2,4-Dinitrotoluene; CASRN 121-14-2

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR 2,4-Dinitrotoluene

File First On-Line 03/01/1991

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I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — 2,4-Dinitrotoluene
CASRN — 121-14-2
Last Revised — 06/01/1992

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of
information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. Oral RfD Summary

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<th>Critical Effect</th>
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<td>Neurotoxicity, Heinz bodies and biliary tract hyperplasia</td>
<td>NOAEL: 0.2 mg/kg/day, LOAEL: 1.5 mg/kg/day</td>
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* Conversion Factors: None

I.A.2. Principal and Supporting Studies (Oral RfD)


Ellis et al. (1985) reported the results of a chronic toxicity study commissioned by the U.S. Army (Ellis et al., 1979) of dogs fed 98% pure 2,4- dinitrotoluene (2,4-DNT) for up to 24 months. Groups of beagle dogs (6/sex/dose) were fed 2,4-DNT in gelatin capsules at 0, 0.2, 1.5, or 10 mg/kg/day. In male dogs fed 10 mg/kg/day, 4 of the 6 males were sacrificed moribund by study week 19 after exhibiting progressive paralysis. Neurotoxic effects, characterized by incoordination and paralysis, were exhibited by all dogs at this dose level within 6 months of study initiation and during month 16 in one dog receiving 1.5 mg/kg/day. CNS lesions included vacuolization, endothelial proliferation, and gliosis of the cerebellum. In dogs fed 1.5 and 10 mg/kg/day, there was methemoglobinemia with associated reticulocytosis and Heinz bodies; biliary tract hyperplasia; and pigmentation of the gallbladder, kidneys, and spleen. The hematologic effects were minimal during year 2, presumably due to an adaptive response. No males had testicular effects. The LOAEL in this study is 1.5 mg/kg/day based on neurotoxicity and the presence of Heinz bodies and biliary tract hyperplasia. The NOAEL is 0.2 mg/kg/day.
In a separate study (reported in Lee et al., 1978), groups of dogs (2/sex/dose) were given 2,4-DNT in capsules at doses of 0, 1, 5, or 25 mg/kg/day for 13 weeks. There was no apparent toxicity in the low- and mid-dose groups. In the high-dose group, 2,4-DNT was toxic after 12-22 days and was lethal after 22 or more days. There was great variation in individual susceptibility. All affected dogs exhibited decreased food consumption, weight loss, urine stains on the fur, pale gums, neuromuscular incoordination, and paralysis. Hematological indices showed methemoglobinemia, anemia, and Heinz bodies. The dogs were in fair to poor nutritional condition with little or no body fat. Histologically, there was hemosiderosis in the liver and spleen, cloudy swelling of the kidneys in males and females, and aspermatogenesis in males. Dogs sacrificed during weeks 6 and 7 had brain lesions characterized by gliosis, edema, and demyelination of the cerebellum, spinal cord, and brain stem. After 4 weeks, dogs partially recovered from the various effects. The LOAEL is 25 mg/kg/day based on body weight loss, hematological abnormalities, neurological signs, and histopathology. The NOAEL is 5 mg/kg/day because no DNT-related effects were observed at this and lower doses.

Lee et al. (1985) reported the results of a chronic toxicity study commissioned by the U.S. Army (Ellis et al., 1979) of rats fed 98% pure 2,4-DNT in the diet for up to 24 months. Groups of CD (Sprague-Dawley) rats (38/sex) were provided an average 2,4-DNT intake of 0, 0.57, 3.9, or 34 mg/kg/day for males, and 0, 0.71, 5.1, or 45 mg/kg/day for females. After 12 months, 8 animals/sex/group were killed for necropsy; the remaining rats were sacrificed after 24 months. Four animals/sex/group were sacrificed at 13 and 25 months after being returned to normal diets for 1 month.

Cumulative deaths in high-dose males and females were significantly higher than in controls; 50% mortality occurred in high-dose rats by month 20 and in controls by month 23. Weight gains were reduced in high-dose animals (approximately 30-40%) and mid-dose (approximately 6-7%) animals compared with controls. Low-dose rats exhibited growth rates comparable to those of controls. Anemia and reticulocytosis occurred in mid- and high-dose males and in high-dose females after 12 months. The incidence of hyperplastic liver foci was increased in high-dose males (16/29) and mid-dose females (19/27). At 12 months, 6/7 high-dose males had marked atrophy of the testes with severe atrophy of the seminiferous tubules and almost complete lack of spermatogenesis. This lesion is common in geriatric rats, but is not normally seen in rats of this age. Beyond 12 months, severe atrophy of the seminiferous tubules occurred in 16% (4/25) of the controls, 26% (7/27) of the low-dose males, 33% (6/19) of the mid-dose males, and 81% (22/27) of the high-dose males. The authors did not report the statistical significance of these effects. However, only the highest dose effect is significant by Chi square (p = 0.01) and Fischer's Exact Test (p = 0.004). The LOAEL is 34 mg/kg/day based on the incidence of changes in the seminiferous tubules of male rats. The NOAEL is 3.9 mg/kg/day.
In a separate study (Lee et al., 1978), groups of CD rats (16/sex/dose) were fed diets containing 0, 0.07, 0.20, or 0.7% 2,4-DNT (98% pure) for up to 13 weeks. The corresponding daily intakes were 0, 34, 93, or 266 mg/kg/day for males, and 0, 38, 108, or 145 mg/kg/day for females. Four animals/sex/group were sacrificed at 4 and 13 weeks after being returned to normal diets for 1 month. All high-dose females died within 3 weeks. One male in the mid-dose group and 6 in the high-dose group died between weeks 4 and 13. All surviving animals exhibited dose-dependent decreases in body weight gain, which ranged from approximately 9-55% when compared with controls. Food consumption was decreased in all dose groups. Orange to yellowish urine stains were observed on the fur of high-dose rats, and one male had widespread and stiff hind legs. Mid- and high-dose animals of both sexes were anemic, characterized by decreases in erythrocyte count, hematocrit, and hemoglobin, and concurrent reticulocytosis. Absolute liver and kidney weights were slightly increased in mid-dose males, and relative weights of these organs were significantly increased. There was splenic hemosiderosis in mid- and high-dose males and females. Spermatogenesis was decreased in mid-dose males and completely arrested in high-dose males. One high-dose male showed some signs of neuromuscular effects with demyelination in the cerebellum and brain stem. The LOAEL was 34 mg/kg/day based on decreased body weight gain and food consumption in male rats. There was no NOAEL because effects occurred at all doses tested.

Hong et al. (1985) reported the results of a chronic toxicity study commissioned by the U.S. Army (Ellis et al., 1979) of mice fed 98% pure 2,4- dinitrotoluene (2,4-DNT) in the diet for up to 24 months. Groups of 38 male and 38 female CD-1 mice were administered 2,4-DNT in their diets at average doses of 0, 14, 95, or 898 mg/kg/day. Both sexes of the high-dose animals and the males of the mid-dose groups had decreased weight gain that was approximately 10-22% lower than that of controls. High-dose males and females exhibited toxic anemia, reticulocytosis, and significant (p<0.05) increases in spleen and liver weights. All treated mice had an increased dose-related pigment in many tissues and organs including the liver, spleen, lungs, and kidney. High-dose females demonstrated ovarian atrophy. Mid- and high-dose males exhibited testicular atrophy.

In a separate study (Lee et al., 1978), groups of 16 male and 16 female CD-1 mice were fed diets containing 0, 0.07, 0.20, or 0.7% 2,4-DNT (98% pure) for 13 weeks. The corresponding daily intakes were 0, 47, 137, or 413 mg/kg/day for males, and 0, 52, 147, or 468 mg/kg/day for females. Five mice died during the study. Compared with controls, treated males exhibited a dose-dependent decrease in body weight (3, 11, and 19% from low to high dose) and, in the high-dose group only, there was decreased food consumption. The high-dose group of both sexes were anemic (decreased erythrocyte count, hematocrit, and hemoglobin) with concurrent reticulocytosis, mild hepatocellular dysplasia, and Kupffer cell dysplasia. High- and mid-dose males had mild degeneration of the seminiferous tubules or testicular degeneration. After 4
weeks off treatment, mice recovered completely. The LOAEL was 47 mg/kg/day, based on body weight loss in males. There was no NOAEL because effects occurred at all doses tested.

Groups of 10 male Sprague-Dawley rats were administered 2,4-DNT (purity not reported) in corn oil by oral gavage at 0, 60, 180, or 240 mg/kg/day for 5 days (Lane et al., 1985). Significant reductions in the mating index and a sharp decrease in sperm-positive and pregnant females were observed in the 240-mg/kg/day dose group. Because of this finding, statistical evaluation of the reproductive results was difficult. No dominant lethal effects, characterized by early fetal deaths, were observed. Dose levels at or below 180 mg/kg/day did not result in changes in fertility or fetal death.

Bloch et al. (1988) fed groups of 9-10 Sprague-Dawley rats 2,4-DNT (97% pure) at dietary levels of 0, 0.1, or 0.2% (0, 1000, or 2000 ppm, respectively; or 0, 100, or 200 mg/kg/day, respectively). Effects observed in the highest dose group included significant body weight reduction (p <0.05), significant increases in serum follicle stimulating hormone and luteinizing hormone (p <0.05), significantly reduced sperm count (p <0.01), disruption of spermatogenesis, and histological alterations or degeneration in Sertoli cells, spermatocytes, and spermatids. No significant effects were observed in the low-dose rats.

In a 3-generation study conducted by Ellis et al. (1979), groups of 10-24 Sprague-Dawley rats/sex were fed diets containing 0, 15, 100, or 700 ppm (approximately 0, 0.75, 5, or 35 mg/kg/day, respectively) 2,4-DNT (98% pure) for up to 6 months prior to mating. Each parental generation produced two sets of offspring (Fa and Fb litters). The study was terminated during the third generation after weaning of the second litter (Fb). The highest dose was associated with reduced parental body weight, reduced pup survival, reduced fertility in F1 animals, and slightly lower mean litter size and pup weight. At mid- and low-dose levels there were slight reductions in body weight for first and third generation pups; however, parental fertility and offspring viability were not affected. The LOAEL is 700 ppm, based on severe reductions in fertility. The NOAEL is 100 ppm.

Technical grade DNT (76% 2,4-DNT; 19% 2,6-DNT; 5% other isomers) was administered in corn oil by gavage to groups of 5-20 time-mated female Fischer 344 rats on gestation days 7-20 (Price et al., 1985). The doses were 0, 14, 35, 37.5, 75, 100, or 150 mg/kg/day. In the 150 mg/kg/day group there was 46% mortality and clinical signs of toxicity began on gestation day 11. Mortality for the other treatment groups was similar to that of the control group. Corrected body weight gain (minus gravid uterine weight) was significantly reduced in dams receiving 14, 100, or 150 mg/kg/day. Relative liver weight was increased significantly in the 75- and 100-mg/kg/day groups. Relative spleen weight was significantly increased at all doses except 14 mg/kg/day. There were no treatment-related effects on the number of corpora lutea, implantations, live and dead fetuses, litter size, sex ratio, fetal weight, crown rump length,
placental weight, or incidences of malformations and variations. There was a statistically insignificant increase in the percent resorptions in the 150-mg/kg/day group, which was considered to be indicative of a compound-related effect. Developmental effects noted in the fetuses were reduced liver weight at 14 mg/kg/day, and increased spleen weight at 35 and 75 mg/kg/day.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF — This uncertainty factor includes a factor of 10 for interspecies variability and a factor of 10 for intraspecies variability.

MF — None

I.A.4. Additional Studies/Comments (Oral RfD)

Reported human health effects from DNT exposure are from occupational exposure studies in which workers were exposed primarily by inhalation with some contribution assumed from dermal absorption and ingestion (Etnier, 1987; Turner, 1986; Turner et al., 1985; Woollen et al., 1985). Major effects from chronic exposure include methemoglobinemia, characterized by Heinz body formation and compensatory reticulocytosis; cyanosis; neurotoxicity; and possible excess mortality from ischemic heart disease and residual circulatory system effects. Neurotoxicity is characterized by vertigo, paresthesia, tremors, unconsciousness, and paralysis. Humans appear to metabolize DNT qualitatively similar to animals with rapid absorption and urinary excretion of metabolites.

Heinz body formation has been observed in humans, dogs, and rodents that were exposed to DNT. Heinz bodies are thought to consist of denatured hemoglobin, possibly sulfhemoglobin, that may form disulfide bonds with red blood cell membranes and thus lead to impaired ion transport resulting in hyperpermeability and hemolysis (Smith, 1986). Cat, mouse, dog, and human erythrocytes are thought to be particularly susceptible to Heinz body formation.

Monitoring and production data indicate that the occurrence of 2,6-DNT is usually found in the presence of 2,4-DNT with the latter more significant by volume. Subchronic (13 week) studies in dogs, rats, and mice indicate that 2,4- and 2,6-DNT systemic toxicity may be qualitatively and quantitatively similar. Oral dosing studies with technical grade DNT (tg-DNT; approximately 75% 2,4-DNT, 20% 2,6-DNT, and 5% other isomers) do not elucidate the relative contribution of the various isomers to toxic effects.

Dinitrotoluene isomers are metabolized initially by liver oxidation (Rickert et al., 1984). Some metabolites are conjugated with sulfate or glucuronate and subsequently excreted in the urine or
bile. The bile metabolites are hydrolyzed and reduced further by intestinal microflora. The bacterial metabolites are reabsorbed from the gut into the systemic circulation, oxidized in the liver, and excreted either in the urine or the bile for additional reduction by intestinal bacteria. There are species qualitative and quantitative differences; however, typical urinary metabolites of orally administered 2,4-DNT\([\text{ring-14C}]\) in female CD rats, CD-1 mice, New Zealand white rabbits, beagle dogs, and rhesus monkeys were the glucuronide conjugates of 2,4-dinitrobenzyl alcohol and 2-amino-4-nitrobenzyl alcohol. Smaller amounts of 2,4-diaminotoluene, 2,4-diaminobenzyl alcohol, 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, and 2,4-dinitrobenzoic acid were also recovered from each species. Several studies demonstrated similar urinary metabolites in male rats and mice. Humans exposed occupationally (via inhalation and assumed dermal routes) to tg-DNT excreted some of the same urinary metabolites demonstrated in animals (e.g., the unchanged parent compound, 2,4-dinitrobenzyl alcohol, 2,4-dinitrobenzyl alcohol glucuronide, and 2,4-dinitrobenzoic acid) (Levine et al., 1985; Turner, 1986; Turner et al., 1985; Woolen et al., 1985). Other 2,4-DNT metabolites detected in the workers include 2-amino-4-nitrobenzoic acid, 4-amino-2-nitrobenzoic acid, 2-acetylamino-4-nitrobenzoic acid, and 4-acetylamino-2-nitrobenzoic acid.

I.A.5. Confidence in the Oral RfD

Study — High
Database — High
RfD — High

The toxic effects observed in the 2-year dog study are based on an adequate number of animals of both sexes. In addition, a variety of gross, histological, hematologic, and clinical endpoints were evaluated. These effects are consistent with those reported to occur in exposed humans. The database is rated high to medium because there are numerous acute, subchronic, chronic, and lifetime studies in several mammalian species. However, developmental toxicity studies with 2,4-DNT are lacking. Several rodent strains have been tested, and both sexes have been tested in all species. Pharmacokinetics and toxic effects demonstrated in laboratory animal species are consistent with observations from human exposure studies. The ratings for both the study and the database result in a high to medium level of confidence in the RfD.

I.A.6. EPA Documentation and Review of the Oral RfD


Other EPA Documentation — None

Verification Date — 08/14/1991

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for 2,4-Dinitrotoluene conducted in September 2002 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — 2,4-Dinitrotoluene
CASRN — 121-14-2

The health effects data for 2,4-dinitrotoluene were reviewed by the U.S. EPA RfD/RfC Work Group and determined to be inadequate for derivation of an inhalation RfC. For additional information on health effects of this chemical, interested parties are referred to the EPA documentation listed below.


Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfC for 2,4-Dinitrotoluene conducted in September 2002 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

EPA Contacts:

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — 2,4-Dinitrotoluene
CASRN — 121-14-2

This substance/agent has not undergone a complete evaluation and determination under US EPA's IRIS program for evidence of human carcinogenic potential.

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Substance Name — 2,4-Dinitrotoluene
CASRN — 121-14-2

VI.A. Oral RfD References


VI.B. Inhalation RfC References


VI.C. Carcinogenicity Assessment References

None
VII. Revision History

Substance Name — 2,4-Dinitrotoluene
CASRN — 121-14-2

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VIII. Synonyms

Substance Name — 2,4-Dinitrotoluene
CASRN — 121-14-2
Last Revised — 03/01/1991

- 121-14-2
- BENZENE, 1-METHYL-2,4-DINITRO-
- 2,4-DINITROTOLUENE
- 2,4-DINITROTOLUOL
- 2,4-DNT
- 1-METHYL-2,4-DINITROBENZENE
- TOLUENE, 2,4-DINITRO-