

## **2. HEALTH EFFECTS**

### **2.1 INTRODUCTION**

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

### **2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE**

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

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or 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 is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant































TABLE 2-1 Levels of Significant Exposure to 2,4,6-Trinitrotoluene - Oral (continued)

| Key<br>to<br>figure | Species/<br>(Strain)      | Exposure/<br>Duration/<br>Frequency<br>(Specific Route) | System | NOAEL<br>(mg/kg/day) | LOAEL  |   | Reference              |
|---------------------|---------------------------|---|--------|----------------------|--|---|------------------------|
|                     |                           |   |        |                      | Less Serious<br>(mg/kg/day)  | Serious<br>(mg/kg/day)  |                        |
| 18                  | Dog<br>Beagle             | 13 wk<br>1x/d<br>(F)                                    |        | 2.0                  | 20 (increased spleen weight<br>and globulin levels,<br>splenic hemosiderosis)              |   | Dilley et al. 1982b    |
| 19                  | Dog<br>Beagle             | 6 mo<br>1x/d<br>(C)                                     |        | 2                    | 8M (enlarged spleen)   |   | Levine et al.<br>1990b |
| <b>Neurological</b> |                           |   |        |                      |  |   |                        |
| 20                  | Rat<br>Fisher 344         | 13 wk<br>(F)  |        | 25                   | 125 (slight lethargy and<br>ataxia)  | 300 (brain lesions: focal<br>vacuolation and/or malacia<br>of cerebellar folia)                   | Levine et al. 1984     |
| 21                  | Dog<br>Beagle             | 13 wk<br>1x/d<br>(F)                                    |        | 2.0                  | 20M (inactivity)   |   | Dilley et al. 1982b    |
| 22                  | Dog<br>Beagle             | 6 mo<br>1x/d<br>(C)                                     |        | 8                    | 32 (slight ataxia)   |   | Levine et al.<br>1990b |
| <b>Reproductive</b> |                           |   |        |                      |  |   |                        |
| 23                  | Rat<br>Sprague-<br>Dawley | 13 wk<br>1x/d<br>(F)                                    |        | 34.7                 |  | 160 M (testicular atrophy,<br>hyperplasia of interstitial<br>cells, atrophy of the<br>epididymis) | Dilley et al. 1982b    |
| 24                  | Rat<br>Wistar             | 6 wk<br>6d/wk<br>(G)                                    |        |                      |  | 200 M (significantly decreased<br>testes weight)  | Jiang et al. 1991      |
| 25                  | Rat<br>Wistar             | 6 wk<br>6d/wk<br>(G)                                    |        |                      | 200M (decreased serum<br>ceruloplasmin and<br>decreased serum zinc<br>levels at six weeks) |   | Jiang et al. 1991      |



TABLE 2-1 Levels of Significant Exposure to 2,4,6-Trinitrotoluene - Oral (continued)

| Key <sup>a</sup><br>to<br>figure | Species/<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Specific Route) | System  | NOAEL<br>(mg/kg/day) | LOAEL                       |  | Reference           |
|----------------------------------|----------------------|---|---------|----------------------|-----------------------------|--|---------------------|
|                                  |                      |   |         |                      | Less Serious<br>(mg/kg/day) | Serious<br>(mg/kg/day)   |                     |
| 26                               | Rat<br>Fisher 344    | 13 wk<br>(F)  |         | 25                   |                             | 125 M (atrophic seminiferous tubules, degenerated germinal epithelium)       | Levine et al. 1984  |
| 27                               | Rat<br>Fisher 344    | 13 wk<br>1x/d<br>(F)                                    |         | 5                    |                             | 125 M (degenerated germinal epithelium)                                      | Levine et al. 1990a |
| <b>CHRONIC EXPOSURE</b>          |                      |   |         |                      |                             |  |                     |
| <b>Systemic</b>                  |                      |   |         |                      |                             |  |                     |
| 28                               | Rat<br>Fischer 344   | 24 mo.<br>(F)   | Hemato  | 10 F                 | 50 F                        | (bone marrow fibrosis)   | Army 1984a          |
|                                  |                      |   | Hepatic | 2                    | 10                          | (increased cholesterol levels, hepatomegaly, hepatocellular hyperplasia)     |                     |
|                                  |                      |   | Renal   | 2                    | 10                          | (increase in pigment in epithelial cells of proximal convoluted tubules)     |                     |
|                                  |                      |   | Bd Wt   | 2                    | 10                          | (14% decrease in body weight gain)   |                     |
| 29                               | Mouse<br>B6C3F1      | 24 mo.<br>(F)   | Hemato  | 10                   | 70                          | (mild anemia)  | Army 1984b          |
|                                  |                      |   | Hepatic | 10                   | 70                          | (increased liver weight, hypotriglyceridemia, reduced serum globulin levels) |                     |
|                                  |                      |   | Bd Wt   | 1.5                  | 10                          | (10-15% decrease in body weight gain)  |                     |

TABLE 2-1 Levels of Significant Exposure to 2,4,6-Trinitrotoluene - Oral (continued)

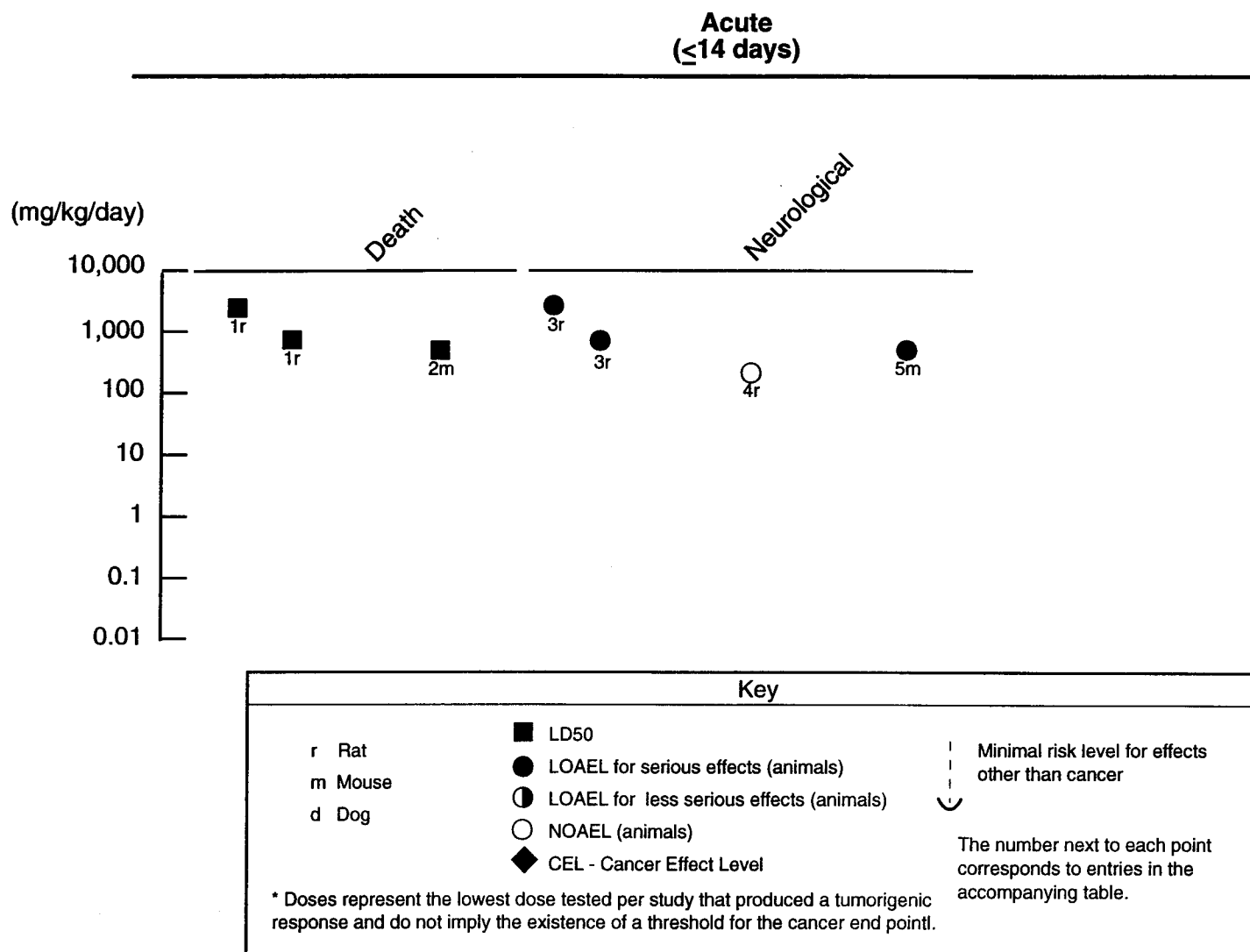
| Key <sup>a</sup><br>to<br>figure | Species/<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Specific Route) | System | NOAEL<br>(mg/kg/day) | LOAEL                       |  | Reference  |
|----------------------------------|----------------------|---|--------|----------------------|-----------------------------|--|------------|
|                                  |                      |   |        |                      | Less Serious<br>(mg/kg/day) | Serious<br>(mg/kg/day)   |            |
| <b>Immuno/Lymphoret</b>          |                      |   |        |                      |                             |  |            |
| 30                               | Rat<br>Fisher 344    | 24 mo.<br>(F)   |        | 2                    | 10                          | (sinusoidal congestion,<br>extramedullary<br>hematopoiesis,<br>hemosiderin-like pigment) | Army 1984a |
| 31                               | Mouse<br>B6C3F1      | 24 mo.<br>(F)   |        | 10 F<br>70 M         | 70 F                        | (enlargement of the<br>spleen and lymph nodes)   | Army 1984b |
| <b>Cancer</b>                    |                      |   |        |                      |                             |  |            |
| 32                               | Rat<br>Fisher 344    | 24 mo<br>(F)  |        |                      |                             | 50 F (CEL- 22% females with<br>carcinoma and 9% with<br>papilloma of urinary bladder)    | Army 1984a |
| 33                               | Mouse<br>B6C3F1      | 24 mo<br>(F)  |        |                      |                             | 1.5 F (CEL-28% females with<br>leukemia and/or lymphoma<br>of spleen)                    | Army 1984b |

<sup>a</sup>The number corresponds to entries in Figure 2-1

<sup>b</sup>Used to derive intermediate oral Minimal Risk Level (MRL); dose divided by an uncertainty factor of 1000 (10 for extrapolation for animals to humans, 10 for human variability, and 10 for use of a LOAEL) resulting in an MRL of 0.0005 mg/kg/day.

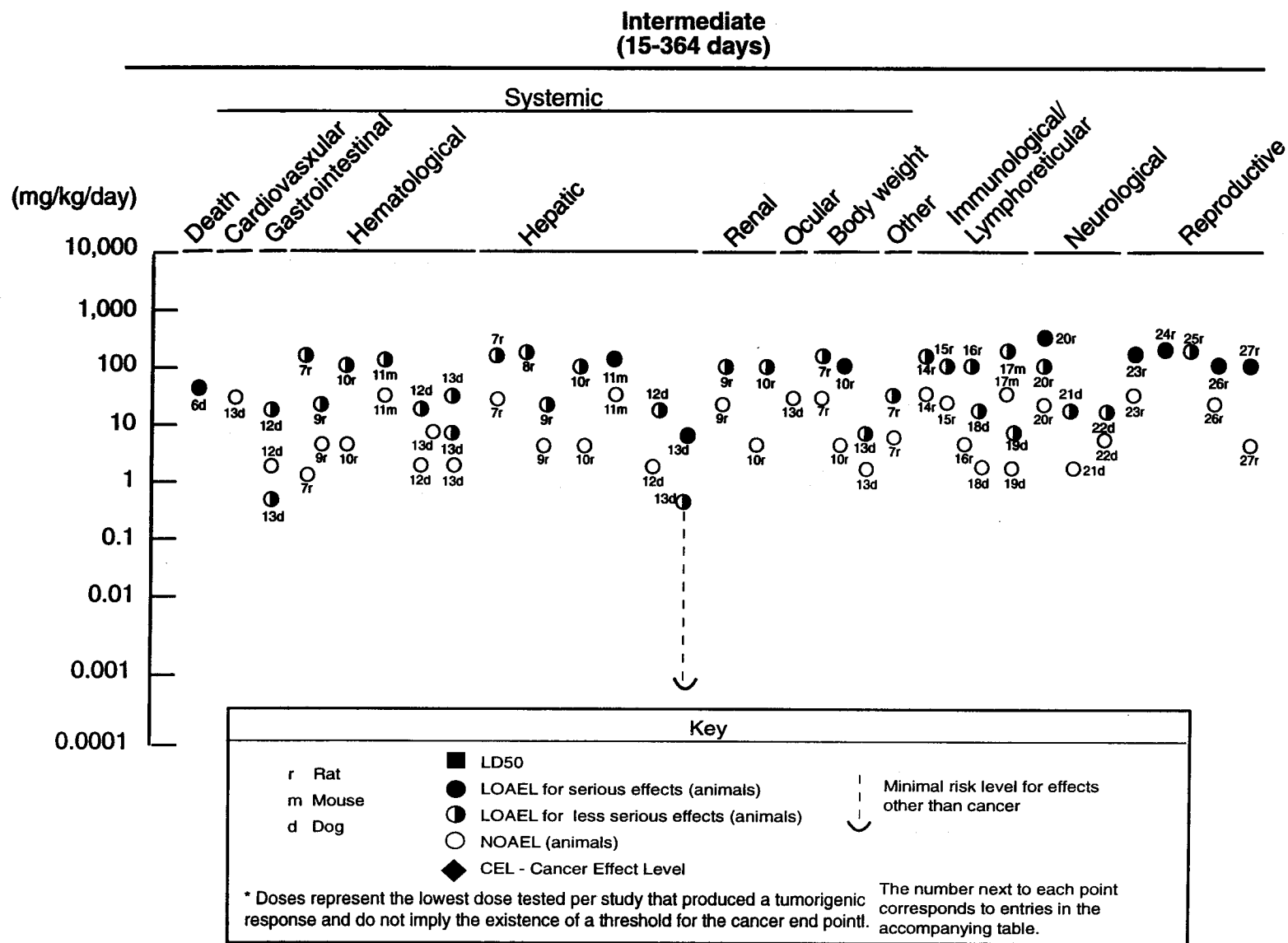
(C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); (F) = feed; F = females; (G) = gavage; Gastro = gastrointestinal; (GO) = gavage in oil; Hb = hemoglobin; Hct = hematocrit; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = males; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; RBC = red blood cell; SGPT = serum glutamic pyruvate transaminase; wk = week(s); x = time(s); < = decrease; > = increase

**Figure 2-1. Levels of Significant Exposure to 2,4,6-Trinitotoluene – Oral**



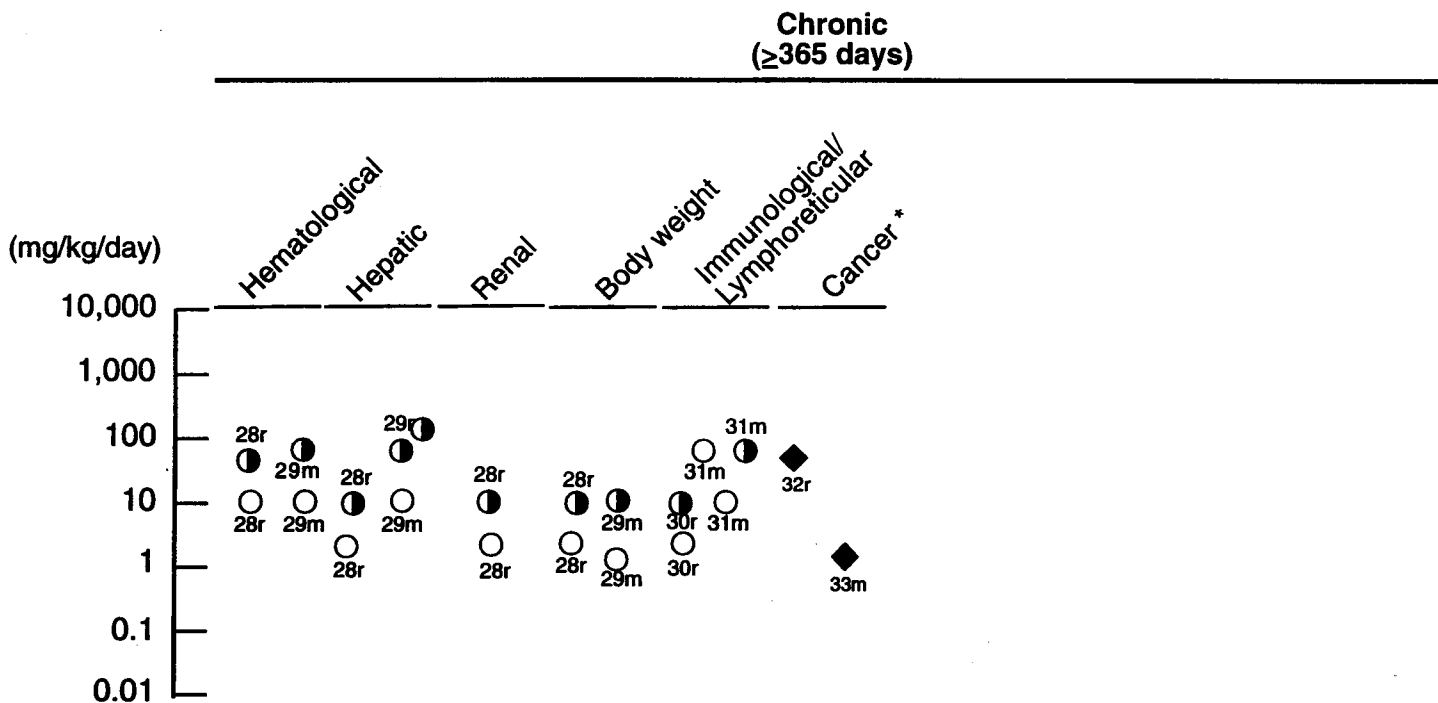
**Figure 2-1. Levels of Significant Exposure to 2,4,6-Trinitotoluene – Oral (continued)**

2,4,6-TRINITOTOLUENE



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Figure 2-1. Levels of Significant Exposure to 2,4,6-Trinitrotoluene – Oral (continued)



**Key**

|         |  |   |
|---------|--|---|
| r Rat   | ■ LD50                                     | Minimal risk level for effects other than cancer                                |
| m Mouse | ● LOAEL for serious effects (animals)      |   |
| d Dog   | ◐ LOAEL for less serious effects (animals) | The number next to each point corresponds to entries in the accompanying table. |
|         | ○ NOAEL (animals)                          |   |
|         | ◆ CEL - Cancer Effect Level                |   |

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

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

Very limited information is available regarding cardiovascular effects in animals after oral exposure to 2,4,6-trinitrotoluene. Intermediate exposure of beagle dogs to a 2,4,6-trinitrotoluene dose as high as 32 mg/kg/day for 26 weeks did not cause any changes in electrocardiogram results or heart rates (Levine et al. 1990b).

**Gastrointestinal Effects.** No studies were located regarding gastrointestinal effects in humans after oral exposure to 2,4,6-trinitrotoluene.

Adverse gastrointestinal effects were reported in dogs after intermediate oral exposure to 2,4,6-trinitrotoluene. Dogs receiving 20 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks had mucoid stools and diarrhea (Dilley et al. 1982b). In another longer intermediate exposure study, histopathology revealed inflammation of a part of the small intestine in beagle dogs fed 0.5, 2, 8, or 32 mg/kg/day 2,4,6-trinitrotoluene for 6 months. Although not dose related, the observed enteritis was more frequent in dogs treated with the highest dose of 2,4,6-trinitrotoluene. None of the control animals had enteritis (Levine et al. 1990b).

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

Anemia is one of the frequent signs of 2,4,6-trinitrotoluene toxicity. Adverse effects on standard hematologic parameters were observed in rats (Dilley et al. 1982b; Jiang et al. 1991; Levine et al. 1984, 1990a), mice (Dilley et al. 1982b), and dogs (Dilley et al. 1982b; Levine et al. 1990b) after intermediate oral exposures to 2,4,6-trinitrotoluene. Compensatory responses occurring as a result of anemia (including reticulocytosis, macrocytosis, and increased levels of nucleated erythrocytes) were observed in Fischer-344 rats fed 125 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Levine et al. 1990a). Dose-related anemia was also observed in Fischer-344 rats fed 10 or 50 mg/kg/day

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2,4,6-trinitrotoluene for 24 months (Army 1984a). In this chronic exposure study, both male and female rats had reduced hematocrit, hemoglobin, and red blood cells. These hematological effects were observed throughout the entire duration of the study in male rats, but only for the first year in female rats. Methemoglobin was observed in male rats at doses of 10 and 50 mg/kg/day. Howell-Jolley and Heinz Bodies occurred at 50 mg/kg/day in male rats. Therefore, male rats seemed somewhat more sensitive than female rats. Reticulocytosis, but not macrocytosis, was present as a compensatory response to the anemic state in all animals. Histopathology revealed splenic lesions consisting of sinusoidal congestion, extramedullary hematopoiesis, and increased amounts of hemosiderin-like pigment (Army 1984a). The findings are consistent with the hypothesis that 2,4,6-trinitrotoluene induces anemia by causing hemolysis through oxidative damage which is mediated by 2,4,6-trinitrotoluene and/or its metabolites. This conclusion is further supported by the presence of methemoglobinemia, produced by the oxidation of the heme iron, observed when 2,4,6-trinitrotoluene was fed to rats at 300 mg/kg/day for 13 weeks (Levine et al. 1984), to rats at 10 or 50 mg/kg/day for 24 months (Army 1984a), and to dogs at 32 mg/kg/day for 6 months (Levine et al. 1990b). Mild anemia was also noted in B6C3F<sub>1</sub> mice fed 70 mg/kg/day 2,4,6-trinitrotoluene for 24 months (Army 1984b). Anemia, as indicated by dose-dependent decreases in hematocrit and hemoglobin levels and decreased erythrocyte counts, was observed in dogs administered 2,4,6-trinitrotoluene (via capsules) for 6 months (Levine et al. 1990b). The anemia was compensated by reticulocytosis, macrocytosis, and an increased number of nucleated erythrocytes. There was an elevated level of methemoglobin in all dogs treated with 32 mg/kg/day of 2,4,6-trinitrotoluene. No effects on methemoglobin levels, or blood cells and Heinz body counts were found in monkeys following gavage administration of 2,4,6-trinitrotoluene at dose levels as high as 1.0 mg/kg/day (Martin and Hart 1974).

Bone marrow fibrosis was present in a significant number of female rats fed 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a).

Dogs treated daily with 8 mg/kg/day of 2,4,6-trinitrotoluene for 6 months had approximately a 68% and 22% increase in platelet levels over the control animals for males and females respectively (Levine et al. 1990b). A similar increase was noted in Fischer-344 rats treated with 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a). This increase occurred during the 2nd year of treatment and was not present at the end of the study period, week 104 (Army 1984a). Although the increase in

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the number of platelets appeared to be related to 2,4,6-trinitrotoluene treatment, its significance is not clear.

**Hepatic Effects.** No studies were located regarding hepatic effects in humans after oral exposure to 2,4,6-trinitrotoluene. However, toxic jaundice was often fatal in workers exposed to 2,4,6-trinitrotoluene in ammunition plants during World War I (Army 1978a). Between 1916 and 1941, 475 cases of toxic jaundice were recorded in a British ammunition plant; 125 of these were fatal (Army 1978a). During World War II, only eight fatal cases of toxic hepatitis were recorded in the United States because industrial hygiene techniques had improved since the first world war (Army 1978a).

Limited information is available for adverse hepatic effects in animals after acute oral exposure to 2,4,6-trinitrotoluene. The most common adaptive change observed in mice, rats, and dogs during intermediate exposure was an increase in liver weight and/or size. A significant increase in liver weight was noted in rats receiving 200 mg/kg/day of 2,4,6-trinitrotoluene for 6 weeks (Jiang et al. 1991) or 125 mg/kg/day for 13 weeks (Levine et al. 1984). A similar observation was made in male mice treated with 193 mg/kg/day for 13 weeks (Dilley et al. 1982b). Dogs treated with 20 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks also had increased liver weight (Dilley et al. 1982b). Another adaptive response to 2,4,6-trinitrotoluene-induced hepatotoxicity was a reduction in serum glutamicpyruvic transaminase (SGPT) in rats treated with 160 mg/kg/day and dogs treated with 20 mg/kg/day for 13 weeks (Dilley et al. 1982b). No change in serum glutamic-oxaloacetic transaminase (SGOT) was noted in those same animals (Dilley et al. 1982b). Dose-related changes such as hepatocytomegaly and cloudy swelling were present in dogs after exposure to doses of 0.5 mg/kg/day or greater of 2,4,6-trinitrotoluene for 6 months (Levine et al. 1990b). In addition, a reduction in SGPT activity was noted in dogs administered 8 or 32 mg/kg/day. Necrotic lesions in the liver were found in mice treated with 193 mg/kg/day for 13 weeks (Dilley et al. 1982b), while hepatic cirrhosis was seen in dogs treated with 8 mg/kg/day of 2,4,6-trinitrotoluene for 6 months (Levine et al. 1990b). Monkeys administered 1 mg/kg/day 2,4,6-trinitrotoluene (by gavage) for 90 days displayed ironpositive material in the liver (Martin and Hart 1974). However, results of the bromosulphthalein (BSP) dye test revealed no effects on liver function. This study was limited because only three monkeys per sex per were used, precluding statistical analysis of the data. In addition, there was a



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high frequency of emesis in treated animals. These results indicate that there is a species difference regarding 2,4,6-trinitrotoluene hepatotoxicity. Dogs seem to respond to lower concentrations of 2,4,6-trinitrotoluene than do mice or rats.

The hepatotoxicity of 2,4,6-trinitrotoluene was reflected in elevated levels of cholesterol present in the serum after intermediate and chronic exposures. Increased serum cholesterol levels were present in rats treated with 25 (Levine et al, 1984), 125 (Levine et al. 1990a), and 160 mg/kg/day (Dilley et al. 1982b) of 2,4,6-trinitrotoluene for 13 weeks. A similar finding was seen in dogs fed 20 mg/kg/day of 2,4,6-trinitrotoluene for the same time period (Dilley et al. 1982b). Increased cholesterol levels were also found in male and female rats treated with 50 mg/kg/day for 24 months (Army 1984a).

Evidence for adverse hepatic effects has also been revealed in studies of chronic exposure. Dose-related hepatomegaly resulting from hepatocellular hyperplasia was observed in Fischer-344 rats given 10 or 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a).

Serum lipids were affected by chronic administration of 2,4,6-trinitrotoluene to rats (Army 1984a). The levels of serum triglycerides were affected differently in male and female rats exposed to 2, 10, or 50 mg/kg/day of 2,4,6-trinitrotoluene (Army 1984a). Treatment-related hypotriglyceridemia was observed in females at 2 mg/kg/day; this change was statistically significant at 10 and 50 mg/kg/day during week 104 of treatment. In male rats treated with 50 mg/kg/day, a significant reduction in triglyceride levels was seen after 104 weeks of treatment (Army 1984a). The results indicate that female rats were more susceptible to 2,4,6-trinitrotoluene-induced reduction in serum triglyceride levels than male rats that showed reduced triglycerides only at the highest treatment doses. Hypoglyceridemia was also observed in mice treated with 70 mg/kg/day 2,4,6-trinitrotoluene for 24 months (Army 1984b), and a decrease in glucose levels was observed in dogs treated with 8 or 32 mg/kg/day 2,4,6-trinitrotoluene (Levine et al. 1990b).

Jaundice (icterus) was observed in beagle dogs treated with 32 mg/kg/day for 26 weeks (Levine et al. 1990b). The presence of jaundice was evidenced by elevated bilirubin levels in both serum and urine and increased urobilinogen values (Levine et al. 1990b). Histopathological analysis of these animals

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revealed hemosiderosis in Kupffer's cells in all dogs receiving 8 and 32 mg/kg/day and in one female receiving 2 mg/kg/day for 26 weeks (Levine et al. 1990b).

One of the proposed mechanisms of 2,4,6-trinitrotoluene-induced toxicity is an increase in free radical levels which occurs after exposure. Superoxide radicals and hydrogen peroxide were measured in mitochondria and microsomes from livers of monkeys treated with 0, 60, or 120 mg/kg/day of 2,4,6-trinitrotoluene for 12 weeks (Kong et al. 1989). The amount of superoxide radicals was indirectly measured by the formation of adrenochrome from adrenalin, and hydrogen peroxide production was evaluated by the conversion of methanol to formaldehyde. There was a dose-dependent increase in superoxide radicals and hydrogen peroxide production in liver mitochondria and microsomes (Kong et al. 1989). These findings were confirmed when mitochondria and microsomes obtained from various organs were treated in vitro with 0, 0.04, 0.2, or 1 mmol of 2,4,6-trinitrotoluene and then tested for adrenochrome and formaldehyde production. Different amounts of hydrogen peroxide were produced in the mitochondria of the various organs. The highest amount of hydrogen peroxide was present in the liver followed by brain, testicle, kidney cortex, and kidney medulla (Kong et al. 1989).

**Renal Effects.** Discoloration of the urine is among the first indications of 2,4,6-trinitrotoluene intoxication in humans. The color of urine ranges from abnormal amber to a deep red, and in most cases the results are positive for Webster's test (a qualitative urine test for 2,4,6-trinitrotoluene based on the formulation of purple color in acidified urine samples following extraction with ether and treatment with potassium hydroxide) (Army 1978a).

Accumulation of yellowish-brown pigment in the renal cortex of rats treated with 125 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks was seen during histopathological analysis. The authors suggest that this pigment may have represented known photolytic decomposition products of 2,4,6-trinitrotoluene (Levine et al. 1984, 1990b). The same observation was made in female rats treated with 10 or 50 mg/kg/day for 24 months (Army 1984a). A dose-related increase in granular pigment within the cytoplasm of epithelial cells of proximal convoluted tubules was observed at the end of the treatment period in male rats receiving either 10 or 50 mg/kg/day for 24 months (Army 1984a). Increased filtration rate was also present in these chronically exposed animals (Army 1984a).

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### 2.2.2.3 Immunological and Lymphoreticular Effects

Limited information was located regarding immunological effects in humans after oral exposure to 2,4,6-trinitrotoluene. However, an early reaction to 2,4,6-trinitrotoluene intoxication was an increase in the number of mononuclear leukocytes found in the blood counts of 105 exposed individuals (Army 1978a). This increase seems to precede any other symptom and remains positive for 2-3 months; therefore, it would be helpful in the differential diagnosis of 2,4,6-trinitrotoluene poisoning, especially when Webster's test is negative (Army 1978a). The route of exposure and precise dose were not defined in this report.

An increase in lymphocyte numbers was also seen in nine fatal cases of 2,4,6-trinitrotoluene toxicity in humans. The normal range for lymphocytes is 20-40% of the total white blood cell count. In the affected patients, the average was 78% of the total white blood count (range, 61-92%) (Army 1978a). Patients who recovered after 2,4,6-trinitrotoluene intoxication had lymphocyte counts which were approximately 46% of the total white cell count (Army 1978a).

No adverse lymphoreticular effects as assessed by changes in lymphocyte levels and histological changes in the spleen were seen in dogs treated with 2.0 mg/kg/day, rats treated with 34.7 mg/kg/day, or mice treated with 35.7 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Dilley et al. 1982b).

Increased spleen weight was seen in mice, rats, and dogs after an exposure of 13 weeks to 2,4,6-trinitrotoluene. The splenomegaly is possibly related to an increased clearance of hemolysed cells (Dilley et al. 1982b; Levine et al. 1984, 1990a). Male and female dogs exposed to 20 mg/kg/day, mice exposed to 193 mg/kg/day (Dilley et al. 1982b), and rats exposed to 125 mg/kg/day (Levine et al. 1984, 1990a) and 160 mg/kg/day (Dilley et al. 1982b) all had increased spleen weights after 13 weeks of exposure. Spleen enlargement related to 2,4,6-trinitrotoluene administration was also noted in dogs treated with 8 or 32 mg/kg/day for 26 weeks. The ratios of myeloid to erythroid cells were also significantly lower in these dogs, and they had varying degrees of splenic congestion (Levine et al. 1990b). Splenic hemosiderosis related to 2,4,6-trinitrotoluene treatment was also present in rats receiving 160 or 300 mg/kg/day (Dilley et al. 1982b; Levine et al. 1984), mice receiving 193 mg/kg/day (Dilley et al. 1982b), and dogs receiving 20 mg/kg/day (Dilley

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et al. 1982b) of 2,4,6-trinitrotoluene for 13 weeks. Lymphopenia was present in mice treated with 193 mg/kg/day for 13 weeks (Dilley et al. 1982b). Increased globulin levels and leukocytosis were noted in the dogs treated with 20 mg/kg/day after a 4 week recovery period. Leukocytosis was observed in rats fed 160 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Dilley et al. 1982b). Sinusoidal congestion, extramedullary hematopoiesis, and hemosiderin-like pigment in the spleen were observed in male and female rats fed 10 or 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a). Enlargement of the spleen and lymph nodes was noted in female mice treated with 70 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984b).

### 2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to 2,4,6-trinitrotoluene.

In acute exposure studies, rats fed 182 mg/kg/day of 2,4,6-trinitrotoluene for 4 days showed no signs of neurotoxicity as measured by changes in zoxazolamine paralysis time and hexobarbital sleeping time (Short and Lee 1980). However, in a single-dose oral LD<sub>50</sub> study in rodents, rats and mice showed signs of inactivity, were tremulous, developed convulsions, and died (Dilley et al. 1982b).

Similar observations were made in intermediate-duration studies. No signs of neurotoxicity were seen after 13 weeks of 2,4,6-trinitrotoluene treatment in dogs receiving 0.2 mg/kg/day (Dilley et al. 1982b), monkeys receiving 1 mg/kg/day (Martin and Hart 1974), or rats receiving 1.42 mg/kg/day (Dilley et al. 1982b). Dogs treated with 32 mg/kg/day for 6 months were ataxic (Levine et al. 1990b), while inactivity was observed in dogs after treatment with 20 mg/kg/day for 13 weeks (Dilley et al. 1982b). Dose-related changes in behavior such as lethargy and/or ataxia were seen in rats treated with 34.7 or 125 mg/kg/day for 13 weeks (Dilley et al. 1982b; Levine et al. 1984). Brain lesions with focal vacuolation were seen in rats receiving 300 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Levine et al. 1984).

No significant signs of neurotoxicity were seen in Fischer-344 rats treated with up to 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a). The combined results of acute and intermediate

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exposure studies indicate species differences in 2,4,6-trinitrotoluene-induced neurotoxicity, with dogs being more sensitive than rats or mice.

### **2.2.2.5 Reproductive Effects**

No studies were located regarding reproductive effects in humans after oral exposure to 2,4,6-trinitrotoluene.

Significantly decreased testes weights and testes zinc and copper concentrations were observed in male rats exposed to 200 mg/kg/day 2,4,6-trinitrotoluene for 6 weeks (Jiang et al. 1991). In addition, the serum ceruloplasmin concentration was significantly lower in the male rats. Zinc metabolism seems to be affected more than copper metabolism by 2,4,6-trinitrotoluene treatment. Although there was a close correlation between testicular weight and testicular zinc concentration, the role of zinc in decreasing testicular weight is not clear (Jiang et al. 1991). However, zinc is known to be essential for maintenance of normal testicular function (Jiang et al. 1991). No adverse reproductive effects were noted in rats exposed to 1.42 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Dilley et al. 1982b). However, male rats treated with 125, 160, or 300 mg/kg/day for the same period of time had serious reproductive effects such as degenerated germinal epithelium, testicular atrophy, and atrophic seminiferous tubules (Dilley et al. 1982b; Levine et al. 1984, 1990a). Testicular atrophy was not reversible in rats allowed 4 weeks of recovery (Dilley et al. 1982b).

### **2.2.2.6 Developmental Effects**

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

### **2.2.2.7 Genotoxic Effects**

No studies were located regarding genotoxic effects in humans after oral exposure to 2,4,6-trinitrotoluene

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Results were negative from in vivo studies employing the oral route of exposure to ascertain whether 2,4,6-trinitrotoluene has the potential to induce clastogenic effects in somatic cells or increase the frequency of unscheduled deoxyribonucleic acid (DNA) synthesis (UDS) in liver cells. However, the findings from somatic cell cytogenetic assays with rats were compromised and, therefore, do not fully support a negative conclusion.

In the bone marrow test, groups of five male Sprague-Dawley rats were administered dietary concentrations of 0.002% or 0.25% 2,4,6-trinitrotoluene for 28 days; two additional groups of five rats each were similarly treated and allowed a 28-day recovery period (Army 1978c). At the conclusion of the treatment or recovery period, animals were sacrificed; bone marrow cells were harvested and examined for abnormal chromosome morphology. No animals died prior to the scheduled sacrifice. The study authors attributed the reduced body weight observed in the high-dose group to the palatability of the test material rather than to a toxic effect. The slight depression in the mitotic indices for high-dose animals at the conclusion of treatment was not considered indicative of a cytotoxic effect on the target organ (bone marrow cells). Although no chromosome aberrations were scored in the exposure groups immediately after treatment or following the 28-day recovery period, the failure to demonstrate overt toxicity in the test animals or cytotoxic effects on the target organ renders the study insufficient to fully support the conclusion that 2,4,6-trinitrotoluene was negative in this in vivo cytogenetics assay.

2,4,6-Trinitrotoluene was administered by oral gavage at doses of 100, 200, 500, or 1,000 mg/kg to male Alderley Park rats and at doses of 200, 500, and 1,000 mg/kg to male Fischer-344 rats (up to three rats/group/strain) to investigate UDS in liver cells (Ashby et al. 1985). There was no evidence of a cytotoxic or genotoxic effect on the hepatocytes of either strain 12 hours after 2,4,6-trinitrotoluene exposure.

Other genotoxicity studies are discussed in Section 2.4.

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**2.2.2.8 Cancer**

No studies were located regarding cancer in humans after oral exposure to 2,4,6-trinitrotoluene. However, a preliminary study of a German population living near the sites of two World War II munitions plants indicates an association between increased rates of some types of leukemia and living in a town near 2,4,6-trinitrotoluene waste from these plants (Kolb et al. 1993). The study shows increased relative risk of acute myelogenous leukemia (AML) for adult males and females living near the former explosives plants when compared with adults in a neighboring county. The relative risk is particularly high for individuals over 65 years of age. However, study case numbers are very small. The relative risk for chronic myelogenous leukemia (CML) is also increased for males but there was only one case among females so comparisons could not be made. The relative proximity of the cases of leukemia to the sites of 2,4,6-trinitrotoluene manufacture or disposal is not known, nor are any 2,4,6-trinitrotoluene concentrations in the environment reported. No investigation of confounding variables (i.e., benzene exposure or occupational exposure to carcinogens) has been done. The study concludes that a causal relationship is suggested, but further investigation of the living and working conditions of the populations is required (Kolb et al. 1993).

In a chronic study, groups of 150 (75 males and 75 female) Fischer-344 rats were exposed to 0, 0.4, 2.0, 10.0, and 50.0 mg/kg/day of 2,4,6-trinitrotoluene in their food for 24 months (Army 1984a). A statistically significant number of female rats (12/55 or 21.8%) exposed to 50-mg/kg/day doses developed urinary bladder carcinomas. Urinary bladder papillomas were present in 1/55 and 5/55 female rats fed 10 and 50 mg/kg/day, respectively. No metastases were observed in any of the animals. Histopathologic lesions included increased incidence of hyperplastic, preneoplastic, and neoplastic changes of the mucosal epithelium of the urinary bladder. The cancer incidence observed in this chronic exposure study is further supported by renal and urinary bladder hyperplasia observed in treated animals. None of the control animals developed lesions of the urinary bladder. In a similar study conducted in groups of 150 B6C3F<sub>1</sub> mice (75 males and 75 females), a statistically significant incidence ( $p < 0.01$ ) of leukemia and/or malignant lymphoma of the spleen was present in female mice receiving 70 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984b). Leukemia and/or malignant lymphoma of the spleen was noted in 28% and 32% of the female mice administered 1.5 and 10 mg/kg/day, respectively. The increase in the cancer incidence in the female mice receiving 1.5

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or 10 mg/kg/day of 2,4,6-trinitrotoluene was not statistically significant. Histopathology revealed that leukemia was of granulocytic or lymphocytic type, while lymphoma was histiocytic, lymphocytic, or of a mixed type. All the lesions were treatment related and systemic in nature. The neoplasias involved other organs and tissues such as adrenals, bone marrow, brain, gastrointestinal tract, eyes, kidneys, liver, lungs, and lymph nodes. The occurrence of combined leukemia/malignant lymphoma seemed to be dose related but was not statistically significant. Based on the information from these two chronic animal studies, EPA has classified 2,4,6-trinitrotoluene as a possible human carcinogen (Group C) (EPA 1989b).

### 2.2.3 Dermal Exposure

In many occupational studies, it is often difficult to make a definitive distinction between dermal and inhalation exposures, as was indicated in the section on inhalation exposure. Therefore, some of the findings described in the inhalation section will be repeated in this section.

#### 2.2.3.1 Death

Adverse effects of 2,4,6-trinitrotoluene on the liver and hematopoietic system have caused the greatest number of deaths among munitions workers. There were 475 deaths out of about 17,000 cases of 2,4,6-trinitrotoluene poisoning in the United States within 7.5 months during World War I (McConnell and Flinn 1946). In the same report, 22 cases of death due to occupational exposure to 2,4,6-trinitrotoluene during World War II were described (McConnell and Flinn 1946). In this series of fatal cases, 8 died from toxic hepatitis, 13 died from aplastic anemia, and 1 died probably from the combination of both these conditions (McConnell and Flinn 1946). The authors indicated that exposure occurred by dermal contact and inhalation.

No studies were located regarding death in animals after dermal exposure to 2,4,6-trinitrotoluene.



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**2.2.3.2 Systemic Effects**

No studies were located regarding respiratory, cardiovascular, gastrointestinal, or musculoskeletal effects in humans or animals after dermal exposure to 2,4,6-trinitrotoluene.

**Hematological Effects.** Acute hemolytic disease was described in three ammunition plant workers who filled shells with 2,4,6-trinitrotoluene (Djerassi and Vitany 1975). All three were deficient in glucose-6-phosphate dehydrogenase (G6PD), an enzyme that catalyzes the oxidation of glucose 6-phosphate to 6-phosphoglucono-lactone. All three cases developed acute severe hemolysis 2-3 days after being exposed to 2,4,6-trinitrotoluene and had very similar symptoms: paleness, weakness, and vertigo. They also had decreased hemoglobin levels, decreased hematocrit, and increased reticulocyte numbers (Djerassi and Vitany 1975). All three recovered and had no further complications when examined 5 and 10 years later. Limitations of this report are that the routes of exposure are not specified, although it seems that there was dermal and inhalation exposure, and that the level of exposure is not known. However, the authors note that the air levels of 2,4,6-trinitrotoluene were higher than the allowed daily exposure limit, which was  $1.5 \text{ mg/m}^3$  at that time.

No studies were located regarding hematological effects in animals after dermal exposure to 2,4,6-trinitrotoluene.

**Hepatic Effects.** A modified MacLagen test (thymol turbidity test) was used to give evidence of cirrhosis or hepatitis resulting from 2,4,6-trinitrotoluene exposure in ammunition workers (Goodwin 1972). In a retrospective study spanning 20 years, the data showed that 40 out of 4,641 workers had  $>5$  MacLagen units (2.9 MacLagen units is considered to be normal); the length of exposure and dose of 2,4,6-trinitrotoluene were not specified. However, the hepatotoxicity was reversible. All the workers with a MacLagen test result of  $>5$  units were transferred to other jobs, and their readings returned to normal within 3 weeks (Goodwin 1972).

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

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**Renal Effects.** No adverse renal effects were reported in humans after acute exposure to 2,4,6-trinitrotoluene as measured by Webster's reaction and the aminodinitrotoluene (ADNT) test (Hassman and Hassmanova 1976). In this study, the exposure route was not clearly specified, but it seems that dermal contact and inhalation were the major routes of exposure.

No studies were located regarding renal effects in animals after dermal exposure to 2,4,6-trinitrotoluene.

**Dermal Effects.** Allergic contact dermatitis with erythema (Goh 1988) and erythematous papillar rash with edema (Goh and Rajan 1983) were reported in two ammunition workers after intermediate exposures to 2,4,6-trinitrotoluene. In both cases, patch tests were used to determine the allergen. The results showed that 5% is the most suitable concentration for patch testing in order to avoid false positive or negative results (Goh and Rajan 1983). In both affected workers, dermal reactions developed on parts of the body that were exposed and in direct contact with 2,4,6-trinitrotoluene, such as hands and forearms. These reactions subsided and disappeared when the workers were transferred to different jobs and were no longer in contact with 2,4,6-trinitrotoluene.

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

**Ocular Effects.** The development of cataracts in humans is believed to be specific to 2,4,6-trinitrotoluene exposure (Hathaway 1985) and is often associated with chronic exposures. Equatorial lens opacities/cataracts were reported in 6 out of 12 Finnish workers (mean age,  $39.5 \pm 8.9$  years) exposed to 2,4,6-trinitrotoluene for an average of  $6.8 \pm 4.7$  years (Harkonen et al. 1983). The principal routes of exposure were dermal and inhalation, although this fact is not clearly indicated in the report. Therefore, it is not known whether cataracts were a systemic or a local effect. The opacities were detectable only on the periphery of the lens and appeared continuous or discontinuous. The opacities of the lens were bilateral and symmetrical and did not affect visual fields or visual acuity. The workroom 2,4,6-trinitrotoluene air concentration was about  $0.3 \text{ mg/m}^3$  with a range of  $0.14\text{-}0.58 \text{ mg/m}^3$  (Harkonen et al. 1983). There was no control population in the study. The formation of cataracts did

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not progress further after exposure to 2,4,6-trinitrotoluene was terminated; however, the cataracts were not reversible. The mechanism of 2,4,6-trinitrotoluene cataract formation is not understood, but the authors raised the possibility that oxidative damage may play a role since cellular defense mechanisms protective against oxidative damage (i.e., glucose-6-phosphate dehydrogenase and glutathione concentration) may be deficient after exposure to 2,4,6-trinitrotoluene (Harkonen et al. 1983).

No studies were located regarding ocular effects in animals after dermal exposure to 2,4,6-trinitrotoluene.

### **2.2.3.3 Immunological and Lymphoreticular Effects**

Two ammunition plant workers developed an allergic contact dermatitis with erythema (Goh 1988) and erythematous papillar rash with edema (Goh and Rajan 1983) after intermediate exposures to 2,4,6-trinitrotoluene. Patch tests were used to identify the allergen using 5% as the most suitable concentration (Goh and Rajan 1983). Dermal reactions were observed to occur on the exposed parts of the body such as the hands and forearms. Once removed from environments containing 2,4,6-trinitrotoluene, the workers sensitivity subsided and the dermatitis disappeared.

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

### **2.2.3.4 Neurological Effects**

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

### **2.2.3.5 Reproductive Effects**

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

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### 2.2.3.6 Developmental Effects

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

### 2.2.3.7 Genotoxic Effects

Two studies involving genotoxic effects in individuals occupationally exposed to 2,4,6-trinitrotoluene suggested that in addition to inhalation exposure, dermal exposure may have occurred (Ahlborg et al. 1985, 1988a). These studies are discussed in detail in Section 2.2.1.7. No other studies were located regarding genotoxic effects in humans or animals after dermal exposure to 2,4,6-trinitrotoluene.

Other genotoxicity studies are discussed in Section 2.4.

### 2.2.3.8 Cancer

In a case of chronic occupational exposure to 2,4,6-trinitrotoluene, a 61-year-old male died of hepatocellular carcinoma (Garfinkel et al. 1988). The routes of exposure were not specified in the study, and it is possible that the worker was exposed via the inhalation and/or dermal route.

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

## 2.3 TOXICOKINETICS

Occupational studies indicate humans readily absorb 2,4,6-trinitrotoluene dusts via inhalation or dermal contact, but quantitative studies have not been done. Toxicokinetic data from animal studies are limited in that the fate of the radiolabelled dose of 2,4,6-trinitrotoluene was followed, and it is not possible to differentiate between parent compound and metabolites. However, studies in dogs, rabbits, mice, and rats indicate that more than 60% of the administered dose of 2,4,6-trinitrotoluene is absorbed when ingested; dermal exposure these animals results in significantly lower (16-68%) absorbance than oral exposure.

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No studies of the distribution of 2,4,6-trinitrotoluene in humans were located. However, in animal studies the highest concentrations of 2,4,6-trinitrotoluene are found in the liver, skeletal muscle, blood, and fat.

Studies indicate 2,4,6-trinitrotoluene is metabolized to several identifiable intermediates in the urine of exposed workers, including the major reduction product aminodinitrotoluene (ADNT). In several studies of oral exposure of rats and other laboratory animals, 2,4,6-trinitrotoluene was rapidly metabolized to molecules too low in concentration to be identified by chromatographic analysis of urine. Trace amounts of 2,4,6-trinitrotoluene could be detected in the urine of exposed animals in isolated cases. After a single dermal exposure of 2,4,6-trinitrotoluene administered to dogs, rabbits, mice, and rats, more unchanged 2,4,6-trinitrotoluene was found in the urine than was found after oral exposure. This result indicates the route of exposure may influence the rate and extent of 2,4,6-trinitrotoluene metabolism.

No studies of the excretion of 2,4,6-trinitrotoluene in humans were located. However, studies indicate that 2,4,6-trinitrotoluene and its metabolites are primarily eliminated in the urine in laboratory animals. In most studies excretion is rapid.

The mechanisms by which 2,4,6-trinitrotoluene and its derivatives exert their toxic effects are largely unknown. A theory of the mechanism of toxicity by 2,4,6-trinitrotoluene is that the chemical and some of the metabolic intermediates of 2,4,6-trinitrotoluene generate reactive oxygen species that cause lipid peroxidation in the liver and injury of the lens resulting in cataracts.

### **2.3.1 Absorption**

#### **2.3.1.1 Inhalation Exposure**

Studies that directly measure the absorption of 2,4,6-trinitrotoluene in humans following inhalation exposure of known amounts of this chemical were not located. The amount of 2,4,6-trinitrotoluene in the urine as measured by Webster's reaction and the amount of 2-ADNT in the urine were compared in 88 factory workers working with 2,4,6-trinitrotoluene (Hassman and Hassmanova 1976). The

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concentration of 2,4,6-trinitrotoluene in the air varied from 0.045 to 0.93 mg/m<sup>3</sup> in different parts of the plant. The results showed that there was a good correlation between the amounts of 2,4,6-trinitrotoluene and its main metabolite ADNT in the urine of exposed workers. The levels of ADNT increased rapidly during the workday and then declined within 24 hours to close to the levels at the beginning of the workday. These results suggest that 2,4,6-trinitrotoluene is rapidly absorbed and eliminated in the course of an acute inhalation exposure. The study is limited in that there was no information on the route of exposure, the length of exposure in the course of a working day, or the exposure dose.

In an attempt to simulate inhalation exposure, 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene suspended in methyl cellulose was instilled into the trachea of anesthetized, tracheotomized Sprague-Dawley rats (Army 1981d). At the same time, another group of rats was treated orally with the same dose of radiolabelled 2,4,6-trinitrotoluene. Both groups were sacrificed 4 hours later, and tissue and urine samples were collected for radioactivity analysis. The rate of absorption was faster after intratracheal instillation than after oral administration of 2,4,6-trinitrotoluene. Urinary excretion averaged 19.3% of the dose after intratracheal administration and 14.6% of the dose after oral administration. These results indicate that there are differences in the absorption rate of 2,4,6-trinitrotoluene depending on the administration route.

### 2.3.1.2 Oral Exposure

Discoloration of the urine is among the first indications that metabolism has occurred after 2,4,6-trinitrotoluene absorption in humans. The color of urine ranges from abnormal amber to deep red (Army 1978a).

Similar observations were made in rats and mice. Sixty minutes after a single exposure to 10,000 mg/kg/day of 2,4,6-trinitrotoluene, the urine of mice and rats becomes red in color (Dilley et al. 1982b). This is an indirect indication of 2,4,6-trinitrotoluene absorption. A more direct estimate of absorption of 2,4,6-trinitrotoluene was done in rats, mice, rabbits, and dogs after a single oral dose of 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene (Army 1981d). Twenty-four hours later, the recovery of radiolabel was measured in rats, mice, dogs, and rabbits. The largest percentage of radioactivity

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was recovered from urine: 59.5%, 59%, and 61% for rats, mice, and dogs, respectively. Rabbits had a slightly higher recovery (74.3%) of radiolabelled 2,4,6-trinitrotoluene in their urine, with a proportional decrease in the radioactivity recovered from the gastrointestinal tract and feces (Army 1981d). Total recovery of the radioactivity (including feces, gastrointestinal tract, and urine) was 92.2%, 94.4%, 94.2%, and 103.6% in rats, mice, dogs, and rabbits, respectively. The red pigment in the urine was not detected in rabbits or dogs. The results of this study indicate that 2,4,6-trinitrotoluene is relatively quickly absorbed after oral administration and that a majority of the ingested compound is excreted within 24 hours.

Findings in rats, mice, and dogs support the observations made in humans. Discoloration of urine was noted in both rats and mice exposed to 34.7 and 35.7 mg/kg/day, respectively, for 13 weeks (Dilley et al. 1982b). Dogs treated with 20 mg/kg/day for 13 weeks had urine that was orange in color (Dilley et al. 1982b), while dogs receiving 8 or 32 mg/kg/day for 26 weeks had light to dark brown urine throughout the treatment period (Levine et al. 1990b). No adverse hepatic effects and no change in urine color were observed in monkeys treated with 1 mg/kg/day for 90 days (Martin and Hart 1974). The Martin and Hart (1974) study was limited in that only three monkeys per sex per group were utilized, thereby precluding statistical analyses of the data. Also, a high frequency of emesis was observed in the treated monkeys. It is believed that the species differences in urine color are due to the presence of unidentified metabolites of 2,4,6-trinitrotoluene. Species differences in 2,4,6-trinitrotoluene toxicity may be attributed to the different metabolic pathways of 2,4,6-trinitrotoluene and its metabolites. Once identified, these urine metabolites may be used as markers of 2,4,6-trinitrotoluene exposure.

### 2.3.1.3 Dermal Exposure

Although data are limited regarding absorption of 2,4,6-trinitrotoluene following dermal exposure in humans, it appears that it occurs rapidly (Woollen et al. 1986). 2,4,6-Trinitrotoluene absorption was assessed by measuring the urinary concentration of one of its metabolites, ADNT, in 25 exposed workers. There were wide variations between individual workers in the rate of clearance of ADNT from the body. Furthermore, when urine samples were collected from a subgroup of workers from the original group of 25, eight out of nine subjects had detectable ADNT levels in their urine even though

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these workers had been away from the workplace for 17 days. This is an indication that a portion of absorbed 2,4,6-trinitrotoluene or its metabolites is slowly excreted (Woollen et al. 1986). Additionally, when five workers from the total group of 25 exposed workers were monitored more closely during two workshifts, it was shown that 2,4,6-trinitrotoluene was absorbed rapidly during the exposure period. The limitations of this study are that the dermal exposure dose was not measured and workers' were also exposed to 2,4,6-trinitrotoluene via the inhalation route.

The differences in absorption and excretion of radiolabelled 2,4,6-trinitrotoluene after dermal and oral exposures were investigated in mice, rabbits, rats, and beagle dogs (Army 1981d). Rats and mice were exposed dermally and orally (by gavage) to 50 mg/kg, and dogs and rabbits to 5 or 50 mg/kg. Twenty-four hours after exposure, animals were sacrificed and urine, gastrointestinal tract, feces, and various tissues were analyzed for radioactivity. Total recovered radioactivity was significantly lower in all species after dermal exposure as compared to after oral exposure (Army 1981d). The highest degree of dermal absorption was observed in rabbits and mice, in which 68.3% and 41.7% of the administered radiolabel was recovered, respectively. The total recovery of radiolabel in dogs and rats was much lower, 17% and 24%, respectively. These results suggest that absorption of radiolabelled 2,4,6-trinitrotoluene is species-dependent and is significantly lower after dermal rather than oral exposure.

### 2.3.2 Distribution

#### 2.3.2.1 Inhalation Exposure

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

In rats that received 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene by intratracheal administration, the highest tissue concentrations of radiolabel after 4 hours were found in both male and female animals in the fat (82 and 155  $\mu\text{g eq/g}$ , respectively) and gastrointestinal tract (82 and 40  $\mu\text{g eq/g}$ , respectively) (Army 1981d). Since the gastrointestinal tract contained considerable amounts of radioactivity, some of the rats in this experiment were bile-duct cannulated in order to collect bile and estimate the amount



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of radiolabel. The results were given as a percent of the administered dose. The radioactivities recovered in bile-duct cannulated male and female rats were 20% and 15%, respectively, from the bile, and 18% and 13%, respectively, from the urine (Army 1981d). When these results were compared to excretion results following oral administration, the percent of radioactivity recovered in the urine and bile was significantly higher after intratracheal exposure in both male and female rats. The opposite was true for the amount of radioactivity recovered in the gastrointestinal tract; it was significantly higher in the orally treated rats regardless of cannulation (Army 1981d). These results indicate that the route of administration contributes to the differences in 2,4,6-trinitrotoluene distribution.

### 2.3.2.2 Oral Exposure

No studies were located regarding distribution following oral exposure to 2,4,6-trinitrotoluene in humans.

Twenty-four hours after administration of a single oral dose of radiolabelled 2,4,6-trinitrotoluene to rats and mice (100 mg/kg), and rabbits and dogs (5 mg/kg), the blood and different tissues were analyzed for radioactivity. The blood and liver, kidney, spleen, lungs, brain, and skeletal muscle of dogs contained a higher percentage of radioactivity than the blood and tissues of rats, mice, and rabbits (Army 1981d). Recovery of radioactivity was greatest in the liver, skeletal muscle, and blood in all four species. However, the amount of radioactivity recovered from tissues was small, ranging from <0.1% to 5.4% of the dose, because the majority of the label is excreted in urine (an average of 60% of the dose) and feces (an average of 11% of the dose) (Army 1981d). This indicates both rapid absorption and rapid distribution in different species after oral exposure to 2,4,6-trinitrotoluene. The limitations of this study are that a small number of animals was analyzed for distribution of radiolabel and that the amount of unchanged 2,4,6-trinitrotoluene was not discussed.

### 2.3.2.3 Dermal Exposure

No studies were located regarding distribution following dermal exposure to 2,4,6-trinitrotoluene in humans or animals. However, the recovery of radiolabel after a single dermal application of 50 mg/kg of 2,4,6-trinitrotoluene was significantly lower in rats, mice, dogs, and rabbits than in those exposed

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orally (Army 1981d). Skin and fat around the site of dermal application were not included in the final radiolabel recovery estimates. This may account for the lower radiolabel recovery obtained after the dermal exposure (Army 1981d). The recovery of radiolabel from urine, feces, gastrointestinal tract, blood, and tissue differed among the four species examined: rabbits (56.9%) > mice (41.7%) > rats (22.8) > dogs (15.9%).

### 2.3.3 Metabolism

#### 2.3.3.1 Inhalation Exposure

No studies were located regarding metabolism following inhalation exposure to 2,4,6-trinitrotoluene in humans or animals. However, in a retrospective study (covering a 5-year period) of 2,4,6-trinitrotoluene workers, no correlation was found between the presence in urine of one of the main 2,4,6-trinitrotoluene metabolites, ADNT, and the results of Webster's reaction, which measures urine 2,4,6-trinitrotoluene levels (Hassman and Hassmanova 1976). This result provides indirect evidence that 2,4,6-trinitrotoluene is metabolized completely and that no detectable amounts of unchanged compound are present in the urine. This study is limited in that it does not clearly define the route of exposure and does not specify the dose or the length of exposure.

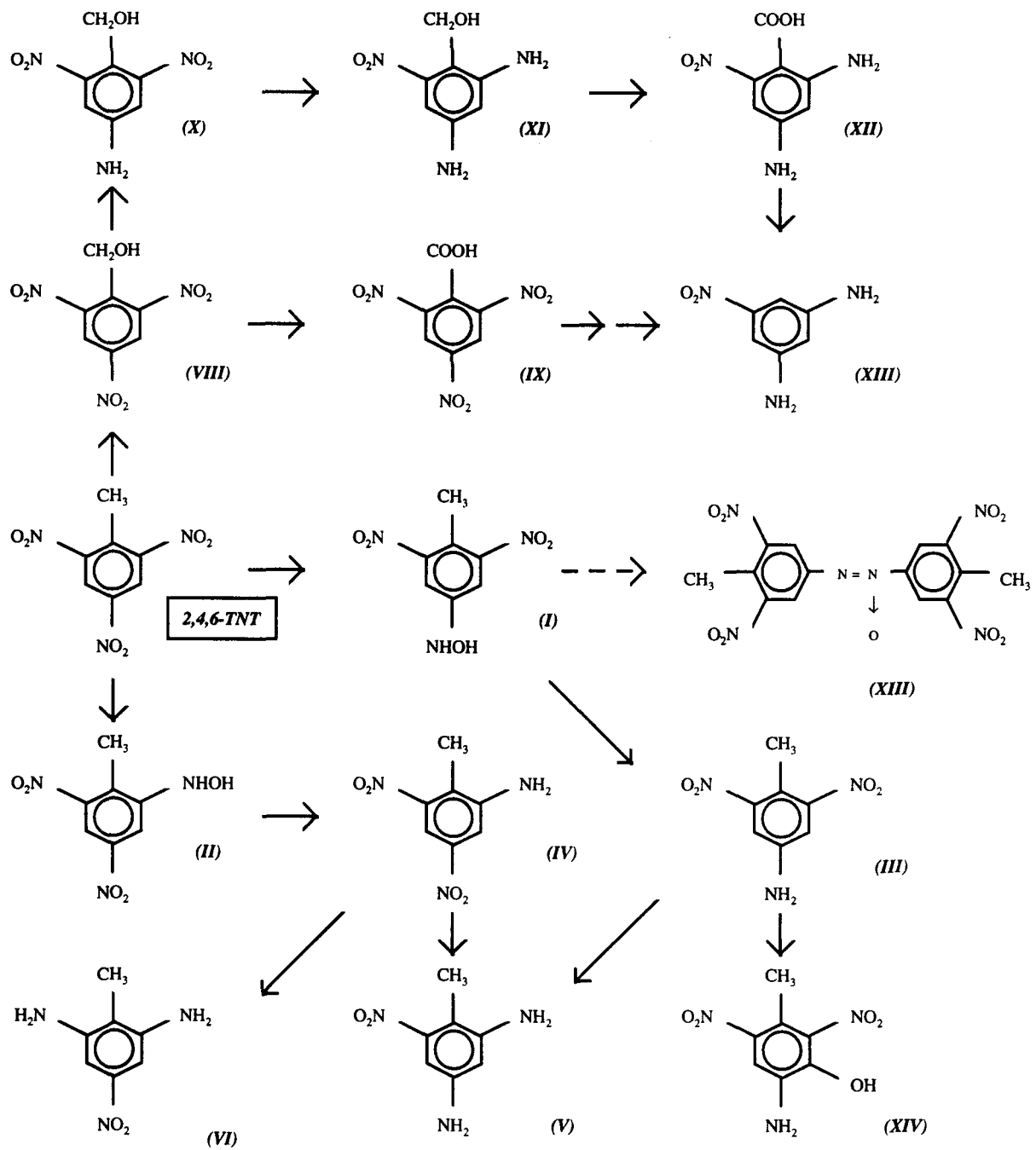
#### 2.3.3.2 Oral Exposure

No studies were located specifically addressing metabolism following oral exposure to 2,4,6-trinitrotoluene in humans.

The 2,4,6-trinitrotoluene molecule may undergo various metabolic transformations, such as oxidation of the methyl group, oxidation of the benzene ring, reduction of the three nitro groups, and conjugation (EPA 1989b). A metabolic pathway illustrating some possible transformation products of 2,4,6-trinitrotoluene is shown in Figure 2-2 (Army 1981d). The failure to detect unmetabolized 2,4,6-trinitrotoluene in the urine of humans (Hassman and Hassmanova 1976) provides indirect evidence that 2,4,6-trinitrotoluene is extensively metabolized. Trace amounts of unmetabolized 2,4,6-trinitrotoluene were found in the urine of rats, mice, rabbits, and dogs (Army 1981d). Several

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Figure 2-2. Possible Biotransformation Products of 2,4,6-TNT\*



\*Adapted from Army 1981d

(I) 4-hydroxylamino-2,6-dinitrotoluene; (II) 2-hydroxylamino-4,6-dinitrotoluene; (III) 4-amino-2,6-dinitrotoluene; (IV) 2-amino-4,6-dinitrotoluene; (V) 4,6-diamino-2-nitrotoluene; (VI) 2,6-diamino-4-nitrotoluene; (VII) 2,6,2',6'-tetranitro-4,4'-azoxytoluene; (VIII) 2,4,6-trinitrobenzylalcohol; (IX) trinitrobenzoic acid; (X) 4-amino-2,6-dinitrobenzylalcohol; (XI) 2,4-diamino-6-nitrobenzylalcohol; (XII) 2,4-diamino-6-nitrobenzoic acid; (XIII) 5-nitro-*m*-phenylenediamine; (XIV) 4-amino-2,6-dinitro-*m*-cresol

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metabolites have been identified in human urine: 4-ADNT, 2-ADNT, 2,4-diatnino-6-nitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene, and amino-nitrocresol (Army 1986c; Channon et al. 1944; Lemberg and Callaghan 1945).

2,4,6-Trinitrotoluene was extensively metabolized in rats, mice, dogs, and rabbits after a single oral dose of 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene (Army 1981d). Only minute amounts of the unmetabolized 2,4,6-trinitrotoluene were found in urine. The majority of urinary metabolic products have high polarity and very low extractability in organic solvents. It was therefore difficult to identify them. The metabolic profiles of urine from the four species differed only quantitatively (Army 1981d). 4,6-Diamine, 2,6-diamine, and monoamines of 2,4,6-trinitrotoluene were the predominant metabolites detected in the urine of rats. Smaller quantities of 2- and 4-hydroxylamines and azoxytoluene were present. In contrast to rat urine, greater amounts of the monoamines and hydroxylamines and smaller quantities of polar metabolites and diamines were found in the urine of mice. The urine of dogs contained appreciable amounts of diamines and monoarnines and small amounts of the 4-hydroxylamine and 2-hydroxylamine. Substantial amounts of monoarnines, hydroxylamines, and diamines were noted in rabbit urine (Army 1981d). Treatment of urine of all species with  $\beta$ -glucuronidase increased the amount of extractable radioactivity, indicating that conjugation of 2,4,6-trinitrotoluene metabolites with UDP-glucuronic acid is an important route of metabolism. Urine from treated mice contained the least amount of glucuronide conjugates (Army 1981d).

### 2.3.3.3 Dermal Exposure

No studies were located regarding metabolism following dermal exposure to 2,4,6-trinitrotoluene in humans.

The differences in metabolic profiles from the urine of mice, rats, dogs, and rabbits after a single dermal dose of 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene were only quantitative (Army 1981d). There was an increased amount of unchanged 2,4,6-trinitrotoluene in urine after a single dermal exposure which was not found after a single oral exposure (Army 1981d). This suggests that the exposure route plays a role in the extent of 2,4,6-trinitrotoluene biotransformation.

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### 2.3.4 Excretion

#### 2.3.4.1 Inhalation Exposure

4-ADNT, which is considered to be a major metabolite of 2,4,6-trinitrotoluene, was shown to be present in the urine of munitions workers exposed to 0.045-0.93 mg/m<sup>3</sup> of 2,4,6-trinitrotoluene by both the Webster reaction and by a polarographic technique (Hassman and Hassmanova 1976). Similar findings were made using a sensitive gas chromatographic method (Almog et al. 1983). No unchanged 2,4,6-trinitrotoluene was detected in this study. However, no detail was provided regarding the exposure dose or route.

Recovery of radiolabel from urine of Sprague-Dawley rats 4 hours after intratracheal instillation of 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene was similar to the recovery after oral exposure. The amount of radiolabel in the urine, expressed as a percentage of the dose, was 19% and 13% in male and female animals, respectively (Army 1981d).

#### 2.3.4.2 Oral Exposure

No studies were located regarding excretion following oral exposure to 2,4,6-trinitrotoluene in humans.

The results from animal studies indicate that urine is the major excretion route after a single oral dose of radioactive 2,4,6-trinitrotoluene. The excretion of radioactive label was studied in Sprague-Dawley rats after a single oral dose of 100 mg/kg (Army 1981d). In the course of 24 hours after exposure, 53-65% of the radioactivity was recovered from the urine; 2-8% from the feces; and 30-34% from the gastrointestinal tract and its contents. Similar results on the recovery of radiolabel in urine were obtained after oral exposure of albino CD1 mice (100 mg/kg), rabbits (5 mg/kg), and dogs (50 mg/kg) to radioactive 2,4,6-trinitrotoluene (Army 1981d). These results were confirmed when a 24-hour recovery of radiolabelled 2,4,6-trinitrotoluene was evaluated in rats, mice, dogs, and rabbits after a single oral exposure of 50 mg/kg (Army 1981d). The percentages of radiolabel in urine were 59.5%, 59%, and 61% for rats, mice, and dogs, respectively. The highest amount of radiolabel was recovered from the urine of rabbits, 74.3%. The amounts of label from feces were 11%, 24%, 5%, and 22% for

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rats, mice, rabbits, and dogs, respectively. Although the number of animals in these studies was relatively small, the results indicate that there are species differences regarding excretion after acute oral exposure to 2,4,6-trinitrotoluene. The urine of rats and mice in these studies was bright red in color, indicating formation of some species-specific, unknown metabolite(s); no such color was observed in dogs and rabbits (Army 1981d).

### 2.3.4.3 Dermal Exposure

No studies were located regarding excretion following dermal exposure to 2,4,6-trinitrotoluene in humans.

Total recovery of radioactive label (from blood, liver, kidneys, lungs, spleen, brain, muscle, gastrointestinal tract, feces, and urine) after a single dermal exposure of 50 mg/kg of radiolabel 2,4,6-trinitrotoluene was evaluated in rats, mice, dogs, and rabbits and compared to total recovery after oral exposure (Army 1981d). In all species, the total recovery of the radiolabel was significantly lower after dermal exposure as compared to oral exposure. Total radioactivity recovered after dermal exposure was 16%, 23%, 42%, and 57% in dogs, rats, mice, and rabbits, respectively. For comparison, the recovery after oral exposure was 92%, 94%, 94%, and 104% in rats, dogs, mice, and rabbits (Army 1981d). These results indicate that the total recovery of the label after a single dermal exposure to 2,4,6-trinitrotoluene varies depending on the species. Recovery is also affected by the exposure route; recovery after oral exposure is significantly higher than after dermal exposure (Army 1981d).

### 2.3.5 Mechanisms of Action

Although the mechanisms by which 2,4,6-trinitrotoluene and its derivatives exert their toxic effects on organ systems (including the blood, liver, and eye lens) are largely unknown, a general theory has been developed to explain the toxicity of 2,4,6-trinitrotoluene.

As discussed in Section 2.3.1, in limited studies of humans and animals it appears 2,4,6-trinitrotoluene is readily absorbed by inhalation, ingestion, or dermal routes of exposure. Also, it has been

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demonstrated that 2,4,6-trinitrotoluene is lipid soluble; tracer studies have shown accumulation in subcutaneous fat, and it would be expected to be present in lipids in the liver and marrow. A theory of the mechanism of toxicity by 2,4,6-trinitrotoluene is that the parent compound and some metabolic intermediates are active oxygen generators (Kong et al. 1989) and are involved in lipid peroxidation in the liver and in oxygenation of the lens to form cataracts (Liu et al. 1992; Savolainen et al. 1985). In addition, it is postulated that in the rat 2,4,6-trinitrotoluene undergoes rapid reduction to hydroxylamine and that this intermediate can be bioactivated to bind sulfhydryl proteins (Liu et al. 1992). This bioactivation is postulated to occur in the liver or in the blood via different pathways. In the liver, bioactivation of hydroxylamine is postulated to involve oxidation by NADPH-dependent hepatic microsomal enzymes. In the blood, bioactivation of hydroxylamine is postulated to involve a reaction with oxyhemoglobin (Liu et al. 1992).

### 2.4 RELEVANCE TO PUBLIC HEALTH

The general public is not likely to be exposed to 2,4,6-trinitrotoluene. However, there is a possibility that populations will be exposed in the vicinity of munitions fabrication plants, demilitarization facilities, and particularly at incinerator facilities and former and current open-bum and opendetonation facilities. Occupational or accidental exposure to 2,4,6-trinitrotoluene may occur by the oral, inhalation, or dermal routes.

Oral exposure to 2,4,6-trinitrotoluene in aquatic environments in the vicinity of ammunition plants is not likely because 2,4,6-trinitrotoluene stays unchanged for only a short period because of photolysis (half-life of less than 24 hours) and biological degradation (half-life of less than 65 days). However, if there is 2,4,6-trinitrotoluene-contaminated soil in the vicinity of a munitions plant, oral exposure through terrestrial food products (especially homegrown produce or locally grazed animals) cannot be completely ignored as a possible exposure pathway. Dermal exposure through contaminated soil is more likely since the degradation of 2,4,6-trinitrotoluene in soil is less effective than in water (Army 1986c). Volatilization of 2,4,6-trinitrotoluene from surface water is negligible, making inhalation exposure unlikely.

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Information on the effects that occur in humans after exposure to 2,4,6-trinitrotoluene comes from case reports of accidental intoxication and from studies of occupationally exposed workers in the manufacture of high explosives. Because of improvements in the work environment, numerous adverse health effects caused by exposure to 2,4,6-trinitrotoluene, such as anemia, liver function abnormalities, respiratory complications, and possibly aplastic anemia, have been greatly reduced.

Historically, adverse effects on the liver have caused the greatest number of deaths among munitions workers; 475 deaths were reported in the United States during World War I (McConnell and Flinn 1946). Cases of aplastic anemia, which is usually fatal, were also reported during that time. As stated in the previous paragraph, these adverse effects have been almost eliminated with the introduction of more effective protection measures for workers handling 2,4,6-trinitrotoluene.

The major effects observed in animals after intermediate or chronic exposures to 2,4,6-trinitrotoluene are reduced number of red blood cells, reduced hemoglobin and hematocrit, anemia, testicular damage, hepatomegaly, and splenomegaly.

### ***Inhalation***

No MRLs were derived for acute, intermediate, or chronic exposure by the inhalation route.

### ***Oral***

An MRL of 0.0005 mg/kg/day has been derived for intermediate oral exposure to 2,4,6-trinitrotoluene. This MRL is based on the occurrence of dose-related liver effects (cloudy swelling and hepatocytomegaly) noted in dogs administered 0.5 mg/kg/day by capsule for 6 months (Levine et al. 1990b). More severe liver injury (hemosiderosis in Kupffer's cells and hepatic cirrhosis) was observed at high doses, supporting the sensitivity of the selected end point. In addition, liver toxicity (jaundice, elevated serum and urine bilirubin levels, hepatocellular hyperplasia, cloudy swelling, focal necrosis, changes in the levels of serum triglycerides, and increased serum cholesterol levels) have been reported in animals orally



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exposed to 2,4,6-trinitrotoluene for intermediate or chronic durations (Army 1984a; Dilley et al. 1982b; Levine et al. 1984).

Insufficient information was available to calculate an acute-duration oral MRL for neurological effects. Additional studies in acute oral exposures to 2,4,6-trinitrotoluene are needed to determine the threshold level for neurological effects. Chronic-duration exposure to the intermediate-duration oral MRL of 0.0005 mg/kg/day, which is the same value as the EPA's chronic oral Reference Dose (RfD), would not be anticipated to cause adverse health effects.

**Death.** In the United States, 475 deaths were reported among munitions workers during World War I (McConnell and Flinn 1946). One death from hepatocellular carcinoma was reported in a case of chronic occupational exposure to 2,4,6-trinitrotoluene (Garfmkel et al. 1988). It is not clear, however, if 2,4,6-trinitrotoluene played a role in the carcinogenic process. Death has been observed in rats, mice, and dogs (Dilley et al. 1982b; Levine et al. 1990b) after oral exposure to sufficient amounts of 2,4,6-trinitrotoluene. Reported oral LD<sub>50</sub> values are 1,010 and 1,320 mg/kg/day for male rats, and 820 and 795 mg/kg/day for female rats (Army 1978b; Dilley et al. 1982b). Acute oral LD<sub>50</sub> values in male and female mice are 1,012 and 660 mg/kg/day, respectively (Army 1978b; Dilley et al. 1982b). 2,4,6-Trinitrotoluene was lethal in beagle dogs receiving 32 mg/kg/day orally for 26 weeks (Levine et al. 1990b).

It is extremely unlikely that sufficient levels of 2,4,6-trinitrotoluene could be ingested acutely by persons living in the vicinity of an ammunition plant to cause death. Furthermore, the low levels of 2,4,6-trinitrotoluene that are likely to be present in the soil near the plants are substantially below the levels that are necessary to cause death.

### **Systemic Effects**

**Respiratory Effects.** Extremely limited information was located regarding respiratory effects in humans after exposure to 2,4,6-trinitrotoluene. One study of occupational exposure (Morton et al. 1976) reported several cases of respiratory difficulties in workers exposed to 2,4,6-trinitrotoluene levels

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in the air that were above the TLV of 0.5 mg/m<sup>3</sup> (ACGIH 1993). However, there are several limitations to this study. The report does not state the exact air concentration of 2,4,6-trinitrotoluene, the exposure duration, or the number of exposed workers reporting difficulties. No details are given about the nature of the reported difficulties. No studies in animals were located that described potential respiratory effects of 2,4,6-trinitrotoluene. Therefore, insufficient evidence exists to assess the relevance of these findings to public health.

**Cardiovascular Effects.** No studies were located regarding cardiovascular effects in humans. Intermediate oral exposure to doses as high as 32 mg/kg/day of 2,4,6-trinitrotoluene for 26 weeks did not cause any changes in electrocardiogram or heart rates in beagle dogs (Levine et al. 1990b). The available information is not sufficient to evaluate the effects of 2,4,6-trinitrotoluene on populations living close to ammunition plants.

**Gastrointestinal Effects.** There are no studies on gastrointestinal effects in humans after exposure to 2,4,6-trinitrotoluene. However, two studies in dogs reported adverse gastrointestinal effects following intermediate oral exposure to 2,4,6-trinitrotoluene. Dogs receiving 20 mg/kg/day for 13 weeks had mucoid stools and diarrhea (Dilley et al. 1982b), while inflammation of a part of the small intestine was observed in beagle dogs fed 0.5, 2, 8, and 32 mg/kg/day of 2,4,6-trinitrotoluene for 25 weeks (Levine et al. 1990b). The inflammation was dose-dependent and was more pronounced in dogs receiving the highest dose. Based on the available information, it is possible, although unlikely, that oral exposure to 2,4,6-trinitrotoluene may cause some adverse gastrointestinal effects, but it is not known if such effects would occur after dermal exposure in the vicinity of an ammunition plant or a demilitarization facility. Although dermal exposure has been indicated as the most likely route of exposure in occupational situations, limited exposure to 2,4,6-trinitrotoluene may result from ingestion of produce contaminated by deposition from fugitive particles or resuspension of contaminated soil, or from ingestion of animal products from animals that graze in the vicinity of an ammunition or demilitarization facility (Army 1986d).

**Hematological Effects.** Fatal cases of aplastic anemia among workers engaged in the production of explosives have not been reported in the recent literature, although they occurred in England

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(Hathaway 1985) and other countries involved in World War I. The incidence of adverse health effects of 2,4,6-trinitrotoluene including aplastic anemia have decreased dramatically because of improvements in protective measures in munitions factories.

Dose-related reductions in hemoglobin and hematocrit and a 50% increase in reticulocyte counts were noted in 626 workers exposed to 2,4,6-trinitrotoluene air levels ranging from  $<0.1$  to  $1.49 \text{ mg/m}^3$  (Army 1976). The duration of exposure was not specified. In another study, no abnormal values for hemoglobin were found in 43 workers employed in the manufacture of 2,4,6-trinitrotoluene who were monitored for 5 months (Morton et al. 1976).

Acute hemolytic disease was described in three ammunition plant workers (Djerassi and Vitany 1975) who were also glucose-6-phosphate dehydrogenase (GGPD) deficient. This study is limited in that the exposure route is not specified, so it is not clear how relevant the finding is for general public health.

Anemia (consisting of reduced number of red blood cells and reduced hemoglobin and hematocrit) is one of the major signs of 2,4,6-trinitrotoluene toxicity. These adverse effects were observed in rats (Dilley et al. 1982b; Jiang et al. 1991; Levine et al. 1984, 1990a), mice (Dilley et al. 1982b), and dogs (Dilley et al. 1982b; Levine et al. 1990b) after intermediate oral exposure to 2,4,6-trinitrotoluene. Similar observations were made in Fischer-344 rats fed 10 or 50 mg/kg/day for 24 months (Army 1984a). In this chronic exposure study, male rats were somewhat more sensitive than female rats to the toxic effects of 2,4,6-trinitrotoluene. Reticulocytosis was present as a compensatory response to the anemic state in all animals. Methemoglobinemia was noted in rats fed 300 mg/kg/day for 13 weeks (Levine et al. 1984), in rats fed 10 or 50 mg/kg/day for 24 months (Army 1984a), and in dogs fed 32 mg/kg/day for 6 months (Levine et al. 1990b).

Bone marrow fibrosis and leukocytosis were present in rats orally exposed to 2,4,6-trinitrotoluene for 24 months or 13 weeks; these animals were also anemic (Army 1984a; Dilley et al. 1982b).

Dogs and rats had increased platelet levels after exposure for 6 and 24 months, respectively (Army 1984a; Levine 1990b). The significance of this finding was not discussed.

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Based on this information, it seems unlikely that sufficient amounts of 2,4,6-trinitrotoluene would be present near ammunition plants to cause adverse hematological effects in the population living in the vicinity. Individuals who are G6PD deficient may need to be evaluated as a potentially susceptible population.

**Hepatic Effects.** Toxic hepatitis has been the principal manifestation of 2,4,6-trinitrotoluene toxicity in humans, and many cases recorded during World War I were fatal (Army 1987a).

Reports on adverse hepatic effects of 2,4,6-trinitrotoluene in humans have been located. Increases in hepatic enzymes (SGOT and LDH) were noted in ammunition plant workers exposed when air levels of 2,4,6-trinitrotoluene rose from 0.3 to 0.8 mg/m<sup>3</sup> (Morton et al. 1976). The duration of the study was 5 months. In another study, no significant differences in liver function (LDH, bilirubin, alkaline phosphatase, SGOT and SGPT) were noted in a cross-sectional study on 626 munitions workers exposed to 0.5 mg/m<sup>3</sup> of 2,4,6-trinitrotoluene (Army 1976).

In a retrospective study spanning 20 years, liver cell irritation (measured with the MacLagen thymol turbidity test) was present in 40 munitions workers (Goodwin 1972). The report did not specify the exposure dose or route. Exposure of animals to moderate-to-high levels (0.5-200 mg/kg/day) of 2,4,6-trinitrotoluene over intermediate-to-chronic periods has been reported to cause adverse effects such as jaundice, elevated serum and urine bilirubin levels, hyperplasia, cloudy swelling, focal necrosis and cirrhosis of the liver, changes in the levels of serum triglycerides, and increased serum cholesterol levels (Army 1984a; Dilley et al. 1982b; Levine et al. 1984, 1990b). It is not known if chronic exposure to the levels of 2,4,6-trinitrotoluene described above would cause similar adverse hepatic effects in exposed humans.

These degenerative effects are distinct from the adaptive changes observed in livers of a number of nonhuman species in response to exposure to 2,4,6-trinitrotoluene. The most common adaptive change observed in several animal species during intermediate exposure to 2,4,6-trinitrotoluene was an increase in liver weight and/or size (Dilley et al. 1982b; Jiang et al. 1991; Levine et al. 1984). It is not known if these effects would occur in humans.

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**Renal Effects.** No studies were located regarding renal effects in humans after exposure to 2,4,6-trinitrotoluene. However, studies have shown that discoloration of the urine is among the first indications of 2,4,6-trinitrotoluene exposure in humans and is due to the presence of 2,4,6-trinitrotoluene metabolites. The color of urine ranges from abnormal amber to deep red (Army 1978a).

Discoloration of the urine from the presence of 2,4,6-trinitrotoluene metabolites also occurs in rats, mice, and dogs. Sixty minutes after acute exposure to 10,000 mg/kg/day of 2,4,6-trinitrotoluene, the urine in mice and rats became red in color because of the presence of 2,4,6-trinitrotoluene metabolites (Dilley et al. 1982b). The same effect was observed in rats and dogs treated with higher doses and for a longer period (Dilley et al. 1982b). Increased filtration rate was present in rats chronically exposed to 10 and 50 mg/kg/day of 2,4,6-trinitrotoluene (Army 1984a).

In rats treated with higher doses of 2,4,6-trinitrotoluene for 13 weeks (Levine et al. 1984; 1990b) or 24 months (Army 1984a), histopathological analysis revealed the accumulation of yellowish-brown pigment in the renal cortex and in the epithelial cells of proximal convoluted tubules. It is therefore possible that persons exposed to extremely high levels of 2,4,6-trinitrotoluene may be at increased risk of renal toxicity.

**Dermal Effects.** Exposure to 2,4,6-trinitrotoluene can cause dermatitis in workers handling the compound (Morton et al. 1976). Two incidences of allergic contact dermatitis were reported in two ammunition plant workers after intermediate-duration exposure to 2,4,6-trinitrotoluene (Goh 1988; Goh and Rajan 1983). These findings indicate that prolonged exposure to relatively low levels of 2,4,6-trinitrotoluene may cause an allergic reaction manifested by dermatitis appearing in the areas of contact with the chemical.

**Ocular Effects.** The appearance of irreversible cataracts is believed to be specific to 2,4,6-trinitrotoluene exposure. It is often associated with chronic exposures to relatively low levels of 2,4,6-trinitrotoluene (Hathaway 1985). Equatorial lens opacities/ataracts were reported in 6 out of 12 workers exposed to 2,4,6-trinitrotoluene for an average of 6.8 years (Harkonen et al. 1983). The average

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concentration of 2,4,6-trinitrotoluene in the air was  $0.3 \text{ mg/m}^3$ , which is well below the threshold limit of  $0.5 \text{ mg/m}^3$  (ACGIH 1993). It is therefore possible that chronic exposure to low levels of 2,4,6-trinitrotoluene in the vicinity of ammunition plants may have adverse ocular effects.

**Immunological and Lymphoreticular Effects.** An increase in the number of mononuclear leukocytes was found in reviewing blood cell counts of 105 individuals exposed to 2,4,6-trinitrotoluene (Army 1978a). This increase precedes any other symptom, remains positive for 2-3 months, and could be helpful in differential diagnosis. Also increased were lymphocyte numbers in nine cases of fatal 2,4,6-trinitrotoluene poisoning (Army 1974). Since the doses necessary to produce these effects were not established, the possibility that susceptible persons living in the vicinity of ammunition plants may be exposed to sufficient amounts of 2,4,6-trinitrotoluene to trigger such immunological effects cannot be excluded.

Exposure to 2,4,6-trinitrotoluene can cause dermatitis in workers handling the compound (Morton et al. 1976). Two incidences of allergic contact dermatitis were reported in two ammunition plant workers after intermediate exposure to 2,4,6-trinitrotoluene (Goh 1988; Goh and Rajan 1983). These findings indicate that prolonged exposure to relatively low levels of 2,4,6-trinitrotoluene may cause an allergic reaction manifested by dermatitis appearing in the areas of contact with the chemical.

Increased spleen weight was the most common effect seen in several nonhuman species after intermediate exposure to medium-to-high doses of 2,4,6-trinitrotoluene (Dilley et al. 1982b; Levine et al. 1984, 1990a). Other changes, such as splenic congestion and hemosiderosis, reduced lymphocyte counts, increased lymphocyte counts, and increased globulin levels, were also noted. Thus, persons exposed to sufficiently high levels of 2,4,6-trinitrotoluene near ammunition plants may be at risk of developing immune system or lymphoreticular effects including splenomegaly with splenic congestion and hemosiderosis, lymphocytosis due to reduced lymphocyte counts, and higher globulin levels.

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**Neurological Effects.** Only minor neurological effects, such as altered taste, were noted in humans after an inhalation exposure of  $0.3 \text{ mg/m}^3$  of 2,4,6-trinitrotoluene (Morton et al. 1976). On the basis of this limited information, it is difficult to speculate on possible adverse neurological effects that may occur in 2,4,6-trinitrotoluene-exposed people living in the vicinity of ammunition plants.

Rats showed no signs of neurotoxicity after acute exposure to  $182 \text{ mg/kg/day}$  (Short and Lee 1980). However, when fed an extremely high dose of  $10,000 \text{ mg/kg/day}$ , both rats and mice showed signs of inactivity; some developed convulsions and died (Dilley et al. 1982b). Similar observations were made in the intermediate-duration studies in dogs, rats, and monkeys fed low doses of 2,4,6-trinitrotoluene (Dilley et al. 1982b; Martin and Hart 1974). When higher doses were used ( $32 \text{ mg/kg/day}$  for 26 weeks), dogs became ataxic (Levine et al. 1990b). In rats exposed to  $300 \text{ mg/kg/day}$  of 2,4,6-trinitrotoluene for 13 weeks, brain lesions (consisting of focal vacuolation and malacia of the white tracts of the cerebellar folia) were seen in histopathological analysis (Levine et al. 1984). In contrast, no significant signs of neurotoxicity were seen in rats treated with up to  $50 \text{ mg/kg/day}$  for 24 months (Army 1984a). Since these results indicate species differences after acute, intermediate, and chronic exposures, it is difficult to estimate potential neurotoxic effects for humans living close to ammunition plants.

**Reproductive Effects.** A preliminary case control study of workers in two 2,4,6-trinitrotoluene plants in China indicates exposure to 2,4,6-trinitrotoluene may have adverse effects on several indicators of male reproductive status (Li et al. 1993). Workers exposed to 2,4,6-trinitrotoluene had significantly lower semen volumes and a smaller percentage of motile spermatozoa as well as a significantly higher incidence of sperm malformation than the control group. However, exposure concentrations and route of exposure are not known. Possible important variables which are not discussed include exposure to other chemicals and heat in the workplace.

Serious reproductive effects, such as testicular atrophy and atrophic seminiferous tubules, were observed in rats treated with high doses of 2,4,6-trinitrotoluene for 13 weeks (Dilley et al. 1982b; Levine et al. 1984, 1990a). These changes were not reversible after a 4-week recovery period. Based

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on this limited information, adverse reproductive effects after exposure of males to sufficiently high concentrations of 2,4,6-trinitrotoluene cannot be excluded.

**Developmental Effects.** No studies were located regarding developmental effects in humans or animals following exposure to 2,4,6-trinitrotoluene by any exposure route. It is therefore not possible to predict the potential developmental toxicity of 2,4,6-trinitrotoluene at hazardous waste sites or near ammunition plants.

**Genotoxic Effects.** No studies were found that directly assess the potential of 2,4,6-trinitrotoluene to induce genotoxic effects in humans. However, there is convincing evidence that the urine of individuals occupationally exposed to 2,4,6-trinitrotoluene contains mutagenic components (Ahlborg et al. 1985, 1988a). The primary metabolite of 2,4,6-trinitrotoluene appears to be 4-aminodinitrotoluene (6ADNT); major intermediate forms, including 4-ADNT, are weakly mutagenic in bacteria (Spanggord et al. 1982b). By contrast, 2,4,6-trinitrotoluene is a confirmed mutagen in bacterial and mammalian cells *in vitro* and 2,4,6-trinitrotoluene-induced mutagenesis is either markedly diminished or abolished by the inclusion of exogenous metabolic activation into these test systems (see discussion of *in vitro* results below).

The detection of unmetabolized 2,4,6-trinitrotoluene in the urine of exposed workers exhibiting a high level of mutagenic activity (Ahlborg et al. 1985) tends to support the assumption that the parent compound rather than its derivatives was responsible for the observed response. However, a more detailed follow-up study found no correlation between mutagenicity and 2,4,6-trinitrotoluene concentration in urine (Ahlborg et al. 1988a). It is nevertheless possible that these conflicting results could be resolved if more appropriate concentration procedures improved the detection of 2,4,6-trinitrotoluene. This would elucidate the possible connection between mutagenesis and the concentrations of 2,4,6-trinitrotoluene and/or its metabolites in the urine of exposed workers.

Only one *in vitro* study employing a human cell line (WI-38 human fibroblasts) was found in the existing literature (Army 1978~). In this study, target cells were exposed to 2,4,6-trinitrotoluene doses ranging from 2 to 2,000 µg/mL without S9 activation and from 6 to 6,000 µg/mL in the presence of an



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uninduced mouse liver homogenate. Precipitation of the test material occurred at  $\geq 200$   $\mu\text{g/mL}$  (without S9) and at 375  $\mu\text{g/mL}$  (in the presence of S9). At nonactivated levels of 500 and 1,000  $\mu\text{g/mL}$ , a significant ( $p < 0.05$ ) increase in UDS was obscured by discoloration of the samples; however, significant ( $p < 0.05$ ) effects were also obtained at 250  $\mu\text{g/mL}$ . No discoloration of the test samples occurred in the S9-activated phase of testing, and no evidence of a genotoxic response was uncovered. Although a definitive conclusion could not be reached because of compound interference with the nonactivated assay results, the data do not suggest that 2,4,6-trinitrotoluene was genotoxic in this human cell line. Similarly, the lack of an effect in the presence of auxiliary metabolic activation is consistent with other *in vitro* assay results.

The single *in vivo* animal study assessing potential adverse effect on the chromosome structure of somatic cells following oral administration of 2,4,6-trinitrotoluene was negative but compromised because neither a toxic effect in the rats nor a cytotoxic effect on the target organ (i.e., bone marrow cells) was demonstrated (Army 1978c). However, the results of a well-conducted mouse micronucleus assay, which evaluated 2,4,6-trinitrotoluene at a level (80 mg/kg) that approximated 80% of the maximum tolerated dose, provided no indication of a clastogenesis (Ashby et al. 1985). For this study, groups of five male CBA x Balb C mice received single intraperitoneal injections of 40 or 80 mg/kg 2,4,6-trinitrotoluene; animals were sacrificed 24, 48, and 72 hours post-treatment, and bone marrow cells were examined for the presence of micronucleated polychromatic erythrocytes (MPEs). Results indicated that there were no significant increases in the frequency of MPEs in bone marrow cells sampled over the entire hematopoietic cycle. Similarly, the *in vivo* / *in vitro* rat liver UDS assay performed by the same investigators was negative.

As the above discussion indicates, deleterious genetic events resulting from exposure to 2,4,6-trinitrotoluene have not been extensively investigated in either humans or animals. Since the only available studies in humans were from occupational settings, inhalation must be considered an important pathway of 2,4,6-trinitrotoluene exposure. However, quantifiable dermal absorption indicates that 2,4,6-trinitrotoluene is absorbed through the skin. It also suggests that dermal absorption plays an important role in 2,4,6-trinitrotoluene uptake, which may be more important than uptake through inhalation (Ahlborg et al. 1988a). The identification of the agent(s) responsible for the

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mutagenic activity observed in the urine of workers exposed to 2,4,6-trinitrotoluene is of considerable importance, because human metabolism of 2,4,6-trinitrotoluene has not been fully characterized. It is possible that the parent compound, a known mutagen, and its metabolites, which are also mutagens in *S. typhimurium* TA98, are contributors to 2,4,6-trinitrotoluene-induced mutagenesis (Spanggord et al. 1982b).

Although only three *in vivo* studies were found, the overall results provided no evidence that 2,4,6-trinitrotoluene is genotoxic in whole animals. This assumption is supported by the results of the *in vitro* UDS assay with human cells indicating that 2,4,6-trinitrotoluene-induced UDS was abolished by the inclusion of exogenous metabolic activation (Army 1978c). Similar results, as discussed below, were obtained in other test systems using both cultured bacterial and mammalian cells.

The implications of both the whole animal and *in vitro* human cell assay findings are highly relevant to human health. If 2,4,6-trinitrotoluene can be reduced to nonmutagenic metabolic products, the potential health hazard to humans would be greatly reduced. Refer to Tables 2-2 and 2-3 for a further summary of these studies.

In contrast to the absence of genotoxicity in animal studies, numerous investigators (Ahlborg et al. 1985, 1988a; Army 1978a, 1978c, 1979b, 1980d; Kaplan and Kaplan 1982c; Pearson et al. 1979; Spanggord et al. 1982b; Whong and Edwards 1984; Won et al. 1976) have demonstrated that 2,4,6-trinitrotoluene is a microbial mutagen. There is good agreement that 2,4,6-trinitrotoluene primarily causes frameshift mutations in *S. typhimurium* TA1537, TA1538, and TA98 and that the mutagenic response is not dependent on auxiliary metabolic activation nor substantially influenced by microbial nitroreductase activity. Data also exist showing that 2,4,6-trinitrotoluene is mutagenic in *S. typhimurium* strains TA1535 and TA100, which detect agents that cause base-pair substitution mutations (Army 1978c, 1979b, 1980d; Whong and Edwards 1984). The weight of evidence, however, is consistent with a frameshift mutagen; appreciably higher levels of 2,4,6-trinitrotoluene ( $\geq 30$   $\mu\text{g}/\text{plate}$ ) were required to achieve positive responses in TA1535 and TA100 as compared to the reactivity ( $\geq 2$   $\mu\text{g}/\text{plate}$ ) of 2,4,6-trinitrotoluene with strains TA1538 and/or TA98. Metabolites of 2,4,6-trinitrotoluene have not been shown as consistently to be mutagens in *S. typhimurium*. While the

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

| Species (test system)   | End point                    | Results         |                    | Reference                  |
|---|------------------------------|-----------------|--------------------|----------------------------|
|   |                              | With activation | Without activation |                            |
| <b>Prokaryotic organisms:</b>   |                              |                 |                    |                            |
| <i>Salmonella typhimurium</i><br>(TA1535, TA1537, TA1538, TA98,<br>TA100NR) | Gene mutations               | - <sup>a</sup>  | + <sup>b</sup>     | Whong and Edwards<br>1984  |
| <i>S. typhimurium</i> (TA1523, TA1537, TA1538,<br>TA98, TA100NR3)           | Gene mutations               | + <sup>c</sup>  | + <sup>b</sup>     | Spanggord et al. 1982b     |
| <i>S. typhimurium</i> (TA1538, TA98)  | Gene mutations               | No data         | +                  | Kaplan and Kaplan<br>1982c |
| <i>S. typhimurium</i> (TA1535, TA1537, TA1538,<br>TA98, TA100)              | Gene mutations               | +               | +                  | Pearson et al. 1979        |
| <i>S. typhimurium</i> (TA1535, TA1537, TA1538,<br>TA98, TA100)              | Gene mutations               | + <sup>d</sup>  | + <sup>d</sup>     | Army 1978a, 1980c          |
| <i>S. typhimurium</i> (TA1535, TA1537, TA1538,<br>TA98, TA100)              | Gene mutations               | + <sup>d</sup>  | + <sup>d</sup>     | Army 1978c, 1979b          |
| <i>S. typhimurium</i> (TA98)  | Gene mutations               | No data         | + <sup>e</sup>     | Won et al. 1976            |
| <b>Mammalian cells:</b>   |                              |                 |                    |                            |
| TK <sup>+</sup> Mouse lymphoma cells  | Gene mutations               | -               | +                  | Styles and Cross 1983      |
| Human (WI-38 fibroblasts)   | Unscheduled DNA<br>synthesis | -               | +/-                | Army 1978c                 |

<sup>a</sup>Tested only with strain TA1538

<sup>b</sup>Strongest response in strains TA98, TA1538, TA1537; negative in strains TA1535, TA100NR

<sup>c</sup>Stronger response without S9 activation; negative with or without S9 in strains TA1535, TA100NR3

<sup>d</sup>Stronger response without S9 activation; response was also stronger with strains TA98, TA1538, TA1537

<sup>e</sup>Parent compound but not seven metabolites were positive

- = negative result; + = positive result; +/- = inconclusive result; DNA = deoxyribonucleic acid; NR = nitroreductase deficiency; TK = thymidine kinase

TABLE 2-3. Genotoxicity of 2,4,6-Trinitrotoluene *In Vivo*

| Species (test system)               | End point   | Results        | Reference            |
|-------------------------------------|---|----------------|----------------------|
| Mammalian cells:                    |   |                |                      |
| Rat (bone marrow)                   | Chromosome aberrations  | - <sup>a</sup> | Army 1978c           |
| Mouse (bone marrow)                 | Micronuclei induction   | - <sup>b</sup> | Ashby et al. 1985    |
| Mouse (liver)                       | Unscheduled DNA synthesis   | - <sup>c</sup> | Ashby et al. 1985    |
| Human (occupational exposure/urine) | Gene mutations in<br><i>Salmonella typhimurium</i><br>TA98 and TA98NR                           | + <sup>d</sup> | Ahlborg et al. 1988a |
| Human (occupational exposure/urine) | Gene mutation in<br><i>S. typhimurium</i> TA98,<br><i>Escherichia coli</i> WP <sub>2</sub> uvrA | + <sup>e</sup> | Ahlborg et al. 1985  |

<sup>a</sup>Negative after oral exposure but the study was compromised

<sup>b</sup>Intraperitoneal exposure

<sup>c</sup>Oral exposure

<sup>d</sup>Positive without auxiliary metabolism; response stronger in strain TA98

<sup>e</sup>Positive without auxiliary metabolism in strain TA98; negative in strain WP<sub>2</sub> uvrA with or without auxiliary metabolism

- = negative result; + = positive result; DNA = deoxyribonucleic acid; NR = nitroreductase deficiency

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parent compound induced a dose-related increase in mutant colonies of *S. typhimurium* TA98 over a concentration range of 2-10 µg/plate, the seven investigated metabolites were negative (Won et al. 1976). In a study using the same assay system, one of the tested metabolites (2-amino-4,6-dinitrotoluene) was positive, while the second one (4-amino-2,6-dinitrotoluene) was only slightly positive; both needed nitroreductase to induce mutagenicity (Spanggord et al. 1982b). Similarly, in a third study the four possible mono- and diamino metabolites of 2,4,6-trinitrotoluene were all less mutagenic than the parent compound in TA98 or TA100 (Tan et al. 1992). Mutagenicity in *Salmonella* tester strains seems dependent on endogenous nitroreductase activity. Strains deficient in nitroreductase show decreased sensitivity to 2,4,6-trinitrotoluene while strains constructed with increased nitroreductase activity show increased sensitivity (Einisto et al. 1991).

2,4,6-Trinitrotoluene is also capable of causing gene mutations in mammalian cells (Styles and Cross 1983). In a well-conducted study, 2,4,6-trinitrotoluene (8-1,000 µg/mL) caused dose-dependent cytotoxicity and significant increases in mutation at the TK<sup>+/-</sup> locus in mouse lymphoma cells. In agreement with other *in vitro* assay findings, S9 activation was not required to demonstrate the response. 2,4,6-Trinitrotoluene was negative under conditions of exogenous metabolic activation. No studies investigating potential clastogenic effects *in vitro* were found.

Although the database for *in vitro* genetic toxicology testing with 2,4,6-trinitrotoluene is limited, a high degree of concordance exists among different assay systems. Based on the existing information, there is sufficient valid *in vitro* data to conclude that 2,4,6-trinitrotoluene is a direct-acting mutagen in bacterial and mammalian cells. There is also suggestive evidence that 2,4,6-trinitrotoluene is a directacting genotoxic agent in cultured human cells. Refer to Table 2-2 for a further summary of these results.

**Cancer.** One preliminary epidemiological study of German populations living in the proximity of former munitions plants suggests 2,4,6-trinitrotoluene may increase leukemia rates in exposed adult human populations (Kolb et al. 1993). However, the case numbers in this study are very small. Also the proximity of the cases of leukemia to sites where 2,4,6-trinitrotoluene was manufactured during World War II or to disposal sites is not reported nor are any environmental 2,4,6-trinitrotoluene

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concentrations. Therefore, there is only slight circumstantial evidence linking the observed leukemia cases to possible 2,4,6-trinitrotoluene exposure. Furthermore, no investigation of confounding variables (e.g., benzene exposure or occupational exposure to carcinogens) has been done. Several female Fisher-344 rats developed urinary bladder carcinoma after exposure to 10 or 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a). A similar study conducted in B6C3F<sub>1</sub> mice showed that a statistically significant ( $p < 0.01$ ) incidence of leukemia and/or malignant lymphoma of the spleen was present in female mice receiving 70 mg/kg/day for 24 months (Army 1984b). On the basis of this result, EPA has classified 2,4,6-trinitrotoluene as a possible human carcinogen (Group C) (EPA 1989b).

### 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 2,4,6-trinitrotoluene are discussed in Section 2.5.1.

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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 2,4,6-trinitrotoluene are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, 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 2,4,6-Trinitrotoluene**

The availability of biomarkers is useful in estimating the degree of exposure in cases where the exposure is suspected or known. Identification of 2,4,6-trinitrotoluene in blood and urine is the most direct confirmation that exposure to 2,4,6-trinitrotoluene has occurred. Sensitive methods have been developed for determination of 2,4,6-trinitrotoluene and its metabolites in human blood and urine (for more information see Chapter 6).

Detection of 2,4,6-trinitrotoluene in the blood or urine is an indication of a recent dermal, oral, or inhalation exposure. However, since 2,4,6-trinitrotoluene is rapidly metabolized, it may be difficult to determine trace amounts of the unchanged compound in blood or urine. In such cases, identification of major 2,4,6-trinitrotoluene metabolites such as 4-ADNT and 2-ADNT in the urine can be used as an indication of exposure. In one case of acute, primarily dermal exposure to 2,4,6-trinitrotoluene, these two metabolites were present in the urine of exposed workers 17 days after exposure (Woolen et al. 1986). This finding indicates that they can be used as indicators of not only recent, but also past, acute exposures to 2,4,6-trinitrotoluene. Another early sign of 2,4,6-trinitrotoluene exposure is the

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change of urine color that can range in humans from abnormal amber to deep red (Army 1978a). The identification of the metabolite responsible for this color change would represent a good biomarker for early detection of 2,4,6-trinitrotoluene exposure.

In the study on distribution of 2,4,6-trinitrotoluene after oral, inhalation and dermal exposure of rats, mice, rabbits and dogs it was found that 2,4,6-trinitrotoluene distributes to fat (Army 1981d). Therefore the lipid concentration of 2,4,6-trinitrotoluene could be used as a potential biomarker of exposure, provided adequate methodology is available.

No information was found on tissue levels of 2,4,6-trinitrotoluene after relatively long-term exposure to constant levels of 2,4,6-trinitrotoluene. However, since absorption, biodegradation, and excretion occur rapidly, it can be assumed that the presence of 2,4,6-trinitrotoluene metabolites may be used to identify exposure. Because of rapid conversion of 2,4,6-trinitrotoluene into its metabolites, it is also reasonable to assume that long-term exposure will not lead to a steady state of 2,4,6-trinitrotoluene levels.

### **2.5.2 Biomarkers Used to Characterize Effects Caused by 2,4,6-Trinitrotoluene**

Prior to the use of blood and urine levels to monitor exposure to 2,4,6-trinitrotoluene (during the two World Wars, and especially during World War I), jaundice was one of the main indicators of 2,4,6-trinitrotoluene intoxication. However, jaundice is a sign of serious hepatic toxicity, that develops over a period of time, and it is not useful as an early indicator compared to other signs such as urine discoloration. Since jaundice is a latent phenomenon, many cases had fatal outcomes before the jaundice was observed. In addition, jaundice is not specific for 2,4,6-trinitrotoluene exposure and may be caused by other factors.

Decreased hemoglobin and hematocrit levels and increased reticulocyte numbers are among the first changes to occur after exposure to sufficiently high levels of 2,4,6-trinitrotoluene (Army 1976). The levels of these three blood parameters reflected in a complete blood count can be used as nonspecific biomarkers; their determination is rapid, relatively inexpensive, and useful for monitoring cohorts of persons possibly exposed to 2,4,6-trinitrotoluene.



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An early reaction to 2,4,6-trinitrotoluene intoxication is an increase in mononuclear leukocytes, which seems to precede any other symptom and remains positive for 2-3 months (Army 1978a). Also found in nine fatal cases of 2,4,6-trinitrotoluene poisoning was a significant increase in lymphocyte counts (Army 1974a). The extent and significance of these findings need further elucidation since both are commonly present in a number of other pathological states.

Changes in the hepatic enzymes SGOT and LDH were noted after the levels of 2,4,6-trinitrotoluene increased from 0.3 to 0.8 mg/m<sup>3</sup> (Morton et al. 1976). In another study, however, no changes in liver function were seen in 626 munitions workers exposed to an average of 0.5 mg/m<sup>3</sup> (Army 1976). An explanation for these different findings may be that new or increased exposure to 2,4,6-trinitrotoluene causes more liver toxicity in potentially susceptible workers, while in cases of longer exposure liver cells may adapt to moderate exposure levels (Hathaway 1985). Although many other substances and diseases can cause changes in the levels of hepatic enzymes, a record of pre-exposure levels could resolve these problems and allow for the use of hepatic enzymes as effective exposure markers. It is possible that in the future a battery of tests to indicate liver disease could be used to identify the causal agent. For example, cholyglycine is a bile acid that accumulates in serum in cases of hepatic dysfunction, and interleukin-1 is indicative of inflammation. Both of these markers were found elevated in all patients with viral hepatitis, but only 37.5% or 25% were positive, respectively, in 2,4,6-trinitrotoluene-induced liver damage (Li et al. 1992).

Another potential adverse effect of 2,4,6-trinitrotoluene exposure is the formation of cataracts. It is believed to be specific to 2,4,6-trinitrotoluene and is often associated with chronic, low-level exposure (Hathaway 1985). Bilateral, symmetrical equatorial lens opacities were reported in workers exposed to 2,4,6-trinitrotoluene for an average of 6.8 years (Harkonen et al. 1983).

Two cases of contact dermatitis were reported in workers after an intermediate exposure to 2,4,6-trinitrotoluene (Goh 1988; Goh and Rajan 1983). On the basis of this information, skin patch testing could be done to detect individuals potentially hypersensitive to 2,4,6-trinitrotoluene.

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### 2.6 INTERACTIONS WITH OTHER CHEMICALS

Limited information was located regarding the influence of other chemicals on the toxicity of 2,4,6-trinitrotoluene. However, one extensive animal study evaluated the acute and intermediate effects of a mixture of 2,4,6-trinitrotoluene and 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX) on rats, mice, dogs, and rabbits (Army 1978c). The mixture of the two compounds is designated as LAP (load, assemble, and pack) mixture. The ratio of 2,4,6-trinitrotoluene and RDX in the LAP mixture in this study was 1.6:1. Acute oral toxicity of the LAP mixture was investigated in rats, mice, and rabbits. The results indicate that there was a distinct species difference regarding acute oral toxicity after exposure to the LAP mixture. The acute oral LD<sub>50</sub> values indicate that rats were more susceptible to toxic effects of the LAP mixture than to 2,4,6-trinitrotoluene alone. The opposite was true for mice which were more resistant to the LAP mixture than to 2,4,6-trinitrotoluene (Army 1978c). LAP applied to the eyes of rabbits produced conjunctivitis, iritis, and/or corneal opacity. Intermediate oral toxicity was determined in a 90-day exposure study in rats, mice, and dogs. The results indicate that the main target organs for LAP toxicity are the same as those for 2,4,6-trinitrotoluene, namely blood and liver. Mild-to-moderate hemolytic anemia, enlarged spleens and livers, hemosiderosis of the spleen, and colored urine were common effects of intermediate exposure to LAP seen in all three species. LAP-induced testicular atrophy (dogs and rats), uterine hypoplasia (rats), and numerous neurological signs (dogs) were also observed. These observations indicate that 2,4,6-trinitrotoluene was the principal, but not the only factor, contributing to the intermediate oral toxicity of LAP; some of the observed toxicity is due to RDX (Army 1978c).

Because 2,4,6-trinitrotoluene is rapidly degraded in the environment, it is possible that it would interact with its degradation products to amplify adverse health effects. However, it is not known how the interaction of 2,4,6-trinitrotoluene with these co-contaminants affects or alters predicted health effects.

### 2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 2,4,6-trinitrotoluene than will most persons exposed to the same level of 2,4,6-trinitrotoluene in the environment. Reasons include

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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 that the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. 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 that are known to be unusually susceptible to toxic effects of 2,4,6-trinitrotoluene. However, in the review of the literature one report described the occurrence of acute hemolytic disease (Djerassi and Vitany 1975) in three individuals deficient in GGPD enzyme. All three developed hemolytic crisis with similar symptoms 2-4 days after being exposed to 2,4,6-trinitrotoluene. All three also recovered and were complication free at 5- and 10-year followup. Populations that may show increased sensitivity to 2,4,6-trinitrotoluene would include very young children, who have immature hepatic detoxification systems; individuals with impaired liver function, including alcoholics (Li et al. 1991), or impaired kidney function; and those who are prone to anemia or who are anemic. Also at increased risk may be individuals with such genetic traits as G6PD deficiency, sickle cell trait, genetically induced unstable hemoglobin forms, or congenital hypercholesterolemia. Another subpopulation that may be at increased risk is comprised of individuals with a potential immune reaction to 2,4,6-trinitrotoluene.

### **2.8 METHODS FOR REDUCING TOXIC EFFECTS**

This section will describe the clinical practice and research concerning methods for reducing toxic effects of exposure to 2,4,6-trinitrotoluene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to 2,4,6-trinitrotoluene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

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### 2.8.1 Reducing Peak Absorption Following Exposure

No chemical-specific recommendations have been reported for reducing absorption following 2,4,6-trinitrotoluene exposure via any route (Haddad and Winchester 1990; HSDB 1994). General methods for reducing exposure can be found for explosives (Bronstein and Currance 1988). Other sources recommend the same treatment for overexposure to 2,4,6-trinitrotoluene as for aniline (Gosselin et al. 1984) or aromatic nitro compounds (Stutz and Ulin 1992). General procedures suggested for reducing absorption following accidental industrial exposure include moving the exposed person into fresh air, removing contaminated clothing and shoes, and flushing exposed skin or eyes with running water (HSDB 1994).

In recent years, there have been very few reported cases of overexposure via inhalation or dermal contact because simple industrial hygiene methods are used to effectively prevent contact with high concentrations of 2,4,6-trinitrotoluene in the workplace. However, during both World Wars some cases of industrial exposure have resulted in fatalities (Haddad and Winchester 1990).

Oral exposure to toxic quantities of 2,4,6-trinitrotoluene have not been reported for humans, although it is possible that some of the cases of overexposure of workers were caused in part by inadvertent ingestion via hand-to-mouth contact as well as by inhalation and dermal contact with 2,4,6-trinitrotoluene. In general, only supportive treatment has been recommended (HSDB 1994). In some cases, gastric lavage, activated charcoal, and emetics have been suggested as useful in reducing absorption of the general class of nitro compounds to which 2,4,6-trinitrotoluene belongs (Gosselin et al. 1984; Stutz and Ulin 1992). Other sources state emesis should not be used for explosives (Bronstein and Currance 1988).

### 2.8.2 Reducing Body Burden

No quantitative studies of human retention or elimination of 2,4,6-trinitrotoluene were located. Acute laboratory studies of animals show rapid elimination in the urine of 2,4,6-trinitrotoluene and its metabolites when it is administered orally or dermally. Historically, only a small proportion of the munitions workers during World War I and II who were exposed to high concentrations of

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2,4,6-trinitrotoluene experienced hepatic disease. The only treatment was to remove the affected workers from areas where they could be exposed to 2,4,6-trinitrotoluene. Because the onset of symptoms was frequently delayed for 1 to 4 months following exposure, in some cases no symptoms appeared until days or weeks after exposure to 2,4,6-trinitrotoluene had ended (Haddad and Winchester 1990).

A more recent study of workers exposed to 2,4,6-trinitrotoluene found that a subgroup of workers showed signs of liver cell irritation. These workers were removed from areas with 2,4,6-trinitrotoluene exposure and fed diets high in protein and calories. After 3 weeks, signs of liver cell irritation disappeared, and they were returned to work requiring contact with 2,4,6-trinitrotoluene (Goodwin 1972).

In some cases of exposure to 2,4,6-trinitrotoluene, methemoglobinemia has been reported. This effect is independent of hepatic damage. Some sources suggest treatment of methemoglobinemia with methylene blue (Ellenhorn and Barceloux 1988; Gosselin et al. 1984; Stutz and Ulin 1992). However, it has been noted that methylene blue should be used with caution (Stutz and Ulin 1992) especially if there is a possibility of glucose-6-phosphate dehydrogenase (G6PD) deficiency (Ellenhorn and Barceloux 1988).

### **2.8.3 Interfering with the Mechanism of Action for Toxic Effects**

The mechanism of toxic action by 2,4,6-trinitrotoluene and the compounds formed by the metabolism of 2,4,6-trinitrotoluene are not known. A theory is that, at a biochemical level, 2,4,6-trinitrotoluene or its metabolites generate active oxygen species (Kong et al. 1989; Liu et al. 1992; Savolainen et al. 1985). No information was located on established therapies designed to interfere with this possible mechanism of action of 2,4,6-trinitrotoluene. Because 2,4,6-trinitrotoluene is known to cause liver damage and may decrease glutathione concentrations (Liu et al. 1992), it is possible an intervention that acts by increasing the cellular concentrations of antioxidants (especially glutathione, glutathione peroxidase, and those that are lipid soluble such as vitamin E) could reduce liver damage caused by 2,4,6-trinitrotoluene.

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The impetus to search for an agent that reduces the toxicity of 2,4,6-trinitrotoluene or its metabolites is generally lacking since the use of good hygiene in the workplace has eliminated the health effects formerly detected in the United States and Western Europe. It should be noted that with industrial or military accidents involving large quantities of 2,4,6-trinitrotoluene, the most immediate critical hazard is one of explosion or fire (HSDB 1994; Stutz and Ulin 1992).

### 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 2,4,6-trinitrotoluene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 2,4,6-trinitrotoluene.

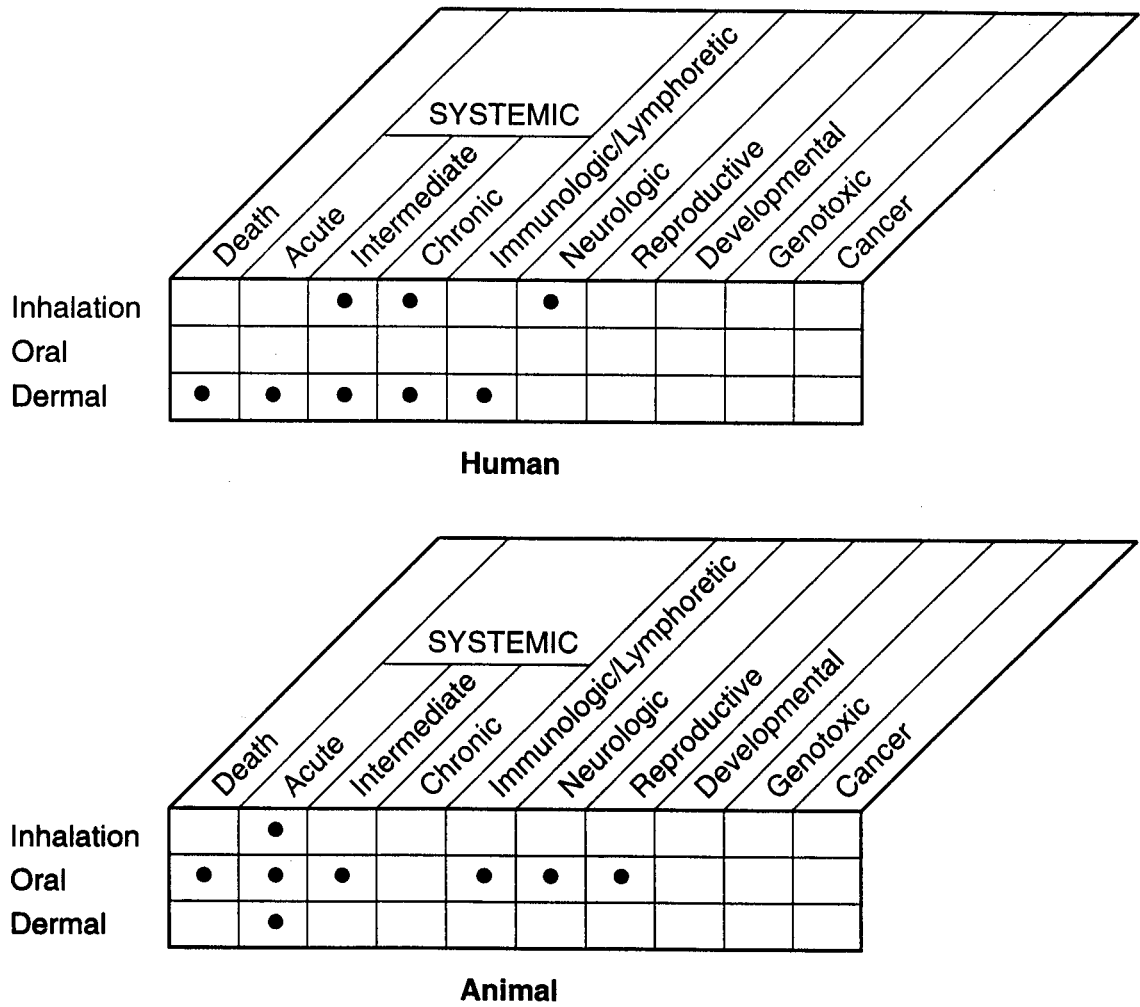
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. This definition should not be interpreted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

#### 2.9.1 Existing Information on Health Effects of 2,4,6-Trinitrotoluene

The existing data on health effects of inhalation, oral, and dermal exposure of humans to 2,4,6-trinitrotoluene are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of 2,4,6-trinitrotoluene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Missing information in this figure should not be interpreted as “data needs” information (i.e., data gaps that must necessarily be filled).

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**FIGURE 2-3. Existing Information on Health Effects of 2,4,6-Trinitrotoluene**



● Existing Studies

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Recent literature pertaining to the health effects of 2,4,6-trinitrotoluene in humans described case reports and retrospective, intermediate- and chronic-duration studies of workers employed in the manufacture of ammunition. No acute human exposure data were located. For those employed in the manufacturing process using 2,4,6-trinitrotoluene, the major routes of exposure are dermal and inhalation. Thus, information on intermediate- and chronic-duration exposures comes exclusively from dermal and inhalation exposure data. Information from occupational and retrospective studies is frequently limited by a lack of data regarding quantification of doses and precise duration of exposure. No information was located regarding developmental, reproductive, genotoxic, or cancer effects in humans by any route. Information on any effects in humans after oral exposure to 2,4,6-trinitrotoluene was also not found.

Virtually all of the data on animals regarding health effects from 2,4,6-trinitrotoluene exposure were obtained from studies in which 2,4,6-trinitrotoluene was administered orally. Over the past 25 years, the majority of the extensive studies done in several mammalian species (i.e., rat, mouse, and dog) were performed for the Army. One of those studies addresses the effects of 2,4,6-trinitrotoluene after dermal exposure and intratracheal (performed under anesthesia) instillation.

Releases from hazardous waste sites or from military installations involved in the manufacture of 2,4,6-trinitrotoluene and the processing of munitions that contain the compound are the main sources of potential exposure of the general population to 2,4,6-trinitrotoluene. Because persons living in the vicinity of these two types of sites may be exposed by oral, dermal, or inhalation routes, additional information on the effects via these routes would be valuable.

### 2.9.2 Identification of Data Needs

**Acute-Duration Exposure.** Available data are not sufficient to derive acute oral or inhalation MRLs. There are no data regarding acute-duration exposure of humans to 2,4,6-trinitrotoluene by inhalation or oral routes. Additional studies in acute oral exposures to 2,4,6-trinitrotoluene are needed to determine the threshold level for neurological effects because the only available data demonstrate serious neurological effects in a single oral LD<sub>50</sub> study in rodents (Dilley et al. 1982b). Populations



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living in the vicinity of ammunition plants or hazardous waste disposal sites may be exposed to 2,4,6-trinitrotoluene. Exposure would most probably occur via the dermal route, but there is a possibility that inhalation and oral exposures may occur. A dermal patch test for 2,4,6-trinitrotoluene was positive in one case of contact dermatitis in a worker previously exposed to 2,4,6-trinitrotoluene (Goh 1988). In a week-long study of workers handling 2,4,6-trinitrotoluene, two metabolites (aminodinitrotoluenes) were present in the urine: 4-ADNT and 2-ADNT. No other effects of exposure were reported (Woolen et al. 1986). Renal effects were observed in mice and rats (discoloration of the urine) after oral exposure to a relatively high dose of 2,4,6-trinitrotoluene (Dilley et al. 1982b). Other signs of exposure were inactivity, tremors, and death in both species. Since populations living in the vicinity of ammunition plants may be exposed to 2,4,6-trinitrotoluene by all three routes, animal studies of exposure via all three routes would be useful in elucidating possible effects in humans after acute exposure.

**Intermediate-Duration Exposure.** One occupational intermediate-duration study of inhalation exposure to low levels of 2,4,6-trinitrotoluene in workers found a significant increase in the hepatic enzymes SGOT and LDH when the air concentration was increased from 0.3 to 0.8 mg/m<sup>3</sup> (Morton et al. 1976). Unspecified respiratory difficulties were reported in some workers. Hemoglobin levels remained within a normal range. Because of the very low exposure level, it is difficult to determine the major target organ since adaptation can mask changes in circulating hemoglobin levels. Another occupational report identified the immunological system as the target organ in a case of contact dermatitis (Goh and Rajan 1983). However, studies in laboratory animals following intermediateduration oral exposure did confirm the blood as a major target organ (Dilley et al. 1982b; Jiang et al. 1991; Levine et al. 1984, 1990a, 1990b). Other target organs identified in intermediate-duration oral studies include liver, kidney, gastrointestinal tract, spleen, central nervous system, and reproductive system (Dilley et al. 1982b; Levine et al. 1984, 1990a, 1990b). An intermediate-duration oral MRL for 2,4,6-trinitrotoluene has been calculated based on a study that noted dose-related liver effects in dogs receiving 0.5 mg/kg/day for 6 months (Levine et al. 1990b).

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**Chronic-Duration Exposure and Cancer.** Few studies have been conducted on workers that have been chronically exposed to 2,4,6-trinitrotoluene. Increased levels of zinc and copper were found in hair samples of workers exposed for 3-7 years, mainly by the inhalation route (Jiang et al. 1991). Three other studies, two of inhalation (Harkonen et al. 1983; Savolainen et al. 1985) and one of dermal exposure (Harkonen et al. 1983), showed the occurrence of varying degrees of cataracts in exposed workers. Another effect after chronic inhalation exposure was the decreased activity of two mitochondrial enzymes necessary for heme synthesis: heme synthase and S-aminolevulinic acid synthase (Savolainen et al. 1985). More studies are needed to elucidate the meaning of that finding and its role in the possible development of anemia. One case of cirrhosis was reported after chronic occupational exposure (35 years) to 2,4,6-trinitrotoluene (Garfinkel et al. 1988). 2,4,6-Trinitrotoluene is known to cause hepatic damage, but it is not known whether it caused the cirrhosis,

The major adverse effects observed in Fisher rats fed 50 mg/kg/day for 24 months were anemia, hepatotoxicity and splenic lesions (Army 1984a). On the basis of this study's finding, chronicduration exposure to the intermediate-duration oral MRL of 0.0005 mg/kg/day (same value as the RfD) would not be anticipated to cause adverse health effects.

One case of hepatocellular carcinoma was reported after chronic occupational exposure (35 years) to 2,4,6-trinitrotoluene (Garfinkel et al. 1988). Carcinoma of the urinary bladder was observed in Fisher rats (in females only) fed 50 mg/kg/day for 24 months (Army 1984a). On the basis of these findings of bladder carcinoma, 2,4,6-trinitrotoluene was classified by EPA as a possible human carcinogen-- Group C (EPA 1989b). Additional animal studies on chronic dermal and inhalation exposures would clarify if bladder carcinoma occurs after exposure by those routes, and if it is sex related.

**Genotoxicity.** Evidence that mutagenic substances are present in human urine comes from studies of occupationally exposed individuals (Ahlborg et al. 1985, 1988a). These exposures occurred via both inhalation and dermal routes, although the latter one appears to be more important in 2,4,6-trinitrotoluene uptake. The lack of a correlation between mutagenesis and 2,4,6-trinitrotoluene concentration in the urine of exposed workers is of considerable relevance and should be thoroughly investigated. The major metabolites of 2,4,6-trinitrotoluene are known to exert a mutagenic effect in

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bacteria. It therefore appears likely that biotransformation products are responsible for the genotoxic activity in the urine of exposed workers since 2,4,6-trinitrotoluene concentrations are low. Similarly, the dose-response relationship between 2,4,6-trinitrotoluene exposure and the mutagenicity of exposed worker urine should be fully characterized in order to establish the urine assay as a reliable biomarker for exposure screens.

The limited whole animal studies provide assurance that 2,4,6-trinitrotoluene is not genotoxic in somatic cells. However, no information related to potential adverse effects on germinal cells was found; therefore, studies (e.g., dominant lethal mutation assay) should be considered to ensure that all relevant genetic end points have been investigated. There is reliable evidence that 2,4,6-trinitrotoluene induces mutations in bacterial (Army 1978a,c, 1979b, 1980c; Spanggord et al. 1982b) and mammalian cells (Styles and Cross 1983) and inconclusive evidence that 2,4,6-trinitrotoluene causes UDS in cultured human fibroblasts (Army 1978c). The relevance of these *in vitro* findings to human health should not be underestimated. The weight of evidence clearly suggests that the genotoxic activity of 2,4,6-trinitrotoluene is markedly inhibited or abolished in the presence of exogenous metabolic activation systems. Thus, the potential hazard to human genetic material resulting from exposure to 2,4,6-trinitrotoluene is very limited. It is, therefore, doubtful whether the performance of additional *in vitro* assays would substantially alter the established genetic toxicology profile of 2,4,6-trinitrotoluene. Confirmation of the mammalian cell assay findings is, nevertheless, desirable to establish full confidence in the validity of the existing data.

**Reproductive Toxicity.** Significantly lower semen volumes, a smaller percentage of motile spermatozoa, and a higher incidence of sperm malformation were reported in a case-control study in two 2,4,6-trinitrotoluene plants in China (Li et al. 1993). However, exposure to 2,4,6-trinitrotoluene was not estimated. No studies were found describing reproductive effects of 2,4,6-trinitrotoluene in human females. Studies in laboratory animals show dose-dependent reproductive toxicity after intermediate oral exposure to 2,4,6-trinitrotoluene. Testicular atrophy, degenerated germinal epithelium, and atrophic seminiferous tubules were effects observed in male rats after exposure to high doses of 2,4,6-trinitrotoluene (Levine et al. 1984). Additional studies of these effects after dermal and inhalation exposure would be helpful in determining if the effects are specific for the oral exposure

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route. The results from one rat study indicate that there is a close correlation between reduction of testes weight and decreased zinc concentration (Jiang et al. 1991). The role of zinc and its possible effect on the male sex function needs further investigation. No studies were located that examined reproductive toxicity in females.

**Developmental Toxicity.** No human or animal studies were located on developmental effects for any exposure route. Studies in animals assessing postnatal survival after maternal exposure by all three routes would be useful.

**Immunotoxicity.** Very little information was located on immunological effects in humans after dermal and inhalation exposure to 2,4,6-trinitrotoluene. An increase in mononuclear leukocytes and lymphocytes was noted in a retrospective study of blood counts of exposed munitions workers (Army 1978a). The significance of this finding needs further investigation since no such data were presented in animal studies. Two isolated cases of allergic contact dermatitis were described in workers handling 2,4,6-trinitrotoluene (Goh 1988; Goh and Rajan 1983). The importance of 2,4,6-trinitrotoluene as an allergen needs to be examined further in order to understand the mechanisms involved in the possible development of hypersensitivity to 2,4,6-trinitrotoluene.

Dose-dependent immunological reactions were seen in mice, rats, and dogs after intermediate oral exposure to 2,4,6-trinitrotoluene. Spleen enlargement and/or increased weight was the most often observed effect (Dilley et al. 1982b; Levine et al. 1984, 1990a). Histopathology revealed hemosiderosis and varying degrees of splenic congestion. Further studies of immunological effects in animals after chronic exposure would be important for estimating human susceptibility for populations potentially exposed in the vicinity of ammunition plants.

**Neurotoxicity.** Limited information regarding neurological effects in humans indicates only minor effects such as altered taste (Morton et al. 1976). Dose-related changes in behavior were observed in several animal species after acute and intermediate oral exposure to 2,4,6-trinitrotoluene. Most common were depression (Dilley et al. 1982b), lethargy, and ataxia (Levine et al. 1990b). Brain lesions were present in rats receiving the highest dose of 2,4,6-trinitrotoluene (Dilley et al. 1982b;

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Levine et al. 1984). The results also show species differences in 2,4,6-trinitrotoluene-induced neurotoxicity, with dogs being more sensitive than rats or mice. Dose-response studies in animals and studies focusing on the mechanism of 2,4,6-trinitrotoluene-induced neurotoxicity would be useful in better understanding the possible neurotoxicity of 2,4,6-trinitrotoluene in humans.

**Epidemiological and Human Dosimetry Studies.** Human studies on 2,4,6-trinitrotoluene consist of either retrospective studies of occupational exposure or case reports of workers employed in the manufacture of munitions. Exposures in both cases are primarily dermal and by inhalation. Locating populations for future epidemiological studies will be possible as long as 2,4,6-trinitrotoluene is produced and used in the manufacture of munitions. The two subgroups with the greatest possible exposure to 2,4,6-trinitrotoluene are those employed in the manufacturing process and those living in the vicinity of military installations/ammunition plants. If such groups are located, information regarding the immunologic, reproductive, developmental, genotoxic, and cancer effects and correlation of these effects with blood or urine levels of 2,4,6-trinitrotoluene that are associated with exposure would be extremely useful.

**Biomarkers of Exposure and Effect.** Exposure to 2,4,6-trinitrotoluene is currently measured by determining the level of 2,4,6-trinitrotoluene in the blood or urine. However, since 2,4,6-trinitrotoluene is rapidly metabolized it may be difficult to determine trace amounts of unchanged compound in either blood or urine. In such cases, the presence of major 2,4,6-trinitrotoluene metabolites such as 4-ADNT and 2-ADNT metabolites, which are present in the urine for over 2 weeks after acute exposure, can be used to indicate recent and past exposures (Woolen et al. 1986). Although the sensitivity of these biomarkers seems to be sufficient at the present time, it would be useful to determine the metabolite responsible for urine color change that occurs after exposure to 2,4,6-trinitrotoluene. Identification of this metabolite in urine would provide an early biomarker of 2,4,6-trinitrotoluene exposure. Since 2,4,6-trinitrotoluene has been found to bind to blood and liver proteins, the identity of these adducts and their tissue lifetimes would greatly enhance the use of these biomarkers as a measure of exposure.

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Decreases in hemoglobin and hematocrit levels and increases in reticulocyte numbers can be monitored and accurately determined after exposure to 2,4,6-trinitrotoluene. In some cases of prolonged exposure, these changes lead to the development of an anemic state. However, the changes in these blood parameters are not specific for exposure to 2,4,6-trinitrotoluene. What would be useful is a better understanding of the fundamental mechanism by which 2,4,6-trinitrotoluene causes changes in hemoglobin and hematocrit levels. Such an understanding might ultimately lead to the development of antidotes to decrease or completely alleviate some of the toxic effects caused by 2,4,6-trinitrotoluene.

There are no tests that are specific for 2,4,6-trinitrotoluene-induced hepatic toxicity, but standard liver function tests should be able to identify hepatic toxicity caused by 2,4,6-trinitrotoluene. Although not specific for 2,4,6-trinitrotoluene exposure, more information is needed on changes in indicators of 2,4,6-trinitrotoluene-induced hepatotoxicity, for example, SGOT and LDH levels.

Jaundice may develop as a result of 2,4,6-trinitrotoluene exposure. Since jaundice is a late phenomenon, it is important to be sure that it is due to serious hepatic injury and not merely to the yellowing of the body's surface exposed to the compound.

Cataract formation is believed to be a specific 2,4,6-trinitrotoluene effect, developing primarily after chronic exposures (Harkonen et al. 1983; Savolainen et al. 1985). Since initial changes are small and often difficult to detect, development of more sensitive detection techniques would allow for earlier detection and prevention of potential adverse ocular effects due to 2,4,6-trinitrotoluene exposure. This is important because in the course of chronic occupational exposure, cataract formation may be the only sign of 2,4,6-trinitrotoluene toxicity. Additional studies are also needed to establish if cataract formation is a dose-response phenomenon.

**Absorption, Distribution, Metabolism, and Excretion.** The majority of information regarding the rapid absorption of 2,4,6-trinitrotoluene in humans and animals comes indirectly from detectable levels of 2,4,6-trinitrotoluene metabolites in the urine after inhalation, oral, or dermal exposures. Occupational studies indicate that humans readily absorb 2,4,6-trinitrotoluene dusts via inhalation or dermal contact, but quantitative data are lacking. Animal studies indicate that 2,4,6-trinitrotoluene is

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absorbed relatively quickly and that the absorbed amount is dose related for the oral route (Army 1981d). Information concerning absorption rates for all three routes is needed. Because there are few absorption studies for all three routes, additional quantitative data in animals would be useful as a basis for estimates of absorption in humans.

No studies were located regarding distribution following inhalation, oral, or dermal exposure to 2,4,6-trinitrotoluene in humans; limited information is available regarding distribution in animals after acute exposure to radiolabelled 2,4,6-trinitrotoluene via all three routes. These studies in animals indicate that 2,4,6-trinitrotoluene is rapidly distributed to blood, liver, fat, and skeletal muscle, but in very small amounts because the majority of the label was recovered from the gastrointestinal tract and urine (Army 1981d). Additional animal distribution studies on dermal exposure would be valuable to establish the biological half-lives in relevant tissues and because there is a potential for human exposure to occur via this route.

No studies were located regarding the metabolism of 2,4,6-trinitrotoluene in humans after oral exposure. Data for the oral route are important because there is a potential for human exposure to occur via this route near waste sites containing 2,4,6-trinitrotoluene. Limited information comes from the analysis of urine of munitions workers after dermal (Woolen et al. 1986) or inhalation exposures (Hassman and Hassmanova 1976). The results from human and animal studies indicate that 2,4,6-trinitrotoluene is readily metabolized and that very small amounts of unchanged compound are present in the urine. More studies are needed to define sex-related and species-related metabolic differences. Since differences in metabolism may occur with differences in the route of exposure, additional information is needed from inhalation and dermal metabolic studies in order to fully characterize the metabolic pathway of 2,4,6-trinitrotoluene.

No studies were located regarding excretion after oral or dermal exposure to 2,4,6-trinitrotoluene in humans. The results from animal studies indicate that urine is the major site of radiolabel recovery after a single oral dose of 2,4,6-trinitrotoluene and that differences in excretion rate are exposure routedependent (Army 1981d). The recovery of radiolabel indicates that excretion was most efficient after inhalation exposure, followed by oral, and was least efficient after dermal exposure. Additional, more

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detailed studies on excretion after dermal exposure would be useful since that is an expected major exposure route for humans in the vicinity of waste sites and workers handling 2,4,6-trinitrotoluene.

**Comparative Toxicokinetics.** Studies using different animal species (rats, mice, dogs, rabbits) indicate the kinetics of 2,4,6-trinitrotoluene differ across species. The observed differences are primarily quantitative (Army 1981b). On the basis of kinetic data alone, it is not possible to identify target organs common to humans and animals, but distribution data together with toxicity data after oral exposure suggest similar target organs: blood, liver, spleen, the kidneys and gastrointestinal tract. Interspecies differences, especially in metabolism and excretion have been noted in rats, mice, dogs, and rabbits (Army 1981b). Further animals studies covering all three exposure routes would be helpful in determining similarities and differences in absorption, metabolism and excretion between humans and animals.

**Methods for Reducing Toxic Effects.** Animal studies addressing the possibility of diminishing or alleviating toxic effects of 2,4,6-trinitrotoluene are needed since no information was located on possible 2,4,6-trinitrotoluene antidotes.

### 2.9.3 On-going Studies

On-going studies regarding the health effects of 2,4,6-trinitrotoluene were reported in the Federal Research in Progress File (FEDRIP 1991) database. One study addressing health effects of 2,4,6-trinitrotoluene in humans, "Deposition of volatile aerosols in the respiratory tract," is being investigated at the University of Rochester, Rochester, New York, by Sidney Soderholm, principal investigator. The sponsoring organization is the National Institute of Environmental Health Sciences,

No additional studies have been reported in the FEDRIP 1994, but it is likely that research in China on industrial exposure will continue (Li et al. 1991, 1992, 1993). The studies in Germany of populations surrounding former munitions manufacturing and disposal areas are being refined (Kolb et al. 1993).