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 1,3-dinitrobenzene and 1,3,Minitrobenzene (1,3-DNB and 1,3,5-TNB) and a depiction of significant exposure levels associated with various adverse health effects. It contains descriptions and evaluations of studies and presents levels of significant exposure for 1,3-DNB and 1,3,5-TNB based on toxicological studies and epidemiological investigations.

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 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 and lymphoreticular, 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 lowestobserved-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 in 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 serous” 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 to determine 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 tables and figures may differ depending on the user’s perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons may be interested in levels of exposure associated with “serious” effects. Public health officials and project managers 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 (NOAEL) 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.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for 1,3-DNB and 1,3,5-TNB. 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 noncancer health effects only and do not reflect a consideration of carcinogenic effects.. Marls 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 1990b), 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.

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

2.2.1 lnhalation Exposure

1,3-DNB and 1,3,5-TNB are nitrobenzene compounds that are structurally similar. The only difference in structure between 1,3-DNB and 1,3,5-TNB is the presence of an additional nitro group in 1,3,5-TNB. There is no information on 1,3,5-TNB exposure by the inhalation route. There is also very little information regarding inhalation exposure to 1,3-DNB.

The one study found on inhalation exposure is a report of an occupational exposure to 1,3-DNB (Okuba and Shigeta 1982). The study indicates that inhalation was the main route of exposure, while skin absorption was much less important. Since precise labels of exposure are not known, the results are not presented in a table or figure.

2.2.1.1 Death

No studies were located regarding lethal effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

2.2.1.2. Systemic Effects

No studies were located regarding gastrointestinal, musculoskeletal, dermal, or ocular effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

Respiratory Effects. No studies were located regarding respiratory effects in humans after inhalation exposure to 1,3,5-TNB. One retrospective study (Okuba and Shigeta 1982) of acute occupational exposure to 1,3-DNB dust particles was located. Six workers were removing crystallized 1,3-DNB from tank and were protected with gauze masks and rubber gloves. Exposure occurred over a period of 6 days. By the end of the exposure period, some of the workers complained of slight dyspnea upon exertion. Inhalation was considered to be a primary route of exposure because a relatively small skin area (face and neck) was exposed. Limitations of this study include lack of

information on the concentration of 1,3-DNB in the air, the amount of particulate 1,3-DNB deposited on workers' skin, and the exact duration of exposure.

No studies were located regarding respiratory effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to 1,3,5-TNB. One retrospective study (Okubo and Shigeta 1982) of acute occupational exposure to 1,3-DNB dust particles was located. Six workers were removing crystallized 1,3-DNB from a tank and were protected with gauze masks and rubber gloves. Exposure occurred over a period of 6 days. By the end of the exposure period, some of the workers complained of palpitations upon exertion. Inhalation was considered to be a primary route of exposure because a relatively small skin area (face and neck) was exposed. Limitations of this study include lack of information on the concentration of 1,3-DNB in the air, the amount of particulate 1,3-DNB deposited on workers' skin, and the exact duration of exposure.

Hematological Effects. No studies were located regarding hematological effects in humans after inhalation exposure to 1,3,5-TNB. Cyanosis was the first symptom noticeable within a day after an acute human exposure to I,3-DNB. Slight-to-moderate anemia with a decrease in specific gravity of the whole blood was also observed in all six workers engaged in the clean-up of crystallized 1,3-DNB (Okubo and Shigeta 1982). It should be noted however that measurement of specific gravity of blood is not common practice and represents an indirect measurement of anemia. No information on methemoglobin levels was available. It is important to stress that pathological effects (cyanosis due to methemoglobin formation) observed after exposure to 1,3-DNB in one system can be manifested as symptoms in another. Other limitations of this study include lack of information on 1,3-DNB concentration in the air and the fact that data were collected 10 days after exposure. No long-term adverse effects were noted in any of the workers that were followed for up to 10 years after exposure to 1,3-DNB.

No studies were located regarding hematological effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

Hepatic Effects. No studies were located regarding hepatic effects in humans after inhalation exposure to 1,3,5-TNB. Data on the effects of 1,3-DNB on the liver are inconclusive. Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels in humans were within normal limits after a single acute exposure to 1,3-DNB (Okubo and Shigeta 1982). Bilirubin was found in the urine of all workers who were strongly positive for urobilinogen indicating an unspecified degree of hepatobiliary disease. This study is limited by the fact that there are no data on the concentration of 1,3-DNB in the air.

No studies were located regarding hepatic effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

Renal Effects. No studies were located regarding renal effects in humans after inhalation exposure to 1,3,5-TNB. Elevated levels of urobilinogen were found in all workers exposed to 1,3-DNB indicating hemolysis (Olcubo and Shigeta 1982). Limitations of the study are that data were collected 10 days after exposure and information is lacking on the dose of 1,3-DNB.

No studies were located regarding renal effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

2.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans after inhalation exposure to 1,3,5-TNB. Limited information is available regarding the neurological effects of 1,3-DNB. Slight headache, nausea, dizziness, and fatigue were the symptoms reported in workers after inhalation exposure to 1,3-DNB (Okubo and Shigeta 1982). These symptoms diminished gradually, and the recovery period was different in each worker. No long-term effects due to inhalation exposure to 1,3-DNB were found in any of the workers for up to 10 years after exposure.

No studies were located regarding neurological effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

No studies were located regarding the following health effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB:

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

Genotoxiciey studies are discussed in Section 2.4.

2.2.1.8 Cancer

No studies were located regarding cancer effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

The oral LD_{50} values for 1,3,5-TNB and 1,3-DNB in rats were 275 mg/kg and 59 mg/kg, respectively (Desai et al. 1991); no further details were provided in that abstract. The oral LD_{50} value for 1,3-DNB in adult male and female Carworth Farms rats was 91 mg/kg and 81 mg/kg, respectively (Cody et al. 1981). Increased mortality in Sprague-Dawley rats (Linder et al. 1990) and rabbits (Parke 1961) was observed at 48 and 100 mg/kg/day, respectively. The age of the animals appeared to influence the acute toxicity of 1,3-DNB, the older animals appearing more susceptible to general toxicity than younger animals (Linder et al. 1990). Increased mortality was observed in prepubertal mice treated with 40 mg/kg/day (Evenson et al. 1989a). Decreased survival in animals consuming 1,3-DNB over longer periods was seen at lower doses in Sprague-Dawley rats. Rats exposed to

1,3-DNB for 11 weeks exhibited an increase in mortality at 6 mg/kg/day (Linder et al. 1986). At slightly a higher doses of 1,3-DNB (12-14 mg/kg/day), increased mortality in rats (Carworth Farms) occurred between weeks 4 and 7 (Cody et al. 1981). These results indicate that there may be a difference in susceptibility to 1,3-DNB toxicity associated with the age of animals.

The LD_{50} value and all reliable LOAEL values for death in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.2.2 Systemic Effects

The highest NOAEL value and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

Respiratory Effects. The only information regarding respiratory effects of 1,3-DNB or 1,3,5-TNB in humans is that of a man who swallowed 30-40 mL of a varnish containing a nitrobenzene dye (Kumar et al. 1990). Upon admission to the hospital he was comatose and his breathing was described as very shallow. This breathing pattern may have been indirectly caused by a significant increase in methemoglobin.

Data in animals are limited to a report in which no histopathologic alterations were seen in the lungs of rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981).

Cardiovascular Effects. The only information available regarding cardiovascular effects in humans is that from a case report in which low blood pressure (80154 mm Hg) and tachycardia (160 beats per minute) were observed in a man shortly after he swallowed 30-40 mL of a varnish containing a nitrobenzene dye (Kumar et al. 1990). After gastric lavage, his blood pressure increased to 100/70 mm Hg and his heart rate decreased to 120 beats per minute. Blood pressure further increased to 106/74 and heart rate decreased to 99 beats per minute following an intravenous injection of methylene blue.

TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Rat (Carworth Farms)	once (GO)				91 M (LD50) 81 F (LD50)	Cody et al. 1981
2	Rat (Sprague-Dawley)	once (GO)				48 M (death in 10/24)	Linder et al. 1990
3	Mouse (B6C3F1/J)	once (GO)				40 M (10% mortality, but group size not given)	Evenson et al. 1989a
4	Rabbit (NS)	once (GW)				100 (death in 3 days, group size not given)	Parke 1961
	Systemic						
5	Rat (Alderley - Park)	once (GO)	Hemato	10M	15M (enlarged spleen, congestion, increased erythropoietic activity)		Blackburn et al. 1988
6	Rat (Sprague-Dawley)	once (GO)	Hemato	8M		16 M (cyanosis)	Linder et al. 1990
			Endocr	48M			
			Bd Wt	48M			
7	Rat (F-344)	once (GO)	Hemato			25 M (28% methemoglobin; cyanosis)	Philbert et al. 1987b

TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
8	Rat (Alderley - Park)	once (G)	Hemato	10M		20 M (cyanosis)	Reader et al. 1991
			Bd Wt	30M			
9	Mouse (B6C3F1/J)	once (GO)	Bd Wt	48M			Evenson et al. 1989a
	Neurological						
10	Rat (Sprague-Dawley)	once (GO)		32M		48 M (ataxia, loss of equilibrium)	Linder et al. 1990
11	Rat (F-344)	once (GO)				20 M (splayed hind limbs and flaccid paralysis of fore limbs in germ-free rats)	Philbert et al. 1987b
	Reproductive						
12	Rat (Alderley - Park)	once (GO)		10M		15 M (damaged germinal epithelium, degeneration and loss of spermatocytes and spermatids)	Blackburn et al. 1988
13	Rat (Sprague-Dawley)	once (GO)		16M		32 M (increase in diploid and decrease in haploid cells; abnormal chromatin)	Evenson et al. 1989b

TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
14	Rat (Sprague-Dawley)	once (GO)				48 M (decreased sperm counts, decreased sperm motility, abnormal sperm morphology, degeneration and sloughing of both spermatocytes and spermatids)	Linder et al. 1988
15	Rat (Sprague-Dawley)	once (GO)		8[b] M		16 M (damaged testicular epithelium, decreased spermatozoa, morphological changes in spermatozoa, histological changes in epididymis)	Linder et al. 1990
16	Rat (Sprague-Dawley)	once (GO)		12M		30 M (decreased relative testes weight, altered testicular histopathology)	Moore et al. 1992
17	Rat (Alderley-Park)	once (G)			10M (increase in plasma LDH-C4 indicative of germ cell loss)	30 M (Sertoli cell cytoplasmic retraction and vacuolation; spermatocytes loss)	Reader et al. 1991
18	Mouse (B6C3F1/J)	once (GO)		32M		48 M (decreased testes weight, reduced numbers of N and 4N testicular cells, abnormalities in chromatin stucture from epididymal sperm.)	Evenson et al. 1989a

TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	INTERMEDIATE EXPOSURE						
	Death						
19	Rat (Carworth Farms)	8 wk ad libitum (W)				12.5 M (4/6 died) 14.4 F (2/6 died)	Cody et al. 1981
20	Rat (Sprague-Dawley)	12 wk 5 d/wk (GO)				6 M (2/12 died)	Linder et al. 1986
	Systemic						
21	Rat (Carworth Farms)	16 wk ad libitum (W)	Resp	3.1 F			Cody et al. 1981
			Cardio	3.1 F			
			Gastro	3.1 F			
			Hemato	1.13M	2.64M (moderate decrease in hemoglobin)		
			Musc/skel	3.1 F			
			Hepatic	3.1 F			
			Renal	3.1 F			
			Endocr	3.1 F			
			Derm	3.1 F			
			Ocular	3.1 F			
			Bd Wt	1.32 F	3.1 F (significant, but unspecified decrease in bw gain after week 8)		

TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
22	Rat (Carworth Farms)	8 wk ad libitum (W)	Resp	14.4 F			Cody et al. 1981
			Cardio	14.4 F			
			Gastro	14.4 F			
			Hemato		4.7 M (mild decrease in hemoglobin) 6.0 F		
			Musc/skel	14.4 F			
			Hepatic	14.4 F			
			Renal	14.4 F			
			Endocr	14.4 F			
			Derm	14.4 F			
			Ocular	14.4 F			
			Bd Wt			4.7 M (bw gain reduced 43%) 6.0 F (bw gain reduced 23%)	
23	Rat (Sprague-Dawley)	12 wk 5 d/wk (GO)	Hemato		0.75 [c] M (splenic hemosiderosis)		Linder et al. 1986
			Endocr	6M			
			Bd Wt	3M	6M (16% bw loss during breeding period)		
	Immuno./Lymp						
24	Rat (Carworth Farms)	16 wk ad libitum (W)		0.40 M 0.48 F	1.13 M (increased spleen weight) 1.32 F		Cody et al. 1981

TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
25	Rat (Carworth Farms)	8 wk ad libitum (W)			4.7 M (increased spleen weight) 6.0 F	12.5 M (spleen atrophy and 14.4 F fibrosis, hemosiderin deposition)	Cody et al. 1981
26	Rat (Sprague-Dawley)	12 wk 5 d/wk (GO)		0.75 M	1.5 M (increased spleen weight)		Linder et al. 1986
	Neurological						
27	Rat (Carworth Farms)	90 day ad libitum (W)		1.13 M			Cody et al. 1981
28	Rat (Carworth Farms)	16 wk ad libitum (W)		3.1 F			Cody et al. 1981
29	Rat (Carworth Farms)	8 wk ad libitum (W)		14.4 F			Cody et al. 1981
30	Rat (Sprague-Dawley)	12wk 5 d/wk (GO)		3 M		6 M (ataxia, paresis equilibrium loss, muscle rigidity)	Linder et al. 1986
	Reproductive						
31	Rat (Carworth Farms)	8 wk ad libitum (W)		14 F		4.7 M (testicular atrophy; decreased spermatogenesis; reduced testicular weight)	Cody et al. 1981

TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
32	Rat (Carworth Farms)	16 wk ad libitum (W)		1.13 M 3.1 F	2.64 M (decreased testes weight, decrease in spermatogenesis)		Cody et al. 1981
33	Rat (Sprague-Dawley)	12 wk 5 d/wk (GO)		0.75 M	1.5 M (decreased testicular spem counts)	3 M (no sperm in testis and epididymis cauda; decreased testis and epididymal weight; infertility)	Linder et al. 1986

[a]The number corresponds to entries in Figure 2-1. Differences in levels of health effects between males and females are not indicated in Figure 2-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive an acute oral Minimal Risk Level (MRL) of 0.008 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability)
[c]Used to derive an intermediate oral MRL of 0.0005 mg/kg/day; dose adjusted for intermittent exposure by multiplying by 5/7 and divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; bw = body weight (LOAEL column); Cardio = cardiovascular; CV = conventional; d = day(s); Derm = dermal; Endocr = endocrine; F = female; (G) = gavage; (GO) = gavage in oil; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LD50 = lethal dose, 50% kill; LDH-C4 = lactate dehydrogenase isozyme C4; LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; N = haploid cells; 4N = polyploid cells; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; (W) = water; wk = week(s)

Figure 2-1. Levels of Significant Exposure to 1,3-Dinitrobenzene

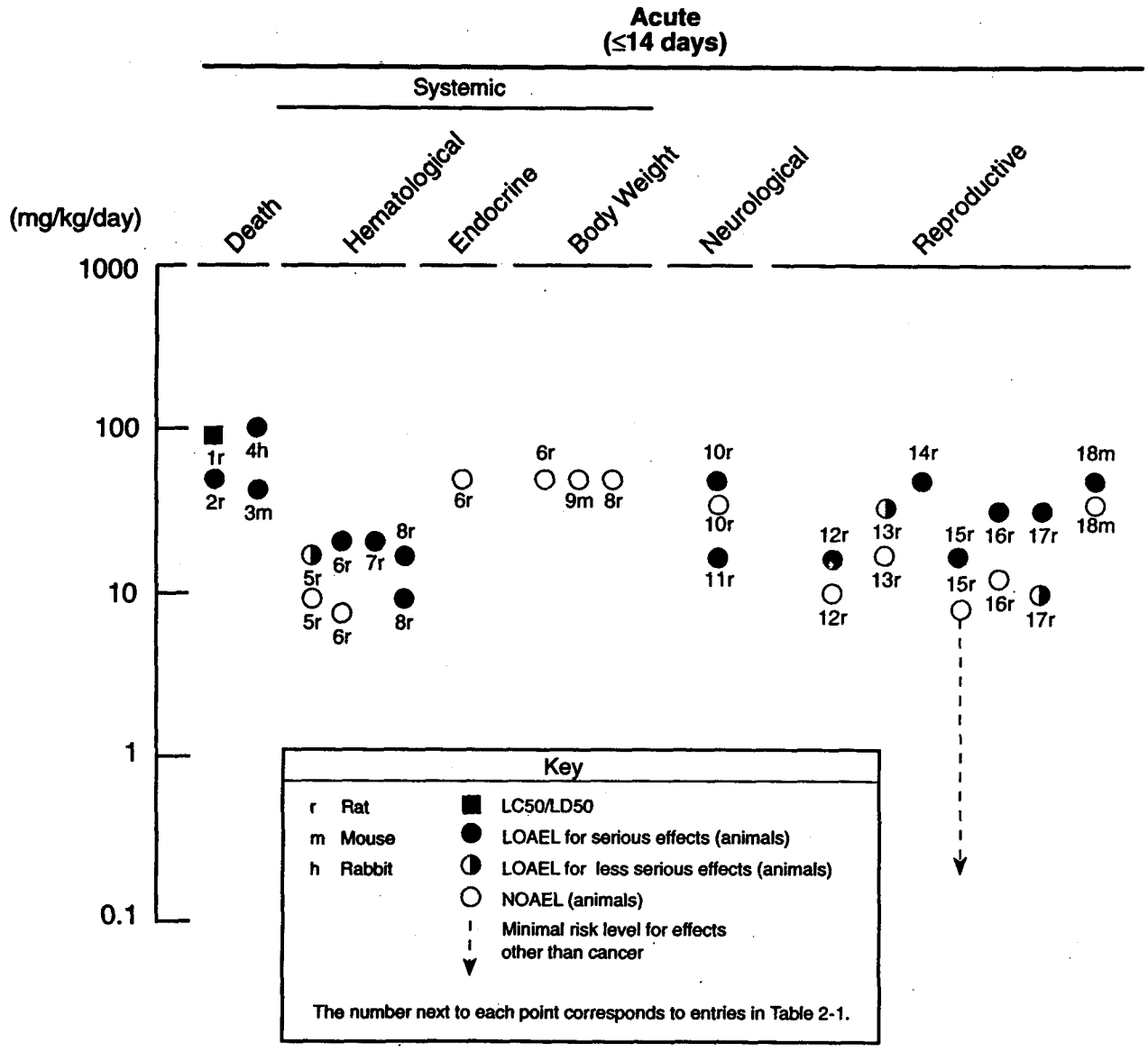
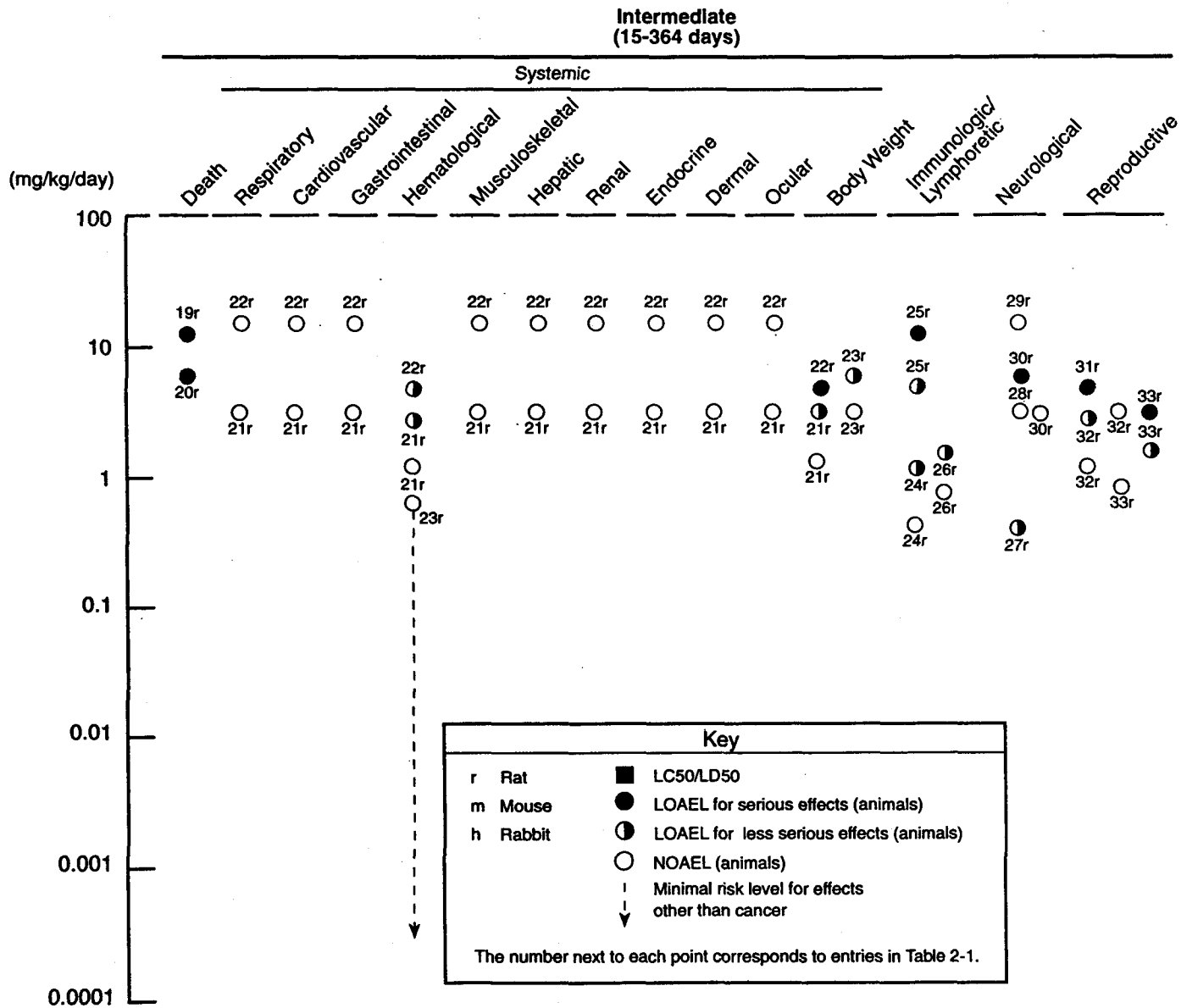


Figure 2-1. Levels of Significant Exposure to 1,3-Dinitrobenzene – Oral (continued)



Information in animals is restricted to a single intermediate-duration study in rats in which no histopathologic alterations were observed in the heart and aorta of rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981).

No studies were located regarding the cardiovascular effects in humans or animals after oral exposure to 1,3,5-TNB.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

No histopathologic alterations were observed in the stomach, duodenu*m*-pylorus, ileum, and colon of rats administered up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981). No further data were located regarding 1,3-DNB and no information was available regarding 1,3,5-TNB.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to 1,3,5-TNB.

The primary effect of 1,3-DNB absorbed into blood is the formation of methemoglobin. For a detailed discussion on the mechanism of methemoglobinemia induction please see Section 2.3.5.

The only information regarding hematological effects of 1,3-DNB in humans is that of a man who swallowed 30-40 mL of nitrobenzene dye and was admitted to the hospital with peripheral and central cyanosis (Kumar et al. 1990). Evidence of hemolytic anemia was present. Methemoglobin was 37.2% after gastric lavage was performed, and was reduced to 5.7% after two injections of methylene blue.

No studies were located regarding hematological effects in animals after oral exposure to 1,3,5-TNB. In acute-duration studies in rats treated with 1,3-DNB, cyanosis was the first sign of acute toxicity and deficient blood oxygenation. The exposure doses in rats ranged from 16 to 180 mg/kg (Blackbum et al. 1988; Cody et al. 1981; Linder et al. 1988, 1990; Philbert et al. 1987b; Reader et al. 1991). In general, the effect was readily reversed when treatment with 1,ZDNB was discontinued. Mice treated once with up to 48 mg/kg 1,3-DNB did not appear to develop cyanosis, but the scope of this study

was primarily the assessment of reproductive end points and not hematological parameters (Evenson et al. 1989a).

When rats were exposed to low doses (0.4-3.1 mg/kg/day) of 1,3-DNB for 16 weeks, no overt signs of acute toxicity were seen (Cody et al. 1981). There was, however, a moderate decrease in hemoglobin levels after weeks 5 and 10, but they returned to control levels by week 14 (Cody et al. 1981). Splenic hemosiderosis was observed in all groups of rats treated with 0.75-6 mg 1,3-DNB by gavage for 12 weeks, including controls (Linder et al. 1986). Splenic hemosiderosis, which was minimal in controls and moderate to moderately severe at the highest dose level, is consistent with hemolytic anemia. No chronic-duration studies were located.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

Information in animals is limited to a study in which no histopathologic alterations were observed in skeletal muscle (unspecified) of rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981). No information was located regarding 1,3,5-TNB.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

No histopathological alterations were seen in the liver of rats treated with up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981). No information was available regarding 1,3,5-TNB.

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

Only one study was located that examined the effect of 1,3-DNB on the kidneys (Cody et al. 1981). In that study, no histopathologic alterations were observed in the kidneys of rats administered up to

3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water. No information was located regarding 1,3,5-TNB.

Endocrine Effects. Levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (Prl), hypothalamic gonadotropin releasing hormone (GnRH), testosterone, and androgenbinding protein (ABP) were evaluated in male Sprague-Dawley rats from 3 hours to 2 weeks after treatment with a single oral dose of 32 mg/kg 1,3-DNB (Rehnberg et al. 1988).

Pituitary weights and weights of androgen-dependent accessory sex organs did not differ in treated animal as compared to the controls at any time point. Serum and pituitary levels of LH and Prl were not affected by I,3-DNB treatment at any time point examined. FSH concentration in serum was significantly higher 2 weeks after treatment, while pituitary FSH levels remained unchanged. These results led the investigators (Rehnberg et al. 1988) to suggest that 1,3-DNB exerts a direct effect on the testes and not through alterations in hypothalamic and pituitary control of gonadal function. No significant changes in pituitary weight were observed over a 14-day period in male rats exposed to a single oral dose of 8-48 mg/kg 1,3-DNB (Linder et al. 1990).

In intermediate-duration studies, no histopathologic alterations were observed in the pancreas, thyroids, adrenals, and pituitary of rats given up to 14 mg/kg/day 1,3-DNB for 8 weeks or 3 mg/kg/day 1,3-DNB for 16 weeks in the drinking water (Cody et al. 1981). Administration of up to 6 mg/kg/day 1,3-DNB by gavage for 12 weeks to rats did not result in alterations of the adrenal's weight (Linder et al. 1986). No further endocrine end point was assessed in the latter study.

Dermal Effects. No studies were located regarding dermal effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

Data in animals are provided by only one intermediate-duration study in rats (Cody et al. 1981). In that study, the investigators reported that no histopathological alterations were observed in the skin of rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water. No data were available for 1,3,5-TNB.

Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to **1,3-DNB** or 1,3,5-TNB.

Very little information was available regarding ocular effects in animals. Exophthalmia was observed in rats given a single lethal dose of 1,3-DNB (Cody et al. 1981). This effect, according to the investigators, appeared to reflect a condition of general congestion prior to death. In an intermediateduration study (Cody et al. 1981), no histopathologic alterations were observed in the eyes of rats treated with up to 14 mg/kg/day 1,3-DNB for 8 weeks or 3 mg/kg/day for 16 weeks in the drinking water. No data were found regarding 1,3,5-TNB.

Body Weight Effects. No studies were located regarding body weight effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

In an acute-duration study, male Sprague-Dawley rats treated with a single dose of 848 mg/kg 1,3-DNB and examined over a 32-day period did not show significant decreases in body weight (Evenson et al, 1989b). In addition, the same group of investigators (Evenson et al. 1989a) noted no significant differences in body weight between treated and control groups of adult and pubertal (26 days old) male mice exposed to a single oral dose of 8-48 mg/kg 1,3-DNB. No significant changes in body weight were noted in male rats exposed to a single oral dose of 8-48 mg/kg 1,3-DNB and observed for 14 (Linder et al. 1990) or 175 days (Linder et al. 1988).

A significant reduction in body weight gain was noted in male and female rats exposed for 8. weeks to 4.7 and 6 mg/kg/day 1,3-DNB, respectively, via drinking water (Cody et al. 1981). Higher doses, 12.5 mg/kg/day in males and 14.4 mg/kg/day in females, induced frank weight loss (Cody et al. 1981). In another study by the same group of investigators (Cody et al. 1981), female rats exposed to 3 mg/kg/day in the drinking water for 16 weeks had a reduced rate of growth after 8 weeks and, at the end of 16 weeks, weight was significantly lower than for control females; growth rate of males was not affected by treatment with 1,3-DNB. Also, male rats treated by gavage with 6 mg/kg/day 1,3-DNB for 12 weeks experienced a significant decrease (16%) in body weight during a breeding period of a week (Linder et al. 1986). No information was located regarding 1,3,5-TNB.

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after exposure to 1,3-DNB or 1,3,5-TNB.

Splenic enlargement and congestion, and increased erythropoietic activity were observed in the spleen from male rats (females were not tested) treated' with a single dose of 15 or 25 mg/kg 1,3-DNB and sacrificed at various intervals (2-96 hours) after dosing (Blackbum et al. 1988). This, however, is most likely an extramedullary response and is consistent with hemolytic anemia.

In intermediate-duration studies, spleen enlargement occurred in male and female rats treated with approximately 1 mg/kg/day 1,3-DNB in the drinking water for 16 weeks (Cody et al. 1981). Similar results were reported in rats administered 1.5 mg/kg/day 1,3-DNB by gavage for 12 weeks (Linder et al. 1986). Treatment of rats with doses of about 12-14 mg/kg/day 1,3-DNB in the drinking water for 8 weeks induced hemosiderin deposits in the spleen and spleen atrophy and fibrosis (Cody et al. 1981). No data were located for 1,3,5-TNB.

All reliable LOAEL values for immunological effects in each species and duration category for 1,3-DNB are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.4 Neurological Effects

The only information available regarding neurological effects in humans comes from a case report of an accidental poisoning of a man who swallowed varnish containing nitrobenzene dye and was admitted to the hospital in a deep coma. Based on the known properties of nitrobenzenes in general, the development of coma may have been secondary to methemoglobinemia (37.2%) and cyanosis.

No studies were located regarding neurological effects in animals after oral exposure to 1,3,5-TNB. Physical signs of neurotoxicity following acute-duration exposure to 1,3-DNB were manifested in slow movement, loss of movement, loss of equilibrium, and general hypoactivity in rats given single oral doses of 1,3-DNB ranging from 20 to 48 mg/kg (Linder et al. 1988, 1990; Philbert et al. 1987b).
Older adult rats seemed to be more susceptible to neurotoxicity following a single dose of 48 mg/kg of 1,3-DNB (Linder et al. 1990).

Studies of intermediate duration evaluating neurological effects are inconclusive. Administration of 6 mg/kg/day 1,3-DNB by gavage to male Sprague-Dawley rats (females not tested) for 12 weeks caused severe neurotoxic effects (impaired movement, paresis, loss of equilibrium, and muscle rigidity) in all animals (Linder et al. 1986). In another study, the activity level of male Cat-worth Farms rats (females not tested) given 0.4 and 1.1 mg/kg/day 1,3-DNB in drinking water for 16 weeks was measured with both activity wheels and activity platforms (Cody et al. 1981). In both treated groups, there was a significant increase in activity wheels relative to controls, but activity in platforms was not significantly greater than the level of activity of the control group. The same group of investigators (Cody et a!. 1981) found no histopathologic alterations in the brain and spinal cord from rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks.

All reliable LOAEL values for neurological effects in each species and duration category for 1,3-DNB are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

No studies were located regarding reproductive effects in animals after oral exposure to 1,3,5-TNB. Reproductive toxicity in the form of reduced testes and epididymis weight was consistently observed in rats exposed to a single oral dose (ranging from 25 to 50 mg/kg) of 1,3-DNB (Blackbum et al. 1988; Evenson et al. 1989a; Linder et al. 1988; Rehnberg et al. 1988). Reduced testicular weight was observed in two groups of adult Sprague-Dawley rats-younger and older animals-after a single 24 mg/kg dose of 1,3-DNB (Linder et al. 1990). However, older adult rats were more sensitive to 1,3-DNB-induced toxicity. Epididymal weight, testicular sperm head count, and cauda sperm reserves in older rats treated with 16 mg/kg of 1,3-DNB were all significantly lower than controls (Linder et al. 1990). In another study, male Sprague-Dawley rats (3-5/group) were given a single oral dose of 1,3-DNB in 1.5% dimethyl sulfoxide (DMSO) in corn oil at 0, 12, 30, or 60 mg/kg; animals were sacrificed 48 hours later (Moore et al. 1992). The lowest dose had no effect on the testis. At 30 mg/kg, degeneration and depletion of some of the late pachytene spermatocytes (phagocytosis and exfoliation) were observed. At 60 mg/kg, all of the pachytene spermatocytes and round spermatids were absent or degenerate. Relative testis weight was reduced at 30 and 60 mg/kg in a dose-related

manner. Based on the finding that urinary creatine was significantly increased at 60 mg/kg in the 24-hour period following dosing (a period consistent with Sertoli cell damage), the authors (Moore et al. 1992) concluded that a substantial proportion of testicular creatine is associated with the cells of the seminiferous epithelium and that creatinuria may serve as a marker for damage to these cells.

In contrast to these findings in .rats are observations made in prepubertal mice that testicular growth and development were not affected by 40 or 48 mg/kg of 1,3-DNB (Evenson et al. 1989a). In pubertal and adult mice, however, abnormal spermatogenesis and an increase in chromatin structure abnormality were apparent after a single dose of 48 mg/kg 1,3-DNB (Evenson et al. 1989a).

Testicular histopathology revealed that major early changes after exposure to 1,3-DNB consisted of degeneration of germinal epithelium and sloughing of both spermatocytes and spermatids which in turn resulted in reduced sperm counts and reduced sperm mobility (Blackbum et al. 1988; Evenson et al. 1989b; Linder et al. 1988, 1990; Reader et al. 1991). Disrupted spermatogenesis was also evidenced by a decrease in the number of seminiferous tubules in rats treated with 48 mg/kg of 1,3-DNB (Hess et al. 1988). At 5 weeks, these changes caused decreased fertilizing ability of spermatozoa and 91% of treated rats lost their fertilizing capability (Linder et al. 1990). However, these changes were partially reversible since at 5 months after exposure only 18% of rats had not recovered their reproductive capability (Linder et'al. 1990).

An indication that Sertoli cells may be targets in the seminiferous epithelium for early damage by 1,3-DNB came with the observatton of significantly increased levels of androgen-binding protein (ABP, released from Sertoli cells) in.seminiferous tubule fluid, interstitial fluid, and serum in rats treated with 15 and 32 mg/kg of 1,3-DNB, respectively (Reader et al. 1991; Rehnberg et al. 1988). Further examination of early toxic effects of 15 mg/kg of 1,3-DNB revealed vacuolization and cytoplasmic retraction in Sertoli cells within the first 24 hours after exposure (Blackbum et al. 1988). Similar observations of Sertoli cell damage were made when 1,3-DNB was administered at a dose of 30 mg/kg (Reader et al. 1991). Data from these studies support the notion that Sertoli cells may be first and primary targets of the toxic effects of I,3-DNB in seminiferous epithelium.

Plasma hormones and enzymes of testicular origin were used as markers for evaluation of acute testicular toxicity in rats treated with 1,3-DNB. Lactate dehydrogenase isozyme C4 (LDHC4) and ABP were both elevated after treatment with doses between 10 and 25 mg/kg of 1,3-DNB (Reader et

al. 1991). Testosterone levels were reduced after treatment with 10 and 32 mg/kg of 1,3-DNB (Reader et al. 1991; Rehnberg et al. 1988).

Adverse reproductive effects were also observed in rats exposed to 1,3-DNB in intermediate-duration studies. Significantly decreased spermatogenesis and atrophy of seminiferous tubules were observed after 12 weeks of treatment with 3 mg/kg/day 1,3-DNB by gavage (Linder et al. 1986). Testicular atrophy was also observed at 4.7 mg/kg/day after 8 weeks of treatment with 1,3-DNB in the drinking water (Cody et al. 1981). A slightly lower dose, 2.64 mg/kg/day, given for 16 weeks induced a decrease in testes weight and decreased spermatogenesis (Cody et al. 1981).

In female rats, administration of up to 3 mg/kg/day 1,3-DNB in drinking water for 16 weeks or up to 14 mg/kg/day for 8 weeks caused no significant alterations in the weight or histopathologic appearance of the ovaries (Cody et al. 1981).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in animals after acute- or intermediate-duration oral exposure are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after oral exposure to 1,3-DNB or 1,3,5-TNB.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after oral exposure to 1,3-DNB or 1,3,5-TNB.

Genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer effects in humans or animals after oral exposure to 1,3-DNB or 1,3,5-TNB.

2.2.3 Dermal Exposure

Human studies presented in the section on dermal exposure are reports of accidental occupational exposure and one volunteer case study. Since precise levels of exposure in these studies are not known, the results in this section are not presented in a table.

2.2.3.1 Death

No studies were located regarding death, in humans after dermal exposure to 1,3-DNB or 1,3,5-TNB.

In an early study, a single dermal application of an ointment containing 25% 1,3-DNB to 3 cats resulted in the death of a female cat 12 hours after dosing (White and Hay 1901). Limitations of this study include small sample size and lack of information on the amount applied. Information located in an abstract indicates that the derrnal LD_{50} for 1,3-DNB in rabbits was 1,990 mg/kg, and that a dose of 2,000 mg/kg 1,3,5-TNB was not toxic when applied for 24 hours to the skin of rabbits, but no further details were provided (Desai et al. 1991).

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, gastrointestinal, or musculoskeletal, effects in humans or animals after dermal exposure to 1,3-DNB or 1,3,5-TNB.

Cardiovascular Effects. The only information available is the case of an investigator who selfapplied an amount of ointment containing 100 mg 1,3-DNB three times over a 24-hour period (White and Hay 1901). After the third application, he noticed that his heart rate had increased to about 100-120 beats per minute and did not return to pre-exposure rate (not specified) until 3 days later. No further information was provided.

Hematological Effects. No studies were located regarding hematological effects in humans after dermal exposure to 1,3,5-TNB. Limited information is available regarding hematological effects in humans after dermal exposure to 1,3-DNB. The case of an investigator who self-applied an amount of ointment containing 100 mg of 1,3-DNB 3 times over a 24-hour period is described in an early study (White and Hay 1901). After only two applications, he noticed that his lips, tongue, and fingernails

were blue. After the third application, cyanosis was evident; he recovered three days later. 1,3-DNB is easily absorbed through skin when in aqueous solution (Ishihara et al. 1976) and its main effect is induction of methemoglobin formation. A female worker who handled electronics parts immersed in a chemical mixture containing 0.5% weight-to-weight (w/w) 1,3-DNB became cyanotic and showed signs of anemia upon admission to the hospital 10 days later. The exposure conditions of the above case were duplicated with a volunteer worker (Ishihara et al. 1976). Methemoglobin levels reached a maximum of 11% four hours after exposure to 1,3-DNB. It was also confirmed that the main exposure route was dermal since 1,3-DNB readily permeated latex gloves used to handle immersed parts and 1,3-DNB was not detected in the volunteer's breathing-zone air. The fact that 1,3-DNB readily permeated the latex gloves used for protection has enormous implications in the occupational setting because it shows that this kind of protection is ineffective. Limitations of this study include small sample size, concomitant exposure to other chemicals, and lack of complete information *on* exposure dose.

No studies were located regarding hematological effects in animals after dermal exposure to 1,3,5-TNB. In one of the earliest reports on 1,3-DNB exposure, an ointment containing 25% (w/w) 1,3-DNB was applied to the backs of 3 cats (White and Hay 1901). All three developed classical symptoms of methemoglobinemia and cyanosis.

Methemoglobinemia also was observed in guinea pigs when a solution containing 0.5% (w/w) 1,3-DNB in a mixture of solvents characterized as water soluble was applied for 4 hours (Ishihara and Ikeda 1979). Methemoglobinemia did not develop when the solvent mixture contained less than 77.5% (w/w) ethylene glycol.

Hepatic Effects. No studies were located regarding hepatic effects in humans after dermal exposure to 1,3,5-TNB. In one case report of occupational exposure to 1,3-DNB (Ishihara et al. 1976), the exposed worker had palpable liver while her liver function tests were negative. This study is limited in that only a single case was described and functional tests were performed 10 days after the exposure.

No studies were located regarding hepatic effects in animals after dermal exposure to 1,3,5-TNB. In one of the earliest studies with 1,3-DNB, the investigators indicate that necropsy of a cat (1 out of 3)

to which an ointment containing 25% (w/w) 1,3-DNB was applied 8 days earlier showed fatty degeneration in the liver (White and Hay 1901). No further information was provided.

Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to 1,3-DNB or 1,3,5-TNB.

Kidney inflammation was reported in a cat (1 out of 3) that received two applications of an ointment containing 25% (w/w) 1,3-DNB over a lo-day period (White and Hay 1901). According to the, investigators (White and Hay 1901), this was probably due to toxic nephritis. No further information was provided.

Dermal Effects. No studies were located regarding dermal effects of 1,3-DNB or 1,3,5-TNB in humans.

Data in animals are limited to a study in which no irritation was observed in the skin of guinea pigs after application of a formulation containing 0.5% 1,3-DNB (w/w) for 4 hours (Ishihara and Ikeda 1979). Data located in an abstract indicate that neither 1,3-DNB nor 1,3,5-TNB caused skin irritation when applied to the skin of rabbits, but no further details were provided (Desai et al. 1991).

Ocular Effects. No studies were located regarding ocular effects of 1,3-DNB or 1,3,5-TNB in humans.

Limited information was presented in an abstract indicating that 1,3-DNB caused mild eye irritation in rabbits, whereas 1,3,5-TNB caused severe irritation; no further details were provided (Desai et al. 1991).

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after dermal exposure to 1,3-DNB or 1,3,5-TNB.

Limited data located in an abstract indicate that 1,3-DNB was not a skin sensitizer in guinea pigs, but 1,3,5-TNB caused a mild allergic reaction; no further details were provided (Desai et al. 1991).

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to 1,3,5-TNB. Very limited information is available regarding the neurological effects of 1,3-DNB. Headache (presumably of vascular origin) and general malaise were reported in a female w.orker who handled electronics parts immersed in a chemical mixture containing 0.5% (w/w) 1,3-DNB (Ishihara et al. 1976). Limitations of this study include small sample size, concomitant exposure to other chemicals, and lack of complete information on dose and duration of exposure.

No studies were located regarding neurological effects in animals after dermal exposure to 1,3-DNB or 1,3,5-TNB.

No studies were located regarding the following health effects in humans or animals after dermal exposure to 1,3-DNB or 1,3,5-TNB:

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding cancer effects in humans or animals after dermal exposure,to 1,3-DNB or 1,3,5-TNB.

2.3 TOXICOKINETICS

Data regarding the toxicokinetics of 1,3-DNB or 1,3,5-TNB in humans are limited to information derived from two occupational studies and from a report in which the experimenter self-administered 1,3-DNB. These data provide qualitative evidence that 1,3-DNB may be absorbed in humans by the inhalation and dermal routes. There are no data regarding oral absorption of 1,3-DNB or 1,3,5-TNB

in humans. In animals, 1,3-DNB is rapidly absorbed by the oral route;. data from one study indicate that at least 70% of a single oral dose was absorbed. In animals, depending on the vehicle, 1,3-DNB can also be readily absorbed through the skin. It appears that polar vehicles facilitate absorption. No information was located regarding absorption of 1,3-DNB in animals by the inhalation route or of 1,3,5-TNB by any route of exposure. The mechanism by which 1,3-DNB and 1,3,5-TNB are transported to the tissues is not completely understood, but there is some evidence indicating that dinitrobenzenes can penetrate the red blood cell membrane.- No information was located regarding distribution patterns for 1,3-DNB or 1,3,5-TNB in humans for any route of exposure or in animals after inhalation or dermal exposure. The metabolism of 1,3-DNB in animals include both oxidative and reductive biotransformations, followed by conjugation. No information is available regarding metabolism in humans. Following oral exposure, the main route of excretion of 1,3-DNB metabolites .in animals is the urine. This also seems to be the case for humans after de'rmal exposure. No data were located regarding excretion of 1,3-DNB or metabolites after inhalation and oral exposure in humans or after inhalation and dermal exposure in animals. The toxicity of 1,3-DNB is related to its methemoglobin forming capacity in the red blood cells. A reactive metabolic intermediate has been .postulated as the responsible agent for the toxicity to the male reproductive organs, but the exact mechanism has not been elucidated.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Studies undertaken specifically to evaluate absorption of either 1,3-DNB or 1,3,5-TNB in humans after an inhalation exposure were not located. However, a study of an occupational exposure to 1,3-DNB showed that workers developed cyanosis within the first 24 hours after exposure (Okubo and Shigeta 1982). Inhalation was considered the major exposure pathway since skin contact was with 1,3-DNB in solid form. There was no information, however, on the amount of 1,3-DNB present in the air or on the amount of particulate 1,3-DNB deposited on the workers' skin.

Studies on the absorption of 1,3-DNB or 1,3,5-TNB in animals following inhalation exposure were not located.

2.3.1.2 Oral Exposure

There-are no quantitative data that describe the absorption of 1,3-DNB or 1,3,5-TNB following oral exposure in humans.

Limited information was located regarding quantitative absorption of 1,3-DNB in animals after oral exposure. Rabbits treated with randomly labeled ^{14}C-1,3-DNB in arachis oil in singles doses of 50-100 mg/kg excreted 65-93% of the administered radioactivity in the urine within 2 days of dosing (Parke 1961). This indicated that at least that amount was absorbed from the gastrointestinal tract; Similar results were reported in rats in which excretion data suggested that at least 63% of a single oral dose was absorbed (Nystrom and Rickert 1987). Further evidence that 1,3-DNB is readily absorbed by the oral route is provided by the many studies which examined the toxicological effects of this compound administered orally (see Section 2.2.2). No information was located regarding 1,3,5-TNB.

2.3.1.3 Dermal Exposure

No studies were located regarding absorption of 1,3,5-TNB following dermal exposure in humans.

Data are very limited regarding absorption of 1,3-DNB following dermal exposure in humans. Evidence of dermal absorption was found in an early report in which an experimenter became cyanotic after self-applying an ointment containing 25% (w/w) 1,3-DNB (White and Hay 1901). Similar findings were described in a case of .a woman exposed to a solution containing 0.5% (w/w) 1,3-DNB at work and in a male volunteer (Ishihara et al. 1976).

No studies were located regarding absorption of 1,3,5-TNB following dermal exposure in animals.

The role that the solvent mixture plays in the absorption of 1,3-DNB was investigated in Hartley guinea pigs exposed to solutions of varying composition, but each containing 0.5% (w/w) 1,3-DNB (Ishihara and Ikeda 1979). The solvents were ethylene glycol and diethylene glycol at various concentrations, along with two different co-existing solutes, ammonium adipate and ammonium sebacate (Ishihara and Ikeda 1979). Animals were sacrificed immediately after a 4-hour dermal exposure. The animals that received 1,3-DNB in a solvent mixture containing 77.5% (w/w) or more

of ethylene glycol developed methemoglobinemia. Methemoglobinemia did not occur when the solvent mixture contained less than 77.5% ethylene glycol. Six dicarboxylic acids were also tested as co-existing solutes and only two, malonic and adipic acid in the presence of ,ethylene glycol and diethylene glycol, were able to induce methemoglobin formation. These two acids were also more water soluble than others and allowed water impregnation of stratum corneum by ethylene glycol. The authors suggested that the increased water content of stratum comeum in the presence of higher concentrations of ethylene glycol may enhance dermal absorption of 1,3-DNB (Ishihara and Ikeda 1979).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding the distribution of 1,3-DNB or 1,3,5-TNB following inhalation exposure in humans or animals.

2.3.2.2 Oral Exposure

No studies were located regarding distribution following oral exposure to 1,3-DNB or 1,3,5-TNB in humans.

Limited information was located regarding distribution of 1,3,5-TNB in animals. 1,3,5-TNB-DNA adducts were detected in the spleen of rats one day after being gavaged once with ^{14}C-1,3,5-TNB (Reddy et al. 1991). DNA adducts were also found in the stomach and liver three days after dosing. Twenty-eight days after treatment, the residual adduct level in the liver and stomach was 25%, whereas in the spleen was still 100%. Tissue distribution of ^{14}C after a single oral dose of 25 mg/kg of ^{14}C-1,3-DNB was examined in Fischer 344 conventional (C) and ger*m*-free (GF) rats (Philbert et al. 1987b). The amount of ^{14}C label in whole blood, plasma, pancreas, lungs, liver, kidney, adrenal, testis, quadriceps femoris muscle, sciatic nerve, white and brown fat, spinal cord, and brain stem was examined and was found to be higher in GF animals. The relative distribution of label in different organs was as follows: liver > white fat > brown fat > kidney > sciatic nerve > whole blood > plasma > testis > brain stem. The amount of label contained in the liver and brain of GF rats was 20 and 13 times greater, respectively, than in C rats (Philbert et al. 1987b). This finding illustrates the

importance of gastrointestinal tract microflora in the initial phases of 1,3-DNB biotransformation. The study is limited in that there is a lack of information on statistical significance of the data.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of I,3-DNB or 1,3,.5-TNB foIlowing dermal exposure in humans or animals.

2.3.2.4 Other Routes of Exposure

The administration of 1,3-DNB by the intraperitoneal route allows for almost complete absorption. The level of 1,3-DNB in blood was evaluated in rats and hamsters after a single intraperitoneal injection of 25 mg/kg of radioactive 1,3-DNB (^{14}C-1,3-DNB) (McEuen and Miller 1991). The peak blood concentration of ^{14}C-1,3-DNB was 99.5 nmol/mL in rats and was reached within 1 hour of exposure. Rats had twice the blood level of ^{14}C-1,3-DNB found in hamsters.

2.3.3 Metabolism

2.3.3.1 Inhalation Exposure

No studies were located regarding metabolism following inhalation exposure to 1,3-DNB or 1,3,5-TNB in humans or animals.

2.3.3.2 Oral Exposure

No studies were located regarding metabolism of 1,3-DNB or 1,3,5-TNB following oral exposure in humans.

Both oxidative and reductive biotransformations, followed by conjugation, have been demonstrated for the metabolism of 1,3-DNB in mammals. The metabolism of the three isomeric dinitrobenzenes administered as single oral doses to rats (25 mg/kg) has been determined and compared (Nystrom and Rickert 1987). Products formed through reduction of the nitro group predominated, and the major metabolites were 3-aminoacetanilide (22%), 4-acetamidophenylsulfate (6%), 1,3-diacetamidobenzene

(7%), and 3-nitroaniline-N-glucuronide (4%). These products and their proposed intermediates are diagramed in Figure 2-2.

The metabolism of an oral dose of 50-100 mg/kg ^{14}C-1,3-DNB was followed in rabbits (Parke 1961). Of the metabolites detected in urine, 30% were conjugated with glucuronic acid and 6% with sulfate. The major urinary metabolites of 1,3-DNB were 2,4-diaminophenol (31%), 1,3-phenylenediamine (25%), 1,3-nitroaniline (18%), and 2-amino-4-nitrophenol (14%). Other minor metabolites comprising about 20% of the label were oxidation and reduction products and azoxy dimers.

Several *in vitro* metabolic studies on 1,3-DNB support the above in viva findings. 1,3-DNB appears not to be a substrate for rat hepatic or erythrocyte glutathione transferases (Cossum and Rickert 1985 1987) since no 1,3-DNB glutathione conjugates were identified. However, the chemical reaction of 3-nitrosonitrobenzene with glutathione has been shown to form the corresponding hydroxylamino and anilino metabolites (Ellis et al. 1992). Studies using rat testicular cells or a co-culture of testicular and Sertoli cells showed that 1,3-DNB is metabolized by nitro reduction to 1,3-nitroaniline through nitrosonitrobenzene and nitrophenyl hydroxylamine intermediates, without being conjugated to glutathione (Cave and Foster 1990; Foster 1989; Lloyd and Foster 1987).

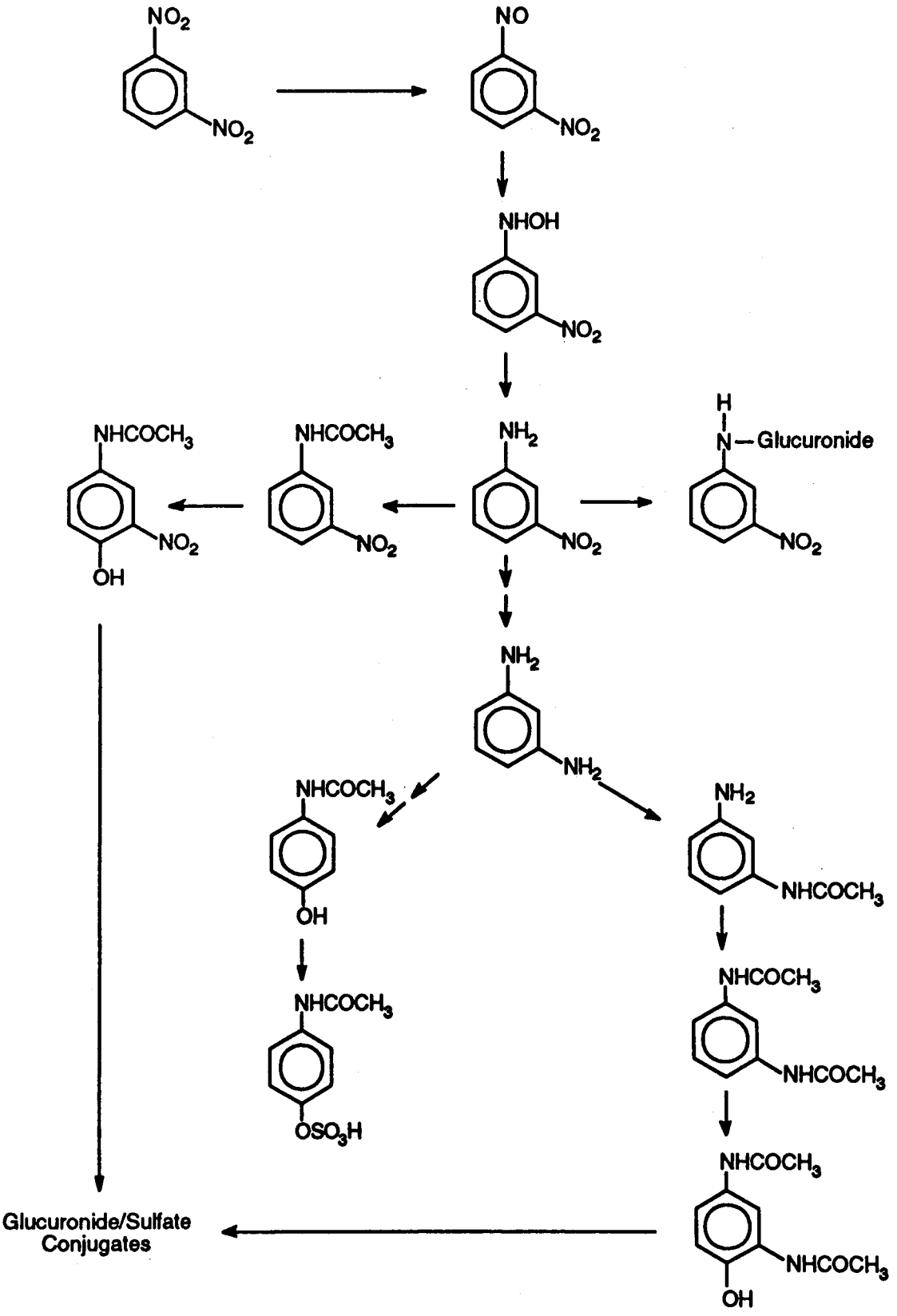
The relative rates of conversion of 1,3-DNB to nitroanilines were calculated in rat hepatocytes and microsomes from the slope of semilogarithmic plots of percentage 1,3-DNB remaining versus time. The half-life of 1,3-DNB was estimated to be 12 and 7 minutes in hepatocytes and microsomesi respectively, indicating a relatively rapid conversion rate (Cossum and Rickert 1985).

No studies were located regarding metabolism following oral exposure to 1,3,5-TNB in animals.

2.3.3.3 Dermal Exposure

No studies were located regarding metabolism of 1,3,5-TNB following dermal exposure in humans. In the only study that measured 1,3-DNB metabolite production in humans after dermal exposure, the total production of both amino and nitro metabolites in urine was reported using 2,4-dinitrophenol as a standard (Ishihara et al. 1976). The results indicate that 1,3-DNB (in solution) rapidly penetrated skin and was also rapidly converted and excreted in urine. A maximum amount of amino and nitro metabolites was reached within the first hour after exposure and returned to normal levels after

FIGURE 2-2. Proposed Pathway of Metabolism of 1,3-DNB in Rats and Hamsters*



* Adapted from Nystrom and Rickert 1987

10 hours. The limitations of this study are a small sample size (one person) and no detailed information on the nature of 1,3-DNB metabolites.

No studies were located regarding metabolism of 1,3-DNB or 1,3,5-TNB in animals after dermal exposure.

2.3.3.4 Other Routes of Exposure

In mammals, the differences in metabolic processing of 1,3-DNB may play an important role in susceptibility to toxicity. The metabolism of 1,3-DNB was examined in rats and hamsters after a single intraperitoneal injection of 25 mg/kg of ^{14}C-1,3-DNB (McEuen and Miller 1991). Hamsters were found to be much less sensitive than rats to the toxic effects of 1,3-DNB. Elimination of 1,3-DNB from blood was biphasic. The initial rapid phase was followed by a much slower one. Maximal blood levels of 1,3-DNB were 46 and 99 nmol/mL in hamsters and rats, respectively. In the urine, rats excreted more unconjugated metabolites and less phenolic metabolites than hamsters. The presence of unconjugated reductive metabolites in rats may in part be responsible for increased toxicity of 1,3-DNB. In another study, Sprague-Dawley rats and Syrian hamsters were exposed to increasing concentrations of 1,3-DNB. It was found that Syrian hamsters were more resistant to the toxic effects of 1,3-DNB (Obasaju et al. 1991). At the lowest dose (25 mg/kg), methemoglobin was 15% in hamsters compared to 83% in rats. The same was true for testicular damage observed within 48 hours in rats and absent in hamsters even when the dose was 50 mg/kg. This difference in susceptibility to toxic effects between the two species, rats and hamsters, is again probably due to differences in metabolism of 1,3-DNB.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion of 1,3-DNB or 1,3,5-TNB after inhalation exposure in humans or animals.

2.3.4.2 Oral Exposure

No studies were located regarding excretion following oral exposure to 1,3-DNB or 1,3,5-TNB in humans.

No studies were located regarding excretion following oral exposure to 1,3,5-TNB in animals. Following administration of a single oral dose of ^{14}C-1,3-DNB to rabbits and rats, radioactivity accounting for more than 80% and 63% of the dose, respectively, was excreted in urine, indicating that the main route of excretion is via the urine (Nystrom and Rickert 1987; Parke 1961). Elimination of 1,3-DNB metabolites in urine was rapid and occurred within 48 hours. The major urinary metabolites in rabbits were 2,4-diaminophenol, 1,3-phenylenediamine, and 1,3-nitroaniline (Parke 1961).

2.3.4.3 Dermal Exposure

No studies were located regarding excretion of 1,3,5-TNB after dermal exposure in humans. In the only study that evaluated 1,3-DNB urinary metabolites in humans after dermal exposure, amino and nitro metabolites were reported as a single value using 2,4-dinitrophenol as a standard (Ishihara et al. 1976). Amino and nitro metabolites reached maximum levels within the 1st hour after exposure and returned to normal levels within 10 hours. The results indicate that 1,3-DNB was rapidly absorbed through skin and was also rapidly converted and excreted in urine. This study is limited by the small sample size (one person) and lack of information on the specific nature of 1,3-DNB metabolites.
No studies were located regarding excretion following dermal exposure to 1,3-DNB or 1,3,5-TNB in animals.

2.3.4.4 Other Routes of Exposure

Excretion of ^{14}C-1 ,3-DNB was followed in urine and feces of Sprague-Dawley rats and Syrian hamsters after a single intraperitoneal dose of 25 mg/kg (McEuen and Miller 1991). More than 80% of the label was excreted in urine by both species within the first 24 hours. Rats needed less time to complete the 1,3-DNB elimination than did hamsters.

2.3.5 Mechanisms of Action

Two major systems have been identified as toxicity targets for 1,3-DNB: the red blood cell and the male reproductive system (see Section 2.2.2). In the red blood cell, 1,3-DNB induces formation of methemoglobin leading to cyanosis (Blackbum et al. 1988; Linder et al. 1988, 1990; Reader et al. 1991). In the male reproductive system, 1,3-DNB causes disruption of spermatogenesis resulting in hypospermia, poor sperm quality, and infertility (Blackburn et al. 1988; Hess et al. 1988; Linder et al. 1988). Whether adverse hematological and reproductive effects are caused by the same mechanism of action remains unresolved.

Reduction of the nitrogroup(s) of 1,3-DNB is a reaction that predominates over oxidative pathways in mammals. Reduction of the nitro groups produces reactive nitroaromatic radical anions which redox cycle to produce other reactive, toxic species such as superoxide anion (Mason and Holzman 1975; Wardman and Clarke 1976). Redox cycling of these intermediates probably causes the methemoglobinemia associated with exposure to 1,3-DNB (Kiese 1974). Methemoglobinemia is defined as a methemoglobin concentration of greater than 1%, and it results from iron in the normal ferrous state being oxidized to the ferric state at a rate that exceeds the. erythrocyte's reducing capacity. Methemoglobin is unable to combine reversibly with oxygen and carbon dioxide and also causes a shift in the oxygen dissociation curve toward increased oxygen affinity, preventing the transfer of oxygen from the blood to the tissues.

Within the reproductive system the prime target for 1,3-DNB toxicity appears to be the Sertoli cell. Results from numerous studies support this hypothesis (Blackbum et al. 1988; Hess et al. 1988; Linder et al. 1988). As previously mentioned, some investigators have suggested that testicular damage may be related to tissue hypoxia, which results from impaired oxygen transport as a consequence. of methemoglobinemia (Linder et al. 1988). It appears, however, that reduction of 1,3-DNB to reactive species such as nitrophenylhydroxylamine and nitrosonitrobenzene. are involved in the testicular toxicity of 1,3-DNB (Cave and Foster 1990; Ellis and Foster 1992). In studies using rat testicular cells or a co-culture of testicular and Sertoli cells, 1,3-DNB was metabolized by nitro reduction to 1,3-nitroaniline through nitrosonitrobenzene and nitrophenyl hydroxylamine intermediates, without being conjugated to glutathione (Cave and Foster 1990; Foster 1989; Lloyd and Foster 1987). Of all the intermediates tested, only nitrosonitrobenzene was able to induce histological changes similar to

those seen with 1,3-DNB when reintroduced into cell cultures (Foster 1989). The specific mechanism by which the reactive intermediate might induce cell damage is unknown.

2.4 RELEVANCE TO PUBLIC HEALTH

The general population is not likely to be exposed to either 1,3-DNB or 1,3,5-TNB. Exposure to both compounds is possible around Army ammunition plants. Occupational or accidental exposure to 1,3-DNB and 1,3,5-TNB may also occur in industries using these two compounds in manufacturing processes (e.g., explosives, plastics, dyes).

The major effects observed in animals after exposure to 1,3-DNB are methemoglobin formation and testicular damage at doses that are higher than 1 mg/kg. Both effects are common to other nitroaromatic compounds. The biochemical changes that occur in the blood, primarily methemoglobin formation, lead to oxygen deprivation in the tissues, and then to cyanosis and neurotoxicity.

For the general population, oral exposure to 1,3-DNB and 1,3,5-TNB is the most likely exposure route. It can occur through ingestion of contaminated water; however, the solubility of these compounds in water is quite low (500 ppm and 3,500 ppm, respectively). Inhalation exposure and dermal exposure to 1,3-DNB and 1,3,5-TNB present in air are less likely because of their low volatility.

No deaths have been reported in humans from exposure to either 1,3-DNB or 1,3,5-TNB. Information on the effects that occur in humans in response to 1,3-DNB exposure comes from case reports of accidental poisoning, from studies of occupationally exposed workers and from a study in which a subject self-administered 1,3-DNB for research purposes.

No information has been located regarding human exposure to 1,3,5-TNB. Acute exposure of humans to 1,3-DNB causes symptoms that are the result of increased levels of methemoglobin in the blood which in turn causes oxygen deprivation in the tissues. Among the first of these symptoms is cyanosis. Other signs of exposure of humans in occupational settings have been associated with mild central nervous system intoxication manifested by headaches and general malaise. No studies were located regarding chronic exposure to 1,3-DNB in humans.

Studies in animals support the observations of the toxic effects in humans. Moreover, results from animal studies indicate that other toxic effects could be associated with exposure to 1,3-DNB. These include testicular damage, decreased reproductive function, splenomegaly, and/or spleen atrophy.

Minimal Risk Levels for 1,3-DNB and 1,3,5-TNB.

Inhalation MRLs.

No inhalation MRLs were derived for 1,3-DNB or 1,3,5-TNB due to lack of human and animal data.

Oral MRLs.

An MRL of 0.08 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to 1,3-DNB.

The acute oral MRL was based on a NOAEL for testicular toxicity in male rats administered a single dose of ≥16 mg 1,3-DNB/kg in corn oil and sacrificed 14 days later (Linder et al. 1990). No adverse effects were observed at 8 mg 1,3-DNB/kg. Effects observed at ≥16 mg/kg included substantial damage to the testicular germinal epithelium, reduction in epididymal weigh, and decreased number and morphological changes in spermatozoa. Histological changes, including luminal debris and atypical cells and hypospermia were noted at ≥16 mg/kg. Cyanosis was seen at dose levels ≥16 mgJkg, and neurotoxicity and increased mortality occurred at 48 mg/kg. These results are supported by a number of studies in animals that have identified the male reproductive system as a target for 1,3-DNB toxicity (Blackburn et al. 1988; Moore et al. 1992; Reader et al. 1991).

An MRL of 0.0005 mg/kg/day has been derived for intermediate oral exposure (15-364 days) to 1,3-DNB.

The intermediate oral MRL was based on a LOAEL for splenic hemosiderosis in male rats administered 0.75 mg/kg/day 1,3-DNB by gavage in acetone/corn oil solution 5 days/week for 12 weeks (Linder et al. 1986). This dose-related response was minimal in controls and moderate to moderately severe at the highest dose level tested, 6 mg/kg/day. Splenic enlargement was also reported at 1.5 mg/kg/day. Adverse testicular effects were observed with doses of 1,3-DNB

≥1.5 mg/kg/day. Altered spermatogenesis was noted at ≥3 mg 1,3-DNB/kg/day. The observed splenic effects are considered secondary to the hematoxicity of 1,3-DNB and are supported by increased erythropoietic activity in rats in a study by Blackburn et al. (1988) and hemosiderosis in rats in a study by Cody et al. (1981), and are consistent with hemolytic anemia. The hematological effects of 1,3-DNB are consistent with effects produced by other nitroaromatic compounds, reinforcing the toxicological significance.

No MRL has been derived for chronic oral exposure to 1,3-DNB, or for acute-, intermediate-, or chronic-duration oral exposure to 1,3,5-TNB due to lack of data.

Death. No deaths have been reported in humans from exposure to either 1,3-DNB or 1,3,5-TNB. Death has been observed in rats, rabbits, and mice after oral exposure to 1,3-DNB (Cody et al. 1981; Evenson et al. 1989a; Linder et al. 1990; Parke 1961). Death has also been reported in rats after oral exposure to 1,3,5-TNB (Desai et al. 1991). However, it is unlikely that amounts of 1,3-DNB or 1,3,5-TNB sufficient to cause death could be ingested by humans from environmental exposures such as living close to ammunition plants or those employed in the dyestuffs, plastics, rubber, and other industries.

Systemic Effects.

Respiratory Effects. Slight dyspnea upon exertion was reported in some of the workers after ah inhalation exposure to 1,3-DNB (Okubo and Shigeta 1982). Six factory workers were cleaning crystallized 1,3-DNB from a tank and had only gauze masks and rubber gloves for protection. No exposure data was available in that study. Shallow breathing was reported in a subject that ingested a varnish containing a nitrobenzene dye (Kumar et al. 1990), but this may have been secondary to the fact that the subject was in a coma and cyanosis had developed. No studies in animals were found on respiratory effects after exposure to 1,3-DNB other than a report in which no histopathologic alterations were seen in the lungs of rats exposed orally for 8-16 weeks (Cody et al. 1981). Data were not located for 1,3,5-TNB. The information available; although scant, do not seem to indicate that the respiratory system is a target for 1,3-DNB or 1,3,5-TNB.

Cardiovascular Effects. Palpitations, low blood pressure, and tachycardia were described in subjects exposed to 1,3-DNB by the inhalation (Oknbo and Shigeta 1982), oral (Kumar et al. 1990), and

dermal (White and Hay 1901) routes of exposure. These responses are consistent with effects of organic nitrates. 1,3-DNB is an organic nitrate and shares many of the cardiovascular properties of therapeutic nitrates. Organic nitrates induce relaxation of the vascular smooth muscle which can result in peripheral vasodilation and a fall in blood pressure followed by a compensatory vasoconstriction (Abrams 1980). The general information available on organic nitrates suggests that exposure to 1,3-DNB or 1,3,5-TNB at ammunition waste sites or at work places where these chemicals are used may lead to adverse cardiovascular effects.

Hematdogicd Effects. Induction of methemoglobin formation is one of the first hematological effects to occur after exposure to nitrobenzene compounds, including 1,3-DNB, by any route of administration (Ishihara et al. 1976; Kumar et al. 1990; Okubo and Shigeta 1982). As a result of oxygen deprivation and increased methemoglobin levels, cyanosis becomes apparent within the first 24 hours after exposure (White and Hay 1901). The mechanism of methemoglobin formation is discussed in section 2.3.5. After exposure to 1,3-DNB, mild-to-moderate anemia may occur and its severity depends on the duration of the exposure (Ishihara et al. 1976; Kumar et al. 1990; Okubo and Shigeta 1982). This small number of studies indicate that 1,3-DNB causes hematological effects in humans shortly after exposure. Although there are no data on high-level or intermediate exposure, it is reasonable to expect that higher levels of 1,3-DNB and longer exposure times would cause severe toxic effects. Studies in animals support findings of these toxic effects in humans and suggest that metabolic processing of 1,3-DNB plays an important role in susceptibility. to hematological effects. For example, an intraperitoneal dose of 25 mg/kg 1,3-DNB caused 15% methemoglobin in hamsters compared with 80% in rats (Obasaju et al. 1991). Another study found that. after administration of the same. dose of 1,3-DNB to hamsters and rats, blood levels of 1,3-DNB in hamsters were half those found in rats (McEuen and Miller 1991). Other differences in the metabolic disposition of 1,3-DNB between hamsters and rats were that rats had higher blood levels of the metabolite nitroaniline and excreted more unconjugated and less phenolic metabolites in the urine (McEuen and Miller 1991). All together, these data suggest that metabolic activation may be needed for hematotoxicity and that this metabolic activation is species-specific. No information was found regarding hematological effects in humans or animals after exposure to 1,3,5-TNB. An intermediate oral MRL was derived based on hematological effects in male rats administered 0.75 mg/kg/day 1,3-DNB by gavage for 12 weeks (Linder et al. 1986).

Hepatic Effects. Results of the studies on hepatic effects in humans after exposure to 1,3-DNB are inconclusive. In one case report of occupational exposure to 1,3-DNB, the exposed worker had palpable liver and jaundice while her liver function tests were negative (Ishihara et al. 1976). This study is limited in that it describes only one case and there is no information on the dose. In addition, functional tests were performed 10 days after the exposure occurred. In another study, hepatic transaminase levels (SGOT and SGPT) were within normal limits after a single acute-duration exposure to 1,3-DNB (Okubo and Shigeta 1982). Bilirubin was also found in all urine samples from exposed workers who were strongly positive for urobilinogen, indicating an unspecified degree of hepatobiliary disease. All exposed workers were followed for 10 years after exposure and showed no long-term adverse effects. This study is limited in that there are no data on the dose of 1,3-DNB and the functional tests were performed 9 days after exposure. Studies in animals provided little information. Exposure of rats to 1,3-DNB for 8 or 16 weeks in the drinking did not result in histopathologic alterations in the liver (Cody et al. 1981). Based on the available information and the lack of chronic-duration exposure data in humans or animals, it is difficult to estimate whether exposure to 1,3-DNB at hazardous waste sites or in industrial settings will lead to adverse hepatic effects. No information was found regarding hepatic effects in humans or animals after exposure to 1,3,5-TNB.

Renal Effects. Although no adverse effects on renal function have been reported, elevated levels of urobilinogen were found in workers after inhalation exposure to an unspecified amount of 1,3-DNB (Okubo and Shigeta 1982). It took approximately 50 days for urobilinogen to return to normal .levels. The only information located regarding renal toxicity in animals after exposure to 1,3-DNB was from an early study in which kidney inflammation was observed in a cat after dermal application of 1,3-DNB (White and Hay 1901); the dose applied was lethal. Persons exposed to high levels of 1,3-DNB by any of the three routes may have an increased risk of renal toxicity. It is not clear whether chronic exposure to very low levels of 1,3-DNB by any of the three routes might cause renal toxicity.

No studies were located regarding renal toxicity in humans or animals after exposure to 1,3,5-TNB. Therefore, it is not known if adverse renal effects would occur following inhalation, oral, or dermal exposure to 1,3,5-TNB.

Body Weight Effects. No information was located regarding body weight effects in humans after inhalation, oral, or dermal exposure to 1,3-DNB or 1,3,5-TNB. One single oral intermediate-duration study showed that 1,3-DNB administered in drinking water for 8 weeks can lead to reduced growth rate (4-6 mg/kg/day) or even to body weight loss (12-14 mg/kg/day) (Cody et al. 1981). These responses could not be explained solely by reduced food consumption, but reduced water intake and dehydration may have played a role. The relevance of these findings to effects in humans is difficult to ascertain.

Immunological and Lymphoreticular Effects. No studies were located regarding immunological effects in humans after exposure to 1,3-DNB or 1,3,5-TNB. Studies in animals have not assessed the immune response after exposure to 1,3-DNB or 1,3,5-TNB, but spleen enlargement was reported in rats in acute- (Blackbum et al. 1988) and intermediate-duration (Cody et al. 1981; Linder et al, 1986) oral studies. Spleen enlargement, however, was probably a secondary response to the methemoglobinemia resulting from 1,3-DNB intake. Results from tests in guinea pigs showed that 1,3-DNB was not a skin sensitizer and that 1,3,5-TNB was mildly allergenic (Desai et al. 1991). Based on the information available, it is not known whether adverse immunological effects would occur in humans following inhalation, oral, or dermal exposure to 1,3-DNB or 1,3,5-TNB.

Neurological Effects. Very limited information is available regarding the neurological effects of 1,3-DNB. Slight headache, nausea, dizziness, and fatigue were symptoms reported in workers after inhalation exposure to 1,3-DNB (Okubo and Shigeta 1982). Headache accompanied a single dermal exposure to 1,3-DNB (Ishihara et al. 1976). These symptoms are probably the result of oxygen deprivation due to the presence of increased methemoglobin in the blood or to vasodilation of cerebral blood vessels. Acute-duration studies in animals have reported ataxia (weakness, loss of balance, flaccid paralysis) at doses that induced cyanosis (Cody et al. 1981; Linder et al. 1988, 1990; Philbert et al. 1987b). There was also ,a difference in susceptibility to the neurotoxic effects of 1,3-DNB between older and younger rats, older adult animals being more sensitive (attributed to reduced metabolism of 1,3-DNB) than younger ones (Linder et al. 1990). Results from intermediate-duration oral studies provided conflicting data. Ataxia was reported in rats given 6 mg/kg/day 1,3-DNB by gavage for 12 weeks (Linder et al. 1986), but neither ataxia nor histopathological alterations in the brain or spinal cord were observed in rats treated with 12-14 mg/kg/day 1,3-DNB in drinking water for 8 weeks (Cody et al. 1981). The different manner of administering the compound between these two studies may have contributed to the different responses. Also, increased activity in a platform was

seen in rats treated with a relatively low dose (0.4 mg/kg/day) of 1,3-DNB for 90 days in drinking water (Cody et al. 1981). This indicates that low 1,3-DNB doses may induce subtle neurological effects, which were not assessed in other studies. The existing information would suggest that adverse neurological effects appear at hematotoxic exposure levels. The information available is insufficient to determine whether long-term exposure to low levels of 1,3-DNB might affect the nervous system.

No studies were located regarding neurological effects after exposure to 1,3,5-TNB in humans or animals. Therefore, it is not known if inhalation, oral, or dermal exposure to 1,3,5-TNB would cause adverse neurological effects.

Reproductive Effects. Studies in humans have not addressed whether adverse reproductive effects occur after exposure to either 1,3-DNB or 1,3,5-TNB. However, adverse reproductive effects were observed in male rats, mice, and ‘hamsters (females were not tested) after a single or repeated oral administration of 1,3-DNB. The Sertoli cell has been suggested as the prime target for 1,3-DNB toxicity (Blackbum et al. 1988; Hess et al. 1988; Reader et al. 1991). Because the Sertoli cells have been shown to be involved in the control of spermatogenesis, damage to them could precipitate the wide range of effects seen in germ cells (Blackbum et al. 1988). Reduced testes and epididymis weights, disruption of spermatogenesis, hypospermia, poor sperm quality, and infertility were consistent findings regarding reproductive toxicity (Blackbum et al. 1988; Cody et al. 1981; Evenson et al. 1989a; Linder et al. 1986, 1988; Moore et al. 1992; Reader et al. 1991; Rehnberg et al. 1988). Susceptibility to reproductive toxicity of 1,3-DNB appears to be different in older and younger animals (Linder et al. 1990); the authors suggested that reduced metabolism in older animals led to greater bioavailability, implying that the parent compound may be the toxic entity. The specific mechanism of 1,3-DNB toxicity has not been elucidated. Some have suggested that testicular damage may be related to tissue hypoxia (Linder et al. 1988), which is the result of increased methemoglobin formation.

Different susceptibility among species to reproductive effects seems also related to the metabolism of 1,3-DNB. Rats were much more susceptible to adverse reproductive effects of 1,3-DNB than hamsters (McEuen and Miller 1991; Obasaju et al. 1991). This was correlated with the fact that blood levels of 1,3-DNB in the hamster reached only half those found in the rat and that blood levels of the metabolite 1,3-nitroaniline were higher in the rat (McEuen and Miller 1991). Furthermore, rats excreted more unconjugated and less phenolic metabolites than hamsters. Results from studies with rat

Sertoli/germ cell cocultures suggest that reactive metabolic intermediates such as nitrosonitrobenzene and nitrophenylhydroxylamine may be responsible for the testicular toxicity of 1,3-DNB (Cave and Foster 1990). An acute oral MRL was derived based on reproductive effects in male rats treated with a single dose of 16 mg/kg 1,3-DNB by gavage (Linder et al. 1990).

Based on the findings reported in these studies, the possibility of adverse effects occurring in human males following exposure to sufficiently high levels of 1,3-DNB cannot be excluded. As stated earlier, these higher 1,3-DNB levels are not likely to be present in the vicinity of ammunition plants.

Developmental Effects. Studies in humans or animals have not investigated whether adverse developmental effects occur as a result of exposure to 1,3-DNB or 1,3,5-TNB. Therefore, it is not known if inhalation, oral, or dermal exposure to 1,3-DNB or 1,3,5-TNB would cause adverse developmental effects.

Genotoxic Effects. There were no studies available regarding the genotoxicity of 1,3-DNB or 1,3,5-TNB in either humans or animals *in vivo.* One study was located that tested the effects of 1,3-DNB on rat liver cells. No significant increase in deoxyribonucleic acid (DNA) damage was observed (Probst et al. 1981). The remaining studies were eithef bacterial or fungal assays for mutagenicity, DNA damage, or mitotic recombination. The results for the *Salmonella typhimurium* mutagenicity tests were dependent on the strain of bacteria and test used. Positive responses were observed in strains TA98, TA100, TA1538, TA1537, TA1535, and D3052. Since strains TA98, TA1538, and TA1537 are sensitive to frameshift mutations and strains TA100 and TA1535 are sensitive to base-pair substitutions, positive responses in each of these strains suggest that 1,3-DNB produces both types of gene mutations in S. typhimurium (Chiu et al. 1978; Furukawa et al. 1985; Gamer and Nutman 1977; Kaden et al. 1979; Kerklaari et al. 1987; McGregor et al. 1980; Melnikow et al. 1981; Probst et al. 1981; Shimizu et al. 1983; Spanggord et al. 1982b). Two groups of investigators compared mutagenicity results using normal strains and strains deficient in nitroreductase (TA100NR or TA100NR3). The results were positive for the normal strains (TA100, TA98, and TA1538) but negative for the nitroreductase-deficient strains (Kerklaan et al. 1987; Spanggord et al. 1982b). This supports a well-documented notion that the mutagenicity observed in normal strains is due to endogenous bacterial reduction of the nitro groups (Chiu et al. 1978; Kerklaan et al. 1987; Probst et al. 1981; Shimizu et al. 1983; Spanggord et al. 1982b). Human intestinal flora contains nitroreducing bacteria, and it is therefore realistic to consider that ingestion of nitrobenzene compounds

may lead to mutagenic effects in humans. *Escherichia coli* was also examined for gene mutations following treatment with 1,3-DNB. Two strains were used: WP2 and WP2 uvrA. The results were negative for both, strains (Probst et al. 1981). Another study tested E**.** coli for DNA damage following 1,3-DNB treatment. According to the paper, concentrations of 1-10 mg/plate produced bacterial toxicity, but it was not clear whether these exposures produced DNA damage or any other form of genotoxicity (McGregor et al. 1980). A fungal study tested the effects of 1,3-DNB on mitotic recombination in *Succharomyces cerevisiae***.** Doses of up to 32 mg/mL were administered, but no genotoxic effects of any kind were observed either with or without metabolic activation (McGregor et al. 1980). Refer to Table 2-2 for a further summary of the genotoxic effects of 1,3-DNB exposure.

A few studies were located that tested the effects of 1,3,5-TNB on mutagenicity, DNA damage, or mitotic recombination, As with 1,3-DNB, 1,3,5-TNB was not significantly mutagenic in the Sulrnonellu strain deficient in nitroreductase, but gene mutations were observed in strains containing the enzyme (Spanggord et al. 1982b). *E. coli* was used to test the DNA-damaging capabilities of 1,3,5-TNB. All concentrations produced bacterial toxicity, but it was unclear whether DNA damage occurred (McGregor et al. 1980). 1,3,5-TNB did not affect mitotic recombination or produce any other observable genotoxic effect in S. *cerevisiue* (McGregor et al. 1980). Refer to Table 2-3 for a further summary of the genotoxic effects of 1,3,5-TNB exposure.

Unfortunately, the lack of human and animal exposure data makes it difficult to determine whether or not 1,3-DNB and 1,3,5-TNB are genotoxic. The available *in vitro* studies indicate that both chemicals have mutagenic potential in S. *typhimurium* bacteria. *S. typhimurium* is a classic system used to evaluate chemicals for their capacity to induce heritable mutations that potentially can occur in humans (Prival 1983). The observed mutagenicity, however, seems to arise from a derivative that is produced from nitroreduction. This nitroreduction occurs in rabbits (Parke 1961), rats (McEuen and Miller 1991; Nystrom and Rickert 1987), hamsters (McEuen and Miller 1991), cultured rat Sertoli cells (Cave and Foster 1990; Cossum and Rickert 1987; Lloyd and Foster 1987), and rat hepatocytes (Cossum and Rickert 1985). Since it is likely that the same nitroreduction process occurs in humans, then 1,3-DNB and 1,3,5-TNB may be considered potential human genotoxins.

Cancer. There is no information regarding carcinogenicity of 1,3,5-TNB or dinitrobenzenes including 1,3-DNB. Because of the lack of data regarding the carcinogenicity of 1,3-DNB and 1,3,5-TNB, EPA has placed them in Group D, not classifiable as to carcinogenic potential (IRIS 1994).

TABLE 2-2. Genotoxicity of 1,3-DNB *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (TA100, TA100/GSH-)	Gene mutation	No data	+	Kerklaan et al. 1987
S. typhimurium (TA100NR)	Gene mutation	No data	–	Kerklaan et al. 1987
S. typhimurium (TA677)	Gene mutation	(+)	No data	Kaden et al. 1979
S. typhimurium (TA1538)	Gene mutation	+[a]	+	Garner and Nutman 1977
S. typhimurium (TA98)	Gene mutation	No data	+	Chiu et al. 1978
S. typhimurium (TA100)	Gene mutation	No data	–	Chiu et al. 1978
S. typhimurium (TA98, TA1538, TA1535, TA1537, TA100)	Gene mutation	No data	+	Shimizu et al. 1983
S. typhimurium (TA98, TA100)	Gene mutation	+	+	Melnikow et al. 1981
S. typhimurium (TA1535, TA1537, TA100NR3)	Gene mutation	–	–	Spanggord et al. 1982b
S. typhimurium (TA1538, TA98, TA100)	Gene mutation	+	+	Spanggord et al. 1982b
S. typhimurium (TA1535, TA1538, TA98, TA100)	Gene mutation	–	No data	Anderson and Styles 1978[b]
S. typhimurium (TA98)	Gene mutation	No data	+	Furukawa et al. 1985
S. typhimurium (TA1538, TA98, TA100)	Gene mutation	+[a]	+	McGregor et al. 1980
S. typhimurium (TA1537)	Gene mutation	(+)	(+)	McGregor et al. 1980
S. typhimurium (TA1535)	Gene mutation	–	–	McGregor et al. 1980
S. typhimurium (TA100, D3052, TA1538, TA98)	Gene mutation	No data	+	Probst et al. 1981
S. typhimurium (G46, TA1535, C3076, TA1537)	Gene mutation	No data	–	Probst et al. 1981
Escherichia coli (WP2, WP2, uvrA)	Gene mutation	No data	–	Probst et al. 1981

TABLE 2-2. Genotoxicity of 1,3-DNB *In Vitro* (continued)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae	Mitotic recombination	–	–	McGregor et al. 1980
Mammalian cells:				
Rat (liver cells)	DNA damage	–	NA	Probst et al. 1981

[a]The presence of metabolic activators lessened the toxicity.
[b]Study did not specify which positional isomer of 1,3-DNB was used.

DNA = deoxyribonucleic acid; NA = not applicable; – = negative result; + = positive result; (+) = weakly positive result

TABLE 2-3. Genotoxicity of 1,3,5-TNB *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (TA 1535, TA1537, TA1538, TA100, TA98)	Gene mutation	+[a]	+	McGregor et al. 1980
S. typhimurium (TA 1535)	Gene mutation	+	–	Spanggord et al. 1982b
S. typhimurium (TA 1537, TA 1538)	Gene mutation	–	+	Spanggord et al. 1982b
S. typhimurium (TA 98, TA100)	Gene mutation	+	+	Spanggord et al. 1982b
S. typhimurium (TA100NR3)	Gene mutation	–	–	Spanggord et al. 1982b
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae	Mitotic recombination	–	–	McGregor et al. 1980

[a]The presence of metabolic activators lessened the toxicity.

– = negative result; + = positive result

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself 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 biologic 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 1,3-DNB and 1,3,5-TNB are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 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 often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by 1,3-DNB and 1,3,5-TNB are discusskd in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism’s ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed

dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, Populations That Are Unusually Susceptible.

2.5.1 Biomarkers Used to Identify or Quantify Exposure to 1,3-DNB and 1,3,5-TNB

Very few methods are available for determining the level of 1,3-DNB and its metabolites in human blood and urine (for more information see Chapter 6). Because 1,3-DNB is rapidly absorbed, metabolized, and excreted, measurement of blood levels of this substance or its metabolites 4s limited to exposures of a very large magnitude and that occur within a few, hours of the time at which the blood sample is obtained. Results from an *in vitro* study in rat hepatocytes and microsomes show that the relative rate of conversion of 1,3-DNB to nitroaniline is 7 and 12 minutes, respectively (Cossum and Rickert 1985). This would make it difficult to accurately determine the level of 1,3-DNB in the blood and use it as a marker of exposure.

Very little information is available about the nature of urinary metabolites of 1,3-DNB in humans. In a study that evaluated 1,3-DNB urinary metabolites after a single dermal exposure, amino and nitro metabolites were grouped together and reported as a single value relative to the level of 2,4-dinitrophenol as a standard (Ishihara et al. 1976). Amino and nitro metabolites may be derived from a variety of nitroaromatic compounds; thus, they are not specific for 1,3-DNB.

In rats, 1,3,5-TNB was found to form adducts with blood proteins such as albumin, globulin, and globin, and with DNA from tissues, and it was suggested that these adducts may be useful as markers for exposure to 1,3,5-TNB (Reddy et .al. 1991). Adducts with albumin and globulin reached a maximum one day after a single oral dose, and by day 7 had almost completely disappeared. Adducts with globin peaked by day 2, and after 28 days, 20% of the adducts remained. DNA adducts were also formed in the spleen, liver and stomach. In the spleen, 100% of the adducts were retained for at least 28 days after dosing.

2.5.2 Biomarkers Used to Characterize Effects Caused by 1,3-DNB and 1,3,5-TNB

One of the earliest effects of 1,3-DNB exposure is induction of methemoglobin formation. The level of methemoglobin in the blood can be used as an indicator of exposure to 1,3-DNB.

There are two questions to be answered in relation to the specificity of methemoglobin formation as a biomarker for exposure to 1,3-DNB. One pertains to the nature of the reaction and whether 1,3-DNB itself or one of its metabolic intermediaries cause methemoglobin formation. The second question relates to the specificity of the reaction, namely the fact that other nitrobenzene compounds and dinitrobenzene isomers may also cause methemoglobin formation. Methemoglobin formation is a common response to exposure to organic nitrates and is not specific for either 1,3-DNB or 1,3,5-TNB. Once these two issues are resolved, it might be possible to select a more specific biomarker for 1,3-DNB exposure. In the meantime, the levels reflected in a complete blood count can be used as a nonspecific biomarker. These are red cell count, hemoglobin concentration, hematocrit, white cell count, and a peripheral blood smear for cell morphology. They are rapid, relatively inexpensive, and useful for monitoring cohorts of persons possibly exposed to particular members of the nitrobenzene class of chemicals.

Another early symptom of exposure to 1,3-DNB is cyanosis due to oxygen deprivation because of the presence of methemoglobin in the blood. These changes are also not specific for 1,3-DNB and may be produced by other nitrobenzene compounds and dinitrobenzene isomers. Therefore, cyanosis is not a good biomarker for 1,3-DNB exposure.

Although little information is available regarding neurotoxicity of 1,3-DNB, slight headache, nausea, dizziness, and general malaise can accompany exposure to 1,3-DNB (Ishihara et al. 1976; Okubo and Shigeta 1982). These symptoms can occur early or be concomitant with cyanosis, but the correlation between them was not investigated. These symptoms are not specific to 1,3-DNB exposure and therefore are not good biomarkers for 1,3-DNB exposure.

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

2.6 INTERACTIONS WITH OTHER CHEMICALS

Limited information is available regarding the influence of other chemicals on the toxicity of 1,3-DNB or 1,3,5-TNB. One study reported that a chemical mixture containing 1,3-DNB was not toxic and did not induce methemoglobin formation. The mixture contained 1,3-DNB (0.5%), ethylene glycol (77.5%

or less), and diethylene glycol (15% or more) (Ishihara and Ikeda 1979). As the mixture was made more polar either by adding water or short-chain dicarboxylic acids, methemoglobin formation was favored. The specific mechanism of this interaction is not known,

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 1,3-DNB/1,3,5-TNB than will most persons exposed to the same level of 1,3-DNB and 1,3,5-TNB in the environment. Reasons include genetic make-up, developmental. stage, health and nutritional status, and chemical exposure history. These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic and renal) or the pre-existing compromised function of target organs. For these reasons we expect the elderly with declining organ function and the youngest of the population with . immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

No information was located on populations unusually susceptible to toxic effects of 1,3,5-TNB.

In. the review of the literature regarding toxic effects of 1,3-DNB, no information on any population that might be unusually sensitive to 1,3-DNB was found. However, populations that may show increased sensitivity include very young children who have an immature hepatic detoxification system (and less efficient fetal hemoglobin), individuals with impaired liver or kidney function, and those persons who are prone to anemia or are anemic. Also at risk of potential 1,3-DNB toxicity are infants with low levels of nicotinamide adenine dinucleotide diaphorase (enzyme that reduces methemoglobin) or persons congenitally deficient in this enzyme. At increased risk for induction of methemoglobinemia due to exposure to the nitrobenzene class of chemicals may be individuals with such genetic traits as glucose 6 phosphate deficiency, sickle cell trait, or genetically induced unstable hemoglobin forms.

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 1,3-DNB or 1,3,5-TNB. 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 1,3-DNB or 1,3,5-TNB. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

No studies were located regarding the reduction of toxic effects of 1,3,5-TNB. In the two occupational studies on human dermal exposure to 1,3-DNB, no treatment was described to diminish or alleviate 1,3-DNB toxicity (Ishihara and Ikeda 1979; Ishihara et al. 1976). In both studies, affected workers were removed from the 1,3-DNB source and recovered without treatment within 40 days (Ishihara et al. 1976).

2.8.1 Reducing Peak Absorption Following Exposure

In order to reduce absorption of I,3-DNB or 1,3,5-TNB following inhalation exposure, patients should be moved to fresh air (HSDB 1994). Following recent ingestion of a substantial amount of either chemical, emesis may be indicated unless the patient is obtunded, comatose, or convulsing (HSDB 1994). Administration of a charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, has also been recommended (HSDB 1994). Following dermal exposure, it is recommended that the exposed area be washed extremely thoroughly with soap and water (HSDB 1994). Eye contamination should be treated by irrigating with copious amounts of tepid water for at least 15 minutes (HSDB 1994).

2.8.2 Reducing Body Burden

No studies were located regarding reducing body burden following exposure to 1,3-DNB or 1,3,5-TNB.

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

No agents are known to interfere with 1,3-DNB or 1,3,5-TNB cyanosis (resulting from methemoglobin production), but procedures are available and have been recommended to counteract these effects. Cyanosis may be treated with high flow (100%) oxygen administration to saturate all remaining normal hemoglobin with oxygen (Donovan 1990; Ellenhorn and Barceloux 1988). Elevated levels of methemoglobin may be decreased by enhancing the rate of conversion of methemoglobin to hemoglobin. Methylene blue is the antidote of choice in this situation. Ascorbate has been suggested

as an alternative reducing agent, but it is believed to have limited efficacy (Donovan 1990; Ellenhorn and Barceloux 1988). Methylene blue is administered intravenously. It is first reduced to leukomethylene blue by NADPH-dependent methemoglobin reductase in the red blood cell. The leukomethylene blue then acts as an electron donor to reduce methemoglobin to hemoglobin nonenzymatically. Use of methylene blue is generally indicated when methemoglobin levels exceed 30% but may be used at lower methemoglobin levels in persons with pulmonary or cardiovascular disease or with preexisting anemia (Donovan 1990; Ellenhom and Barceloux 1988; Goldfrank et al. 1990). Methylene blue is ineffective in persons with glucose-6-phosphate dehydrogenase deficiency and of limited effectiveness in persons with NADPH-dependent methemoglobin reductase deficiencies (Donovan 1990; Ellenhom and Barceloux 1988; Goldfrank et al. 1990). Severe hemolytic anemia may develop if methylene blue is given to persons with glucose-6-phosphate dehydrogenase deficiency. Caution should also be used when administering methylene blue to others because high doses (>7 mg/kg) may increase methemoglobin levels and cause hemolysis (Donovan 1990; Ellenhom and Barceloux 1988). In cases of failure of methylene blue therapy, exchange transfusions have been used to replace hemoglobin and remove the absorbed toxin (Donovan 1990; Ellenhom and Barceloux 1988).

If seizures develop following exposure to 1,3-DNB or 1,3,5-TNB, administration of diazepam IV bolus has been suggested. Administration of phenytoin is recommended if seizures are uncontrollable or recur (HSDB 1994).

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 1,3-DNB and 1,3,5-TNB 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 1,3-DNB and 1,3,5-TNB.

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 or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the

identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of 1,3-DNB and 1,3,5-TNB

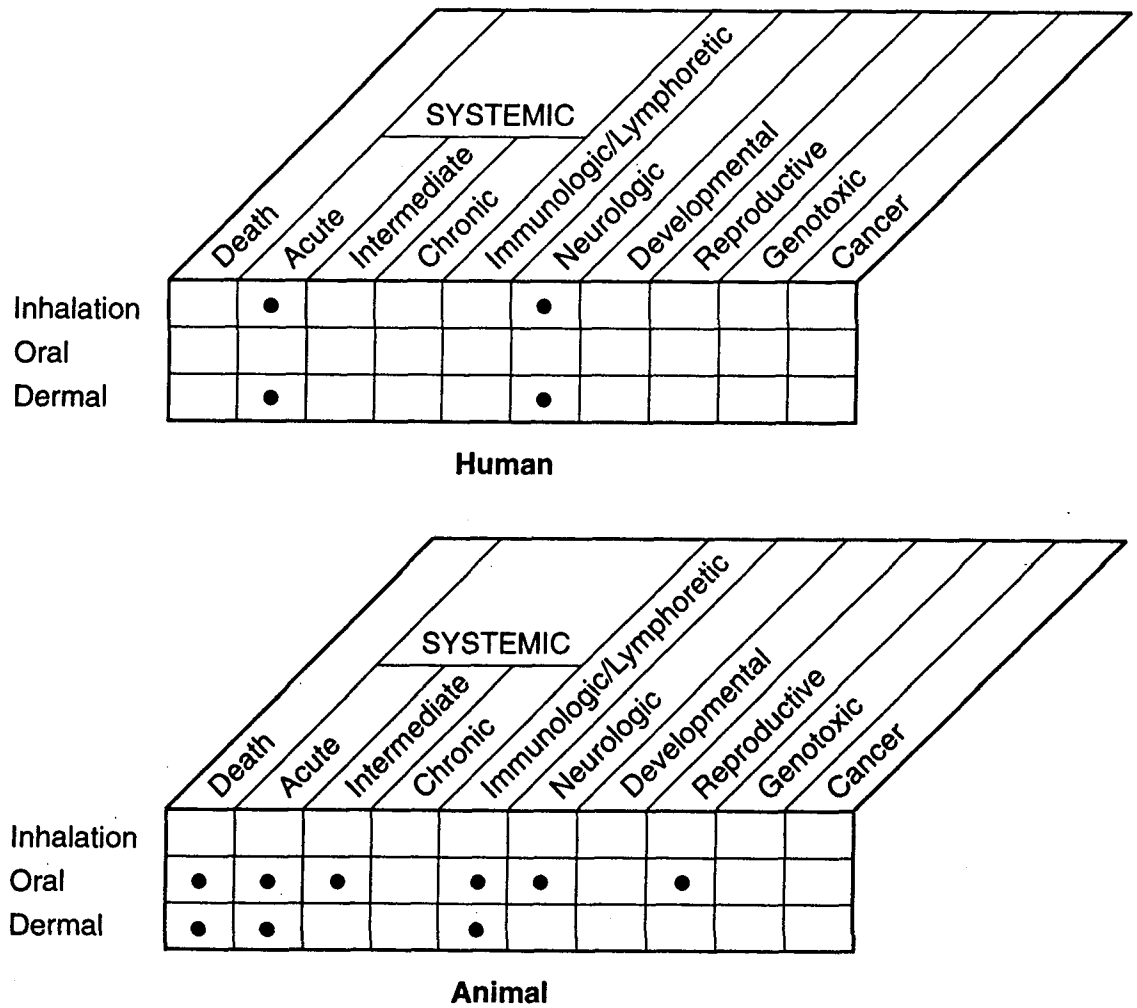
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 1,3-DNB and 1,3,5-TNB are summarized in Figure 2-3 and 2-4, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of 1,3-DNB and 1,3,5-TNB. 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 1989e), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature. In addition to this information, a database is available through the National Institutes of Health on the metabolism and toxicity of 1,3-DNB.

No studies were located concerning the health effects of 1,3,5-TNB in humans. Information on effects in animals was limited to acute oral and dermal data.

With regard to human health effects of 1,3-DNB, the few available studies involved acute-duration occupational exposure to 1,3-DNB by the inhalation and dermal routes, a case of accidental ingestion of a nitrobenzene dye, and a case of an experimenter who self-applied 1,3-DNB dermally for research purposes. No information was located on intermediate- or chronic-duration exposures in humans by any route. No information is available regarding immunologic, developmental, reproductive, genotoxic, or cancer effects in humans by any route of exposure.

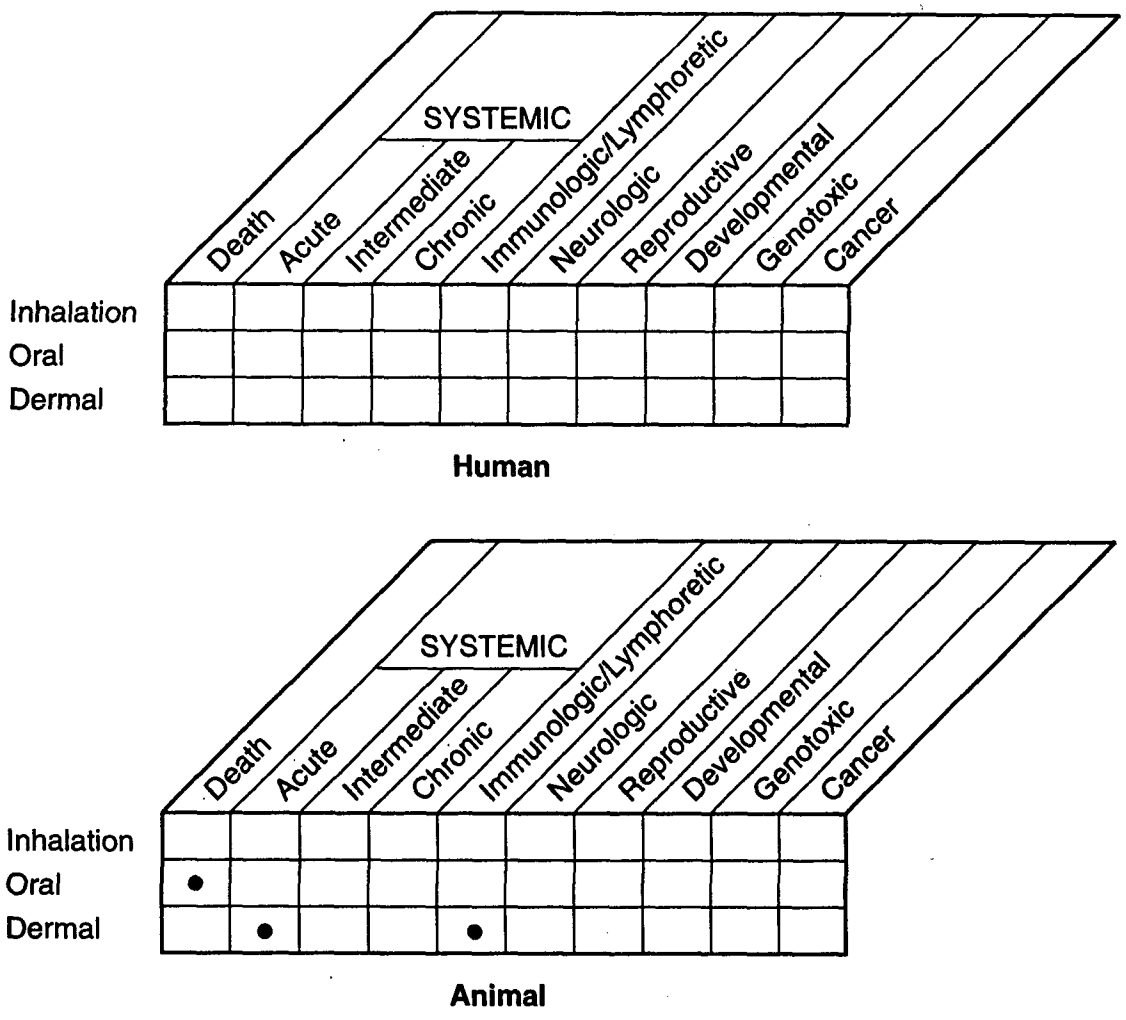
Virtually all of the data regarding the health effects of 1,3-DNB in animals were obtained from studies in which 1,3-DNB was administered orally. No information is available concerning health effects in animals following inhalation exposure, and only two reports on dermal exposure to 1,3-DNB were located. Therefore, information on those two routes of exposure would be useful because of the

FIGURE 2-3. Existing Information on Health Effects of 1,3-DNB



● Existing Studies

FIGURE 2-4. Existing Information on Health Effects of 1,3,5-TNB



● Existing Studies

potential exposure via those two routes for humans living near ammunition plants and workers in the dyestuffs, plastics, rubber, and other industries.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. Populations living in the vicinity of ammunition plants may be exposed to 1,3-DNB or 1,3,5-TNB for a short time. Exposure would probably occur via the oral route, but inhalation and dermal exposures cannot be excluded.

No information regarding health effects of 1,3,5-TNB administered by any route is available in humans. Data presented in abstract form provided limited information on oral and dermal toxicity in animals (Desai et al. 1991). Therefore, studies addressing toxic effects of 1,3,5-TNB in animals after acute oral exposure (since this is the most likely route of exposure for human populations in the vicinity of ammunition plants) would provide needed information for estimation of possible 1,3,5-TNB toxicity in humans. Also needed are acute exposure studies of 1,3,5-TNB after inhalation and dermal exposures because exposure by these routes may occur in spite of the low volatility of these compounds.

The hematological system is the major target of 1,3-DNB toxicity in humans and animals following acute exposure by any route. Biochemical changes that occur in blood are responsible for methemoglobin formation leading to oxygen deprivation in the tissues (Donovan 1990; Ellenhorn and Barceloux 1988). That change further results in cyanosis (Linder et al. 1988, 1990; Philbert et al. 1987b; Reader et al. 1991). Studies in animals further indicate that adverse neurological effects such as ataxia (Cody et al. 1981; Linder et al. 1988, 1990; Philbert et al. 1987b), and reproductive effects, such as altered spermatogenesis and infertility (Cody et al. 1981; Hess et al. 1988; Linder et al. 1988, 1990; Moore et al. 1992; Reader et al. 1991) occur after acute oral exposure to 1,3-DNB. These potential targets have not been studied in humans. An acute oral MRL was derived for 1,3-DNB based on adverse reproductive effects in male rats (Linder et al. 1990) (see Section 2.4).

Respiratory changes have not been studied in either humans or animals after acute inhalation exposure to 1,3-DNB. However, slight dyspnea was observed in humans following an acute inhalation exposure to 1,3-DNB (Okubo and Shigeta 1982). Although the volatility of 1,3-DNB is low and thus the levels in the atmosphere are expected to be low, lung absorption of 1,3-DNB is possible in areas close to

ammunition plants and in occupationally exposed workers. It would therefore be useful to perform studies that examine the respiratory effects after acute inhalation exposure to 1,3-DNB. Limited data indicate that humans can absorb 1,3-DNB through the skin (Ishihara et al. 1976; White and Hay 1901), and although.there was no evidence of adverse respiratory effects, well-conducted studies in animals may provide valuable supportive information.

Intermediate-Duration Exposure. No studies were located on intermediate-duration exposure to 1,3,5-TNB in humans or animals by any route. Therefore, studies in animals would provide useful information. There were also no studies on intermediate-duration exposure by any route to 1,3-DNB in humans. Studies in laboratory animals following intermediate-duration oral exposure to 1,3-DNB showed that a major target is the hematological system (see Section 2.2.2.2), but other targets include the central nervous system (Cody et al. 1981; Linder et al. 1986) (see Section 2.2.2.4) and the male reproductive system (Cody et al. 1981; Linder et al. 1986) (see Section 2.2.2.5). An intermediate oral MRL was derived for 1,3-DNB based on hematological effects in rats exposed for 12 weeks (Linder et al. 1986) (see Section 2.4). Studies to determine whether adverse effects occur in animals after inhalation or dermal intermediate duration exposure would be useful.

Chronic-Duration Exposure and Cancer. No studies were located following chronic-duration exposure to either 1,3-DNB or 1,3,5-TNB in humans or animals. This may be because both compounds were shown to be potent acute toxicants. Animal studies that examine the effects of 1,3-DNB and 1,3,5-TNB after low-level chronic exposure by oral, dermal, and inhalation routes would be of value to determine whether exposures via these routes could cause toxicity in populations living in the vicinity of ammunition plants or in those exposed in industries where these chemical are used.

Because of the lack of data on the carcinogenicity of 1,3-DNB and 1,3,5-TNB, and in the absence of data to adequately describe the mechanism of action, these two compounds are not presently classified as carcinogens. Studies to determine if these two compounds have carcinogenic potential via inhalation, oral and dermal exposure routes would be useful.

Genotoxicity. No human or animal in viva studies on the genotoxicity of I,3-DNB or 1,3,5-TNB were located. However, several bacterial mutagenicity studies were located for both chemicals. Depending on the strain of S. typhimurium used in mutagenicity, testing both compounds caused nonsignificant frameshift mutations and base-pair substitutions (Chiu et al. 1978; Furukawa et al. 1985;

Gamer and Nutman 1977; Kaden et al. 1979; Melnikow et al. 1981; Spanggord et al. 1982b) indicating that both 1,3-DNB and 1,3,5-TNB have a mutagenic potential. The results of several studies have also suggested a link between the mutagenicity of 1,3-DNB/1,3,5-TNB and nitroreduction (Chiu et a1.1978; Kerklaan et al. 1987; Probst et al. 1981; Shimizu et al. 1983; Spanggord et al. 1982b). *S: typhimurium* strains deficient in enzyme nitroreductase had nonsignificant gene mutations after exposure to either 1,3-DNB or 1,3,5-TNB (Spanggord et al. 1982b). Further investigation into this link using mammalian cells/systems would be helpful in establishing if the same processes also occur in mammalian cells.

Reproductive Toxicity. No studies were found describing reproductive effects of 1,3-DNB in humans and 1,3,5-TNB in humans and animals. Studies in laboratory animals exposed orally to 1,3-DNB show that 1,3-DNB is a potent testicular toxicant (see Section 2.2.2.5) (Blackburn et al. 1988; Cody et al. 1981; Evenson et al. 1989b; Hess et al. 1988; Linder et al. 1986, 1988; Reader et al. 1991). An acute-duration oral MRL was derived on the study by Linder et al. (1990) (see Section 2.4). No studies in animals were found regarding the reproductive effects of exposure to 1,3-DNB by inhalation or dermal routes. Therefore, studies examining the effects on reproduction (including exposure of females during gestation) following inhalation, oral, and dermal exposure to 1,3-DNB would be useful, since oral exposure is the most likely route for people living near ammunition plants, but inhalation and dermal exposure may be more relevant in industrial settings. Animal studies following exposure to 1,3,5-TNB by any of the three routes would be useful to establish if there is potential for reproductive toxicity in people living close to ammunition plants or in occupationally exposed workers.

Developmental Toxicity. No human or animal studies on the developmental effects of 1,3-DNB or 1,3,5-TNB for any exposure route were located in this literature review. Animal studies examining postnatal survival and developmental effects following maternal exposure by all routes of exposure would be helpful since potential oral exposure exists for populations living near ammunition plants, and inhalation and dermal exposure may occur in industries involved in dyestuff, plastics, and rubber production.

Immunotoxicity. No information on immunotoxicity after exposure to 1,3,5-TNB by any of the three routes is available in humans or animals. Therefore, animal studies following acute, intermediate, and chronic exposure to 1,3,5-TNB via all three routes would help in estimating the

potential immunotoxic effects in humans. Spleen enlargement was reported in acute- (Blackburn et al. 1988) and intermediate-duration (Cody et al. 1981; Linder et al. 1986) studies in animals. These effects, however, were secondary to adverse hematological effects. Studies in laboratory animals following acute exposure to 1,3-DNB by the oral route would help define possible effects on antibody production and cellular immunity. This information could be used to determine populations sensitive to possible exposure to 1,3-DNB at locations close to ammunition plants or in specific workplaces.

Neurotoxicity. The few human studies available on dermal and inhalation exposure to 1,3-DNB indicate that the central nervous system. may be a target of 1,3-DNB toxicity (Ishihara et al. 1976; Okubo and Shigeta 1982) (see Sections 2.2.1.4 and 2.2.3.4). Studies in animals support this finding although results in animal studies were mostly obtained after acute oral exposure to 1,3-DNB (Linder et al. 1988, 1990; Philbert et al. 1987b). The animal data have also shown that the severity of neurotoxic effects is dose dependent and that neurotoxicity is probably due to decreased oxygenation because of increased levels of methemoglobin (Cody et al. 1981; Linder et al. 1988; Philbert et al. 1987b). Laboratory animal studies that focus on subtle neurological effects following acute, intermediate, or chronic exposure to a range of doses via oral, inhalation, and dermal routes would help to better estimate potential neurotoxic effects in humans living near ammunition plants and in workers who might be exposed in certain occupational settings.

No studies were found describing neurotoxicity of 1,3,5-TNB in humans or animals. Therefore, studies following oral, inhalation, or dermal exposure to 1,3,5-TNB would be very helpful in evaluating potential neurotoxic effects close to ammunition plants.

Epidemiological and Human Dosimetry Studies. No epidemiological studies on exposure to either 1,3-DNB or 1,3,5-TNB have been located. Studies of worker populations and populations living near ammunition plants might be useful to determine effects of low-level acute, intermediate, or possibly chronic exposure to 1,3-DNB or 1,3,5-TNB. If such populations are identified, carefully designed information gathering of immunologic, reproductive, hematologic, neurotoxic, genotoxic, developmental, and carcinogenic effects of the two compounds should be implemented. The correlation of these effects with the levels of methemoglobin associated with exposure would provide useful information regarding potential exposure of populations living near ammunition plants and occupationally exposed workers.

Biomarkers of Exposure and Effect.

Exposure. Exposure to 1,3-DNB is currently measured indirectly by determining levels of methemoglobin in the blood (Donovan 1990). However, increased methemoglobin formation is not a specific response to 1,3-DNB exposure and may occur after exposure to other nitrobenzene compounds such as the other two isomers of dinitrobenzene. Determination of methemoglobin levels is widely used and is a reliable detection method. Very few methods are available for direct evaluation of 1,3-DNB levels, and they are not extensively used, probably because of the relatively rapid rate of conversion of 1,3-DNB to its degradation products (Cossum and Rickert 1985). Preliminary data suggested that the formation of adducts of 1,3,5-TNB with tissue DNA and/or with blood proteins may be useful as markers for exposure to 1,3,5-TNB (Reddy et al. 1991). Further research with both 1,3-DNB and 1,3,5-TNB in the area of adduct formation could provide valuable additional information.

Effect. Cyanosis is also an early symptom of exposure to 1,3-DNB (Okubo and Shigeta 1982) and is a result of oxygen deprivation due to the presence of methemoglobin in the blood. However, it is not specific and may occur after exposure to other nitrobenzene compounds or other non-related chemicals.

Nitroaniline is one of the 1,3-DNB metabolites and animal studies designed to evaluate its level in the urine would give information about the usefulness of nitroaniline as a biomarker of 1,3-DNB exposure.

No studies in humans or animals were located dealing with biomarkers of effects after exposure to 1,3,5-TNB. Therefore, research efforts aimed to identifying such a biomarker would be useful.

Absorption, Distribution, Metabolism, and Excretion. The few studies available in humans indicate that 1,3-DNB can be readily and rapidly absorbed via the dermal and inhalation routes (Ishihara et al. 1976; Okubo and Shigeta 1982). Quantitative information on the rates of absorption of 1,3-DNB in humans and animals following all routes of exposure are limited (see Section 2.3.1). Obtaining additional quantitative data in animals via all exposure routes and using different vehicles would be helpful for estimating absorption in humans.

No studies were located regarding distribution following inhalation exposure to 1,3-DNB and 1,3,5-TNB in humans and animals. Data on distribution via the dermal and oral routes for humans were not located. There is limited information describing distribution following acute oral exposure to 1,3-DNB in animals. Studies indicate that 1,3-DNB is distributed in the blood, liver, kidneys, and fat tissue (Philbert et al. 1987b; Parke 1961). Additional studies regarding acute oral and dermal exposures would help elucidate the distribution pattern of 1,3-DNB. The oral route is the most likely route of exposure near ammunition plants, while workers in the plants would most likely be exposed by inhalation of dusts and through dermal contact. Only one study was located that provided information regarding distribution of 1,3,5-TNB in animals after acute oral exposure (Reddy et al. 1991). Further studies via all three routes of exposure would be valuable to determine the distribution pattern for 1,3,5-TNB.

No information was located regarding metabolism of 1,3-DNB in humans and animals following inhalation exposure. Information on metabolism following oral exposure is available (see Section 2.3.3.2); however, more information would be useful because the potential exists for exposure to occur in humans via this route.

No studies were located on absorption or metabolism following oral inhalation or dermal exposure to 1,3,5-TNB in humans or animals. Therefore, animal studies are needed to elucidate the absorption process and metabolic path following exposure to 1,3,5-TNB.

No information was located regarding excretion in humans or animals following inhalation or dermal exposure to 1,3-DNB or following exposure to 1,3,5-TNB by any route. Studies in these areas would provide useful information since exposure to these compounds can occur by all three routes. Several studies in animals describe excretion following oral exposure to 1,3-DNB (see Section 2.3.4.2). These studies show quantitatively that the metabolites (2,4-diaminophenol, 1,3-phenylenediamine, 1,3-nitroaniline, and 2-amino-4-nitrophenol) (Parke 1961) are excreted primarily in the urine. Differences in excretion of metabolites have been observed in several species following oral exposure. It is not clear which species is best for determining excretion patterns in humans. Therefore, studies to determine which is the best animal model to be used for extrapolation of data on distribution and excretion patterns of 1,3-DNB to humans would be useful.

Comparative Toxicokinetics. Several studies using different animal species (rat, hamster, rabbit) indicate that the kinetics of 1,3-DNB differ across species (McEuen and Miller 1991; Parke 1961; Watanabe et al. 1976). The differences are primarily quantitative. On the basis of kinetic data alone, it is not possible to identify common target organs, but distribution data and toxicity data after oral exposure together suggest similar target/systems organs (hematological and reproductive systems, liver and kidneys. Interspecies differences between rats and hamsters include metabolism and excretion (McEuen and Miller 1991). The interspecies differences, and the lack of data across different routes, point to the possible problem in comparing the toxicokinetics of 1,3-DNB in animals with that in humans. Additional studies using several species exposed to 1,3-DNB by the oral route would help in determining differences and similarities between humans and animals. Also needed are animal studies on 1,3-DNB toxicokinetics after inhalation and dermal exposures. Since no information was located on toxicokinetics of 1,3,5-TNB after inhalation, oral, or dermal exposures, animal studies addressing these issues would be useful in addressing the data needs.

Methods for Reducing Toxic Effects. The most notable clinical sign of exposure to 1,3-DNB (and nitroaromatic compounds in general) is increased formation of methemoglobin that can lead to cyanosis (see Section 2.4). This may occur in humans after inhalation (Okubo and Shigeta 1982), oral (Kumar et al. 1990) or dermal (Ishihara et al. 1976) exposure. Methylene blue is considered the antidote of choice for methemoglobinemia (Donovan 1990; Ellenhorn and Barceloux 1988) and was successfully applied in a case of oral poisoning (Kumar et al. 1990). However, methylene blue is ineffective in populations with certain enzymes deficiencies, or may cause unwanted side effects; therefore, studies aimed at developing alternative antidotes for the treatment of methemoglobinemia would be useful.

2.9.3 Ongoing Studies

Ongoing studies regarding the health effects of 1,3-DNB and 1,3,5-TNB were reported in the Federal Research in Progress File (FEDRIP 1994) database. Table 2-4 summarizes the ongoing studies that address the health effects of 1,3-DNB and 1,3,5-TNB.' The table also includes research communicated in recent abstracts.

TABLE 2-4. Ongoing Studies on 1,3-DNB and 1,3,5-TNB

Investigator	Affiliation	Research description	Sponsor
M.A. Philbert et al.	Rutgers University, New Brunswick, New Jersey	role of antioxidant vitamins and antioxidant enzymes in the etiology of nitrocompound-induced encephalopathies	NIH
M.G. Miller[a]	*University of California,* Davis, California	*Mechanisms of 1,3-DNB testicular toxicity*	*NIEHS*
M.G. Miller[a]	University of California, Davis, California	Male reproductive toxicity of environmental chemicals	USDA
G. Reddy et al.	U.S. Army Biomedical R&D Laboratory, Frederick Maryland	Effect of 1,3,5-TNB on drug metabolizing enzymes in rats	No data
G. Reddy et al.	U.S. Army Biomedical R&D Laboratory, Frederick Maryland	Mechanism of interaction of dinitrobenzenes with hemoglobin	No data
D.E. Ray et al.	University of Leicester, Leicester, United Kingdom	Functional modulation of auditory pathway damage induced by 1,3-DNB	No data
E.R. Kinkead et al.	Mantech Environmental Technology and Wright-Patterson AFB, Dayton, Ohio	Effects of 1,3,5-TNB on reproduction in rats	No data

[a]FEDRIP 1994