Support Document

for

1,3,5-Trinitrobenzene (TNB)

(CAS No. 99-35-4)

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In Support of Summary Information on
INTEGRATED RISK INFORMATION SYSTEM (IRIS)

United States Environmental Protection Agency
Office of Research and Development
National Center for Environmental Assessment
and
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Cincinnati, Ohio 45268

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FOREWORD

This document provides health risk assessment of TNB (1,3,5-trinitrobenzene, CAS Number 99-35-4). Information pertaining to non-cancer effects were previously assessed by the United States Environmental Protection Agency (U.S. EPA) in a 1989 Health and Environmental Effects Document and by the Reference Dose (RfD) Work Group in 1988 (U.S. EPA, 1997). In view of the currently available subchronic/chronic toxicity studies, it became necessary to reevaluate the previously developed RfD for TNB. The new RfD is substantially higher than the one developed in 1988.

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ABSTRACT

Data pertaining to the potential adverse health effects of TNB (1,3,5-trinitrobenzene) are reviewed. TNB has been tested in feeding studies in rats and mice *(Peromyscus leucopus)* of both sexes. Short-term and long-term lifetime exposure studies (F344 rat) did not provide any evidence of carcinogenicity of TNB. TNB has been detected at high levels as an environmental contaminant of ground water and soils near trinitrotoluene (TNT) production sites and at Army installations. The U.S. EPA in 1988 developed an RfD for TNB (5E-5 mg/kg/day) based on analogy to dinitrobenzene (DNB). Recently, however, oral subchronic/chronic studies in rats exposed to TNB conducted for the U.S. Army are available for review and evaluation. Available information on nitroaromatics, such as DNB, TNT and tetryl, have clearly demonstrated potential toxicity such as methemoglobinemia, anemia, reproductive failure and CNS effects in hamsters, mice and rats. TNB showed similar hematological effects in oral rat subchronic and chronic studies and is being reviewed in this document. Based on the results of these studies, a NOAEL of 2.68 mg/kg/day and a LOAEL of 13.31 mg/kg/day (both sexes) for hematological effects were estimated. Using an uncertainty factor of 100 (10 for inter-, 10 for intra-species extrapolation), an RfD for TNB is estimated as 3E-2 mg/kg/day, which is an approximate 600-fold increase in the RfD using new risk-based research.

1. INTRODUCTION

Nitroaromatics, such as 1,3-dinitrobenzene (DNB), 1,3,5-trinitrobenzene (TNB) and N-methyl-N,2,4,6-tetranitroaniline (tetryl), have been detected as environmental contaminants of groundwater and soil near production sites and in some instances at military test grounds. TNB is formed as a by-product during 2,4,6-trinitrotoluene (TNT) production and can be formed through photochemical oxidative degradation of TNT manufacturing (Spanggord et al., 1982). TNB is not easily biodegradable, persists in the environment, eventually leaches out, and contaminates groundwater near waste disposal sites. Exposure to TNB can occur through contact with waste waters and soil at the original production sites and other plants devoted to munitions assembly which contain large quantities of these nitroaromatic compounds (Reddy et al., 1997).

Toxicity data on these compounds are limited. The oral LD_{50} of DNB, TNB and tetryl were 59 mg/kg, 284 mg/kg and greater than 5 g/kg, respectively, in rats for combined sexes. TNB and tetryl were not toxic at 2 g/kg when applied to rabbit skin for 24 hours; however, the dermal LD_{50} of DNB was 1.99 g/kg for combined sexes of rabbits. None of these compounds produced skin irritation but positive (DNB) and severe (TNB, tetryl) eye irritation potentials in rabbits were noted. The sensitization tests showed that DNB and tetryl are not skin sensitizers while TNB caused a mild allergic reaction in guinea pigs (FitzGerald et al., 1992a,b). Some of the toxicological effects of TNB in hamsters, rats and mice are formation of methemoglobin, testicular degeneration and reproductive failure, weight loss and anemia. Neurological and hematological disorders have also been reported in dogs. DNB, TNB and tetryl have been shown to be genotoxic in the *Salmonella* mutagenesis assay (McGregor et al., 1980) while TNB and DNB have been shown to form adducts with blood proteins and tissue DNA in rats (Reddy et al., 1991).

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

1,3,5-Trinitrobenezene is also known as syn-trinitrobenzene and by the acronym TNB. Some relevant physical and chemical properties of TNB are listed below (U.S. EPA, 1989).

CASRN: 99-35-4
Empirical Formula: $C_6H_3N_3O_6$
Molecular Weight: 213.11
Vapor Pressure: 3.2×10^{-6} mm Hg at 20°C
Water Solubility: 0.034 g/100 g at 20°C
Log K_{ow}: 1.18
Conversion Factor: 1 ppm = 8.66 mg/cu.m
1 mg/cu.m = 0.115 ppm

1,3,5-Trinitrobenzene is a yellow crystalline solid at room temperature. It is a dimorphous solid; the most common form melts at 125.5°C and the rare form melts at 61°C. It is soluble in

polar organic solvents, such as alcohol, ether, acetone and methanol, and in nonpolar organic solvents such as benzene, carbon disulfide and petroleum ether.

3. TOXICOKINETIC STUDIES

3.1. TOXICOKINETICS

Male Wistar rats injected intraperitoneally with 100 µmol/kg body weight (21.3 mg/kg) of TNB (in propylene glycol) excreted approximately 3.24-3.42 mg p-aminophenol equivalents per kilogram in the urine 5 hours after dosing (Watanabe et al., 1976). Since these levels were twice the base line levels excreted by vehicle-only treated animals, it was concluded that absorption and metabolism of TNB is slow. Additional details on TNB deposition were not included in the study.

The toxicokinetics (absorption, distribution and elimination) of ^{14}C-TNB were studied in Fischer 344 rats following a single oral dose (Reddy and Gunnarson, 1993). Male (4) and female (4) rats were dosed with ^{14}C-TNB (152 mg/kg, 6-8 mCi) in DMSO. Groups of 2 male and 2 female rats were used in experiments to determine ^{14}C-TNB elimination through expired CO_2. ^{14}C-TNB levels in urine and feces were measured at 24, 48, 72 and 96 hours after dosing. Approximately 10% of the dose was eliminated in the urine of male and female rats in the first 24 hours. Approximately 21% and 36% of the dose appeared in the urine in 4 days in male and female rats, respectively. Excretion via feces was approximately 4% in the same period in both sexes. The expired ^{14}C-CO_2 was about 3% and 5% of the dose in 2 days in male and female rats, respectively. At 4 days after treatment, the radioactive residues were about 0.02-0.03%/g of tissue in the liver, kidney, skin and lungs, whereas other tissues showed lower levels of residues (about 0.001%/g or less). The results showed that a single dose of TNB in the rat was absorbed in the gut (administered in DMSO) and was eliminated mainly in the urine, with low levels in feces in 4 days. The results from this study did not show bioaccumulation of TNB in rats.

3.2. METABOLISM

Bel et al. (1994) determined TNB and its metabolites in the biological fluids of rats exposed to TNB in the diet and identified reductive metabolites, 1,3-dinitro,5-aniline (urine), 1,3-diamino-5-nitrobenzene (urine, feces and blood) and 1,3,5-triaminobenzene (urine and feces). No TNB was found in the checked samples by a GC/MS method. Reddy et al. (1996a) studied the metabolism of ^{14}C-TNB in an *in vitro* rat liver microsomal system and found that TNB (43 µg) is metabolized in 5 minutes. No parent compound and three metabolites were detected by HPLC. The two major peaks were identified as 3-amino-5-nitroaniline (3,5-diamino-nitrobenzene) and 3,5-dinitroaniline by either spiking or coelution with authentic standards. ^{14}C-TNB when administered orally (single dose, 225 mg/kg) to Fischer rats formed stable adducts with blood proteins and tissue DNA. Hemoglobin binding persisted throughout the life span of red blood cells (65 days); 10 weeks later, a significant amount of radioactivity was associated with spleen and kidney DNA (Reddy et al., 1991).

3.3. PERCUTANEOUS ABSORPTION

Kraeling et al. (1995) studied *in vitro* percutaneous absorption of ^{14}C-TNB in acetone or water in viable hairless guinea pig (HGP), F344 rat and human skin, assembled in flowthrough diffusion cells. The absorption was expressed as the percent of the applied dose (0.5 µCi) absorbed (skin and receptor fluid) for 24 hours. Rapid absorption of TNB by rodent skin was found with both vehicles. For HGP, TNB absorption was 73% in acetone and 83% in water. For rat skin, TNB absorption was 61% in acetone and 67% in water. However, the absorption of TNB in acetone was lower (36%) in human skin while the absorption remained high (72%) in water. The rat skin with thicker dermatome sections (350 µm) retained 13-21% of the absorbed radioactivity in skin after 24 hours. There was little tendency of TNB to form a reservoir in skin when thin (200 µm) dermatome sections were used (HGP and human skin).

3.4. STRUCTURE-ACTIVITY RELATIONSHIPS

The LD_{50} of m-dinitrobenzene in rats is 59.5 mg/kg and the LD_{50} of 1,3,5-trinitrobenzene in rats is 284 mg/kg, indicating that its additional nitro group results in reduced toxicity (FitzGerald et al., 1992a,b). Based on subchronic toxicity data, both these compounds showed toxic effects on the hematopoietic system and testis, thus indicating similar target organ effects. TNB is formed as a by-product of 2, 4,6-trinitrotoluene (TNT); however, unlike TNT, it does not cause cancer in rats.

3.5. MECHANISTIC STUDIES

At present data are insufficient on the mechanism by which TNB causes toxicity to the hematopoietic system, kidney, testis or brain. However, available information provided in this report suggests modes of action for methemoglobinemia and hemolytic anemia caused by TNB effects on extramedullary hematopoiesis.

4. HAZARD IDENTIFICATION

4.1. EPIDEMIOLOGIC STUDIES

Pertinent epidemiologic studies of TNB are not available.

4.2. SHORT-TERM STUDIES

4.2.1. Human Toxicity. Information on toxicity of TNB in humans is not available.

4.2.2. Animal Toxicity.

4.2.2.1. Acute Toxicity.

4.2.2.1.1. Oral Toxicity. Korolev et al. (1977) reported 1,3,5-trinitrobenzene oral LD_{50} values of 600 mg/kg for white mice, 450 mg/kg for white rats, and 730 mg/kg for guinea pigs. The toxicity to the animals was characterized by central nervous system and respiratory disorders and cyanosis. They also reported that 2 of 10 rats died following daily oral doses of 90 mg/kg TNB (unspecified vehicle) for 30 days. Timofievskaya and Rodionova (1973) reported an oral LD_{50} of 572 mg/kg in mice. No details are reported in these abstracts (published in Russian).

FitzGerald et al. (1992a) conducted acute oral toxicity tests of TNB in corn oil in male and female rats (F344) and mice (Swiss Albino). Based on the range finding study, three dose levels were chosen for the LD_{50} study: 185, 260 and 335 mg/kg for rats, and 500, 700 and 900 mg/kg for mice. Animals were observed for clinical signs for 14 days following dosing. Clinical signs during the study period included dyspnea, prostration, ataxia and catalepsy in rats and dyspnea and prostration in mice. The oral LD_{50} for male, female and combined sexes of rats were 298, 275 and 284 mg/kg, respectively. The oral LD_{50}s (mg/kg) for mice were >900 for male, 702 for female and 804 for combined sexes. Mice seem to be less sensitive to TNB than rats.

Hematological Effects: Chandra et al. (1995a) studied the hematological effects of single or repeated exposure of TNB in rats. Male F344 rats were gavaged with TNB at 35.5 and 71 mg/kg in corn oil. Blood was collected 5 and 24 hours after a single oral dose or 24 hours after daily oral doses for 4 or 10 days. A dose-dependent methemoglobinemia was present only in blood collected 5 hours after a single dose. A highly significant dose-dependent anemia with reduced cells, hemoglobin and hematocrit was present in rats receiving TNB for 10 days. A dose-dependent decrease in serum triglycerides was also present in rats receiving TNB for 10 days.

Neurotoxicity: Chandra et al. (1995b) studied neurotoxicity of TNB in rats. Administration of TNB by oral gavage to male F344 rats produced encephalopathy after 10 daily doses of 71 mg/kg. The affected areas exhibited severe gliosis and numerous vacuoles (malacia) and petechial hemorrhages in the cerebellar peduncles and brain stem. Rats administered daily oral doses of 35.5 mg/kg of TNB for 10 days and 35.5 and 71 mg/kg of TNB for 1 or 4 days did not have brain lesions.

4.2.2.1.2. Dermal and Ocular Toxicity. Acute dermal toxicity of TNB was studied in rabbits (New Zealand White) by FitzGerald et al. (1992a). A dose of 2.0 g/kg of TNB (ground to a fine powder) was applied to the skin of 10 rabbits (5/sex) under a semi-occlusive patch for 24 hours and observed for clinical signs and toxicity for 14 days. There were no deaths or other signs of toxicity noted during the 14 days. Gross necropsy revealed no unusual lesions in the skin of rabbits. TNB was considered to be nontoxic when applied at 2 g/kg to the skin of rabbits for 24 hours.

Skin and Eye Irritation: Timofievskaya and Rodionova (1973) reported that application of TNB to the shaved skin of mice caused hyperemia, edema and hemorrhages and that instillation of 50 mg of TNB into the eyes of rabbits caused irritation. Further details, however, were unavailable. Primary eye irritation potential of TNB was evaluated by applying 0.1 g of fine powder to each test eye of six rabbits. Eyes were examined at 1, 24, 48, 72 and 96 hours post

application, by the Draize method. The eyes were rinsed with 20 mL of saline at 24 hours. The treated eyes of all animals received scores of severe (grade 3 or 4) for redness, chemosis and opacity through the 96-hour observation period. As reported by FitzGerald et al. (1992a), TNB caused irreversible damage to the ocular tissue of rabbits and was considered to be corrosive.

Primary dermal irritation potential of TNB was studied by applying a dose of 0.5 g of TNB to one intact and one abraded skin site per rabbit (New Zealand White) for 24 hours. Erythema and edema were scored at 30-60 minutes after the exposure period and again at 48 and 72 hours according to the Draize method. There were no signs of dermal irritation. Primary dermal irritation index was judged as 0.0. TNB is considered to be a non-irritant to the skin of rabbits (FitzGerald et al., 1992a).

Dermal Sensitization: FitzGerald et al. (1992a) studied dermal sensitization by the Buehler method. Twenty guinea pigs were exposed three times at weekly intervals to TNB (0.5 g) under semi-occlusive wrap for 6 hours. After a 2-week latent period for development of immunological reactivity, a challenge dose was similarly applied for 24 hours and the skin reaction was scored at 2, 24 and 48 hours. Signs of erythema (score of 0.5-1.0) were observed in 17/20 animals. TNB was considered a mild skin sensitizer.

4.2.2.2. Subacute Toxicity. The toxic effects of subacute (14-day) dietary administration of TNB were studied in Fischer 344 rats (Reddy et al., 1994a, 1996b). Groups of 5 male and 5 female rats were fed diets containing TNB for 14 days at dose levels of 0, 50, 200, 400, 800 and 1200 ppm. The calculated average TNB intake for male rats was 0, 4.41, 17.08, 34.30, 55.87 and 92.33 mg/kg/bw and for female rats 0, 4.42, 17.72, 34.26, 59.19 and 79.68 mg/kg/bw, respectively. These studies were conducted to select suitable doses for subsequent subchronic (90-day) toxicity studies. There were no clinical signs and no early deaths observed during the 14-day feeding period. The mean daily food consumption significantly ($p<0.05$) decreased in female rats fed 400, 800 and 1200 ppm TNB and in male rats fed 800 and 1200 ppm TNB. Average daily water consumption was decreased in males fed on high doses (800 and 1200 ppm). The decreased food intake and water consumption in rats resulted in decreased body weight in the 1200 ppm dose group.

Mean relative organ weights (to final body weight) showed significant changes in male and female rats. Mean relative brain weight (1200 ppm dose) and spleen weight (400, 800 and 1200 ppm dose) for both sexes were significantly increased, while the relative thymus weight was significantly decreased in male rats (1200 ppm dose). The relative testicular weight was decreased significantly in rats fed 800 and 1200 ppm TNB while relative kidney weights increased significantly in rats fed 200-1200 ppm TNB. The relative liver weight was increased in the 400 ppm TNB males but decreased in the 50 ppm TNB females.

TNB produced hematological effects in all dose groups. A significant decrease in total red blood cell count and hematocrit was present in females (50-1200 ppm TNB) and in males (400-1200 ppm TNB). The hemoglobin levels were significantly decreased in females of the 800 and 1200 ppm groups. A significant increase in the percentage of Heinz bodies was present in

800 and 1200 ppm groups in both sexes. There was no difference in the total white cell count among the groups, but there was a relative shift to an increased lymphocyte percentage in all male groups and in females at 1200 ppm. Methemoglobin concentrations were significantly increased in both sexes in the 400, 800 and 1200 ppm groups. The clinical chemistry results showed that there were no biologically significant changes of any analytes except for significantly decreased alkaline phosphatase levels.

Histopathological analysis showed that TNB produced significant changes in the testis, spleen, kidneys and brain. In male rats receiving 800 and 1200 ppm TNB in the diet, the testes were characterized by moderate to severe seminiferous tubular degeneration. The kidneys of male rats in the 200, 400, 800 and 1200 ppm groups exhibited an increased incidence of cortical tubular hyaline droplet deposition. The spleen and bone marrow showed mild to moderate erythroid cell hyperplasia in both sexes at 400, 800 and 1200 ppm TNB. Two females (2/5) from the 1200 ppm TNB dose group exhibited changes in cerebellar peduncles characterized by hemorrhage, malacia, vacuolization and micro gliosis.

4.2.2.3. Subchronic Toxicity. Rats: Reddy et al. (1994b,c) conducted a 90-day subchronic oral toxicity study of TNB (99.83% pure) in 10 male and 10 female Fischer 344 rats that were randomly assigned to each of four dose groups. The dose groups were selected on the basis of 14-day toxicity studies. The rats were fed a diet containing 0, 67, 400 and 800 ppm TNB for 90 days. The average daily TNB consumption for females was 0, 4, 25 and 49 and for males 0, 4, 23 and 44 mg/kg/day. The mean daily food consumption decreased significantly in male and female rats receiving 400 and 800 ppm TNB in the diet, which resulted in significant decrease in body weight gain. However, the water consumption in females in those groups was significantly increased. The mean relative organ weights (g/100 g body weight), liver, spleen (males and females) and brain weights (males), were increased significantly in rats receiving 400 and 800 ppm TNB in the diet. The relative testicular weight was decreased significantly in males in the 400 and 800 ppm TNB dose groups.

TNB produced hematological effects in rats. A significant decrease in total red blood cell count in both sexes receiving 400 and 800 ppm TNB was noted. In contrast, there was a significant increase in the percent of reticulocyte in males (400 and 800 ppm TNB) and in all female groups. A decrease in hemoglobin content and a significant increase in methemoglobin was observed in both dose groups of male and females (400 and 800 ppm TNB). Clinical chemistry results showed no significant changes in any of the analytes studied.

Histopathological analysis revealed significant changes in testes (moderate to severe seminiferous tubular degeneration) in mid- and high-dose groups (400 and 800 ppm TNB) and in kidney (deposition of hyaline droplets) in all male rats receiving TNB in the diet. Possibly in response to decreased hemoglobulin levels, the spleen and bone marrow featured mild to moderate erythroid cell hyperplasia in male and female rats receiving 400 and 800 ppm TNB.

Kidneys in male rats receiving TNB at dose levels of 67, 400 and 800 ppm TNB had an increased incidence of cytoplasmic hyaline droplets in proximal cortical tubular epithelial cells at

all treatment levels. The severity of this change was dose dependent, ranging from moderate in the high-dose group (800 ppm TNB) to mild in the low-dose group (67 ppm TNB). These droplets were occasionally irregularly shaped and had angular contours, but were more often spheroid. These droplets stained positive with Mallory's Heidenhain protein stain. Further characterization of these droplets would require immunohistochemical staining. A diagnosis of alpha-2-µ-globulin nephropathy was not deemed appropriate since there was no significant increase in single cell necrosis, no presence of granular casts or linear papillary mineralization or increased tubular hyperplasia. In addition to the deposition of hyaline droplets, the presence of early chronic progressive nephropathy was evident in both treated as well as control male rats. This change was characterized by an increased incidence of tubular degeneration and regeneration, as well as mineralized foci. Tubular degeneration was the only change that appeared to be dose related as noted by an increased severity (mild) in the high- and mid-dose groups.

From the subchronic toxicity studies, a no-observed-adverse-effect level (NOAEL) of 4 mg TNB/kg/day and lowest-observed-adverse-effect level (LOAEL) of 25 mg/kg/day was established for female rats. In male rats, deposition of hyaline droplets without cell necrosis was observed at all doses tested. Mild to moderate histopathological changes in spleen and testis observed in mid- and high-dose groups, respectively, suggested a LOAEL of 25 mg/kg/day. This 90-day study was conducted independently (Reddy et al., 1994b) and is different from the 90-day interim sacrifice discussed in the 2-year chronic study (Reddy et al., 1996d). In the absence of changes in BUN and creatinine levels, these changes are not considered biologically significant.

White-footed Mice: Reddy et al. (1995) evaluated 90-day subchronic toxicity of TNB (99.83% pure) in White footed mice*(Peromyscus leucopus)*. Groups of 10 males and 10 females were fed diets containing 0, 150, 375 and 750 ppm TNB. The average calculated TNB consumption was 20.2, 64.8 and 108.2 mg/kg/day for females and 23.5, 67.4 and 113.5 mg/kg/day for males. The only significant change noted was an increase in relative kidney weight (females) and an absolute and relative spleen weight (male) in mice of the 750 ppm TNB group, which was considered biologically insignificant. Hematology data showed no significant changes in female mice, while male mice reticulocyte increased significantly in the 150 and 750 ppm TNB groups and white blood cells increased only in the 750 ppm TNB group. Histopathological analysis revealed treatment-related changes in spleen (erythroid cell hyperplasia) and in testis (seminiferous tubule degeneration) in the 750 ppm TNB (113.5 mg/kg-day) group. The only biologically significant findings were present in the male 750 ppm TNB group. From this subchronic toxicity study, a NOAEL of 108.2 mg/kg/day for female and 67.4 mg/kg/day for male mice is recommended.

4.2.3. Mutagenicity. TNB produced reverse mutations in*Salmonella typhimurum* strains (TA98 and 100) in three separate studies (McGregor et al., 1980; Spanggord et al., 1982; Kawai et al., 1987). In each study, the presence of the S-9 activating system reduced, but did not abolish, the mutagenic activity of TNB. TNB did not induce genotoxicity in DNA repair assay with *Escherichia coli* or in mitotic recombination assays with*Saccharomyces cerevisiae* D5 (McGregor et al., 1980).

4.3. LONG-TERM STUDIES

4.3.1. Carcinogenicity. Slaga et al. (1985) studied the carcinogenic activity of TNB in mouse skin and lung tumor assays. A single application of 10 or 50 mg of TNB (in acetone) to the skin of (Sencar) mice increased the incidence of inflammation, epidermal hyperplasia and dark cells. The response elicited by these dose levels was similar to the maximum response obtained with TPA, a potent promoter of two-stage carcinogenic tumors in the skin of SENCAR mice. TNB was found negative in assay for initiation of TPA-promoted skin carcinogenicity. Potentiation for the promotion of the skin cancers was not conducted with TNB.

Intraperitoneal administration of TNB in corn oil (0, 600, 1500 and 3000 ppm, 3 times weekly for 8 weeks) did not cause lung tumors in male A/Jax mice (Slaga et al., 1985). The lungs of five mice/dose were examined 16 weeks after the last injection. TNB did not cause lung tumors in mice, but neither did benzo(a)pyrene and 4-nitroquinoline-N-oxide, known carcinogens in the study.

Górski (1969) studied the biological role of charge transfer complexes of aromatic hydrocarbon oxi-derivatives in chemical carcinogenesis in male BALB/c strain mice. He injected subcutaneously 3-methyl cholanthrene (3MC), its complex forms with TNB and TNB itself (equivalent to 1 mg of 3MC) in 0.4 mL paraffin oil. The charge transfer complex from the oxidation product of 3MC with TNB revealed carcinogenic activity on mice higher than for 3MC by itself. Animals receiving TNB (1 mg equivalent of 3MC) alone were in good condition and remained without tumors throughout the observation period (144 days). This showed that TNB alone is not tumorigenic in mice in this study.

Medium Term Glutathione-S-transferase (placental form)-positive foci (GST-P foci) Bioassay: The carcinogenic potential of TNB (as initiator or promotor) was studied by using a medium term bioassay described by Ito et al. (1988). TNB (600 ppm in the diet for 8 weeks) did not induce gamma-glutamyl transpeptidase or glutathione-S-transferase (placental form) foci nor promote dimethylnitrosamine-initiated GGT and GST-P foci. Similarly, TNB at 60 ppm single oral dose did not induce GI-tract nuclear anomalies in rats nor stimulate dimethylhydrazine-induced GI-tract nuclear anomalies (Heddle et al., 1982). In contrast to reverse mutations reported in Section 1.2.3, TNB at 60 ppm also did not cause DNA strand breaks in the rat liver (Reddy et al., 1996c), suggesting that TNB may not be a genotoxic agent. In the chronic rat toxicity study discussed below, Reddy et al. (1996d) did not observe any neoplastic lesions; however, additional lifetime studies in mice are needed to further support these observations.

4.3.2. **Chronic Toxicity.** In a 2-year toxicity study conducted by the U.S. EPA (Reddy et al., 1996d, 1997), male and female rats (75/sex for treated groups, 60/sex for controls) were fed diets supplemented with 0, 5, 60 and 300 ppm TNB (98.83% pure). Rats (10/dose/sex) were sacrificed and complete histopathological examinations of target organs were performed at 3, 6 and 12 months after exposure. The remaining animals were sacrificed at 24 months and routine toxicological evaluations of target organ toxicity were performed. Based on the food consumption for each exposure time, the authors calculated the average TNB intake for females

to be 0, 0.23, 2.68 or 13.31 mg/kg bw/day, and for males to be 0, 0.22, 2.64 or 13.44 mg/kg bw/day (Table 1).

Exposure of Fischer 344 rats to the aforementioned dietary regimen for 3 months resulted in the following significant findings:

1. The food consumption was reduced in all females receiving TNB diets. The terminal body weights were significantly decreased in both sexes receiving 300 ppm TNB in the diet.

2. The relative weights of spleen, brain and kidneys were increased in females receiving 60 ppm TNB in the diet. Similarly, in female rats receiving 300 ppm TNB in the diet, the relative weights of spleen, liver, lungs and kidneys were increased. In the males, however, the relative weights (organ weight/100 g bw) of spleen, brain and kidneys were increased in the 300 ppm TNB diet group and no significant effect was seen in the 60 ppm TNB diet group (Table 2).

TABLE 1
Estimated TNB Consumption

Exposure Duration (Months)	Female			Male		
	mg TNB/kg (ppm) diet					
	5	60	300	5	60	300
	mg TNB/kg/day					
3	0.27	3.21	15.17	0.35	3.72	17.93
6	0.27	3.23	14.37	0.28	3.22	15.69
12	0.24	2.93	14.90	0.25	2.96	14.61
24	0.23	2.68	13.31	0.22	2.64	13.44

TABLE 2
Organ Weight/100g Body Weight and Percent Methemoglobin Levels in the 90-Day Rat Study

	Dose Groups (mgTNB/kg diet)[a]							
	300		60		5		0	
	Male	Female	Male	Female	Male	Female	Male	Female
Body Wt. (g)	272±8[b]	161±2[b]	310±6	166±2[b]	296±6	167±2	297±4	175±3
Kidneys	0.72±0.01[b]	0.77±0.02[b]	0.71±0.01	0.74±0.01	0.69±0.01	0.74±0.01	0.68±0.01	0.72±0.01
Spleen	0.24±0[b]	0.31±0[b]	0..20±0	0.27±0.01[b]	0.20±0	0.24±0	0.20±0	0.23±0
Met Hb	3.12±0.57[b]	2.22±0.56[b]	1.13±0.30[b]	0.85±0.25[b]	0.60±0.32	0.36±0.20	0.50±0.33	0.38±0.26

[a]Mean ± Standard Deviation
[b]Significantly different for controls ($p \leq 0.05$) by Dunnett's test.

3. Hemoglobin, hematocrit and red blood cell count were significantly decreased in females receiving 60 ppm and decreased in both sexes receiving 300 ppm TNB in the diet (Table 2).

4. Methemoglobin levels were increased in both sexes receiving 60 and 300 ppm TNB in the diet. Erythroid cell hyperplasia was noted in both sexes exposed to 300 ppm TNB in the diet. Similarly, excessive hyaline droplets deposition, without single cell necrosis, was apparent in the cortical renal tubules of male rats receiving 60 and 300 ppm TNB in the diet.

Fischer 344 rats exposed to the various concentrations of TNB (0, 5, 60 or 300 ppm) in the diet for 6 months resulted in the following significant findings:

1. Food consumption was significantly decreased in females in the 60 ppm group and in both sexes at 300 ppm. The terminal body weights were significantly reduced in the 300 ppm females.

2. The relative weights of spleen, brain and liver in female rats, and the relative weights of spleen, liver and kidney in male rats were significantly increased in the 300 ppm groups (Table 3), suggesting hypertrophy.

3. Hemoglobin and red blood cell levels were significantly decreased in both sexes at 60 and 300 ppm while methemoglobin was significantly increased in both sexes receiving 300 ppm, and in females receiving 60 ppm (Table 3).

4. Erythroid cell hyperplasia was prominent in the 60 and 300 ppm groups in both sexes. Excessive hyalinedroplet formation was also present in males in those dose groups.

For Fischer 344 rats exposed to diets containing TNB (0, 5, 60 or 300 ppm) for 1 year, the following significant findings were noted:

1. Terminal body weights were significantly reduced in both males and females receiving 300 ppm. The brain, spleen, liver and heart relative weights were significantly increased in males and females in the 300 ppm group. In the same diet groups, the relative weight of the kidneys were significantly increased for males. In addition, spleen and liver relative organ weights were significantly increased in males at 60 ppm.

2. Hemoglobin and hematocrit levels were significantly decreased in both sexes at 300 ppm while methemoglobin was significantly increased in both the 60 and 300 ppm groups (Table 4).

TABLE 3
Organ Weight/100g Body Weight and Percent Methemoglobin Levels in the 6-Month Rat Study

	Dose Groups (mgTNB/kg diet)[a]							
	300		60		5		0	
	Male	Female	Male	Female	Male	Female	Male	Female
Body Wt. (g)	327±5	172±2	344±9	184±3	358±5	192±3	347±8	190±2
Liver	3.05±0.04	2.91±0.05	2.90±0.04	2.75±0.03	2.78±0.04	2.73±0.03	2.85±0.04	2.77±0.04
Spleen	0.24±0[b]	0.31±0.01[b]	0.20±0	0.26±0.01	0.20±0	0.25±0.01	0.19±0	0.25±0
Met Hb	2.96±0.76[b]	2.27±0.47[b]	1.10±0.42	1.19±0.34[b]	0.84±0.37	0.92±0.35	0.89±0.36	0.57±0.41

[a] Mean ± Standard Deviation
[b]Significantly different for controls ($p \leq 0.05$) by Dunnett's test.

TABLE 4
Organ Weight/100g Body Weight and Percent Methemoglobin Levels in the 1-Year Rat Study

	Dose Groups (mgTNB/kg diet)[a]							
	300		60		5		0	
	Male	Female	Male	Female	Male	Female	Male	Female
Body Wt. (g)	353±6[b]	183±5	409±7	214±6	422±5	224±6	401±5	223±4
Spleen	0.23±0[b]	0.31±0.01[b]	0.21±0[b]	0.25±0	0.20±0	0.23±0.01	0.19±0.01	0.23±0.01
Met Hb	2.35±0.43[b]	1.74±0.55[b]	1.38±0.75[b]	1.01±0.55	1.02±0.30	0.87±0.46	0.71±0.41	0.69±0.48

[a] Mean ± Standard Deviation
[b]Significantly different for controls ($p \leq 0.05$) by Dunnett's test.

3. Increased splenic erythroid cell hyperplasia and/or pigment deposition were evident in both sexes receiving 60 or 300 ppm. Seminiferous tubular degeneration was evident in males at 300 ppm.

4. Cytoplasmic renal tubular droplet deposition was increased in both sexes in the 60 and 300 ppm groups.

Administration of TNB to Fischer 344 rats in the diet at varied concentrations (0, 5, 60, 300 ppm) for 2 years resulted in the following:

1. Terminal body weights were significantly reduced in both males and females at 300 ppm, even though the food consumption in this group was not reduced. In the same dose group the water consumption in both sexes was increased.

2. Relative spleen weights were significantly reduced in both sexes at 300 ppm (Table 5).

3. Increased splenic erythroid cell hyperplasia and pigment (hemosiderin) deposition, along with elevated methemoglobin levels, were present in both sexes at 300 ppm. Increased methemoglobin (Table 5) levels were probably due to increased production of the transformation product of oxyhemoglobin under the influence of TNB oxidation and is the probable initiating cause of the hematopoietic activity. The regenerative anemia previously noted in the interim sacrifices appeared to be nearly fully compensated.

4. Cytoplasmic renal tubular protein droplets were excessive in both sexes at 60 and 300 ppm. BUN creatinine levels were minimally increased in the 300 ppm females. The adverse effects, such as increased relative organ weights, increased methemoglobin, and erythroid cell hyperplasia, observed during interim sacrifices in rats at 60 ppm did not persist and were not detected in rats that were given 60 ppm TNB for 2 years. This suggests that an adaptive mechanism has taken place in order to compensate the adverse effects noted during interim sacrifices. It is also interesting to note the appearance of the renal protein droplets in females in both 1- and 2-year sacrifices, but not in females sacrificed at 3 or 6 months after TNB exposure.

5. Since there were no adverse renal effects caused by the protein droplets, in general, the protein droplets seen in males and females in the 60 ppm groups were not considered biologically significant. Therefore, a NOAEL of 2.68 mg/kg bw/day was established for TNB.

TABLE 5
Organ Weight/100g Body Weight and Percent Methemoglobin Levels in the 2-Year Rat Study

	Dose Groups (mgTNB/kg diet)[a]							
	300		60		5		0	
	Male	Female	Male	Female	Male	Female	Male	Female
Body Wt. (g)	320±9[b]	216±3[b]	354±6	242±6	342±14	257±7	343±12	257±8
Spleen	0.30±0.02[b]	0.41±0.06[b]	0.44±0.08	1.0±0.31	1.02±0.25	0.94±0.20	0.84±0.22	0.71±0.25
Met Hb	1.92±0.55[b]	2.49±0.65[b]	1.10±0.44	1.16±0.28	0.57±0.41	0.87±0.29	0.66±0.30	1.0±0.63

[a]Mean ± Standard Deviation
[b]Significantly different for controls ($p \leq 0.05$) by Dunnett's test.

4.4. REPRODUCTIVE EFFECTS

Kinkead et al. (1994, 1995) conducted a modified Screening Information Data Set (MIDS) for a single generation reproductive study of TNB (99.83% pure). Male and female Sprague-Dawely rats were maintained on a diet containing 30, 150 and 300 ppm TNB. The calculated

average TNB intake was 2, 9 and 19 mg/kg/day for males and 3, 14 and 29 mg/kg/day for females. Groups of 6 male and 6 female rats were used in the reproductive toxicity evaluation. Six male rats were dosed for 14 days prior to the mating and throughout the mating period for a total of 28 days. All female rats were dosed for 14 days prior to mating, during mating, gestation, postpartum (21 days) and 4 weeks post-weaning for a total of 90 days. Pups were maintained on a treated diet through 4 weeks post-weaning.

No mortality occurred in the parental animals during the study. Six male rats were necropsied following the mating period (28 days of treatment). No treatment-related differences were noted in absolute or relative organ weights in male rats. An increase in absolute and relative spleen weights was noted in the high-dose (300 ppm) female rats necropsied 90 days after treatment. Relative liver weight (300 ppm) and relative kidney weight (150 and 300 ppm) were increased in females after 90 days of exposure. Male rats sacrificed following 28 days mating showed reduced number and concentration of motile sperms (300 ppm) and reduced percent of cells traveling a circular pattern (150 and 300 ppm). Histopathological evaluation revealed significant increase in splenic hemosiderosis (150 and 300 ppm) and presence of hyaline droplets (mild) in control and all treated animals after 28 days of treatment, which may not be treatment related.

TNB showed no adverse effects on reproductive indices, on mating (100%) or fertility index (92%) in 300 ppm and both indices at 100% in other groups. No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of offspring per litter. During the 21-day lactation phase, the mean body weights of the TNB-treated pups (male and female) were significantly lower than the control group pups, except at 14 days when the 150 and 300 ppm group pups weighed equal to control. The results of the lactation phase showed that TNB produced decreased organ-to-body weight ratio in pups at 150 and 300 ppm. A LOAEL for reproductive toxicity, however, cannot be ascertained in this study.

EPA research priorities have a special focus on issues affecting children. The data generated in the above studies did not suggest the impact of TNB on children's reproductive health.

4.5. DEVELOPMENTAL EFFECTS

Cooper and Caldwell (1995) studied the developmental toxicity of TNB (99.83% pure) in female Sprague-Dawley Crl:CD BR rats. TNB in 1% agar in sterile water was administered orally by gavage to 24, 26, 24 and 25 bred rats at dose levels of 11, 22, 45 and 90 mg/kg/day, respectively. Animals were identified as sperm positive on day 0 and were dosed on gestation days 6 through 15. A concurrent control group composed of 25 bred females received vehicle. One female in each of the groups (11, 25, 45 and 90 mg/kg/day) died from an intubation error between gestation days 6 and 16. Another female in the 90 mg/kg/day group died from a possible intubation error on gestation day 16. A laparohysterectomy was performed on all surviving animals on gestation day 20. The maternal toxicity was expressed at a dose level of 90 mg/kg/day by significant decreased body weight and food consumption. Clinical changes included disorientation, shaking, unsteadiness and hyperactivity observed in one animal in the 90 mg/kg/day group. Developmental toxicity was exhibited in the 90 mg/kg/day group, which included reduced mean

fetal weight and crown-rump length and increased incidence of one skeletal variation. No maternal toxicity or developmental toxicity was observed at dose levels of 11, 22 and 45 mg/kg/day. Based on this study, a dose level of 45 mg/kg/day was considered to be the NOAEL and 90 mg/kg/day as the LOAEL for maternal toxicity and developmental toxicity.

Lack of developmental toxicity observed at doses below either maternal or systemic toxicity (methemoglobinemia) in this study provided some evidence in support of toxicity threshold in children against risks from TNB exposure.

4.6. SYNTHESIS AND EVALUATION OF MAJOR NON-CANCER EFFECTS

Nitroaromatics such as DNB, TNB and tetryl have been detected as environmental contaminants of ground water and soil near production sites and in some instances at military test grounds. Available information has clearly demonstrated that DNB causes methemoglobinemia, anemia, testicular degeneration, reproductive failure and CNS effects in hamsters, rats and mice (Cody et al., 1981; Korolev et al., 1977). Comparative acute toxicological data indicates that DNB is more potent than TNB, thus by analogy to DNB, a NOAEL of 0.51 mg/kg/day was calculated to develop an RfD for TNB (U.S. EPA, 1997). Since then several rodent studies, as previously discussed, have been conducted for the U.S. Army to better characterize the toxicity of TNB. Subchronic, chronic and developmental studies have clearly demonstrated adverse health effects of TNB that resulted in hematopoietic and testicular abnormalities.

TNB effects on kidney have been reported in the 90-day, 6-month, 1-year, and 2-year studies. Immunohistochemical analysis of rats exposed to TNB for 2 years revealed the presence of alpha-2-µ-globulin in proximal tubular epithelium in male and female rats (Reddy et al., 1996d). However, not all of the droplets stained positive for alpha-2-µ-globulin. The droplets that were treatment-related did not demonstrate a strong positive staining for alpha-2-µ-globulin in female rats. Positive immunohistochemical stain for alpha-2-µ-globulin in the female rat kidney has not been reported. Older female rats (104 weeks) may possibly be masculinized due to hormonal changes. The histopathologic and immuno histochemical findings from the present study do not support the histopathology and lesion progression as described in the EPA's Risk Assessment Forum Guidelines (U.S. EPA, 1991) for alpha-2-µ-globulin associated nephrotoxicity. Furthermore, in the present study, there was no increase in response to TNB treatment (no change in BUN or creatinine levels or water consumption); these results, therefore, preclude consideration of TNB-nephrotoxicity in rats in this study for risk assessment.

Hyaline (protein) droplets accumulation in proximal tubule has been observed in male rats exposed to unleaded gasoline, 2,2,4-trimethylpentane, decaline, 1,4-dichlorobenzene, pentachlorobenzene and d-limonene, the natural product found in citrus oils (Hard et al., 1993). This protein is not found in humans and female rats. Hyaline droplet accumulation may be a nonspecific response to protein overload in the renal tubule and may not be due to alpha-2-µ-globulin (e.g., as with chlorothalonil). The characterization of these droplets will help to differentiate alpha-2-µ-globulin inducers from chemicals that produce renal tumors through other means. There was no pathological sequence of lesions associated with alpha-2-µ-globulin

nephropathy as described in U.S. EPA (1991). Typical lesions include single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules and tubule hyperplasia. These lesions were not observed in the present study.

It is interesting to observe that rats treated with 60 ppm TNB showed decreased hematological parameters and increased relative spleen weights and erythroid cell hyperplasia in earlier exposure periods (3 months to 1 year); in contrast, the hematological changes were moderate to negligible in the 2-year study. Since precursors of red blood cells are found in the spleen, the increased spleen weight and erythroid cell hyperplasia are indicative of a regenerative response to compensate for the loss of oxygen-carrying capacity related to TNB exposure. These effects were equivocal and appeared to have been compensated in the 2-year study. In the 2-year study, decreased spleen weights were observed along with decreased body weights indicative of aging process. At the same higher incidence of mononuclear leukemia, increased pigmentation was reportedly observed in all exposed animals. However, there was about 25% incidence of these lesions in controls, thus indicating aging process.

Available information suggests methemoglobinemia as one of the critical effects of nitroaromatic exposure. In this report, rats exposed to the 60 ppm dose showed significantly increased methemoglobin levels in the 3-, 6- and 12-month periods but the observed ranges were within the range of laboratory controls (0.5-1.5%). Increased methemoglobin levels in the 300 ppm dosage groups were consistently higher during the entire study (greater than 2.5%) and thus considered a LOAEL for TNB. The histopathological changes, interstitial cell tumor incidences, were observed in all 2-year-old rats with increased severity after 1 year of exposure to TNB. These lesions are, however, considered characteristic in aged rodents.

5. DOSE-RESPONSE ASSESSMENT

5.1. INGESTION EXPOSURE

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name --1,3,5-Trinitrobenzene
CASRN -- 99-35-4
Preparation Date -- April 22, 1996

ORAL RfD SUMMARY

Critical Dose -- 2.68 mg/kg-day (NOAEL)
UF -- 100
MF -- 1
RfD -- 3E-2 mg/kg-day
Confidence -- High

Critical Study

Critical Effect -- Methemoglobinemia and spleen-erythroid cell hyperplasia

Study Type -- Rat 2-Year Dietary Study

Reference -- Reddy et al., 1996d, 1997

NOAEL -- 2.68 mg/kg-day
NOAEL(ADJ) --

LOAEL -- 13.31 mg/kg-day
LOAEL(ADJ) --

Conversion Factors and Assumptions -- Based on food consumption data, the authors calculated the intake of TNB from dietary concentrations of 0, 5, 60 and 300 ppm: 0, 0.23, 2.68 and 13.31 mg/kg/day (females) and 0, 0.22, 2.64 and 13.44 mg/kg/day (males).

PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Reddy, T.V., F.B. Daniel, G.R. Olson, B. Wiechman and G. Reddy. 1996d. Chronic toxicity studies of 1,3,5-trinitrobenzene in Fischer 344 rats. U.S. Army, Fort Detrick, MD. (Final Report)

Reddy, G., T.V. Reddy, H. Choudhury, F.B. Daniel and G. Leach. 1997. Assessments of environmental hazards of 1,3,5-trinitrobenzene (TNB). J. Toxicol.Environ. Health. 52:101-114.

Chronic toxic effects of 1,3,5-TNB in male and female Fisher 344 rats were evaluated by feeding powdered certified laboratory chow diet supplemented with varied concentrations of TNB for 2 years. Based on food consumption, the average TNB intake was calculated for both males and females.

The study was conducted in accordance with the U.S. EPA guidelines for chronic toxicity studies as required by the GLP standards. One of the unique features of this study is that 10 animals/sex were sacrificed at the end of 90 days, 6 months and 1 year, and 25 or more rats were sacrificed at 2 years; complete toxicological evaluations were performed during these periods.

High-dose animals showed decreased body weight gains associated with decreased food consumption. Relative organ weight changes for the brain (increase), spleen (increase), liver (increase) and testes (decrease in 90- and 180-day periods) were reported for all treated animals dosed with TNB at levels higher than 3 mg/kg/day; adverse hematological findings (decreased hematocrit and hemoglobin) and increased methemoglobulin) were consistently reported in all animals treated at these levels. Histopathological findings in the 1-year study revealed extramedullary hematopoiesis in rats treated with TNB at doses of 3 mg/kg-day or higher. In the 2-year study, these effects were seen only in rats dosed with TNB at the high dosage level (13.23

mg/kg/day). The adverse effects, such as increased methemoglobin, erythroid cell hyperplasia, and increased relative organ weights, observed during interim sacrifices in rats receiving 60 ppm TNB did not persist and were not detected in rats fed 60 ppm TNB for 2 years, suggesting that an adaptive mechanism has taken place in order to compensate adverse effects observed during interim sacrifices.

Results of this study exhibited clear evidence of toxicity of the hematopoietic system as has been reported for other nitroaroniatics such as, dinitrobenzene and trinitrotoluene. The NOAEL for this study is 2.68 mg/kg/day and the LOAEL for hematological effects is 13.31 mg/kg/day.

UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

Ten for inter- and 10 for intra-species extrapolation.

UF -- 100

MF -- 1

The previous RfD developed in 1988 was based on analogy to dinitrobenzene with a UF of 10,000. Subsequently, several subchronic, chronic, reproductive and developmental toxicity studies were conducted in rats and mice through the collaborative efforts of the U.S. EPA and the U.S. Army; thus reducing uncertainties in the studies and data base for TNB.

The RfD, based on 2-year oral toxicity study, is supported by additional subchronic studies in mice and rats, reproductive, developmental and single-generation studies where adverse effects were observed at doses higher than that reported in the 2-year study; thus precluding uncertainties in database.

ADDITIONAL STUDIES / COMMENTS (ORAL RfD)

Subchronic studies: Reddy et al., 1994b,c; 90-day Subchronic Toxicity Study

Male and female Fisher 344 rats were evaluated by feeding certified laboratory chow diets containing different doses of TNB for 90 days. The estimated doses of TNB were 0, 4, 23 and 44 mg/kg/day for males and 0, 4, 25 and 49 mg/kg/day for females.

Toxicological evaluation of data indicated the following adverse effects:

(a) Increased relative spleen and brain weights and decreased testis weight in rats dosed with 23-49 mg/kg/day TNB. Liver weights were increased in the high-dose animals.

(b) Decreased RBC, hematocrit, and serum alkaline phosphatase and increased methemoglobulin levels in the mid- and high-dose groups.

(c) Histopathological examinations showed extramedullary hematopoiesis and testicular degeneration in the mid- and high-dose groups. The data indicated a NOAEL of 4 mg/kg/day and a LOAEL of 23 mg/kg/day for hematopoietic effects (methemoglobulinemia) and testicular degeneration. Hyaline droplets were observed in all dosed male animals but the clinical significance of this lesion cannot be ascertained in terms of human renal effects.

Kinkead et al., 1994, 1995; 90-day Reproductive Toxicity Study

Male and female Sprague-Dawley rats were dosed for 14 days prior to mating, and continued through a total of a 90-day dosing period. One group of male rats was treated with the high dose for 90 days followed by a 60-day recovery period to ascertain the reversibility of the testicular toxicity. The dosing regimen for females included premating, gestation and lactation and 4 weeks post-weaning; thus achieving a 90-day total exposure period. The consumed doses of TNB were 2, 9 and 19 mg/kg/day for males and 3, 14 and 29 mg/kg/day for females.

Toxicological evaluation of data indicated adverse effects on the spleen and testis. The high dose males showed testicular degeneration during the 90-day exposure, which continued through the recovery period. Increased methemoglobin levels and hemosiderosis were observed in all rats exposed to 10 mg/kg/day or higher doses of TNB. This study indicated 3 mg/kg/day as the NOAEL and 10 mg/kg/day as the LOAEL for hematological (spleen) effects.

Kinkead et al., 1994; Single Generation Reproductive Assessment Study

Male and female Sprague-Dawley rats were dosed with TNB during the 14-day premating period and continuing through the end of lactation. The target administered doses were 0, 2, 23 and 51 mg/kg/day for males and 0, 3, 30 and 60 mg/kg/day for females.

Results:

(a) Both mid- and high-dose males showed testicular degeneration

(b) High-dose females showed CNS toxicity

(c) Neonatal mortality were observed during lactation period in pups born to high-dose animals.

This study indicated a NOAEL of 3 mg/kg/day for females and a LOAEL of 23 mg/kg/day for testicular degeneration.

Cooper and Caldwell, 1995; Developmental Toxicity Study in Rats

Pregnant female Sprague-Dawley rats were gavaged daily with 0, 11.25, 22.50, 45.0 and 90.0 mg/kg-day TNB during 20 days of their gestation period. Complete morphological and

microscopical examinations of fetuses on gestation day 20 was performed; material toxicological evaluations were also conducted. This study indicated maternal toxicity and skeletal abnormalities and decreased fetal body weight in the high dosage group, resulting in a NOAEL of 45 mg/kg/day and LOAEL of 90 mg/kg/day.

Reddy et al., 1995; 90-Day *Peromyscus leucopus* (white footed mice) Toxicity Study

A subchronic toxicity study was conducted in the White-footed mouse dosed with 0, 150, 375 and 750 mg/kg diet. The calculated doses were 0, 20, 65 and 108 mg/kg/day (females) and 0, 23.5, 67.4 and 113.50 mg/kg/day (males) of TNB during a 90-day period.

Results of this study indicated erythroid cell hyperplasia (spleen), increased reticulocytes, increased relative spleen weight, and testicular degeneration in mice exposed to high dose TNB; no other toxicological effects were observed in this study; thus the White-footed mouse was considered to be less sensitive to TNB exposure.

CONFIDENCE IN THE ORAL RfD

Study -- High
Data Base --Medium
RfD -- Medium

The RfD is based on a well-conducted 2-year study that includes interim sacrifices at 3, 6 and 12 months and is supported by subchronic reproductive and developmental toxicity data in rats and subchronic data in mice. High confidence is recommended for the study. The data base contains adequate subchronic studies in rats and mice, reproductive, developmental and chronic studies in rat and lacks additional developmental studies in other species. Medium confidence is therefore recommended for the data base and the RfD.

EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document -- U.S. EPA, 1989

Other EPA Documentation – None

Agency Consensus Date – 08/27/97

5.2. INHALATION EXPOSURE

Pertinent information is not currently available.

5.3. DERMAL EXPOSURE

Pertinent information is not currently available. Available *in vitro* data are discussed in Section 3.3.

6. MAJOR CONCLUSION IN CHARACTERIZATION OF HAZARD AND DOSE-RESPONSE

6.1. HAZARD IDENTIFICATION

1,3,5-Trinitrobenzene (TNB) is a yellow crystalline solid at room temperature. It is soluble in polar organic solvents, such as alcohol, acetone, ether and methanol, and in nonpolar organic solvents such as benzene, carbon disulfide and petroleum ethers. It is also moderately soluble in water (U.S. EPA, 1989). TNB is found at contaminated sites as a by-product during 2,4,6-trinitrotoluene (TNT) production and can be formed through photochemical oxidative degeneration of TNT manufacturing. TNB is not easily biodegradable, persists in the environment, eventually leaches out, and contaminates ground water near waste disposal sites.

Information on human exposure to TNB is unavailable in the current literature. It has been well-documented that exposure to nitroaromatics causes methemoglobinemia in humans (Ishihara et al., 1976) and laboratory animals (Watanabe et al., 1976). In addition, some animal studies have shown that many nitroaromatics, such as dinitrobenzene, are male reproductive toxicants (Cody et al., 1981). Available information provided in this profile showed no adverse reproductive effects, such as mating or fertility index, in rats exposed to 300 mg TNB/kg in the diet. No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index or mean number of offspring per litter (Kinkead et al., 1995). Developmental effects of TNB have not been reported in rats exposed up to 45 mg TNB/kg/day (Cooper and Caldwell, 1995). Additionally, rats are found to be more sensitive to TNB than mice. The oral LD_{50}s were 284 mg/kg for rats and greater than 900 mg/kg for mice (FitzGerald et al., 1992). The NOAELs of 20 mg/kg/day (females) and 23 mg/kg/day (males) for subchronic toxicity in white-footed mice (Reddy et al., 1995) are comparatively higher than the chronic NOAELs (3 mg/kg/day) used for derivation of the RfD. This profile provides adequate information to reduce scientific uncertainty pertinent to reproductive and developmental effects in a second species. Compared to its structural analog DNB, an additional nitro group in TNB resulted in reduced toxicity and a substantially higher RfD (TNB RfD of 0.03 mg/kg/day vs DNB RfD of 0.0001 mg/kg/day).

6.2. DOSE-RESPONSE

Quantitative estimates of human risk are based on animal studies since no adequate human exposure data are available in studies where adverse effects have been observed in people. Hematological effects in rats are observed at the highest dose tested in chronic feeding studies. The human chronic dose of ingested TNB considered to be safe (the RfD) is 0.03 mg/kg/day.

The overall confidence in this RfD assessment is medium. The principal study is a rat chronic oral study performed with large group sizes in which biochemical and histopathological analyses on known target tissues were thoroughly performed at 90 days, 6 months, 12 months and 24 months. The confidence in the data base for TNB is medium. In animal studies, the critical hematological effect was consistent; however, reproductive toxicity observed in other structurally similar nitroaromatics may be a relevant endpoint in chronic human exposures, thus resulting in medium confidence for the data base and the RfD.

Adequate data to develop an RfC or cancer slope factor are inadequate. Based on weight of evidence for carcinogenic endpoints, the U.S. EPA (1989) has classified TNB as a Group D carcinogen.

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Appendix A

Summary of Response to External Peer Review Comments

This Support Document for TNB has undergone both internal peer review performed by scientists within EPA and other federal agencies, (i.e, the Army, the Air Force and the Navy) and a more formal external peer review through a contract with the U.S. Army. Comments made by internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific uncertainty. All three external peer reviewers were in agreement with the choice of the appropriate critical and supporting studies and the pertinent data used in the development of the RfD.

General Comments

Suggested comments with their annotations were all related to editorial and other changes, such as, expanding experimental details of critical studies, effects and biological significance of observed toxicities. All these suggested changes have been incorporated into the text to the extent feasible. Major scientific comments are addressed below.

A. Comment: Experimental Methods

One reviewer commented that the chronic study should be clearly described to show administered doses, estimated doses and interim sacrifice intervals. Another reviewer suggested clarifying the dose and evaluations of the developmental study.

Response to Comment: The experimental protocols were detailed to indicate administered doses in food as ppm and the estimated doses per body weight basis. The interim sacrifice intervals and the toxicity assessments in those intervals were separated and discussed independently. The developmental toxicity study was also expanded to indicate dosing and evaluations of the developmental effects.

B. Comment: Hyaline Droplet Nephropathy and Biological Significance

Two reviewers cautioned that this report is unclear about the nephrotoxicity observed in females and its correlation with hyaline droplets; thus suggestive of the kidney as the target organ.

Response to comments: The discussion on hyaline droplets was expanded to indicate the absence of adverse effects and weak positive staining in the immunofluoroscnce assay in females.

C. Comment: Uncertainty Factor(s)

One reviewer suggested providing appropriate justification for selection of the uncertainty factor(s). Two reviewers thought that an additional uncertainty factor should be applied for lack of a second species for developmental effects. One of them suggested that appropriate statements would be acceptable to him in favor of reduced uncertainty.

Response to Comments: Appropriate text was provided in support of the uncertainty factors used in the development of the RfD.

D. Comment: Confidence in the RfD

One reviewer suggested reducing the confidence in the RfD from high to medium due to inadequate developmental toxicity information.

Response to Comments: The high confidence in the RfD was changed to medium with appropriate statements.