in animal studies to potential adverse liver effects in humans exposed to DNT isomers is uncertain. The negative findings in humans cannot be considered conclusive. Studies of DNT exposure are limited by the small groups of workers studied and by the lack of individual exposure monitoring. The consistent observation of hepatotoxicity in animals indicates that a potential exists for hepatotoxicity in humans.

Endocrine Effects. Although no histopathological effects on adrenal, pituitary, or thyroid glands have been observed in several bioassays, after oral exposure to 2,4- or Tg-DNT (Hazleton Laboratories 1985; McGown et al. 1983), an increase in the incidence and severity of parathyroid hyperplasia, and fatty metamorphoses and vascular ectasia were found in rats fed Tg-DNT in a chronic study (Hazleton Laboratories 1982). No data are available on endocrine effects in humans after exposure to 2,4-, 2,6-, or Tg-DNT. Therefore, the likelihood that exposure to 2,4- or 2,6-DNT will cause deleterious effects on the endocrine system in people cannot be determined.

Renal Effects. Medical surveys of workers exposed to Tg-DNT revealed no significant differences in renal blood chemistry profiles (Ahrenholz 1980; Ahrenholz and Meyer 1982). The limitations of these studies were discussed previously. Adverse effects in the kidney have been observed in laboratory animals exposed to 2,4- and 2,6-DNT, but the observations have not been as consistent as reports of hematological, reproductive, and hepatic effects in exposed animals.

Renal dysplasia was observed in male, but not female, mice exposed chronically (up to 2 years) to 2,4-DNT (Ellis et al. 1979). Cystic degeneration, atypical epithelium lining the cysts, and a variety of tumors were reported by Ellis et al. (1979). Subchronic administration of 2,6-DNT to dogs, but not mice or rats, resulted in less severe renal toxicity that included kidney degeneration (Lee et al. 1976).

The potential for renal toxicity in humans exposed to DNT is uncertain; 2,4-DNT has elicited severe renal effects only in the male mouse. Renal effects were observed in dogs administered 2,6-DNT but not when 2,4-DNT was administered.

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Dermal Effects. Dermatitis was attributed to dermal exposure to 2,4-DNT in a small percentage of munitions workers exposed topically to DNT (McGee et al. 1942, 1947). Both 2,4- and 2,6-DNT are mildly irritating to rabbit skin (Ellis et al. 1978; Lee et al. 1975). Mild sensitization has been observed in guinea pigs after 2,6-DNT exposure but was not observed after exposure to 2,4-DNT (Ellis et al. 1978; Lee et al. 1975). Therefore, although limited data are available, it appears that direct contact with low levels of 2,4- or 2,6-DNT may cause dermatitis and/or dermal sensitization in humans; however, insufficient data are available to state this with certainty.

Ocular Effects. Only animal data are available on ocular effects after exposure to 2,4- or 2,6-DNT. Histopathological examination of the eyes of rats fed Tg-DNT for up to 2 years showed no abnormalities (Hazleton Laboratories 1982). Neither 2,4- nor 2,6-DNT was found to cause eye irritation in rabbits (Ellis et al. 1978; Lee et al. 1975). There was no mention of ocular effects in studies of munitions workers exposed to 2,4- or Tg-DNT (McGee 1942, 1947; Perkins 1919). Therefore, it appears unlikely that low-level exposure to 2,4- or 2,6-DNT would cause ocular effects in people living near hazardous waste sites.

Body Weight Effects. Effects on body weight have not been reported in munitions workers (McGee et al. 1942, 1947; Perkins 1919), but numerous studies have shown body weight loss or decreased body weight gain in rats, mice, and dogs after oral treatment with 2,4-, 2,6-, or Tg-DNT (Bloch et al. 1988; Ellis et al. 1979, 1985; Hazleton Laboratories 1982; Hong et al. 1985; Kozuka et al. 1979; Lane et al. 1985; Lee et al. 1978,1985; Leonard et al. 1987; NCI 1978). However, in most of these studies there was a concurrent decrease in food consumption. Therefore, it is not known whether exposure to low levels of 2,4- or 2,6-DNT found near hazardous waste sites would cause deleterious effects on body weight in humans.

Immunological and Lymphoreticular Effects. Although no data are available regarding immunological or lymphoreticular effects in humans, some data on these end points are available in animals. No effects on serum concentrations of IgE, the antibody associated with allergic or hypersensitive reactions, were reported in rats or dogs exposed to 2,4- or 2,6-DNT (Ellis et al. 1985; Lee et al. 1976, 1978, 1985). Mild sensitization was observed in guinea pigs after dermal exposure to 2,6-DNT, but not after exposure to 2,4-DNT (Ellis et al. 1978; Lee et al. 1975). Therefore, it may be expected that human sensitizing potential to 2,4-DNT would be low but may occur after exposure to 2,6-DNT. Lymphoreticular effects have been reported in rats, mice, and dogs administered 2,6-DNT (Lee et al. 1976) and rats administered Tg-DNT (Hazleton Laboratories 1977, 1982). Splenic effects included alterations in appearance such as discoloration, enlargement, and surface irregularities; thymic involution was also seen in dogs (Hazleton Laboratories 1977,

1982; Lee et al. 1976). Based on these data in several species, it is possible that immunological or lymphoreticular effects may occur in people living near hazardous waste sites exposed to 2,4- or 2,6-DNT, but insufficient data are available to state this with certainty.

Neurological Effects. Early reports of workers occupationally exposed presumably to 2,4-DNT refer to symptoms indicative of neurotoxicity in humans. Perkins (1919) reported headaches, sleepiness, pain in the joints, and dizziness in exposed workers. McGee et al. (1942) reported that occupationally exposed workers complained of nausea, insomnia, headaches, dizziness, and tingling pains in the extremities. More recent occupational studies (Ahrenholz 1980; Ahrenholz and Meyer 1982; Hamill et al. 1982; Levine et al. 1985a) failed to examine workers for symptoms of neurotoxicity.

In animals, the nervous system has been observed to be a major target of 2,4- and 2,6-DNT toxicity (Ellis et al. 1979, 1985; Kozuka et al. 1979; Lee et al. 1978, 1985). Clinical signs have been most pronounced in dogs, and less severe symptoms have been observed in rats and mice (Ellis et al. 1979; Lee et al. 1976, 1978). Initial symptoms in dogs included incoordination and stiffness, especially of the hind legs, resulting in an abnormal gait. More seriously affected dogs had paralysis of the hind legs which progressed to the forelimbs and eventually the neck. CNS lesions, including cerebellar vacuolization, hypertrophy and focal gliosis, and some cerebellar and brain stem perivascular hemorrhage were seen in dogs fed 2,4-DNT (Ellis et al. 1979, 1985). In the mouse, there were signs of depression and hyperexcitability. Neurotoxic symptoms in rats administered 2,4-DNT were less common, but some rats administered 2,6-DNT had neuromuscular symptoms of incoordination and abnormal gait.

There are no data on the biochemical events involved in the toxicity of the nervous system. Based on the available human and animal data, it appears that neurological effects may occur in persons exposed to low levels of 2,4- or 2,6-DNT.

Reproductive Effects. A consistent finding in several studies in rats, mice, and dogs dosed with 2,4- or 2,6-DNT has been the impairment of the male reproductive system. Testicular atrophy, degeneration of the seminal vesicles, and decreased sperm production have been observed in the test animals (Bloch et al. 1988; Ellis et al. 1979; Lee et al. 1976, 1978).

Sertoli cells play a major role in maintaining spermatogenesis in the testis. Bloch et al. (1988) reported that fine structural alterations of Sertoli cells accompanied the diminished sperm count in rats acutely exposed to

2,4-DNT. Other deleterious effects included frayed basal lamina and distortion of peritubular tissue. Sertoli cell cultures prepared from testes of Wistar rats and treated with up to 100 pM 2,4- or 2,6-DNT remained intact, although at 50 FM of 2,4- or 2,6-DNT, some damage was observed as many of the germ cells were absent and some Sertoli cells contained cytoplasmic vacuoles (Reader and Foster 1990). Germ cell detachment from Sertoli germ cell cocultures was significantly increased ($p<0.05$) compared to controls at 10 FM 2,4- or 2,6-DNT (Reader and Foster 1990). Further evidence of disruption of Sertoli cell function was observed as increased production of lactate and pyruvate, although increases in pyruvate production were minimal with 2,6-DNT (Reader and Foster 1990). Thus, it appears that structural changes in the Sertoli cells may be precipitating events responsible for spermatogenic disruption noted in 2,4-DNT exposed rats. Bloch et al. (1988) also reported increased serum levels of FSH. Increased FSH levels have been associated with Sertoli cell misfunction.

In rodent studies measuring the fertility of test animals dosed with 2,4-DNT, there have been marked and significant decreases in the number of fetal implants, which have been attributed to the adverse impacts of 2,4-DNT on sperm production (Ellis et al. 1979; Lane et al. 1985; Lee et al. 1978).

Based on the adverse effects on sperm production and fertility seen in oral studies in animals, assessments of the reproductive effects of 2,4- and 2,6-DNT on occupationally exposed workers have been conducted. As described previously, inhalation is assumed to be the main route of exposure, with probable concurrent dermal and oral exposure. Three of these studies, Hamill et al. (1982), Ahrenholz and Meyer (1982), and Levine et al. (1985a), reported no detectable differences in sperm levels or fertility rates as a result of occupational exposure.

In an earlier, study Ahrenholz (1980) reported a significant reduction in the sperm counts of exposed male workers as well as an increase of marginal statistical significance in the number of spontaneous abortions in the wives of exposed workers.

The small exposure populations and lack of historical individual exposure monitoring limit the effectiveness of the occupational studies to detect adverse effects on reproduction. Although the findings reported by Ahrenholz (1980) are suggestive of a reproductive problem in exposed workers (especially with regard to the findings of reproductive toxicity in animal studies), the human results cannot be considered conclusive, however, neither can they be dismissed as insignificant. Therefore, insufficient data are available to

determine whether people exposed to low levels of 2,4- or 2,6-DNT near hazardous waste sites may have reproductive effects.

Developmental Effects. No defects were found in rat pups after dams were fed 2,4-DNT in the diet during gestation (Ellis 1979). Decreased pup viability in this study was believed to result from a high incidence of maternal death during parturition and maternal neglect.

Tg-DNT was orally administered to pregnant rats during gestation and adverse effects upon blood elements and organ weights were observed in the dams and the fetuses (Jones-Price et al. 1982). The findings of fetal hematotoxicity imply that oxygen supply to developing tissues may be impaired by exposure of mothers to DNT. Exposure to any substance that depletes the amount of oxygen available to developing fetal tissues can have adverse consequences. However, insufficient data are available to predict whether low-level exposure to 2,4- or 2,6-DNT may cause developmental effects in humans.

Genotoxic Effects. Some results of *in vitro* genotoxicity assays are presented in Tables 2-3 (2,4-DNT), 2-4 (2,6-DNT), and 2-5 (Tg-DNT). Results of *in vivo* genotoxicity assays are presented in Table 2-6. DNT causes gene mutations in the reverse mutation assay using *S. typhimurium.* However, the test system has given variable results because of the need for metabolic activation and the sensitivity of the tester strains.

Unscheduled DNA synthesis (UDS) and S-phase synthesis (SPS) were induced *in vitro* in the hepatocytes of Fischer-344 rats treated with 2,4-DNT *in vivo* (Mirsalis et al. 1989). The genotoxicity of Tg-DNT is believed to be due to the potent genotoxicity of the 2,6-DNT component, as was evidenced in an *in vivo-in vitro* hepatocyte UDS system (Mirsalis and Butterworth 1982). 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. 1989b). The putative metabolite 2,6-dinitrobenzaldehyde was a direct acting mutagen, that is, it did not require activation (Sayama et al. 1989b). Urine from Fischer-344 rats administered 75 mg/kg 2,6-DNT by gavage tested positive for mutagenicity using *S. typhimurium* TA 98 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).

TABLE 2-3. Genotoxicity of 2,4-Dinitrotoluene *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Salmonella typhimurium	Reverse mutation	-	+	Couch et al. 1981
TM 677	Forward mutation	+	+	Couch et al. 1981
S. typhimurium	Reverse mutation	NT	-	Chiu et al. 1978
S. typhimurium				
(TA 98)	Reverse mutation	-	-	Dellarco and Prival
(TA 100)	Reverse mutation	-	-	1989
S. typhimurium	Reverse mutation	+	+	Tokiwa et al. 1981
S. typhimurium with flavin mononucleide				
(TA 98)	Reverse mutation	+	-	Dellarco and Prival
(TA 100)	Reverse mutation	-	-	1989
S. typhimurium	Reverse mutation	NT	+	Mori et al. 1982
S. typhimurium	Reverse mutation	+	+	Spanggord et al. 1982b
S. typhimurium				
(TA 100)	Base-pair substitution	+	+	Ellis et al. 1978
(TA 1535)	Base-pair substitution	-	-	
(TA 98, 1537)	Reverse mutation	-	-	
(TA 1538)	Reverse mutation	+	-	

TABLE 2-3. Genotoxicity of 2,4-Dinitrotoluene *In Vitro* (*continued*)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
S. typhimurium	Reverse mutation	+	NT	Pearson et al. 1979
S. typhimurium	Reverse mutation			Einistö et al. 1991
(TA 98)		NT	+	
(TA 98 NR)		NT	+	
(TA 98/1,8-DNP_6)		NT	+	
(YG 1021)		NT	+	
(YG 1024)		NT	+	
E. coli	Reverse mutation	-	-	Dunkel et al. 1985
Chinese hamster ovary cells (CHO)	Sister chromatid exchange	+	-	Loveday et al. 1989
	Chromosomal aberrations	-	-	Loveday et al. 1989
CHO/HEPRT	Forward mutation	-	-	Abernethy and Couch 1982
P388 mouse lymphoma TK	Forward mutation	-	+	Styles and Cross 1983
Syrian hamster embryo cells	Morphological transformation	NT	-	Holen et al. 1990

+ = positive result; - = negative result; NT = not tested

TABLE 2-4. Genotoxicity of 2,6-Dinitrotoluene *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Salmonella typhimurium	Reverse mutation	+	+	Couch et al. 1981
TM 677	Forward mutation	+	+	Couch et al. 1981
S. typhimurium				
TA 100, TA 1535	Base-pair substitution	–	–	Ellis et al. 1978
TA 98, 1537	Reverse mutation	–	–	
TA 1538	Reverse mutation	–	+	
S. typhimurium	Reverse mutation	NT	+	Simmon et al. 1977
S. typhimurium				
TA 98, TA 100	Reverse mutation	–	–	Sayama et al. 1989
S. typhimurium	Reverse mutation	–	+	Tokiwa et al. 1981
S. typhimurium	Reverse mutation	+	+	Spanggord et al. 1982b
CHO/HEPRT	Forward mutation	–	–	Abernethy and Couch 1982
P388 mouse lymphoma TK	Forward mutation	–	–	Styles and Cross 1983
Syrian hamster embryo cells	Morphological transformation	NT	–	Holen et al. 1990

TABLE 2-4. Genotoxicity of Trichloroethylene *In Vitro* (*continued*)

Species (test system)	End point	Results		Reference
		With activation	Without activation	
S. typhimurium	Reverse mutation			Einistö et al. 1991
(TA 98)		NT	+	
(TA 98 NR)		NT	–	
(TA 98/1,8-DNP_6)		NT	+	
(YG 1021)		NT	+	
(YG 1024)		NT	+	
S. typhimurium	Reverse mutation	+	NT	Pearson et al.

+ = positive result; – = negative result; NT = not tested

TABLE 2-5. Genotoxicity of Technical-Grade DNT

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Salmonella typhimurium	Reverse mutation	+	+	Couch et al. 1981
S. typhimurium	Reverse mutation[a]	NT	+	Chadwick et al. 1990
TM 677	Forward mutation	+	+	Couch et al. 1981
CHO/HEPRT	Forward mutation	-	-	Abernethy and Couch 1982
P388 mouse lymphoma TK	Forward mutation	-	-	Styles and Cross 1983

[a]Urine from CD-1 mice or Fischer-344 rats that had been treated *in vivo* with DNT was used.

+ = positive result; - = negative result; NT = not tested

TABLE 2-6. Genotoxicity of 2,4-Dinitrotoluene *In Vivo*

Species (test system)	End point	Results	Reference
Rat hepatocyte	UDS	+	Mirsalis et al. 1989
Rat hepatocyte	SPS	+	Mirsalis et al. 1989
Human lymphocyte	Chromosomal aberration	+	Huang et al. 1995
Mouse bone marrow	Micronucleus	-	Ashby et al. 1985
Rat hepatocyte	UDS	+	Ashby et al. 1985
Rat hepatocyte	UDS	+	Mirsalis and Butterworth 1982
Rat hepatocyte	DNA binding	+	La and Froines 1993
Rat hepatocyte	DNA binding	+	Chadwick et al. 1993
Human lymphocyte	Chromosomal aberration	+	Huang et al. 1995
Rat hepatocyte	UDS	+	Ashby et al. 1985

+ = positive result; - = negative result; DNA = deoxyribonucleic acid; SPS = *S*-phase synthesis; UDS = unscheduled DNA synthesis

2. HEALTH EFFECTS

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, three DNA adducts were found in the liver of Fischer-344 rats (La and Froines 1992). Four DNA adducts, which were not identified, were found in the liver of rats treated with 75 mg/kg 2,6-DNT by gavage (Chadwick et al. 1993). The formation of 4 DNA adducts was also observed after intraperitoneal administration of 219 mg/kg 2,6-DNT to Fischer-344 rats in a study by La and Froines (1992, 1993). One adduct accounts for the majority of the radioactivity measured; about 85% of the total was 1 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 lo-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 more than 40% in the 2 weeks after exposure (La and Froines 1992).

Cancer. 2,4-DNT administered in the diet of mice caused cancer of the kidneys (Ellis et al. 1979); 2,4-, 2,6-, and Tg-DNT caused hepatocellular carcinoma in rats (Ellis et al. 1979; Hazleton Laboratories 1982; Leonard et al. 1987). 2,4-DNT was shown to be a tumor promoter, but not a tumor initiator, using *in vivo* hepatic initiation-promotion protocols (Leonard et al. 1983, 1986; Mirsalis and Butter-worth 1982). 2,6-DNT and Tg-DNT were shown to be complete carcinogens using the same protocols (Leonard et al. 1983,1986). The results of Leonard et al. (1983, 1986, 1987) and Mirsalis and Butterworth (1982) suggest or show that 2,6-DNT is the isomer most responsible for the carcinogenic response observed in animal studies with Tg-DNT. No increase in morphological transformation was observed in Syrian hamster embryo cells (SHE) compared to controls when SHE cells were exposed to 2,4-, 2,6-, or Tg-DNT, or to the DNT metabolites 2,4-diaminotoluene, 2-amino-4-nitrotoluene, 2-amino-6-nitrotoluene, or 2,4-dinitrobenzoic acid (Holen et al. 1990). In addition, none of these isomers had initiator-like or promotor-like effects in this assay when tested with *O*-tetradecanoyl phorbol 13-acetate or benzo[a]pyrene, respectively.

In order to evaluate the possibility that DNT might also cause cancer in humans, Levine et al. (1986b) performed a retrospective cohort mortality study at two army ammunition plants that used Tg-DNT and/or 2,4-DNT. No significant increases in mortality from malignant neoplasms as a whole or from particular cancers (liver, lung, gallbladder, kidney, and connective tissues) were observed. The study was limited by small cohort size, and could have detected only an 8-fold or greater increase in liver or gallbladder cancer. As

a result, the negative findings of Levine et al. (1986b) do not refute the animal cancer findings with regard to the potential for cancer in humans. The cancer classification for 2,4- and 2,6-DNT mixture is B2: probable human carcinogen (EPA 1992). An upperbound q_1* for oral exposure to 2,4- and 2,6-DNT mixture was estimated by EPA (1986d, 1998) to be 0.68 $(mg/kg/day)^{-1}$ based on the combined tumor incidence of liver and mammary gland tumors in rats. Doses associated with excess cancer risks of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} by the oral routes are plotted in Figure 2-1.

2.6 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 due to 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 5.6 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 pre-natal and post-natal 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 (Morselh 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 (Widdowson and Dickerson 1964; Foman et al 1982; Owen and Brozek 1966; Altman and Dittmer 1974; Foman 1966). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis

barrier (Setchell, BP and Waites GMH 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns and 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 (Leeder and Keams 1997; Komori 1990; Vieira et al 1996; NRC 1993). Whether differences in xenobiotic metabolism make the child more or less susceptible also depend 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 the newborn who has a low glomerular filtration rate and has not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; West et al 1948; NRC 1993). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer 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 while others may decrease susceptibility to the same chemical. For example, the fact that infants breathe more air per kilogram of body weight than adults may be somewhat counterbalanced by their alveoli being less developed, so there is a disproportionately smaller surface area for 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 DNT 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 DNT does not cause dominant lethal mutation or increases in the proportion of nonviable conception (Ellis et al. 1979).

2. HEALTH EFFECTS

DNT has 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 *Salmonella typhimurium* strains TA98 and TAlOO 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 DNT is 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, DNT did not cause dominant lethal mutation or increases in the proportion of nonviable conceptions following exposure of male animals (Ellis et al. 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 DNT and its metabolites will accumulate in maternal tissues because of its low octanolwater partition coefficient. No studies are available that demonstrate DNT or its metabolites cross the placenta or get into breast milk. Thus, it is unlikely that the developing fetus or nursing infant would be exposed to DNT as a consequence of maternal exposure prior to gestation. However, developmental toxicity from DNT 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 hematoxicity of DNT 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 DNT 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-l 8 months of age (Leeder and Keams 1997). Thus, the toxicity of DNT may be different in children.

2.7 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/NFX 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic

substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NASLNRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 2,4- and 2,6-dinitrotoluene are discussed in Section 2.7.1.

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 2,4- and 2,6-dinitrotoluene are discussed in Section 2.7.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism’s ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.9, Populations That Are Unusually Susceptible.

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

2. HEALTH EFFECTS

2.7.1 Biomarkers Used to Identify or Quantify Exposure to 2,4- and 2,6-Dinitrotoluene

Spectrophotometric analysis of complexes of primary arylamines in urine, resulting from the reduction of DNT and its metabolites, has been used to biomonitor DNT workers (Smith et al. 1995). The method is specific for primary arylarnines and eliminates interferences from other classes of amines that might be present in the urine. Metabolic intermediates and conjugates of DNT can also be detected if they are present as primary arylamines. Although this method cannot identify or quantify individual metabolites, it is simple and accurate and yields results in about 1 hour.

Workers exposed to DNT 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 DNT 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 DNT ranged from 0.5 to 4.9 mg/day, less than the total excreted. These results indicate that dermal and oral exposure contributed to the body burden of DNT.

Woollen et al. (1985) determined that the urinary concentration of a Tg-DNT metabolite, 2,4-dinitrobenzoic acid, was less than 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.

2.7.2 Biomarkers Used to Characterize Effects Caused by 2,4- and 2,6-Dinitrotoluene

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDRDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

After exposure to DNT, methemoglobin levels in the blood may be elevated (Elleuhorn 1997). The methemoglobinemia present may be quite profound and its onset is often delayed by up to 4 hours (Ellenhorn

1997). Another hematological change that might be present in individuals who have undergone repeated or prolonged exposure to DNT 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 (Block et al. 1988; Ellis et al. 1979). However, decrease in sperm counts in workers exposed to DNT has been reported in only one study (CDC 1981).

2.8 INTERACTIONS WITH OTHER CHEMICALS

Reduced tolerance to alcohol was observed in some workers presumably exposed to 2,4-DNT and other workers reported that alcohol ingestion intensified the symptoms of 2,4-DNT exposure (McGee et al. 1942). Perkins (19 19) 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 Fischer-344 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.

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

2. HEALTH EFFECTS

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 2,4- and 2,6-dinitrotoluene than will most persons exposed to the same level of 2,4- and 2,6-dinitrotoluene 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 may result in reduced detoxification or excretion of 2,4- and 2,6-dinitrotoluene, or compromised function of target organs affected by 2,4- and 2,6-dinitrotoluene. Populations that are at greater risk due to their unusually high exposure to 2,4- and 2,6dinitrotoluene are discussed in Section 5.7, Populations with Potentially High Exposure.

Humans sensitive to DNT 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 DNT 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 19 19), the extent of this compromise has not been determined. The susceptibility of children to the health effects of DNT may be different from that of adults, as discussed in Section 2.6.

2.10 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 2,4- and 2,6-dinitrotoluene. 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 2,4- and 2,6-dinitrotoluene. 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 2,4- and 2,6-dinitrotoluene: Bronstein and Currance 1994; Ellenhom 1997.

2. HEALTH EFFECTS

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.

2.10.1 Reducing Peak Absorption Following Exposure

Limited information from humans indicates that DNT is absorbed after inhalation exposure, while animal data suggest that DNT is rapidly and completely absorbed after oral exposure. Efforts to reduce absorption following acute exposure to DNT 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 (Bronstein and Currance 1994). It is suggested that eyes exposed to DNT be copiously irrigated with water and normal saline (Bronstein and Currance 1994). If ingestion of DNT occurs, it is suggested that the mouth be rinsed and water can be administered for dilution if the patient can swallow, has a good gag reflex, and is not drooling (Bronstein and Currance 1994). In addition, the use of activated charcoal has been suggested (Bronstein and Currance 1994). Induction of emesis is contraindicated (Bronstein and Currance 1994). In patients who present within 2 to 4 hours of DNT ingestion, gastric lavage may be helpful in decreasing peak absorption following exposure (Ellenhom 1997). There may also be some benefit in administering activated charcoal and cathartics after lavage (Ellenhorn 1997).

2.10.2 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 (Ellenhorn 1997).

2.10.3 Interfering with the Mechanism of Action for Toxic Effects

Exposure to DNT can cause profound methemoglobinernia with its sequelae (cyanosis and Heinz body formation), anoxia, and death (Ellenhorn 1997). The antidote used for serious methemoglobinemia is methylene blue (tetramethylthionine chloride), but treatment with methylene blue is not indicated for all patients (Ellenhorn 1997). Treatment with methylene blue is believed to be effective because it acts as a cofactor to increase the erythrocyte reduction of methemoglobin in the presence of NADPH (Ellenhorn 1997). Methylene blue is oxidized and the resulting molecule becomes an electron donor for the nonenzymatic

reduction of methemoglobin to oxyhemoglobin (Ellenhorn 1997). Exchange transfusion and/or packed RBC transfusion may be useful for patients who do not respond to methylene blue or for patients with G6PD- or NADPH-methemoglobin reductase deficiencies (Ellenhorn 1997).

2.11 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 2,4- and 2,6-DNT 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 2,4- and 2,6-dinitrotoluene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.11.1 Existing Information on Health Effects of 2,4- and 2,6-Dinitrotoluene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 2,4-, 2,6-, and Tg-DNT are summarized in Figures 2-7,2-8, and 2-9, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of 2,4-, 2,6, 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* (ATSDR 1989), is substancespecific 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.

2. HEALTH EFFECTS

2.11.2 Identification of Data Needs

As shown in Figures 2-7 (2,4-DNT), 2-8 (2,6-DNT), and 2-9 (Tg-DNT), there are limited data on health effects in humans, primarily for Tg-DNT, following inhalation exposure.

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-life-time studies.

Acute-Duration Exposure. Although there are no human data available from acute-duration oral exposure to 2,4- or 2,6-DNT, the data currently available from animal studies using single-dose exposure to 2,4- and 2,6-DNT are appropriate for evaluation of oral toxicity (Ellis et al. 1978; Lee et al. 1975; Vernot et al. 1977). The LD_{50}s 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 (Ellis et al. 1978; Lee et al. 1975; Vernot et al. 1977). After oral administration of 2,6-DNT, LD_{50}s ranged from 180 to 795 mg/kg in rats and from 621 to 807 mg/kg in mice (Ellis et al. 1978; Lee et al. 1975; Vemot et al. 1977). Ataxia was observed in these animals before death. 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). 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). An acute-duration oral MRL has been derived for 2,4-DNT from a NOAEL for neurotoxicity in dogs (Ellis et al. 1985; Lee et al. 1978). Data were insufficient to derive an acute-duration oral MRL for 2,6-DNT.

There were no acute-duration inhalation or dermal studies in humans available for evaluation. Both 2,4- and 2,6-DNT were shown to be mild primary dermal irritants in rabbits (Ellis et al. 1978; Lee et al. 1975). Acute inhalation and dermal studies would be useful for determination of route-specific toxicity.

FIGURE 2-7. Existing Information on Health Effects of 2,4-DNT

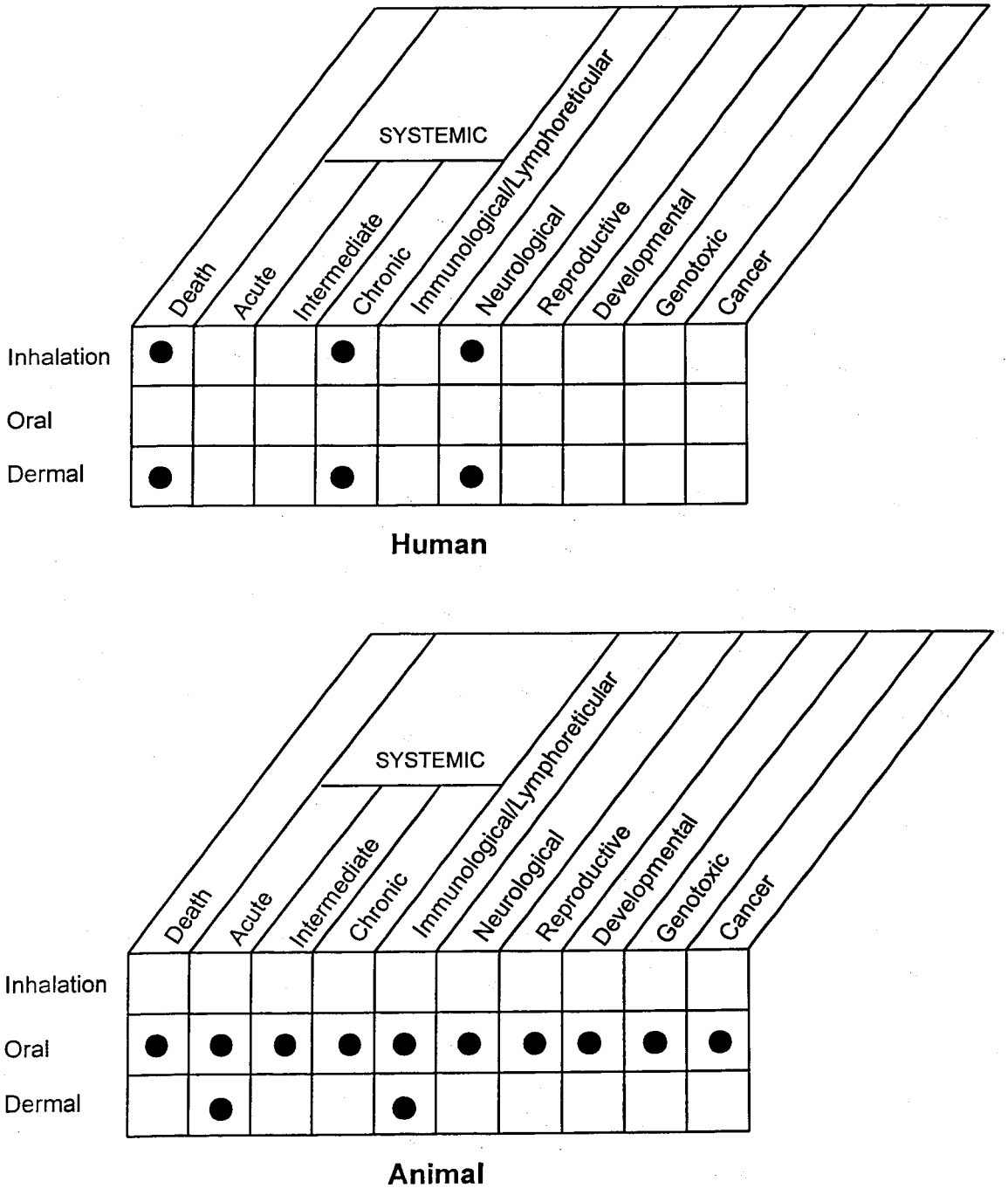
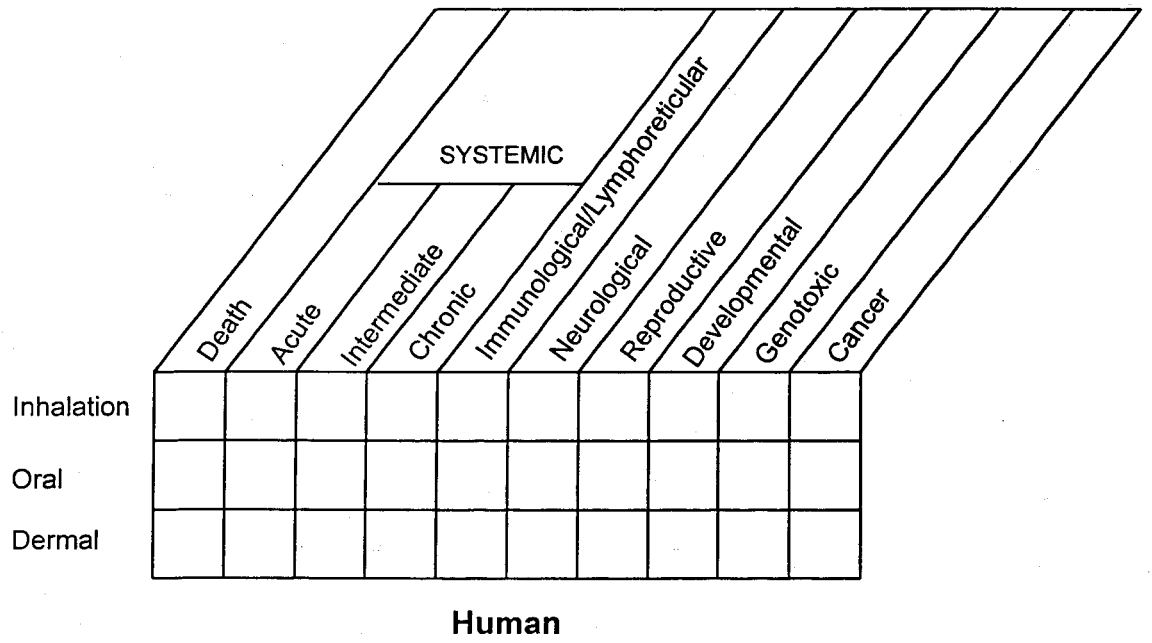
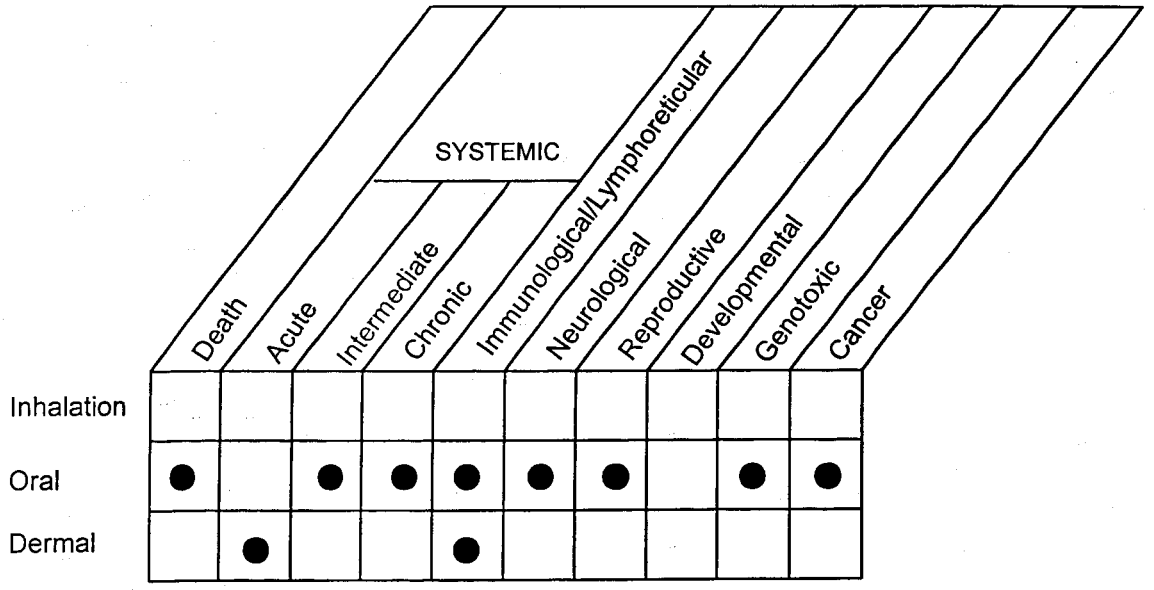


FIGURE 2-8. Existing Information on Health Effects of 2,6-DNT



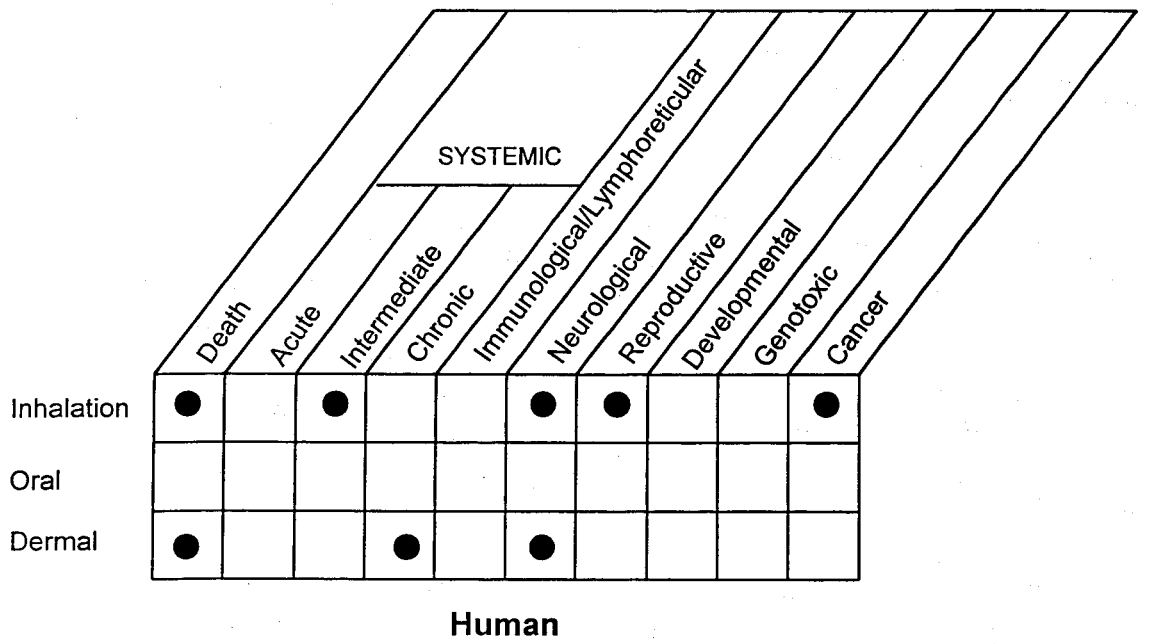
Human



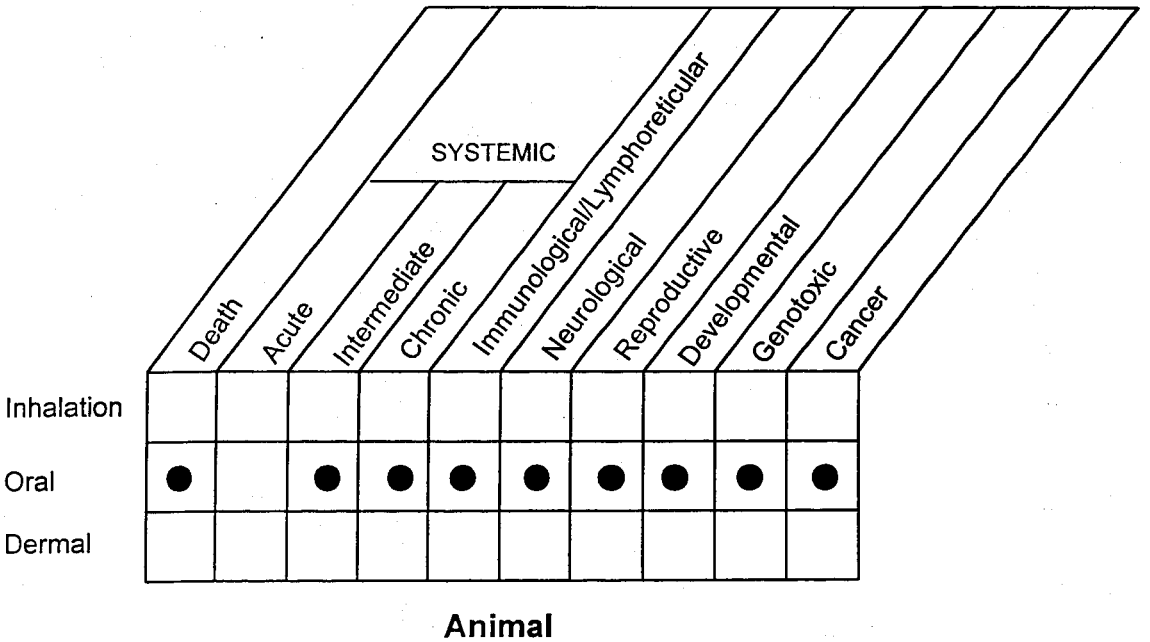
Animal

● Existing Studies

FIGURE 2-9. Existing Information on Health Effects of Tg-DNT



Human



Animal

● Existing Studies

2. HEALTH EFFECTS

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. 1979, 1985; Hazleton Laboratories 1977, 1982; Hong et al. 1985; Jones-Price et al. 1982; Lee et al. 1976,1978,1985; McGown et al. 1983; Smith et al. 1996). 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; Lee et al. 1976,1978). Mild hepatocellular dysplasia was observed in mice fed 2,4-DNT in the diet for 13 weeks (Hong et al. 1985; Lee et al. 1978), but no hepatotoxicity was observed after 2,4-DNT administration to rats or dogs for the same duration (Ellis et al. 1985; Lee et al. 1978). However, treatment with 2,6-DNT did cause bile duct hyperplasia in rats and mice (Lee et al. 1976). This lesion, as well as hepatic degeneration, was observed in dogs dosed with 2,6-DNT (Lee et al. 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; Lee et al. 1978). Administration of 2,6-DNT to dogs for 13 weeks caused renal inflammation and degeneration, which were not observed in rats or mice (Lee et al. 1976). Decreased body weight gain or weight loss was observed in rats and mice after administration of 2,4-DNT (Ellis et al. 1979; Hong et al. 1985; Lee et al. 1978, 1985; Leonard et al. 1987; NCI 1978) and in rats, mice, and dogs after administration of 2,6-DNT (Lee et al. 1976). An intermediate-duration oral MRL has been derived for 2,6-DNT based on a LOAEL for hematological effects in dogs (Lee et al. 1976). Subchronic inhalation and dermal studies 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 2,4- or 2,6-DNT. A retrospective cohort mortality study performed using data from workers at ammunition plants that used 2,4- 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 (Ellis et al. 1979; Leonard et al. 1987). Renal cancer was observed in mice after administration of 2,4-DNT in the diet (Ellis et al. 1979). An upperbound qi* has been derived for oral exposure to 2,4-DNT/2,6-DNT mixture (EPA 1986d, 1998).

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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. Studies performed in nonhuman primates to investigate further the potential cardiovascular effects of both chemicals would help to elucidate the mechanism of heart disease observed.

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. 1979, 1985; Hazleton Laboratories 1982; Lee et al. 1978, 1985; Leonard et al. 1978; NCI 1978). 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. 1979, 1985; Hong et al. 1985; Lee et al,. 1978). A chronic-duration oral MRL has been derived for 2,4-DNT based on a NOAEL of 0.2 mg/kg for hematological and neurological effects and biliary hyperplasia in dogs (Ellis et al. 1979, 1985). 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. 1979, 1985; Hong et al. 1985; Leonard et al. 1987). Renal cystic dysplasia was observed in mice, but not rats or dogs, treated orally with 2,4-DNT for chronic-duration periods (Ellis et al. 1979; Hong et al. 1985). 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. 1979, 1985; Hazleton Laboratories 1982; Hong et al. 1985; Leonard et al. 1987; NCI 1978).

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.

2. HEALTH EFFECTS

Genotoxicity. Both 2,4- and 2,6-DNT cause gene mutations in the reverse mutation assay using S. *typhimurium*. (Couch et al. 198 1; Dellarco and Prival1989; Ellis et al. 1978; Spanggord et al. 1982b; Tokiwa et al. 198 1). 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 unscheduled DNA synthesis 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; Ellis et al. 1979; Lee et al. 1976, 1978). *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 (Ahrenholz and Meyer 1982; Hammill et al. 1982; Levine et al. 1985a). 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 (Ahrenholz 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.

2. HEALTH EFFECTS

Developmental Toxicity. No data are available regarding developmental effects in humans after oral exposure to DNT, 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 endpoints 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 DNT. Mild sensitization has been reported in guinea pigs after dermal exposure to 2,6-DNT, but not 2,4-DNT (Ellis et al. 1978; Lee et al. 1975). 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. 1976, 1978, 1985). Studies have not been performed that would describe effects on immunocompetence following exposure to DNT. 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. 1979, 1985; Kozuka et al. 1979; Lee et al. 1979, 1985). Clinical signs in dogs have included incoordination and stiffness of the hind legs leading to complete paralysis; cerebellar vacuolation, hypertrophy, and focal gliosis; are cerebellar and brain stem hemorrhage. In mice, depression and hyperexcitability were observed, while some rats administered 2,6-DNT showed neuromuscular symptoms. More systematic examination of the neurological effects of these compounds in laboratory animals would be useful to assess fully behavioral abnormalities and morphological damage to the nervous system. The biochemical mechanism of dinitrotoluene 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. 1942; Perkins 19 19). However, the more recent occupational studies performed failed to examine workers for symptoms of

neurotoxicity (Ahrenholz 1980; Ahrenholz and Meyer 1982; Hammill et al. 1982; Levine et al. 1985a). 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 DNT suggest a potential for heart disease in exposed populations (Levine et al. 1986a). Doses of DNT associated with heart disease in humans have not been determined. Further studies with historical cohort monitoring data 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 DNT. 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 endpoints in humans would be useful.

Biomarkers of Exposure and Effect

Exposure. Recently a rapid, accurate method for determining exposure to DNT has been developed using spectrophotometric analysis of complexes of primary arylamines, which result from the reduction of DNT 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 DNT 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 DNT 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 to 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 DNT 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 DNT 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 DNT. Methylene blue treatment is used with patients presenting with serious methemoglobinemia (Ellenhorn 1997). No additional studies are considered necessary at this time to examine further methods for reducing body burden of DNT. 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. No data are available on the health effects of DNT on exposed children. Few usable data are available on whether the developmental process is altered by exposure to DNT; this information is summarized above under the Developmental Toxicity heading.

2. HEALTH EFFECTS

There is inadequate experimental evidence to evaluate if the pharmacokinetics of DNT are different in children. There are no studies on whether DNT or its 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 DNT and its metabolites are stored in maternal tissues and thus might be later mobilized during gestation or lactation; however, DNT and its 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 DNT or its mechanisms of action are different in children. As discussed in section 2.6, newborns are highly sensitive to the methemoglobingenerating effect of DNT 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 DNT. It will also be helpful to have data on the metabolism and mechanism of action of DNT on children to determine if children are more vulnerable than adults to health effects from exposure to DNT, 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 DNT is shown to be genotoxic, it is not known if parental exposure to DNT may affect children via parental germ cells, or if DNT may indirectly affect the fetus during maternal exposure.

Child health data needs relating to exposure are discussed in 5.8.1 Data Needs: Exposures of Children.

2.11.3 Ongoing Studies

No information was located regarding ongoing research related to the potential health effects of 2,4- or Tg-DNT.

A project to develop and apply biomarkers of exposure associated with 2,6-DNT is being conducted by S. Rappaport at the University of North Carolina at Chapel Hill (FEDRIP 1997). The project will ultimately perform epidemiological studies of exposed populations in order to provide exposure-biomarker relationship. It is anticipated that *in vivo* and *in vitro* measurements of the protein adducts will provide data on the disposition of reactive intermediates and mechanisms of toxicity.