Drinking Water Health Advisory for Dinitrotoluenes

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Technical grade dinitrotoluene (TG DNT) is a mixture of six isomers. 2,4- and 2,6-DNT are the most prevalent forms and make up more than 90 percent of the DNT used in commerce. Minor components of TG DNT include the 2,3-, 2,5-, 3,4- and 3,5-DNT isomers. Most toxicological and environmental studies have been conducted using either the technical grade mixture of these isomers, or purified 2,4- or 2,6-DNT. Only a few short-term or *in vitro* studies have been done on the minor isomers. These studies suggest that the minor isomers are toxicologically similar to 2,4- and 2,6-DNT. Effective doses or aquatic concentrations of the six DNT isomers are generally within an order of magnitude of each other. All DNT isomers are capable of inducing cyanosis secondary to methemoglobin formation. At high doses, this is the critical effect that can lead to death. Target tissues include the hematopoetic system, the central nervous system and the male reproductive system. TG DNT, as well as 2,4- and 2,6-DNT are known to cause cancer in animals. Minor isomers have not been tested for this effect. All isomers have shown mutagenic effects in short-term studies. In order to protect against adverse health effects that can result from long-term exposure to DNT, a single health advisory for the summed concentration of all DNT isomers is proposed.

Background

Dinitrotoluenes are produced by nitration of toluene or nitrotoluenes. Approximately 99% of DNT are used for polyurethanes, as an intermediate in the production of toluene di-isocyanate. Manufacturing and processing of DNT is executed in closed systems. However, DNT can be released to the environment as a result of accidental releases and disposal. DNTs are also used as gelatinizing and waterproofing agents in the manufacture of explosives, and in smokeless gunpowders. About 500 US workers are potentially exposed to DNT during the production of munitions and explosives. The main route of exposure at ammunition facilities is inhalation, but dermal contact and inadvertent ingestion can also be substantial.

Human exposure to DNTs has been linked to a variety of health effects, including cyanosis, dizziness, headache, metallic taste, shortness of breath, weakness, loss of appetite, nausea, and vomiting. Other symptoms including pain or paresthesia in extremities, abdominal discomfort, tremors, paralysis, chest pain, and unconsciousness have also been reported. The primary targets of DNT toxicity are the hematopoietic system (pallor, cyanosis, anemia, and leukocytosis), the cardiovascular system (ischemic heart disease), the nervous system (muscular weakness, headache, dizziness, nausea, insomnia, and tingling pains in the extremities) and the reproductive system (reduction of sperm counts, alteration of sperm morphology, and aspermatogenesis). An association

between DNT exposure and increased risk of hepatocellular carcinomas and subcutaneous tumors in rats, as well as renal tumors in mice, has been established.

Dinitrotoluene (DNT) is a potent liver toxin and carcinogen in rats. Prolonged exposure causes methemoglobinemia. DNT is also toxic to the nervous system and has reproductive effects in all species tested. Administered orally, DNT has caused hematological effects, including methemoglobinemia, anemia, reticulocytosis, and increases in the number of Heinz bodies in all species tested.

The oral lethal dose of the various DNT isomers range from 216 mg/kg for 3,5-DNT in female rats to 1,954 mg/kg for 2,4-DNT in male mice [ACGIH 1991].

3,5-DNT is more toxic to male and female rats and mice than other isomers [ACGIH 1991]. Technical-grade DNT fed to rats for 24-months caused liver discoloration at a dose of 3.5 mg/kg/day and liver nodules and malignancies at a dose of 14 mg/kg/day [ACGIH 1991]. An NCI bioassay also showed that technical-grade DNT causes subcutaneous tissue fibromas in male rats and mammary gland fibroadenomas in female rate. DNT administered orally to dogs caused neurotoxic effects, with tremors, loss of coordination, and convulsions; neurotoxic effects are not seen in either rats or mice until much higher doses are given [Hathaway et al. 1991; NLM 1992]. Oral administration of DNT to rats, mice, and dogs causes reproductive effects, including testicular and ovarian atrophy, decreased fertility, and decreased sperm count [ACGIH 1991].

Dinitrotoluene causes methemoglobinemia in workers [ACGIH 1991]. The first symptom of methemoglobinemia is headache, followed by fatigue, nausea, vomiting, and chest pain. Onset may be delayed as long as 4 hours after exposure [ACGIH 1991]. Chronic exposure to dinitrotoluene can cause anemia and jaundice [Clayton and Clayton 1982]. The effects of dinitrotoluene are exacerbated by alcohol consumption [NJDH 1992].

In an occupational setting, DNT can be absorbed through the skin in toxic amounts [ACGIH 1991; NLM 1992].

Table 1. Screening Level Toxicity Information for DNT Isomers

	2,3-DNT	2,4-DNT	2,5-DNT	2,6-DNT	3,4-DNT	3,5-DNT
Synonym	1-Methyl-2,4-dinitrobenzene	2-methyl-1-nitrobenzene	2-Methyl-1,4-dinitrobenzene	2-Methyl-1,3-dinitrobenzene	4-Methyl – 1,2-dinitrobenzene	1-Methyl-3.5-dinitrobenzene
CAS No	602-01-7	121-14-2	619-15-8	606-20-2	610-39-9	618-85-19
% in Tech Grade	1.3%	78%	0.5%	18%%	2.4%	<0.1%
$LD50_{low}$ (rat)	911 mg/kg	270 mg/kg	517 mg/kg	177 mg/kg	177 mg/kg	216 mg/kg
Cancer Class	Not classified	B2 – Known animal carcinogen	Not classified	B2- Known animal carcinogen	Not classified	Not classified
Mutagenicity	Weakly positive with activation	Positive	Positive	Positive	Weakly positive	Weakly positive
NOAEL Subchronic Chronic	 Not available Not available	 0.2 mg/kg/day	 Not available Not available	 4 mg/kg/day 7 mg/kg/day	 Not available Not available	 Not available Not available
Aquatic toxicity*	1.8 mg/L	32.8 mg/L	1.3 mg/L	18.5 mg/L	1.5 mg/L	22.6 mg/L
USEPA RfD	None	0.002 mg/kg/day	None	0.001 mg/kg/day	None	None
USEPA MCL	None	None	None	None	None	None
WI GWES	None	0.05 ug/L	None	0.05 ug/L	None	None
ACGIH – TLV	1.5 mg/cu m	1.5 mg/cu m	1.5 mg/cu m	1.5 mg/cu m	1.5 mg/cu.m	1.5 mg/cu m

*96-hr static LC50 Pimephales promelas Liu, Bailey and Pearson, 1983

Carcinogenicity:

The U.S. EPA classifies both 2,4-DNT and 2,6-DNT as B2 carcinogens (sufficient evidence of carcinogenicity in animals with inadequate or lack of evidence in humans).

In chronic studies, 2,4-DNT produced renal tumors in male mice and was moderately hepatocarcinogenic in rats. 2,6-DNT and technical grade DNT are potent hepatocarcinogens in rats[6]. In all these studies, increases in tumors were statistically significant and clear dose response relationships were observed.

One study of workers in a munitions plant concluded no significant increases in cancer mortality[9]. However, this study was limited by small cohort size.

Mutagenicity:

Both DNT isomers are positive in the *S. typhimurium* histidine reversion assay and the TM 677 forward-mutation assay both with and without metabolic activation but negative in numerous mammalian cell forward reversion assays[10].

Reproductive Effects:

Male reproductive effects resulting from oral administration of 2,4-DNT include decreased spermatogenesis in rats and dogs at 20-25 mg/kg/day and testicular atrophy at 34 mg/kg/day[5,6]. In a multigenerational rat study, decreased neonatal viability was noted at 40 mg/kg/day of 2,4-DNT[6]. A study of DNT's reproductive effects in workers showed decreased sperm counts, slight abnormalities in the sperm of workers, and a slight increase in the rate of spontaneous abortions in their wives [NLM 1992]. A retrospective cohort mortality study of DNT-exposed workers found a significant increase in mortality due to ischemic heart disease. The average DNT exposure of these workers was estimated to be 1 mg/kg/day or less from inhalation, ingestion, and dermal sources [NLM 1992].

Interactive Effects:

In a 1942 study, workers exposed to 2,4-DNT were found to be more sensitive to alcohol. Exposure of rats to 2,6-DNT both increased and decreased the rate of phenobarbital metabolism, dependent on the time of exposure.

Environmental Fate:

Atmospheric
The low vapor pressure of the DNTs (2,4-: 0.005 torr at 20 C; 2,6-: 0.018 torr at 20°C) suggests that volatilization from contaminated surface water or soil are unlikely. In the atmosphere, DNT is degraded by photochemically produced OH radicals. The half-life is calculated to be approximately 84 days.

Aquatic
The solubilities of 2,4- and 2,6-DNT in water are 270 and 180 mg/L, respectively. Major routes of DNT degradation are photo-oxidation and biodegradation. The half life of DNT is 3 to 10 hours in sunlit natural waters and 28 days in anaerobic sewage[14]. No studies of DNT persistence in groundwater have been noted, but disappearance of the compounds under these conditions may be negligible.

Terrestrial
DNTs are poorly adsorbed to soils[1]. As a result, "DNTs in buried munition wastes could potentially be released to groundwater or transported as contaminated soil and sediment.[1]" No studies have been performed on soil DNT biodegradation.

Analytical Laboratory Methods:

DNTs are extracted from water using methylene chloride and subsequently analyzed by gas chromatography/mass spectrometry using EPA method 625. The detection limit for this procedure is about 3 µg/L. Alternatively, high pressure liquid chromatography with an ultraviolet detector can be used. This method has a detection limit of 10 µg/L.

USEPA and USCDC Regulatory Position:

Reference Dose 2,4-DNT (non-cancer effects):	0.002 mg/kg/day
Reference Dose for 2,6-DNT (non-cancer effects):	0.001 mg/kg/day
MCL	None
Cancer slope factor for 2,4-DNT and 2,6-DNT	0.68 per mg/kg/day
Drinking water concentration at 1-in-a-million:	0.05 ug/L
Ambient Water Quality Criteria: Water & Fish	0.11 ug/L
Ambient Water Quality Criteria: Fish only	9.1 ug/L
ATSDR MRL:	0.002 mg/kg/day

Recommendations and Conclusions:

In accordance directives outlined in Chapter 160 of the Wisconsin State Statutes, the Department of Health and Family Services recommends that all isomeric forms of dinitrotoluene be regulated as a single entity and that the health advisory level limit cancer risk to a theoretical level of 1-in-a-million. This recommendation is based on the following findings:

1). A complete toxicological database is available for technical grade DNT, which is a mixture of all isomers, and for the two major isomers (2,4- and 2,6-DNT). Only limited testing has been conducted with the other 4 isomers making independent risk assessments for them impossible. In 2000, the Chemical Manufacturer's Association petitioned the US EPA to remove individual isomers of DNT from the High Production Challenge Program arguing that none of the minor isomers is produced separately in commerce. In a letter to Charles M. Auer, Director of the USEPA's Chemical Control Division, CMA stated, "Separately evaluating each isomer under the HPV program will not result in a better understanding of the adverse health or safety implications of dinitrotoluene." EPA's approval of this request alleviated a requirement for the manufacturers to provide screening level toxicity and environmental fate data for individual DNT isomers and allowed submission of data for technical grade DNT instead.

2). Published studies for the minor isomers indicate that their toxic effects are the same as that of TG DNT and that the minor isomers are as toxic or more toxic than 2,4- and 2,6-DNT.

3). All isomers of DNT have shown mutagenic activity in short-term studies.

4). TG DNT, as well as the purified 2,4- and 2,6- isomers are classified as known animal carcinogens. Minor isomers have not been tested for this effect, but are structurally and toxicologically similar suggesting that they may also have carcinogenic effects.

5) The six isomers of DNT are structurally and toxicological similar. In addition, these isomers have a common commercial source and are frequently found together in the environment.

The recommended Health Advisory for total dinitrotoluene residues in Wisconsin groundwater is 0.05 µg/L.