2,4-/2,6-Dinitrotoluene mixture; no CASRN

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-/2,6-Dinitrotoluene mixture

File First On-Line 09/01/1990

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	not evaluated	
Inhalation RfC (I.B.)	not evaluated	
Carcinogenicity Assessment (II.)	yes	09/01/1990

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

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

Substance Name — 2,4-/2,6-Dinitrotoluene mixture
CASRN —

Not available at this time.

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

Substance Name — 2,4-/2,6-Dinitrotoluene mixture
CASRN —

Not available at this time.

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — 2,4-/2,6-Dinitrotoluene mixture
CASRN —
Last Revised — 09/01/1990

Section II provides information on three aspects of the carcinogenic assessment for the substance in question; the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per ug/L drinking water or risk per ug/cu.m air breathed. The third form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000 or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in The Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

NOTE: The carcinogenicity assessment for dinitrotoluene mixture includes both 2,4-dinitrotoluene and 2,6-dinitrotoluene.

II.A. Evidence for Human Carcinogenicity

II.A.1. Weight-of-Evidence Characterization

Classification — B2; probable human carcinogen

Basis — Based on multiple benign and malignant tumor types at multiple sites in both sexes of rats (2 strains) and malignant renal tumors in male mice. The classification is supported by evidence of mutagenicity.

II.A.2. Human Carcinogenicity Data

None.

II.A.3. Animal Carcinogenicity Data

Sufficient. Ellis et al. (1979) tested 2,4-DNT (98% 2,4-DNT and 2% 2,6- DNT) in a chronic oral study using Charles River CD (Sprague-Dawley) rats (38/sex/dose) and CD-1 Swiss mice (58/sex/dose) for 2 years. Rats and mice were fed dietary concentrations of 0, 15, 100, and 700 ppm and 0, 100, 700, and 5000 ppm, respectively. Mortality was high in all treatment groups; the control group survival rate at 2 years was only 40-45% in rats and 20-30% in mice. In rats the test chemical induced increased incidences of hepatocellular carcinomas in high-dose males (1/25, 2/28, 2/19, 6/30) and a statistically significant increase in the same tumor type in high-dose females (0/23, 0/35, 1/27, 19/35). The incidence of hepatocellular neoplastic nodules was not considered statistically significantly elevated in any of the rat treatment groups. A statistically significant increase in the incidence of benign mammary gland tumors was observed in high-dose female rats (8/23, 9/35, 16/27, 33/35). Most male mice in the high-dose group died before 12 months and were not included in the incidence. In male mice the incidence of kidney tumors (both benign and malignant) was significantly elevated in the mid-dose group (0/20, 4/21, 15/17 for control, low and medium dose groups). No evidence of treatment-related increases in tumor frequency was noted in female mice.

In a 2-year NCI study (1978), 2,4-DNT (greater than 95% purity) was administered in the diet of Fischer 344 rats (50/sex/dose) and B6C3F1 mice (50/sex/dose) at doses of 80 and 200 ppm (rats) and 80 and 400 ppm (mice). Controls consisted of 75 rats/sex and 50 mice/sex. Rats and mice were on test for 78 weeks followed by an additional observation period of 13 to 26 weeks. Survival was adequate in all groups, and a reduced body weight gain in high dose groups indicated that an MTD had been approached; this indicates that the study conditions were valid. Only benign tumors were noted. 2,4-DNT induced a statistically significant increase in fibromas of the skin and subcutaneous tissue in male rats (0/71, 7/49, 13/49) and fibroadenomas of the mammary gland in high-dose female rats (13/71, 12/49, 23/50). No statistically significant increase in incidence of tumors was noted in male or female mice.

A CIIT study (1982) treated F344 rats (130/sex/dose) with technical grade DNT (76% 2,4-DNT and 19% 2,6-DNT) at dietary concentrations of 0, 3.5, 10.0 and 35.0 mg/kg/day. All male and female rats in the high-dose group were sacrificed at 55 weeks because of significantly reduced

survival. Histopathological studies were performed on sacrificed animals (20 rats/sex) with 100% incidence of hepatocellular carcinoma in male rats (20/20) and 55% incidence in females (11/20). Mid- and low-dose animals were kept on test for 104 weeks. The incidences of liver carcinoma in males at 104 weeks were 1/61 for the control group, 9/70 for the low-dose group, 22/23 for the mid-dose group, and 20/20 (at 55 weeks) for the high-dose group; the incidences in females at 104 weeks were 0/57 for the control group, 0/61 for low-dose group, 40/68 for mid-dose group and 11/20 (at 55 weeks) for the high-dose group. The incidence of neoplastic nodules in males was 9/61, 11/70, 16/23, and 5/20, and the incidence in females was 5/57, 12/61, 53/68, and 12/20, at 104 weeks for the control, low-, mid- and (at 55 weeks) for the high-dose groups, respectively. Cholangiocarcinomas, presumably derived from the bile duct epithelium, were also observed in three high-dose males at 55 weeks and two mid-dose males at 104 weeks.

Leonard et al. (1987) treated groups of 20 F344 male rats with either technical-grade DNT, 2,4-DNT, or 2,6-DNT in the diet for 1 year. There was an untreated control group of 20 rats. Technical DNT (76% 2,4-DNT, 19% 2,6-DNT) (35 mg/kg/day) induced hepatocellular carcinomas in 47% (9/19) of the treated males. 2,6-DNT (99.9% purity) induced hepatocellular carcinomas in 100% (19/19) of the high-dose rats (14 mg/kg/day) and 85% (17/20) of the low-dose (7 mg/kg/day). No tumors were found in controls or rats exposed to 2,4-DNT (99.9 purity) at 27 mg/kg/day. Two low-dose males receiving 2,6-DNT and two males receiving technical DNT developed cholangiocarcinoma. Although the duration of these studies was limited to 1 year and the number of animals tested was small, the data suggest that the 2,6-isomer accounts for much of the carcinogenic activity observed in previous mixed-isomer DNT bioassays.

II.A.4. Supporting Data for Carcinogenicity

The mutagenicity of dinitrotoluenes has been tested in numerous systems. 2,4-DNT causes reverse and forward mutations in several strains of Salmonella typhimurium (Couch et al., 1981; Tokiwa et al., 1981). DNA repair, as measured by UDS, was shown to occur in an in vivo male F344 rat hepatocyte assay (Mirsalis and Butterworth, 1982), but negative results were obtained in in vitro assays in rat hepatocytes (Bermudez et al., 1979) and spermatocytes (Working and Butterworth, 1984). Although Lee et al. (1978) observed an increased frequency of chromosomal aberrations in CD rat lymphocyte and kidney cultures, Ellis et al. (1979) observed no increased frequency in CD rat and beagle dog bone marrow and kidney cultures.

In a series of in vivo tumor initiation-promotion tests, Leonard and coworkers (Popp and Leonard, 1983; Leonard et al., 1983, 1986) compared the development of hepatic foci by the 2,4- and 2,6-DNT isomers and technical DNT. Both 2,6- and technical DNT showed comparable initiating activity in partially-hepatectomized male F344 rats. In a promotion experiment, male F344 rats were initiated with a single dose of diethylnitrosamine prior to feeding 27 mg/kg/day 2,4-DNT or 7 mg/kg/day 2,6-DNT for 12 weeks. Positive results were observed for both 2,4 and

2,6-DNT, with the 2,6-isomer yielding a stronger response. These findings suggest the 2,6-isomer may be a complete hepatocarcinogen and 2,4-DNT a promoter.

In a skin-painting study using SENCAR mice, 2,6-DNT and 2,4-DNT were given as initiators (1, 5, or 10 mg) followed by TPA application for 30 weeks. Increased incidence of squamous cell carcinoma (5%) was observed in the 2,6- DNT-treated mice, although these results were not statistically significant (Slaga et al., 1985). When given intraperitoneally at 10 mg/kg followed by weekly TPA applications, 2,6-DNT produced 10% incidence of carcinomas, which was not significantly greater than controls. In the lung tumor bioassay, neither 1200 mg/kg of 2,4- nor 4800 mg/kg of 2,6-DNT administered intraperitoneally 3 times a week for 8 weeks increased the incidence of lung tumors in male A/Jax mice (Slaga et al., 1985). Schut et al. (1982), Stoner et al. (1984) and Maronpot et al. (1983) also reported negative results for 2,4-DNT administered orally or ip in the lung tumor bioassay with A/Jax mice, but positive results were reported using female A/St mice (Maronpot et al., 1983).

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

II.B.1. Summary of Risk Estimates

Oral Slope Factor — 6.8E-1 per (mg/kg)/day

Drinking Water Unit Risk — 1.9E-5 per (ug/L)

Extrapolation Method — Linearized multistage procedure

Drinking Water Concentrations at Specified Risk Levels:

Risk Level	Concentration
E-4 (1 in 10,000)	5 ug/L
E-5 (1 in 100,000)	5E-1 ug/L
E-6 (1 in 1,000,000)	5E-2 ug/L

II.B.2. Dose-Response Data (Carcinogenicity, Oral Exposure)

Tumor Type — liver: hepatocellular carcinomas, neoplastic nodules; mammary gland: adenomas, fibroadenomas, fibromas, adenocarcinomas/carcinomas

Test animals — rat/Sprague-Dawley, female
Route — diet
Reference — Ellis et al., 1979

Administered Dose (ppm)	Human Equivalent Dose (mg/kg)/day	Tumor Incidence
0	0	11/23
15	0.129	12/35
100	0.927	17/27
700	7.557	34/35

II.B.3. Additional Comments (Carcinogenicity, Oral Exposure)

The tumor incidences could be combined for quantitative purposes because the report by Ellis et al. (1979) provided pathology data for the individual animals. Transformed doses reflect the measured weight of the rats for each treatment period (0.425 kg control and low dose, 0.410 kg medium dose, 0.325 kg high dose).

The U.S. Army (ORNL, 1987) has calculated a quantitative risk estimate for the 2,6-isomer based on Leonard et al. (1987).

The unit risk should not be used if the water concentration exceeds 500 ug/L, since above this concentration the slope factor may differ from that stated.

II.B.4. Discussion of Confidence (Carcinogenicity, Oral Exposure)

Relatively few animals were observed for a period of time approximating the lifespan of the animals. A slope factor of 3.9E-1 per (mg/kg)/day, obtained from renal tumors in male CD-1 mice (Ellis, 1979), is supportive of the risk estimate.

II.C. Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

Not available.

II.D. EPA Documentation, Review, and Contacts (Carcinogenicity Assessment)

II.D.1. EPA Documentation

Source Document — U.S. EPA, 1986, 1987

The values in the 1986 Health and Environmental Effects Profile for Dinitrotoluene have received extensive Agency Review.

II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Work Group Review — 04/01/1987, 04/22/1987, 05/25/1988, 11/09/1988, 11/30/1988, 05/03/1989

Verification Date — 05/03/1989

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the cancer assessment for 2,4-/2,6-Dinitrotoluene mixture conducted in August 2003 identified one or more significant new studies. IRIS users may request the references for those studies from the IRIS Hotline at hotline.iris@epa.gov or 202-566-1676.

II.D.3. EPA Contacts (Carcinogenicity Assessment)

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).

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

VI. Bibliography

Substance Name — 2,4-/2,6-Dinitrotoluene mixture
CASRN —

VI.A. Oral RfD References

None

VI.B. Inhalation RfD References

None

VI.C. Carcinogenicity Assessment References

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VII. Revision History

Substance Name — 2,4-/2,6-Dinitrotoluene mixture
CASRN —

Date	Section	Description
09/01/1990	II.	Carcinogen assessment on-line
10/28/2003	II.D.2.	Screening-Level Literature Review Findings message has been added.

VIII. Synonyms

Substance Name — 2,4-/2,6-Dinitrotoluene mixture
CASRN —
Last Revised — 09/01/1990

- 121-14-2
- BENZENE, 1-METHYL-2,4-DINITRO-
- 2,4-DINITROTOLUENE
- 2,4-DINITROTOLUOL
- 2,4-DNT
- HSDB 1144
- HSDB 2931
- 1-METHYL-2,4-DINITROBENZENE
- NCI-C01865
- NSC 7194
- RCRA WASTE NUMBER U105
- RCRA WASTE NUMBER U106
- TOLUENE, 2,4-DINITRO-
- 606-20-2
- 2,6-DINITROTOLUENE
- 2,6-DNT
- BENZENE, 2-METHYL-1,3-DINITRO-
- 2-METHYL-1,3-DINITROBENZENE
- TOLUENE, 2,6-DINITRO-