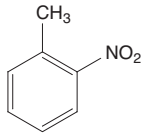
# *o*-Nitrotoluene

## CAS No. 88-72-2

Reasonably anticipated to be a human carcinogen

First listed in the *Twelfth Report on Carcinogens* (2011)

Also known as 2-nitrotoluene




## Carcinogenicity

*o*-Nitrotoluene is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting data on mechanisms of carcinogenesis.

### *Cancer Studies in Experimental Animals*

Oral exposure to *o*-nitrotoluene caused tumors at several different tissue sites in rats and mice and early onset of cancer in male rats. Malignant mesothelioma and mesothelial-cell hyperplasia of the tunica vaginalis of the epididymis were observed in male rats administered *o*-nitrotoluene in their feed for 13 weeks (NTP 1992). Bile-duct cancer (cholangiocarcinoma) was observed after 26 weeks, both in rats exposed to *o*-nitrotoluene for 26 weeks and in rats exposed for 13 weeks and then observed for 13 more weeks without exposure (NTP 1996). *o*-Nitrotoluene caused cancer at several tissue sites in two-year chronic exposure studies of rats and mice of both sexes and in a study in which male rats were exposed to *o*-nitrotoluene for 13 weeks and evaluated at two years (NTP 2002). In rats, *o*-nitrotoluene caused (1) subcutaneous skin tumors and mammary-gland tumors (fibroadenoma) in both sexes, (2) malignant mesothelioma and benign or malignant tumors of the liver (hepatocellular adenoma or carcinoma or cholangiocarcinoma) and lung (alveolar/bronchiolar adenoma or carcinoma) in males, and (3) benign liver tumors (hepatocellular adenoma) in females. In mice, it caused malignant blood-vessel tumors (hemangiosarcoma) in both sexes, malignant tumors of the large intestine (cecal carcinoma) in males, and benign or malignant liver tumors (hepatocellular adenoma or carcinoma) in females (NTP 2002).

### *Studies on Mechanisms of Carcinogenesis*

Following oral administration to rats and mice, *o*-nitrotoluene is absorbed into the blood and rapidly cleared; the serum half-life is 1.5 hours in rats (NTP 2002). In the rat liver, *o*-nitrotoluene is metabolized to *o*-nitrobenzyl alcohol and can follow several metabolic pathways: (1) glucuronidation to *o*-nitrobenzyl glucuronide, (2) sulfation and subsequent reaction with glutathione and acetylcysteine to *o*-nitrobenzyl sulfate, *S*-(*o*-nitrobenzyl)glutathione, and *S*-(*o*-nitrobenzyl)-*N*-acetylcysteine, or (3) metabolism to *o*-aminobenzyl alcohol followed by oxidation to *o*-aminobenzoic acid. The metabolites are eliminated primarily in the urine. The major metabolites are *o*-nitrobenzyl glucuronide and *o*-nitrobenzoic acid major metabolites in rats and mice and *o*-aminobenzyl alcohol and *S*-(*o*-nitrobenzyl)-*N*-acetylcysteine in rats. Female rats excrete less than half as much of the dose in the form of *o*-aminobenzyl alcohol, *o*-nitrobenzyl alcohol, or *S*-(*o*-nitrobenzyl)-*N*-acetylcysteine as male rats (NTP 2002). The glucuronidated form can also be excreted in the bile; when the glucuronidated form in the bile is excreted into the small intestine, intestinal bacteria can deconjugate it and reduce the nitro group to an amino group, forming aminobenzyl alcohol. Aminobenzyl alcohol can be reabsorbed from the intestine and further metabolized by the liver to reactive compounds (carbonium and nitrenium ions) that can covalently bind to DNA or to proteins (Chism and Rickert 1985, NTP 2002, 2008). Thus, microbial metabolism in the intestine is an important step in the carcinogenicity of *o*-nitrotoluene. However, neither *o*-aminobenzyl alcohol nor its metabolites have been detected in mouse urine after exposure to *o*-nitrotoluene (NTP 2002); therefore, other unidentified biochemical pathways leading to tumor formation most likely are involved.

*o*-Nitrotoluene did not cause mutations in bacteria. In studies of its ability to cause genetic damage in cultured mammalian cells, the results were mixed. *o*-Nitrotoluene caused (1) sister chromatid exchange in Chinese hamster ovary (CHO) cells, (2) chromosomal aberrations in Chinese hamster lung (CHL) cells and human peripheral lymphocytes but not in CHO cells, (3) micronucleus formation in CHL cells but not in CHO-K1 cells, and (4) DNA damage in L5178Y mouse lymphoma cells (NTP 2008). It did not induce DNA repair in rat or human hepatocytes (NTP 2008). In rats and mice exposed *in vivo*, *o*-nitrotoluene caused a slight increase in micronucleus formation in peripheral normochromatic erythrocytes in male mice at a high dose level; this finding was not considered conclusive. *o*-Nitrotoluene did not induce micronucleus formation in peripheral normochromatic erythrocytes in female mice or in polychromatic erythrocytes in the bone marrow of male rats or mice (NTP 2002). Following *in vivo* exposure of rats to *o*-nitrotoluene, DNA repair was increased in liver cells isolated from males, but not from females or germ-free males. These results, together with *o*-nitrotoluene's inability to induce DNA repair in hepatocytes *in vitro,* suggest that activation of *o*-nitrotoluene to become genotoxic is sex-specific and depends on both mammalian metabolism and metabolism by intestinal bacteria (Doolittle *et al*. 1983). However, *o*-nitrotoluene also caused tumors in other tissues in rats and mice of both sexes, suggesting that other activation mechanisms exist.

In rats exposed to *o*-nitrotoluene *in vivo*, DNA adducts were detected in the liver of males but not females (NTP 2008). Formation of DNA adducts was consistent with the reaction of intermediate compounds derived from *o*-aminobenzyl alcohol with guanine or adenine bases (Jones *et al.* 2003). The pattern of mutations in oncogenes from *o*-nitrotoluene–induced tumors was also consistent with guanine adduct formation: the majority of *p53* mutations in hemangiosarcomas were G:C to A:T transitions, and almost all the K-*ras* mutations in cecal carcinomas were G:C to T:A transversions (Hong *et al.* 2003, Sills *et al.* 2004). Mutations in the *p53*, β-*catenin*, and K-*ras* genes also were found in hemangiosarcomas from mice exposed to *o*-nitrotoluene, but not in spontaneously occurring hemangiosarcomas from unexposed mice (Hong *et al.* 2003).

In factory workers exposed to *o*-nitrotoluene, *o*-nitrotoluene–hemoglobin adducts were detected in the blood (Jones *et al.* 2005a), and *o*-nitrobenzoic acid and *o*-nitrobenzyl alcohol were detected in the urine (Jones *et al.* 2005b), providing evidence that human exposure to *o*-nitrotoluene results in production of a reactive metabolite(s). In addition, adducts between hemoglobin and 2-methylaniline (a metabolite of *o*-nitrotoluene) were identified in both exposed workers and exposed rats, and the level of 2-methylaniline–hemoglobin adducts in the blood of rats was proportional to the level of 2-methylaniline–DNA adducts in the livers of rats (Jones and Sabbioni 2003, Jones *et al.* 2003).

### *Cancer Studies in Humans*

The data available from epidemiological studies are inadequate to evaluate the relationship between human cancer and exposure specifically to *o*-nitrotoluene. One cohort study of workers involved in

the manufacture of magenta dye mentioned exposure of workers to *o*-nitrotoluene as part of the manufacturing process. A large excess of urinary-bladder cancer was reported; however, the workers were also exposed to other chemicals — *o*-toluidine (2-methylaniline) and 4,4′-methylenebis(2-methylaniline)—that are suspected of causing bladder cancer (Rubino *et al.* 1982). Two other studies of magenta manufacturing workers also reported an excess of urinary-bladder cancer, but did not report whether the workers were exposed to *o*-nitrotoluene (Case and Pearson 1954, Vineis and Magnani 1985).

## Properties

*o*-Nitrotoluene is a nitroaromatic compound. It is one of three isomers of nitrotoluene; the other two are *m*-nitrotoluene (also known as 3-nitrotoluene) and *p*-nitrotoluene (also known as 4-nitrotoluene). At room temperature, *o*-nitrotoluene is a yellow liquid with an odor of bitter almonds. It is slightly soluble in water and soluble in acetone, benzene, chloroform, diethyl ether, ethanol, and petroleum ether. It has a flash point of 106°C (closed cup) and an autoignition temperature of 305°C (PTCL 2003). It does not ignite easily; however, it may burn, and containers of *o*-nitrotoluene may explode when heated (HSDB 2010). Physical and chemical properties of *o*-nitrotoluene are listed in the following table.

| Property | Information |
|---|---|
| Molecular weight | 137.1 |
| Specific gravity | 1.162 at 19°C/15°C |
| Melting point | –9.5°C (needles); –2.9°C (crystals) |
| Boiling point | 222°C at 760 mm Hg |
| Log $K_{ow}$ | 2.30 |
| Water solubility | 650 mg/L at 30°C |
| Vapor pressure (mm Hg) | 0.188 at 25°C |
| Vapor density relative to air | 4.73 |

Source: HSDB 2010.

## Use

*o*-Nitrotoluene is used primarily in the production of *o*-toluidine (also known as *o*-aminotoluene or 2-methylaniline), 2-amino-4-chlorotoluene, 2-amino-6-chlorotoluene, *o*-toluidine-4-sulfonic acid, and other chemicals that are intermediates in the production of various azo dyes (IARC 1996). It is also used in the manufacture of (or the manufacture of intermediates for) other dyes, such as magenta and various sulfur dyes for cotton, wool, silk, leather, and paper (IARC 1996, HSDB 2010). In addition, it is used as an intermediate in the synthesis of (or the synthesis of intermediates for) explosives and a variety of organic chemicals, including compounds used in the agricultural chemical, pesticide, petrochemical, pharmaceutical, and rubber industries (HSDB 2010).

## Production

*o*-Nitrotoluene is produced principally by the nitration of toluene with a mixture of nitric acid and either sulfuric, aromatic sulfonic, or phosphoric acid (IARC 1996). U.S. production of *o*-nitrotoluene was calculated as 29 million pounds for 1981 (HSDB 2010) and estimated at 35.5 million pounds for 1993 (Dunlap 1998). From 1986 to 2002, the U.S. Environmental Protection Agency (EPA) listed *o*-nitrotoluene as a high-production-volume chemical, with combined production and imports totaling 10 million to 50 million pounds (EPA 2004). However, combined U.S. production plus imports of *o*-nitrotoluene reported to EPA in 2015 were less than 25,000 lb (EPA 2016). No data were found on U.S. imports or exports of *o*-nitrotoluene. In 2010, *o*-nitrotoluene was available from 14 U.S. suppliers (ChemSources 2010).

## Exposure

Exposure to *o*-nitrotoluene in the United States is expected to result primarily from dermal and inhalation exposure during its production and use. The general population may be exposed to *o*-nitrotoluene as a result of its occurrence in the environment from (1) inadvertent spills of *o*-nitrotoluene or chemical mixtures containing *o*-nitrotoluene, (2) emissions directly into the environment, or (3) breakdown products of dinitrotoluenes (DNT) and trinitrotoluenes (TNT). *o*-Nitrotoluene has been detected in U.S. air and water. The National Response Center database contains reports of two spills reported as *o*-nitrotoluene in 1990 and one spill reported as nitrotoluene (*o*-, *p*-, and mixtures) in 2000 (NRC 2008). Two ambient-air samples collected in Boise, Idaho, in the winter of 1986–87 contained *o*-nitrotoluene vapor at concentrations of 0.03 and 0.29 ng/m$^3$ (Nishioka and Lewtas 1992). *o*-Nitrotoluene was also detected in a paper-mill waste-treatment lagoon (concentration and location not reported) (HSDB 2010) and at concentrations ranging from 320 to 16,000 µg/L in the effluent of a U.S. plant producing TNT (IARC 1996, HSDB 2010).

DNT and TNT are used in the production of commercial and military explosives, and *o*-nitrotoluene has been found in groundwater, private well water, surface water, and soil at or near munitions production facilities and military training grounds. *o*-Nitrotoluene was found at average concentrations of 42.6 mg/L (42,600 µg/L) (Best *et al.* 2001) and 2.9 mg/L (2,900 µg/L) (Spain *et al.* 1999) in groundwater at a Tennessee munitions arsenal. At a facility that has produced munitions since World War II, *o*-nitrotoluene has been detected sporadically during routine groundwater monitoring of both the Ogallala aquifer, at concentrations of 0.12 to 2.9 µg/L (both measured in 2004), and a perched aquifer above the Ogallala, at concentrations of 0.14 µg/L (in 2003) to 5 µg/L (in 2004) (Pantex 2008). At a former munitions production site in Wisconsin, *o*-nitrotoluene was detected in off-site private well water at concentrations of up to 0.095 µg/L (WDHFS 2002). At a historical testing ground in Idaho, soil contaminated with TNT at a concentration of 39,100 ppm contained *o*-nitrotoluene at a concentration of 1.4 ppm (Radtke *et al.* 2002).

No information was found on the number of U.S. workers potentially exposed to *o*-nitrotoluene in the production of chemical intermediates. *o*-Nitrotoluene was detected at a concentration of 47 ng/m$^3$ in ambient air at a chemical manufacturing plant in New Jersey (IARC 1996) and at air concentrations of up to 2.0 mg/m$^3$ in the nitrotoluene production area of a Swedish plant producing pharmaceuticals and explosives (Ahlborg *et al.* 1985). The American Conference of Governmental Industrial Hygienists considers *o*-nitrotoluene to be an inducer of methemoglobin and recommends that methemoglobin in blood be used as a biological exposure index for *o*-nitrotoluene (and the other nitrotoluene isomers) (ACGIH 2009).

## Regulations

***Coast Guard (Dept. of Homeland Security)***

Minimum requirements have been established for the safe transport of *o*-nitrotoluene on barges.

***Department of Transportation (DOT)***

*o*-Nitrotoluene is considered a hazardous material, and special requirements have been set for marking, labeling, and transporting this material.

Safety measures after spills or leaks are prescribed in accordance with *o*-nitrotoluene being a combustible toxic hazardous material.

***Environmental Protection Agency (EPA)***

*Comprehensive Environmental Response, Compensation, and Liability Act*

Reportable quantity (RQ) = 1,000 lb.
(EPA has not carried out an Integrated Risk Information System assessment for *o*-nitrotoluene.)

*Emergency Planning and Community Right-To-Know Act*

*Toxics Release Inventory:* Listed substance subject to reporting requirements.

***Occupational Safety and Health Administration (OSHA, Dept. of Labor)***

While this section accurately identifies OSHA's legally enforceable PELs for this substance in 2010, specific PELs may not reflect the more current studies and may not adequately protect workers.
Permissible exposure limit (PEL) = 5 ppm (30 mg/m$^3$).
Potential for dermal absorption.

## Guidelines

***American Conference of Governmental Industrial Hygienists (ACGIH)***

Threshold limit value – time-weighted average (TLV-TWA) = 2 ppm.
Potential for dermal absorption.
Biological Exposure Index (BEI): (during or at end of shift) 1.5% of hemoglobin for methemoglobin in blood.

***National Institute for Occupational Safety and Health (NIOSH, CDC, HHS)***

Immediately dangerous to life and health (IDLH) limit = 200 ppm.
Recommended exposure limit (REL) = 2 ppm (11 mg/m$^3$).
Potential for dermal absorption.

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