

Dinitrotoluene (mixtures of isomers)

MAK Value Documentation, addendum – Translation of the German version from 2024

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Keywords

dinitrotoluene (mixtures of isomers); kidney; carcinogenicity; germ cell mutagenicity; skin absorption; genotoxicity; human studies; epidemiology

Abstract

The German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) summarized and re-evaluated the data for dinitrotoluene (mixtures of isomers) [25321-14-6] considering all toxicological end points, especially for a possible re-classification in Carcinogen Category 1. Relevant studies were identified from a literature search. The critical effect of dinitrotoluene (mixtures of isomers) is nephrocarcinogenicity in rats and mice, which was already described in the last evaluation by the Commission. New studies in a collective of workers in a mining company handling explosives with dinitrotoluene suggest a similar effect in humans. The evidence, however, is limited because neither internal nor external exposure was measured. Instead, inhalation and dermal exposure levels were estimated by the former technical director 20 years after cessation of exposure. Additionally, 2 different compositions were given for the ingredients of the explosive rods. Therefore, the Commission regards the data as insufficient for a classification in Carcinogen Category 1 and dinitrotoluene (mixtures of isomers) remains classified in Carcinogen Category 2. Since the publication of the last evaluation, new studies investigating the genotoxicity of dinitrotoluene have become available. Dinitrotoluene is clastogenic in the liver of rodents, but not in the bone marrow or peripheral blood. 2,4-Dinitrotoluene demonstrated no clastogenic effect in a dominant lethal assay, but 2,6-dinitrotoluene was not evaluated. Data investigating point mutations in germ cells are, however, not available. Due to this data gap, and because it seems likely that the substance can reach the germ cells, dinitrotoluene (mixtures of isomers) has been classified in Germ Cell Mutagenicity Category 3B. In a developmental toxicity study in rats, dinitrotoluene (mixtures of isomers) had no adverse effects on the foetus up to 150 mg/kg body weight and day; maternal toxicity (methaemoglobinaemia) occurred at 100 mg/kg body weight and day and above. The designation with “H” is confirmed, as according to the findings of a biomonitoring study in workers and the predictions of skin absorption models, dinitrotoluene penetrates the skin and can increase the carcinogenic risk. Valid data to evaluate the sensitizing potential of dinitrotoluene (mixtures of isomers) are not available for humans or for animals.

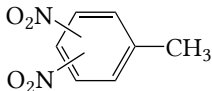
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MAK value	–
Peak limitation	–
Absorption through the skin (1986)	H
Sensitization	–
Carcinogenicity (1985)	Category 2
Prenatal toxicity	–
Germ cell mutagenicity (2023)	Category 3 B
BAT value	–
Synonyms	technical dinitrotoluene
Chemical name (IUPAC)	methyldinitrobenzene
CAS number	25321-14-6
Structural formula	
Molecular formula	C ₇ H ₆ N ₂ O ₄
Molar mass	182.14 g/mol
Melting point	56–59 °C (ECHA 2020)
Boiling point at 1024 hPa	300 °C, decomposes (Henschler 1986, available in German only) >224 °C (ECHA 2020)
Density at 20 °C	1.353 g/cm ³ (ECHA 2020)
Vapour pressure at 25 °C	5.3 × 10 ^{−4} hPa (Henschler 1986) 2,4-dinitrotoluene: 1.593 × 10 ^{−4} hPa (ECHA 2020) 2,6-dinitrotoluene: 3.182 × 10 ^{−4} hPa (ECHA 2020)
log K _{OW}	80/20 mixture: 2 (calculated) (ECHA 2020) 2,4-dinitrotoluene: 1.98 (ECHA 2020) 2,6-dinitrotoluene: 2.1 (ECHA 2020)
Solubility at 22 °C	166 mg/l water (ECHA 2020) 2,6-dinitrotoluene: 145 mg/l water (ECHA 2020)
1 ml/m³ (ppm) ≙ 7.558 mg/m³	1 mg/m³ ≙ 0.132 ml/m³ (ppm)
Hydrolytic stability	no data
Uses	in the production of toluene diamine, toluene diisocyanate, dyes, polyurethanes, solvents, explosives, as gelatinizing or waterproofing agents (impermeability) (ATSDR 2016; IARC 1996; Seidler et al. 2014 a)

Note: The substance can occur simultaneously as vapour and aerosol.

Documentation for the technical mixture “Dinitrotoluene (mixtures of isomers)” was published in 1986. The mixture was classified in Carcinogen Category 2 (at that time Section III A2) and given the designation “H” (for substances which can be absorbed through the skin in toxicologically relevant amounts) (Henschler 1986).

The technical mixture “Dinitrotoluene (mixtures of isomers)” is composed mainly of 2,4-dinitrotoluene (71%–77%) and 2,6-dinitrotoluene (18%–20%). Other isomers such as 3,4-dinitrotoluene (2.4%–4%) and 2,3-dinitrotoluene (1.5%–4.3%) are present in only very small amounts, making up at most 5% of the mixture. The dinitrotoluenes are yellow or yellowish-orange crystalline solids at room temperature and volatile in steam. Their odour remains perceptible at room temperature in spite of their low vapour pressure. Heating releases the toxic vapours of NO_x (ATSDR 2016; Henschler 1986).

1 Toxic Effects and Mode of Action

Exposure at the workplace may cause headaches, fatigue, changes in the sense of taste, loss of appetite, dizziness, nausea, insomnia and muscle weakness. The effects that were observed in humans in workplace studies were changes in haematological parameters (methaemoglobin formation, anaemia, compensatory haematopoiesis) and effects on the nervous system (clinical signs of neurotoxicity, ataxia, tremor, weak legs, cramps). Workplace studies carried out from 1999 to 2014 found signs of carcinogenic effects in the urogenital system of male workers. The target organs in animals are the kidneys, liver, respiratory tract and reproductive system; tumours formed in the kidneys of mice and in the liver, testes and mammary glands of rats.

2,4-Dinitrotoluene and 2,6-dinitrotoluene were mutagenic in the Salmonella mutagenicity test. Several metabolites are mutagenic in strains with high nitroreductase and *O*-acetyltransferase activities. Mutagenicity tests in mammalian cells yielded mainly negative results. In vivo studies did not detect mutations in the reticulocytes of rats after exposure to 2,6-dinitrotoluene, nor mutations in the melanocytes of mice after exposure to 2,4-dinitrotoluene. Oral exposure of rodents led to DNA adducts, unscheduled DNA synthesis, DNA damage (comet assay) and micronuclei in the liver. 2,4-Dinitrotoluene and 2,6-dinitrotoluene did not induce clastogenic effects in the bone marrow; 2,4-dinitrotoluene was not clastogenic in germ cells (dominant lethal test).

Neoplastic nodules and other lesions occurred in the liver of male rats that were given 2,4-dinitrotoluene doses of 0.6 mg/kg body weight and day and above for 2 years; kidney dysplasia was observed in male CD1 mice given 2,4-dinitrotoluene doses of 14 mg/kg body weight and day for 2 years.

No conclusive data are available for the induction of skin sensitizing effects by 2,3-dinitrotoluene, 2,4-dinitrotoluene, 2,5-dinitrotoluene and 3,4-dinitrotoluene. There is evidence that 2,6-dinitrotoluene has skin sensitizing potential and induces photoallergic effects. There are no data for sensitization of the airways.

2 Mechanism of Action

The exact mechanism underlying the carcinogenic effects has yet to be fully explained. As in the activation of aromatic amines, an important first step in bioactivation appears to be the formation of nitroso compounds and hydroxylamines by nitro reduction. Genotoxicity studies in vitro and in vivo showed that dinitrotoluenes initiate gene mutations, DNA adduct formation and chromosomal aberrations and influence DNA repair synthesis. However, the data do not clearly explain how the subsequent promoting effects, which are necessary for the manifestation of the tumours, are initiated (ATSDR 2016; Brüning et al. 2001; Seidler et al. 2014 a).

2.1 Kidneys

The renal tumours induced in mice after exposure to 2,4-dinitrotoluene are thought to be triggered by damage to the proximal tubule of the nephron. Initiation-promotion experiments in the liver identified 2,6-dinitrotoluene as a complete hepatocarcinogen and 2,4-dinitrotoluene as a promoter. The active metabolite was found to be the hydroxylamino sulfate of aminonitrobenzyl alcohol, which is unstable and forms electrophilic carbonium or nitrenium ions, leading subsequently to DNA binding. A number of in vitro and in vivo genotoxicity tests with 2,6-dinitrotoluene yielded negative results and others positive results. 2,6-Dinitrotoluene induced, for example, micronuclei in the liver, but not in the peripheral blood of rats after oral administration. It is postulated that the dinitrotoluenes initiate specific cells of the proximal renal tubules also in humans, leading subsequently to tumours in the proximal renal tubules via a promoting effect. The urothelium of the renal pelvis and the bladder or, generally, the lower urogenital tract, have been identified as further possible target organs based on the metabolic pathways of the dinitrotoluenes (ATSDR 2016; Brüning et al. 2001; Seidler et al. 2014 a).

2.2 Liver

The formation of hepatocellular carcinomas in rats after chronic oral exposure to 2,4-dinitrotoluene, 2,6-dinitrotoluene and technical dinitrotoluene is attributed to toxicity. The initial effects were inflammation, increased liver weights, hepatocellular hyperplasia and hypertrophy, proliferation of the epithelium of the bile ducts and increased liver enzymes in the blood. Tumour formation is probably the result of a subsequent promoting effect (ATSDR 2016).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

As described in the previous documentation, most of the available studies investigated the main constituent of the mixture, 2,4-dinitrotoluene. In rats and mice, this isomer is readily absorbed after intraperitoneal or oral administration, reaching a maximum concentration in the blood after 6 hours. A half-life of 22 hours was determined after oral administration in rats. The isomer is excreted mainly with the urine in the form of metabolites; the primary route of excretion is the faeces only if the isomer is administered as a single high oral dose (Henschler 1986).

Dinitrotoluenes are absorbed after inhalation and dermal exposure. The dinitrotoluenes and their metabolites were found in the urine and blood of humans (Seidler et al. 2014 a). An occupational health study examined the workers at an explosives factory using human biomonitoring tests. The study identified the skin as the principal route of absorption at the workplace because the dinitrotoluenes were present in the air only in low concentrations and the concentrations in the urine of the workers varied greatly and were, in some cases, quite high (Woollen et al. 1985).

The elimination half-life of the metabolites in the urine was 1 to 2.7 hours in workers involved in the production of dinitrotoluene (Turner et al. 1985).

Fluxes of 8.0 and 0.6 $\mu\text{g}/\text{cm}^2$ and hour, respectively, were calculated using the mathematical models of Fiserova-Bergerova et al. (1990) and IH SkinPerm (Tibaldi et al. 2014) assuming a saturated aqueous solution (166 mg/l), a log K_{OW} of 2.0 and a molar mass of 182.14 g/mol. Assuming exposure of 2000 cm^2 of skin for 1 hour, this would result in an absorbed amount of 16 and 1.2 mg, respectively.

3.2 Metabolism

The extensive dataset that is available for the metabolism of the dinitrotoluenes is summarized below based on the review published by ATSDR (2016). Figures 1 to 3 depict the different metabolic pathways in rats and humans and the known metabolites for each pathway.

3.2.1 Animals

Studies in rats have identified complex pathways for the metabolism of 2,4-dinitrotoluene and 2,6-dinitrotoluene. The metabolism occurs in the liver and also in the intestines by microflora.

Oxidative pathway: 2,4-Dinitrotoluene and 2,6-dinitrotoluene are oxidized by the cytochrome P450 enzyme system to their respective dinitrobenzyl alcohols, which are then either converted to glucuronide conjugates or further oxidized to dinitrobenzoic acids as the principal metabolites via dinitrobenzaldehyde. These metabolites may undergo nitroreductase reactions to form various aminonitrobenzoic acids. Dinitrobenzyl glucuronide is excreted with the urine or reaches the intestines via the bile, where it undergoes further reductive metabolism by the gut microflora via glucuronidase and nitroreductase reactions (see reductive metabolism) (ATSDR 2016).

Reductive metabolism: Liver microsomes of rats were used to show that 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene were formed from 2,4-dinitrotoluene via nitroreductase reactions. However, this form of metabolism was less prevalent in the liver than oxidative metabolism. Another reductive metabolic pathway involves the gut microflora. All isomers were reduced by *Escherichia coli* isolated from the human intestine to monoaminonitrotoluenes via hydroxylaminonitrotoluenes. The deconjugated metabolites are reabsorbed and transported back to the liver by enterohepatic circulation. The newly formed amine group is *N*-hydroxylated in the liver by cytochrome P450 and conjugated with sulfate. The sulfate conjugate is unstable and can decompose to form a carbonium or nitrenium ion that can bind to hepatic macromolecules. Sulfation may be involved in the initiation stage of hepatocarcinogenesis. Metabolism by intestinal microflora appears to be essential for the production of metabolites that bind covalently to liver macromolecules. Reduced metabolites may play a role in the development of the dinitrotoluene-induced methaemoglobinaemia and anaemia observed in humans and animals (ATSDR 2016).

The metabolism of 2,4-dinitrotoluene in rats may vary according to sex. In male rats, a larger percentage of the administered dose is excreted with the bile than in female rats. In the females, a larger percentage of the dose is excreted with the urine in the form of the dinitrobenzyl alcohol glucuronide. This may account for the sex differences in the susceptibility of rats to the hepatocarcinogenic effects of 2,4-dinitrotoluene. If larger amounts are excreted with the urine, less of the glucuronide may be available to the intestinal microflora for metabolism to a carcinogenic metabolite (ATSDR 2016).

The results of an *in vitro* metabolism study using rat liver homogenate show that 2,3-dinitrotoluene and 2,5-dinitrotoluene are reduced to monoaminonitrotoluenes and hydroxylaminonitrotoluenes (ATSDR 2016). Although only very little information is available for the metabolism of 2,3-, 2,5- or 3,5-dinitrotoluene, the isomers are probably metabolized in a manner similar to 2,4- and 2,6-dinitrotoluene.

3.2.2 Humans

Workplace studies analysed the metabolites present in the urine of workers in the explosives industry who were exposed to technical mixtures of dinitrotoluenes. The evaluation took into consideration not only inhalation exposure, but also dermal absorption and possible ingestion. The main metabolites, 2,4- and 2,6-dinitrobenzoic acid, constituted about 52% (men) and about 29% (women) of the total metabolites excreted with the urine. The levels fluctuated greatly both between and within individuals. Other metabolites were 2-amino-4-nitrobenzoic acid (men 37%, women 37%) and 2,4- and 2,6-dinitrobenzyl alcohol glucuronide (men 9.5%, women 33.3%). Other metabolites that were found in the urine in small amounts of only a few per cent were 4-amino-2-nitrobenzoic acid, 2-amino-6-nitrobenzoic acid, 2-(*N*-acetyl)amino-4-nitrobenzoic acid (< 1%) and 4-(*N*-acetyl)amino-2-nitrobenzoic acid. Several samples contained also unmetabolized 2,4- and 2,6-dinitrotoluene. The distribution of the metabolites found in workers in China who were

exposed to technical mixtures of dinitrotoluenes and mononitrotoluenes differed from the percentage values determined in cohorts in England and the US (2,6-dinitrobenzoic acid 27.3%, 4-amino-2-nitrobenzoic acid 26%, 2-amino-4-nitrobenzoic acid 21.6%, 2,6-dinitrobenzyl alcohol glucuronide 18.3%). The metabolism in humans is qualitatively similar to that of the rat. Small quantitative differences in the formation of the metabolites are probably due to the different routes of exposure (inhalation in humans, mostly oral in animal studies). As in the test animals, differences were found in humans according to sex: females excreted a higher proportion of the metabolites with the urine in the form of the dinitrobenzyl alcohol glucuronides than did the males (ATSDR 2016).

As in the activation of aromatic amines, an important step in the bioactivation of dinitrotoluenes appears to be the formation of nitroso compounds and hydroxyl amines by nitroreduction (Seidler et al. 2014 a).

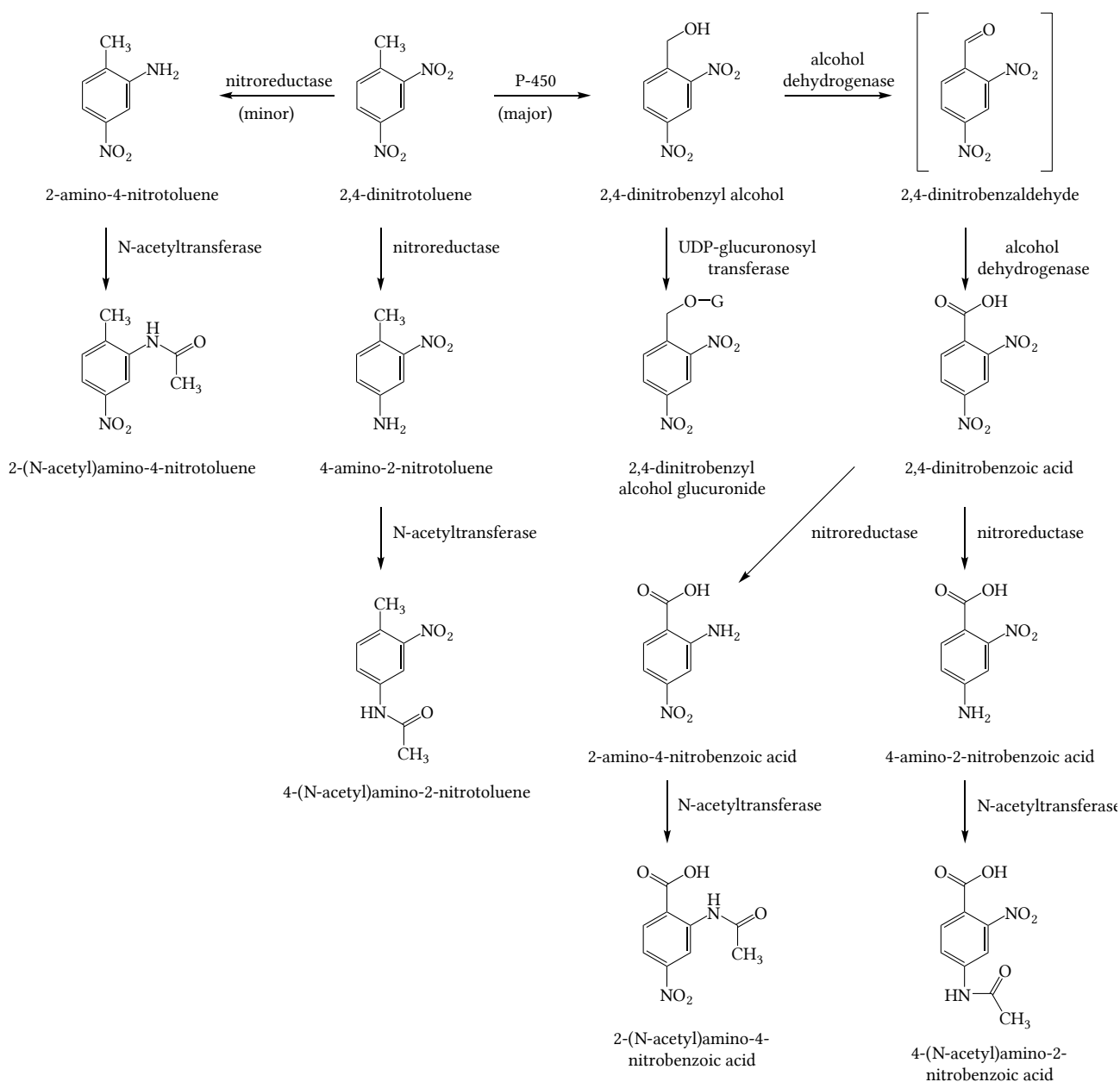
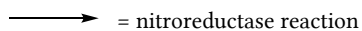


Fig. 1 Diagram of the oxidative and reductive metabolism of 2,4-dinitrotoluene according to ATSDR (2016). Proposed metabolites in parentheses.



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4.1 Single exposures

4.2 Repeated exposure

At the time the documentation was published in 1986 (Henschler 1986), most of the available studies investigating workers exposed to 2,4-dinitrotoluene, 2,6-dinitrotoluene or the technical mixture of dinitrotoluenes had been carried out before 1950. Most of these studies did not provide data for the exposure concentrations and the control groups

and most used small cohorts. Furthermore, the workers were exposed also to other substances. The target organs were identified as the blood (methaemoglobinaemia, anaemia, compensatory haematopoiesis) and nervous system (clinical signs of neurotoxicity such as headaches and vertigo, ataxia, tremors, leg weakness, convulsions). The haematological parameters reacted with greater sensitivity than those relating to the nervous system. None of the studies of workers reported effects on the respiratory tract (ATSDR 2016; Henschler 1986).

4.3 Local effects on skin and mucous membranes

There are no data available.

4.4 Allergenic effects

4.4.1 Contact allergy

A publication described 3 persons suspected of being allergic to nitroglycerin after handling explosives at the workplace. 2,4-Dinitrotoluene was one of the substances tested in the patch tests.

A man developed pruritus, which progressed to dermatitis of the entire hand, over the period of a year after having handled dynamite (containing 60% inorganic nitrates, other organic nitrates and 10% dinitrotoluene) about 10 times during the year leading up to the examination. The worker had previously been employed as a logging supervisor for 14 years and had only worked with explosives for the last 4 years. The patient reacted in the patch test to 2,4-dinitrotoluene (2+ reactions to 2%, 1%, 0.5%, 0.2% and 0.1% formulations; questionably positive reactions to 0.05%, 0.02% and 0.01% formulations) and to dynamite, nitroglycerin and other nitro compounds. The symptoms subsided after contact with nitroglycerin was avoided. Two other persons who had worked for many years in a munitions factory or as blasting engineers did not react to 2,4-dinitrotoluene concentrations between 0.01% and 2% or 1%, respectively (Kanerva et al. 1991). The first case seems plausible; however, the dermatitis cannot be attributed solely to exposure to 2,4-dinitrotoluene because the patient was exposed and produced positive reactions also to other substances.

4.4.2 Photoallergic effects

A man who had worked for 10 years as a rock-blasting engineer developed itchy, confluent vesiculobullous eruptions on his hands. The skin rashes occurred only in the summer and only after his hands had come into contact with dynamite powder. He had no symptoms during the following winter, but the skin rashes re-occurred the next summer. However, the rashes could be prevented by wearing gloves.

Dinitrotoluene (0.5% and 1% in arachis oil) produced a 3+ reaction in the photopatch test (24-hour occlusive application of the test substance followed by UVA radiation, 5 J/cm², reading taken 48 hours after radiation), while negative results were obtained in the non-irradiated patch test. Furthermore, the patient produced a 2+ reaction to sodium nitrate (5%) only in the photopatch test, but did not produce reactions to the 2.5% formulation or to ammonium nitrate (2.5% and 5%). He reacted to ethylene glycol nitrate (0.5% and 1% in ethanol) with a 3+ reaction both on the irradiated area of skin and on the non-irradiated area. Idiopathic photosensitivity was ruled out. The wearing of gloves prevented any further eczema. The authors concluded that the patient had a photocontact allergy to dinitrotoluene and a typical case of contact allergy to ethylene glycol nitrate, among other substances. Photopatch tests and patch tests with the components of dynamite yielded negative results in 5 and 10 control persons, respectively (Emtestam and Forsbeck 1985).

Tab. 1 Patch test and photopatch test results for the components of dynamite 48 hours after irradiation (Emtestam and Forsbeck 1985)

Test substance	Concentration	5 J/cm ² UVA	without UVA
ethylene glycol nitrate (in 96% ethanol)	1%	+++	+++
	0.5%	++	+++
dinitrotoluene (in arachis oil)	1%	+++	–
	0.5%	+++	–
sodium nitrate (in water)	5%	++	–
	2.5%	–	–
ammonium nitrate (in water)	5%	–	–
	2.5%	–	–

4.5 Reproductive and developmental toxicity

In the workers of a chemical plant (21 exposed workers, 9 controls) with long-term exposure via the air to dinitrotoluene and toluene diamine (no other details), sperm counts were decreased with statistical significance by 50% and altered sperm morphology was observed. The study group was very small and was exposed to several chemicals. No data for the concentrations and the length of exposure were provided. Four other studies did not report any such effects in workers at similar workplaces after exposure to technical dinitrotoluene (ATSDR 2016).

A study reported a slight, but statistically significant increase in spontaneous miscarriages in the partners of male workers with long-term exposure to technical dinitrotoluene. However, this finding was obtained in a very small group and no information was provided about the exposure of the workers and their partners to other substances (ATSDR 2016). Therefore, the study has not been included in the evaluation.

4.6 Genotoxicity

In 91 workers employed at a factory manufacturing dinitrotoluene and trinitrotoluene in Liaoning, China, the incidence of chromatid-type chromosomal aberrations, including gaps, was increased with statistical significance in comparison with the values determined in 60 workers employed by the factory who did not have exposure to these substances. The frequency of chromosomal aberrations (chromatid type, chromosome type and gaps) was increased with statistical significance in comparison with the value determined in a different cohort (no other details) that was exposed only to trinitrotoluene. The median length of employment at the factory was 10.5 years for the exposed workers and 17.6 years for the control persons. A total of 71% of the exposed workers and 82% of the control persons were male. The 8-hour time-weighted average concentrations of the substances in the workplace air were 759 µg/m³ for 2-nitrotoluene, 685 µg/m³ for 4-nitrotoluene, 43 µg/m³ for 2,4-dinitrotoluene and 13.9 µg/m³ for 2,6-dinitrotoluene. A number of specific haemoglobin adducts of 2,4-dinitrotoluene and 2,6-dinitrotoluene were used as exposure markers in the blood; these did not correlate with the concentrations in air. The workers were grouped using a job exposure matrix. An odds ratio (OR) of 1.5 (95% CI: 0.75–2.8) was determined for the likelihood of chromosomal aberrations at “high” haemoglobin adduct levels of 2,4-dinitrotoluene in comparison with the likelihood at “low” levels. Another evaluation divided the workers into 3 groups by age (youngest group 15.9 to 31 years, no data for the age structure of the other groups). A statistically significant relationship between the frequency of chromosomal aberrations and the sum of the specific haemoglobin adducts of 2,4-dinitrotoluene (OR 6.5; 95% CI: 1.3–33.3) was found only in the group with the youngest workers. According to the authors, a statistically significant relationship could likewise be established for the main haemoglobin adduct formed from 4-nitroso-2-nitrotoluene, which, in turn, was formed either from 4-amino-2-nitrotoluene by oxidation or from 2,4-dinitrotoluene by reduction (Sabbioni et al. 2006). The authors do not explain why a relationship was found only in the youngest workers. A possible explanation is that any effect that might be induced by dinitrotoluene in the other age groups is obscured by the increased formation of chromosomal aberrations that is a normal part of aging. However, it cannot be determined with certainty whether the increased frequency of chromosomal aberrations can be attributed only to 2,4-dinitrotoluene because the workers were exposed to other nitrotoluenes.

4.7 Carcinogenicity

In 2019, the age-standardized incidence for kidney cancer (ICD-10 C64) in Germany was 6.5 in women and 15.1 in men (per 100 000 persons; age-standardized according to the former European standard) (RKI 2022).

A cohort of 4989 workers employed by a munitions factory in the USA who had worked at least 1 day on a job with probable exposure to dinitrotoluene was compared with 7436 control persons who had worked for at least 5 months at the same company between 1 January 1949 to 21 January 1980, but without exposure. The vital status as of 31 December 1982 was ascertained for 97% of the workers. The standardized mortality ratio (SMR) for all malignant diseases was 0.84 (95% CI: 0.7–1.00). An SMR of 2.67 (95% CI: 0.98–5.83) was determined for the 6 observed cases of hepatobiliary tumours in comparison with the mortality rates determined for the general US population. A standardized rate ratio (SRR) of 3.88 (95% CI: 1.04–14.41) was derived in a comparison with the values determined in the internal control group without exposure. The SMR for hepatobiliary tumours was highest in exposed workers 10 to 19 years after the first exposure (SMR: 4.19; 95% CI: 0.87–12.35) and lower in workers 20 years after their first exposure (SMR: 1.75; 95% CI: 0.21–6.34). The study failed to demonstrate a clear exposure–response relationship between exposure to dinitrotoluene and this end point; however, there were only few workers with long-term exposure, there are no quantitative data for exposure to dinitrotoluene and there are no data for the exposure to other chemicals. Nevertheless, the authors found signs of the carcinogenicity known from animal studies (ATSDR 2016; Stayner et al. 1993).

A cluster of 3 cases of urothelial carcinoma was found among a total of 60 workers employed at a manufacturing facility for nitrotoluene explosives who were exposed to high concentrations of dinitrotoluene. The carcinomas occurred between 1990 and 2002 at an incidence that was 15.9 times higher than the incidence of the population of Saxony-Anhalt, the federal state where the chemical factory was located. Case 1 was additionally exposed to ammonium nitrate, beech wood, nitrobenzene, nitroglycol, nitrate and oil, Case 2 to nitroglycol, nitrate, nitric acid and sulfuric acid, and Case 3 to beech wood, nitrobenzene and nitroglycol (Harth et al. 2005).

A total of 500 mining workers who had been employed in underground copper mines in Mansfeld, in the German Democratic Republic (GDR), were identified based on archive documents as having had possible exposure to technical dinitrotoluene in explosives during the period from 1940 to 1980. It was possible to contact 340 of the miners; 183 of these workers were included in the study. All participants in the study were of German origin and were interviewed for more information about their exposure. They were grouped into 4 qualitative exposure groups. Beginning in the early 1950s, Donarit® was the only explosive that was used in the GDR. It contained 30% technical dinitrotoluene, which consisted of 75% 2,4-dinitrotoluene and 20% 2,6-dinitrotoluene (Brüning et al. 1999). No determined values are available for the concentrations the workers were exposed to during the exposure period. The study did not provide any data for the substances that made up the remaining 70% of the explosive sticks. Donarit explosives vary in composition: for example, gelatinous industrial explosives contain 50% ammonium nitrate, 30% dinitrochlorohydrine with nitroglycol and 20% other aggregates, while ammonium nitrate explosives contain 55% to 84% ammonium nitrate with up to 22% nitroglycol and 11% to 16% of a mixture of dinitrotoluene and trinitrotoluene (Haas and Thieme 2023).

The Commission was informed by personal communication that, according to the former technical director of the copper mine in Mansfeld, Gelamon 22 was the explosive used in the mines at that time (Seidler 2023). A reference work (Roschlau et al. 1983) describes the components of this explosive as 7% dinitrotoluene, 22% nitroglycol, 20% calcium ammonium nitrate, 48% ammonium nitrate, 0.8% nitrocellulose and 2.1% wood meal.

The data for the composition of the explosive sticks used during the exposure period vary, making the evaluation more difficult. Both types of explosives may have been used.

The workers were exposed to dinitrotoluene vapour after a blast and via direct contact with the skin when breaking the 70 cm long sticks into smaller pieces before positioning them in the tunnels (gloves were not worn, 60% of the workers carried out this work for at least 5 hours a day). Six cases of urothelial cell carcinomas and 14 cases of renal cell carcinomas occurred between 1984 and 1997. The length of exposure was between 7 to 37 years and the latency periods were in the range from 21 to 46 years. The data from the cancer register of Saxony-Anhalt show that the incidences of both renal and urothelial cell carcinomas were increased by factors of 4.5 and 14.3, respectively, in comparison with

the incidences found in the general population. The 14 cases of renal cell carcinoma did not show any concentration dependency with respect to the dinitrotoluene concentrations that would be expected based on the job exposure matrix and were similar to the findings in a representative group of employees without exposure. The urothelial cell carcinomas were found only among the groups of workers with high exposure. All identified patients were phenotyped regarding the genetic status of the polymorphic metabolizing enzymes including *N*-acetyltransferase 2 and the glutathione-*S*-transferases M1 and T1. The persons with urothelial cell carcinomas were all slow acetylators. Enzyme status and tumour incidence were not associated in the case of the other enzymes (Brüning et al. 1999).

Workers of the group described above with high levels of exposure to dinitrotoluene ($n = 161$) (Brüning et al. 1999) were examined for signs of subclinical renal damage. The workers were categorized semi-quantitatively in exposure groups (low, medium, high and very high) according to information collected by questionnaire. The exposure concentrations were not determined. The urinary excretion of the biomarkers alpha1-microglobulin and glutathione-*S*-transferase alpha increased with exposure. This was shown by an increase in damage to the proximal renal tubules (Brüning et al. 2001).

These data agree with the proposed mechanism of carcinogenicity: the dinitrotoluene isomers initiate specific cells of the proximal renal tubules and a subsequent promoting effect leads to the formation of tumours in the proximal renal tubules. Due to their metabolism, other possible target organs of the dinitrotoluenes are the urothelium of the renal pelvis and the lower urogenital tract (Brüning et al. 2001; Seidler et al. 2014 a).

The findings of Brüning et al. (1999, 2001) led to a case-cohort study that investigated the cancer incidence in male workers (total cohort $n = 16\,441$, subcohort $n = 999$) who were employed at 1 of 2 underground copper mines in Mansfeld in the German Democratic Republic in the period from 1953 to 1990 (“external analysis”). The cohort probably overlaps with that described above because the workers were employed at the same mines (Brüning et al. 1999). The workers handling the explosive sticks were exposed by inhalation and through the skin, for example when manually breaking the long sticks into smaller pieces, an activity that was regularly done with bare hands. The exposure concentrations during inhalation or dermal exposure were not determined. The exposure was assessed about 20 years after the end of exposure based on a job exposure matrix developed by an expert, the former technical director. The average length of employment was 6.8 years (0.2–46 years). Data were available for the cancer incidence as from 1961 and the workers were followed up until 2005. A total of 1476 cases of cancer were observed in the period from 1961 to 2005. The cancer risk of the workers in comparison with that of the general population of Saxony-Anhalt was calculated based on the standardized incidence ratio (SIR). To check for possible bias due to various losses in the follow-up period, a sensitivity analysis was carried out using individual extrapolation factors for each 5-year birth cohort instead of a global extrapolation factor. The SIR for all cancer types combined was not increased with statistical significance (SIR 1.04; 95% confidence interval (CI): 0.96–1.14), nor was the SIR for renal tumours (SIR 1.01; 95% CI: 0.79–1.27) or for bladder tumours (SIR 1.04; 95% CI: 0.82–1.30). The incidence of lung tumours was increased with statistical significance (SIR 1.29; 95% CI: 1.13–1.46). The risk of developing tumours of the parotid gland and other salivary glands was increased, but not with statistical significance (SIR 1.73; 95% CI: 0.72–4.16); the latter value, however, was based only on 5 cases. An increased SIR of 1.41 (95% CI: 0.85–2.35) was found for bladder cancer after a period of employment lasting more than 20 years. The SIR for renal cancer was not increased with statistical significance after employment for 10 years or more (SIR 1.51; 95% CI: 0.9–2.53) and for more than 20 years (SIR 1.25; 95% CI: 0.72–2.18) (Seidler et al. 2014 b).

The main analysis as a case-cohort study (“internal analysis”; Seidler et al. 2014 b) also took the exposure levels of the workers into consideration. Additional cases of renal and urothelial carcinomas that were not included in the archived health records of the mining company were obtained from the records of a network of pathology institutes and included in the evaluation. A total of 109 cases of renal cancer were evaluated; an exact morphological or topographical diagnosis was available for 88 of these cases. The 109 cases and the 993 miners without cancer were categorized into groups of “never exposed” and “ever exposed” persons. The group of “ever exposed” persons was further divided into groups according to inhalation or dermal exposure and according to low, medium or high cumulative exposure. The relative risk (RR) for renal cancer was increased by a statistically borderline or statistically non-significant degree in the medium and high dermal exposure groups (medium: RR 2.73, 95% CI: 1.00–7.42; high: RR 1.81; 95% CI: 0.75–4.33); the same results were obtained in the group with the highest level of inhalation exposure (RR 1.36; 95% CI: 0.84–2.21). It should be noted that there were only few cases in the medium and high dermal exposure groups (6 and 8 cases, respectively).

When inhalation and dermal exposure were evaluated together, the RR for renal cancer was increased with statistical significance to 2.12 (95% CI: 1.03–4.37) in the “medium/high inhalation and concurrent medium/high dermal” group. By limiting the analysis to workers who were employed for at least 20 years in the copper slate mines in Mansfeld, the hazard ratio was increased to 1.87 (95% CI: 0.97–3.61) based on 36 cases. Furthermore, limiting the analysis to persons who had worked for at least 20 years in the copper slate mines in Mansfeld increased the hazard ratio to 2.77 (95% CI: 1.15–6.71) in workers with combined medium/high inhalation exposure and medium/high dermal exposure to dinitrotoluene. Less than 10 years of the occupational history of 34% of the cases and of 57% of the non-cases was known. The smoking status was determined for 36.7% of the cases and for 40.2% of exposed miners without cancer. Five (6.1%) of the cases with reliable documentation included evidence of possible chronic alcohol abuse. As neither smoking nor alcohol abuse correlated with exposure, the two variables were not included in the analysis (Seidler et al. 2014 b). Overall, the generation and evaluation of the data in the study was very good. All of the available information was well documented. Exposure occurred by handling sticks of explosives containing dinitrotoluene. Two sets of data are available for the composition of the sticks (see study description: Brüning et al. 1999). The data for the composition of the explosive sticks that were used at the time of exposure vary, making the evaluation more difficult. Exposure levels were assessed about 20 years after exposure ended based on a job exposure matrix established by an expert (the former technical director); this took both inhalation and dermal exposure into account. However, there are no determined values for the concentrations in air, there are no biomonitoring data and the amounts absorbed after inhalation and dermal exposure are unknown. Therefore, the actual exposure to the substances and the levels of exposure are not known.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

There are no data available for the technical mixture. The 6-hour LC_{50} for 2,6-dinitrotoluene was 430 mg/m³ (56.8 ml/m³) in male and female F344 rats (ECHA 2020).

5.1.2 Oral administration

The oral LD_{50} values for 2,3-dinitrotoluene were 1072 and 1122 mg/kg body weight for mice and rats, respectively, for technical 2,4-dinitrotoluene 1250 and 1000 mg/kg body weight, for 2,5-dinitrotoluene 1231 and 707 mg/kg body weight, for 2,6-dinitrotoluene 1000 and 177 mg/kg body weight, for 3,4-dinitrotoluene 1414 and 1072 mg/kg body weight and for 3,5-dinitrotoluene 607 (female mice) and 216 mg/kg body weight (female rats) (Henschler 1986).

5.1.3 Dermal application

There are no data available.

5.2 Subacute, subchronic and chronic toxicity

No new data that are relevant for the evaluation in this section have been published. The studies with repeated exposure that are described in Henschler (1986) are not discussed in detail again. The studies below are those that were included in ATSDR (2016) which report the lowest concentrations and effects in the target organs.

5.2.1 Inhalation

There are no data available.

5.2.2 Oral administration

5.2.2.1 Liver

Technical dinitrotoluene and the isomers 2,4- and 2,6-dinitrotoluene induced hepatocellular carcinomas in long-term studies in rats. Effects in the liver were observed in F344 rats given 2,4-dinitrotoluene with the feed for 2 years in doses of 0.6 mg/kg body weight and day and above (hepatocellular changes, neoplastic nodules) and 2,6-dinitrotoluene for 13 weeks in doses of 35 mg/kg body weight and day and above (bile duct hyperplasia, NOAEL (no observed adverse effect level) 7 mg/kg body weight and day). Biliary hyperplasia was observed in dogs given 2,4-dinitrotoluene for 2 years at a dose level of 10 mg/kg body weight and day; the NOAEL was 1.5 mg/kg body weight and day (ATSDR 2016).

5.2.2.2 Kidneys

Renal dysplasia was observed in the male animals after 2,4-dinitrotoluene was given to CD1 mice for 2 years in a dose of 14 mg/kg body weight and day. No renal effects were detected in rats up to a dose of 34.5 mg/kg body weight and day and in dogs up to a dose of 10 mg/kg body weight and day after exposure for 2 years (ATSDR 2016).

5.2.2.3 Nervous system

Effects on the nervous system were observed in animal studies after subchronic and chronic administration of the isomers 2,4-, 2,6-, 3,4-, 3,5-dinitrotoluene and technical dinitrotoluene. The studies described symptoms of weakness, stiffness, rigid paralysis of the hind legs, laboured gait, tremors, ataxia and convulsions that developed in a dose-dependent manner. The lowest dose to induce effects in dogs after oral administration was a 2,4-dinitrotoluene dose of 25 mg/kg body weight, given over periods of 12 days to 13 weeks, and a 2,6-dinitrotoluene dose of 20 mg/kg body weight and day, given for 13 weeks. 2,4-Dinitrotoluene given to rats in doses of 93 mg/kg body weight and day and above for 13 weeks caused demyelination in the cerebellum and brain stem. In a 2-year study with oral doses given to dogs, 1 of 6 animals exhibited a loss of hindquarter control at a 2,4-dinitrotoluene dose of 1.5 mg/kg body weight and day. Vacuolization, hypertrophy, endothelial mitosis, focal gliosis in the cerebellum and perivascular haemorrhage in the cerebellum and brain stem were observed in several animals at doses of 10 mg/kg body weight and day and above (ATSDR 2016).

5.2.2.4 Blood

The haematological parameters that are most commonly affected by dinitrotoluene exposure include the formation of Met-Hb and Heinz bodies, an increased reticulocyte count, a decreased erythrocyte count and decreased haematocrit and haemoglobin levels; these effects are dependent on the dose. Additionally, extramedullary erythropoiesis of the spleen, a compensatory response, may occur. Haematological changes were induced by all 6 isomers of dinitrotoluene in rats given oral doses for 14 days. The lowest oral dose that caused haematological effects after short-term exposure was a 2,6-dinitrotoluene dose of 4 mg/kg body weight; the dose was given to the dogs daily for 14 days. The lowest oral dose that caused haematological effects after long-term exposure was a 2,4-dinitrotoluene dose of 1.5 mg/kg body weight and day; this dose was given to dogs over a period of 3 to 9 or 24 months. Extramedullary erythropoiesis in the spleen was observed in dogs given 2,6-dinitrotoluene doses of 4 mg/kg body weight and day and above for 4 to 13 weeks (ATSDR 2016).

5.2.3 Dermal application

There are no data available.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

A modified Draize assay that tested the different isomers on the skin of rabbits detected either no or mild to moderate irritation of the skin (ECHA 2020; Henschler 1986).

Earlier studies carried out by companies in 1975 and 1978 found that 2,4- and 2,6-dinitrotoluene were slightly irritating to the skin of rabbits (no other details; ATSDR 2016).

The results of a Draize test with the application of 10% 2,4- or 2,6-dinitrotoluene in arachis oil to the shaved dorsal skin of rabbits indicate that 2,4-dinitrotoluene is a “very mild skin irritant” and 2,6-dinitrotoluene a “borderline mild skin irritant” (no other details) (Lee et al. 1975).

5.3.2 Eyes

The isomers do not cause irritation in the eyes of rabbits (ECHA 2020; Henschler 1986).

In earlier studies carried out by companies in 1975 and 1978, unknown concentrations of 2,4- and 2,6-dinitrotoluene did not cause irritation in the eyes of rabbits. A study published in 1981 reported that 2,4-dinitrotoluene induced slight irritation in the eyes of rabbits (no other details; ATSDR 2016).

“Primary eye irritation” was not induced after 10% 2,4- or 2,6-dinitrotoluene in arachis oil was instilled in one eye of a rabbit (no other details) (Lee et al. 1975).

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

Studies that investigated 5 isomers (2,3-dinitrotoluene, 2,4-dinitrotoluene, 2,5-dinitrotoluene, 2,6-dinitrotoluene and 3,4-dinitrotoluene) in guinea pigs are available. No data are given for the positive and negative controls or for the animal strains (Lee et al. 1975). The authors noted that the tests were performed according to Magnusson and Kligman (1969), but did not provide any data for intradermal application. Concentrations between 3% and 5% in arachis oil were tested in groups of 10 guinea pigs (no other details). Two of 10 animals reacted to treatment with 5% 2,6-dinitrotoluene. In spite of the limitations, the test results for 2,6-dinitrotoluene are regarded as positive. The negative test results for 2,3-dinitrotoluene, 2,4-dinitrotoluene, 2,5-dinitrotoluene and 3,4-dinitrotoluene are more difficult to evaluate, as only 10 animals per substance were tested and the test guideline recommends using groups of 20 animals per substance for the evaluation of negative test results.

Tab. 2 Skin sensitization tests with dinitrotoluene isomers in guinea pigs (Lee et al. 1975)

Test substance	Purity	Concentration in arachis oil	Reaction	Evaluation of the authors	Irritation induced by the test formulation on the rabbit skin
2,3-DNT	> 99%	3%	0/10	negative	weak irritation
2,4-DNT	98% 2,4-DNT, 2% 2,6-DNT	4%	0/10	negative	weak irritation
2,5-DNT	95% 2,5-DNT, 1% 2,3-DNT, 4% 2,6-DNT	4%	0/10	negative	necrosis
2,6-DNT	> 99%	5%	2/10	mild	weak irritation
3,4-DNT	> 99%	3%	0/10	negative	weak irritation

DNT: dinitrotoluene

Negative results were obtained for dinitrotoluene (isomer not specified) with the computer-assisted mechanistic prediction model DEREKTM (NIOSH 2011).

5.4.2 Sensitizing effects on the airways

According to the assessment of the registrants that is included in the REACH dossier, there are data for sensitizing effects on the respiratory tract. As a result, classification of the dinitrotoluenes according to the GHS was not carried out. However, the REACH dossier does not include the studies in question (ECHA 2020).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In a 3-generation study, CD rats were given 0, 0.0015%, 0.01% or 0.07% 2,4-dinitrotoluene with the feed (male animals: 0, 0.6, 3.9, 34.5 mg/kg body weight and day, female animals: 0, 0.7, 5.1, 45.3 mg/kg body weight and day). Only few F1 animals mated at the high dose and there was no F2 generation; this is interpreted as an adverse effect on fertility (Ellis et al. 1979; Henschler 1986).

No further generation studies have been carried out since the publication of the 1986 documentation. The effects on the reproductive organs are summarized below based on the ATSDR (2016) report.

A number of animal studies reported effects on the reproductive organs in rats, mice and dogs. Reduced fertility and histological changes in the male and female reproductive organs were induced particularly by oral doses of 2,4-dinitrotoluene. The male reproductive system was more susceptible with findings of decreased sperm production and testicular atrophy, changes in Sertoli cell morphology and degeneration of the seminiferous tubules. NOAELs or LOAELs (lowest observed adverse effect level) of 9 to 34 and 35 to 371 mg/kg body weight and day in rats and 137 to 295 and 413 to 1032 mg/kg body weight and day in mice were determined for effects on the male reproductive organs (testicular atrophy and no or reduced spermatogenesis) in 13-week studies with oral administration of 2,4-dinitrotoluene. In some cases, the absence of spermatogenesis in male rats was irreversible. Ovarian atrophy and non-functioning follicles were observed in the females (ATSDR 2016).

In several dominant lethal tests with oral or intraperitoneal administration in mice and rats, dominant lethal mutations were not induced by technical dinitrotoluene and 2,4- and 3,5-dinitrotoluene up to the high dose of 250 mg/kg body weight and day (see Section 5.6.2; Ellis et al. 1979; Lane et al. 1985; Soares and Lock 1980).

5.5.2 Developmental toxicity

No new studies have been published since the publication of the documentation in 1986.

In a prenatal and postnatal developmental toxicity study, F344 rats were given gavage doses of technical dinitrotoluene of 0, 14, 35, 37.5, 75, 100 or 150 mg/kg body weight and day from gestation days 7 to 20 or from gestation day 7 to birth (5 to 12 animals/dose group, vehicle control group: 20 animals, vehicle: corn oil). The scope of the prenatal examination on gestation day 20 was similar to that of a study carried out later according to OECD Test Guideline 414 (1981 version). No toxic effects on development or teratogenic effects were observed up to the highest dose. Maternal toxicity in the form of methaemoglobin formation was observed at 100 mg/kg body weight and day and above. At the high dose of 150 mg/kg body weight and day, rough fur, lethargy and weakness in the hind limbs were observed in 7/13 animals. Delays in growth, that were not dose-dependent, were detected during the postnatal examination of the offspring (Henschler 1986; Research Triangle Institute 1982).

In the 3-generation study in rats that was described above, reduced viability and delayed eye opening were observed in the offspring at the high dose of 45.3 mg/kg body weight and day. The reduced viability was attributed to maternal neglect and the foetuses that died during parturition. The offspring did not have external anomalies (see Section 5.5.1; Ellis et al. 1979; Henschler 1986).

5.6 Genotoxicity

5.6.1 In vitro

There are a large number of in vitro studies that investigated the isomers and the technical mixture. These were described in detail in the ATSDR (2016) report and the data are therefore only summarized below.

In most of the studies, the dinitrotoluene isomers induced mutations in different *Salmonella typhimurium* strains, but not in mammalian cells (ATSDR 2016). 2,4-Dinitrotoluene and 2,6-dinitrotoluene and several of their reductive metabolites were mutagenic in *Salmonella typhimurium* strains with high levels of nitroreductase activity or, additionally, high levels of *O*-acetyltransferase activity (YG1041 and YG1042, respectively) (Sayama et al. 1998). As in the activation of aromatic amines, an important first step in bioactivation seems to be the formation of nitroso compounds and hydroxyl amines by nitroreduction (Seidler et al. 2014 a). The metabolite 2,6-dinitrobenzaldehyde showed direct mutagenic potential without the addition of a metabolic activation system (Sayama et al. 1989). The urine of workers employed in DNT and TNT production yielded positive results in the *Salmonella typhimurium* strain YG1041 (Sabbioni et al. 2006). The urine of rats given 2,6-dinitrotoluene in a single gavage dose of 75 mg/kg body weight was directly mutagenic in the *Salmonella typhimurium* strain TA98 (ATSDR 2016).

2,4-Dinitrotoluene and 2,6-dinitrotoluene induced chromosomal aberrations in the lung fibroblasts of Chinese hamsters or in human peripheral lymphocytes (Huang et al. 1995), DNA damage (comet assay) in the rat liver (Suzuki et al. 2011) and DNA single strand breaks in the Sertoli cells of rats (Yang et al. 2005).

5.6.2 In vivo

A large number of in vivo studies that investigated the isomers (ATSDR 2016) and the technical mixture (US EPA 2013) have been described and evaluated in detail. An overview of their findings can be found under “Summary” in this section.

The tests described below were not included in the tables published in ATSDR (2016) or US EPA (2013).

5.6.2.1 Clastogenicity tests

2,4-Dinitrotoluene did not induce chromosomal aberrations in the bone marrow or the kidneys (no other details) in 13-week or 24-month studies in CD rats. The male and female animals were given 2,4-dinitrotoluene with the feed for 13 weeks in doses of up to 266 and 145 mg/kg body weight and day, respectively. In the chronic feeding study, the animals were given doses of up to 34.5 or 45.3 mg/kg body weight and day, respectively (Lee et al. 1985).

Groups of 3 male and 3 female SD rats were treated by intraperitoneal injection either on 3 consecutive days (1 × 3) or on 3 consecutive days a week (3 × 3) for a total of 3 weeks. A micronucleus test that examined the reticulocytes of SD rats treated with 2,6-dinitrotoluene doses of 0, 25, 50 or 100 mg/kg body weight and day yielded negative results; however, a dose-dependent increase in micronuclei was observed in the liver that was statistically significant at the lowest dose and above (1 × 3) and at the medium dose and above (3 × 3) (Dertinger et al. 2019).

2,6-Dinitrotoluene given by gavage in doses of 0, 30, 40 or 50 mg/kg body weight and day for 14 consecutive days or in doses of 0, 20, 30 or 40 mg/kg body weight and day for 28 consecutive days led to the statistically significant induction of micronuclei in the hepatocytes of male rats. In the 40 mg/kg group, the increase in the number of micronucleated hepatocytes was higher after treatment for 28 days than after treatment for 14 days. Effects were observed in the histopathological examination even at the low dose, particularly diffuse hypertrophy of the hepatocytes and perilobular proliferation of the oval cells. Necrosis was observed at the low dose and above after 14 days, but not after 28 days. In contrast to these findings, the incidence of anisokaryosis was increased with the dose in all groups after the longer period of exposure, but only in the high dose group after treatment for 14 days. Negative results were obtained in the bone marrow. The fraction of polychromatic erythrocytes and the mitotic index in hepatocytes remained unchanged compared with the values determined in the controls (Imamura et al. 2015).

The same tests were carried out with 2,4-dinitrotoluene in doses of 0, 50, 100 or 200 mg/kg body weight and day. Three of the 6 animals in the high dose group died or were moribund. The incidence of micronucleated hepatocytes was increased with statistical significance at the low dose of 50 mg/kg body weight and day and above after treatment for 14 days and after treatment for 28 days. However, no micronuclei were induced in the bone marrow. The fraction of polychromatic erythrocytes remained unchanged compared with the values determined in the controls (Maeda et al. 2015).

5.6.2.2 Mutations

A Pig-a assay that examined the reticulocytes and erythrocytes of SD rats treated with 2,6-dinitrotoluene doses of 0, 25, 50 or 100 mg/kg body weight and day yielded negative results. Groups of 3 male and 3 female animals were treated by intraperitoneal injection either on 3 consecutive days or on 3 consecutive days a week for a total of 3 weeks (Dertinger et al. 2019).

A coat colour spot test in mice given a 2,4-dinitrotoluene (technical-grade) dose of 100 mg/kg body weight by intraperitoneal injection yielded negative results (Soares and Lock 1980).

5.6.2.3 Germ cells

2,4-Dinitrotoluene induced lethal mutations in *Drosophila melanogaster* (no other details), but no reciprocal translocations (ATSDR 2016).

Several dominant lethal tests obtained negative results in DBA/2J mice administered 2,4-dinitrotoluene (pure), 2,4-dinitrotoluene (technical-grade) or 3,5-dinitrotoluene intraperitoneally or orally in doses of 250 mg/kg body weight (Henschler 1986; Soares and Lock 1980).

In another dominant lethal test, mature male Sprague Dawley rats were given 2,4-dinitrotoluene dissolved in corn oil daily in gavage doses of 0, 60, 180 or 240 mg/kg body weight and day for 5 days. The positive controls were given a single dose of triethylene melamine. 2,4-Dinitrotoluene did not induce early foetal death, but did adversely affect reproductive performance (Lane et al. 1985).

5.6.3 Summary

2,4-Dinitrotoluene and 2,6-dinitrotoluene were mutagenic in *Salmonella* mutagenicity tests. Several of the reductive metabolites were mutagenic in strains with high nitroreductase and *O*-acetyltransferase activities. However, mutagenicity tests in mammalian cells yielded mainly negative results. Mutations were not detected in vivo in the reticulocytes and erythrocytes of rats (Pig-a assay, 2,6-dinitrotoluene) or in the melanocytes of mice (coat colour spot test, 2,4-dinitrotoluene).

After oral administration, DNA adducts, unscheduled DNA synthesis, DNA damage (comet assay) and micronuclei were induced in the liver of rodents, the target organ for carcinogenic effects in rats. The investigated dinitrotoluenes did not induce clastogenic effects in the bone marrow (2,4- and 2,6-dinitrotoluene), the peripheral blood (2,3-, 2,4-, 2,5-, 2,6- and 3,5-dinitrotoluene) and the germ cells (dominant lethal test, 2,4-dinitrotoluene) of rats.

Dominant lethal tests to investigate the clastogenic potential of 2,6-dinitrotoluene and mutagenicity tests in germ cells with 2,4- and 2,6-dinitrotoluene are not available.

5.7 Carcinogenicity

5.7.1 Short-term studies

2,4-Dinitrotoluene and 2,6-dinitrotoluene yielded positive results in a cell transformation test using the embryonal cells of Syrian hamsters (ATSDR 2016).

2,4-Dinitrotoluene is a tumour promoter, but not an initiator, in the liver. By contrast, 2,6-dinitrotoluene and the technical mixture of dinitrotoluene are both promoters and initiators in the liver (ATSDR 2016); however, the initiating activity of the technical mixture was very slight and occurred only 12 hours after a partial hepatectomy (Henschler 1986; OECD 2004).

In short-term initiation-promotion assays, a 2:1 mixture of 2,4- and 2,6-dinitrotoluene did not induce initiating effects in the skin of Sencar mice. Lung tumours were not induced after intraperitoneal administration of this mixture to strain A mice 3 times a week for 8 weeks followed by a 16-week observation period (Henschler 1986; OECD 2004).

5.7.2 Long-term studies

As described in the 1986 documentation (Henschler 1986), a carcinogenicity study found that kidney tumours formed in CD1 mice given 2,4-dinitrotoluene with the feed; the male animals were more susceptible (induction at doses of 13 mg/kg body weight and day and above) than the females (911 mg/kg body weight and day). In CD1 rats, mainly hepatocellular carcinomas formed in male rats at 34 mg/kg body weight and day (6/29) and in female rats at 45 mg/kg body weight and day (18/34). Tumours were induced in the mammary tissues at this dose (33/35) (Ellis et al. 1979).

Hepatocellular carcinomas were observed after 12 months in F344 rats given 2,6-dinitrotoluene with the feed in doses of 7 mg/kg body weight and day and above (ATSDR 2016).

In a feeding carcinogenicity study in F344 rats, the technical mixture of dinitrotoluene (about 76.4% 2,4-dinitrotoluene and 18.8% 2,6-dinitrotoluene) induced hepatocellular carcinomas in male rats at doses of 3.5 mg/kg body weight and day and above (♂ 10/130; ♀ 1/129) and in male and female rats at doses of 14 mg/kg body weight and day (♂ 101/128; ♀ 52/130) (Henschler 1986).

In a 12-month feeding study in male rats, hepatocellular carcinomas formed in 47% of the animals given a mixture of 76% 2,4-dinitrotoluene and 18% 2,6-dinitrotoluene in a dose of 35 mg/kg body weight and day. Hepatocellular carcinomas did not form in animals fed pure 2,4-dinitrotoluene, but did form in 100% of the animals fed pure 2,6-dinitrotoluene in a dose of 14 mg/kg body weight and day (Leonard et al. 1987).

Conclusion: The technical mixture of dinitrotoluene is carcinogenic in the liver of rats and in the kidneys of mice (Table 3).

Tab. 3 Carcinogenicity study in rats with technical dinitrotoluene

Author:	ATSDR 2016; Henschler 1986				
Substance:	technical dinitrotoluene (76.4% 2,4-dinitrotoluene, 18.8% 2,6-dinitrotoluene)				
Species:	rat, F344/N, 130 ♂, 130 ♀				
Administration route:	with the feed				
Dose:	0, 3.5, 14, 35 mg/kg body weight and day				
Duration:	26, 52, 78, 104 weeks, daily (interim necropsy: groups of 10 animals per sex and dose after 26 and 52 weeks, 20 ♂, 20 ♀/dose after 78 weeks); high dose: surviving animals after 55 weeks, remaining dose groups after 104 weeks				
Toxicity:	3.5 mg/kg body weight and above: liver toxicity (nodules, cysts, hyperbasophilia)				
		Dose [mg/kg body weight and day]			
		0	3.5	14	35 (52 or 55 weeks)
tumours and preneoplasia					
12 months					
liver:					
hepatocellular carcinomas	♂	n. d.	n. d.	n. d.	32/40 (80%)
	♀	n. d.	n. d.	n. d.	15/40 (37%)
neoplastic nodules	♂	n. d.	n. d.	n. d.	8/40 (20%)
	♀	n. d.	n. d.	n. d.	9/40 (22.5%)
hepatocellular cholangiocarcinomas	♂	n. d.	n. d.	n. d.	3/40 (7%)
	♀	n. d.	n. d.	n. d.	15/40 (37%)
cholangiocarcinomas	♂	n. d.	n. d.	n. d.	5/40 (12%)
	♀	n. d.	n. d.	n. d.	0/40 (0%)
testes:					
tumours interstitial cells	♂	n. d.	n. d.	n. d.	13/40 (32%)
24 months					
liver:					
hepatocellular carcinomas	♂	1/120 (0.8%)	10/130 (8%)*	98/128 (76%)*	–
	♀	0/120 (0%)	1/129 (0.8%)	45/130 (35%)*	–
neoplastic nodules	♂	8/120 (7%)	14/130 (11%)	64/128 (50%)*	–
	♀	5/120 (4%)	15/129 (12%)*	74/130 (57%)*	–
hepatocellular cholangiocarcinomas	♂			15/128 (12%)	–
	♀			0/130 (0%)	–
mammary glands:					
fibroadenomas	♂	6/91 (6%)	0/130 (0%)	22/79 (28%)*	–
	♀	16/90 (18%)	0/129 (0%)	29/91 (32%)*	–
testes:					
tumours interstitial cells	♂	82/101 (81%)	1/1 (100%)	104/108 (96%)*	–
skin:					
subcutaneous fibromas	♂			44/52 (85%)	–
	♀			11/15 (73%)	–
fibrosarcomas	♂			10/52 (19%)	–
	♀			4/15 (27%)	–

*p ≤ 0.05; subsequently calculated using the one-sided Fisher's exact test; n. d.: no data

Tab. 4 Carcinogenicity study in rats with technical dinitrotoluene

Author:	ATSDR 2016; Henschler 1986; Leonard et al. 1987		
Substance:	76.5 % 2,4-dinitrotoluene and 18.8% 2,6-dinitrotoluene (2.43% 3,4-dinitrotoluene, 1.54% 2,3-dinitrotoluene, 0.69% 2,5-dinitrotoluene, 0.04% 3,5-dinitrotoluene)		
Species:	rat, F344/N, groups of 28 ♂		
Administration route:	with the feed		
Dose:	0, 35 mg/kg body weight and day		
Duration:	52 weeks, daily		
Toxicity:	body weight gains ↓, liver toxicity		
		Dose [mg/kg body weight and day]	
		0	35
surviving animals	♂	n. d.	n. d.
liver:			
hepatocellular carcinomas	♂	n. d.	9/19 (47%)
neoplastic nodules	♂	n. d.	10/19 (53%)
hepatocellular cholangiocarcinomas	♂	n. d.	2/19 (10%)

n. d.: no data

n. d.: no data

Tab. 5 Carcinogenicity study in rats with 2,4-dinitrotoluene

Author:	Ellis et al. 1979				
Substance:	2,4-dinitrotoluene				
Species:	rat, CD, 38 ♂, 38 ♀				
Administration route:	with the feed				
Dose:	0, 0.57/0.71, 3.9/5.1, 34/45 mg/kg body weight and day for ♂/♀				
Duration:	104 weeks, daily				
Toxicity:	3.9/5.1 mg/kg body weight and above: effects, 34/45 mg/kg body weight: survival ↓				
		Dose [mg/kg body weight and day]			
		0	0.57/0.71	3.9/5.1	34/45
surviving animals	♂	32/38	35/38	27/38	34/38
	♀	31/38	36/38	34/38	33/38
liver:					
foci and hepatocellular changes	♂	9/25 (36%)	10/28 (36%)	9/19 (47%)	16/29 (55%)
	♀	7/23 (30%)	18/35 (51%)	19/27 (70%)*	13/34 (38%)
hepatocellular nodules	♂	1/25 (4%)	2/28 (7%)	1/19 (5%)	2/29 (7%)
	♀	0/23 (0%)	3/35 (9%)	2/27 (7%)	6/34 (18%)*
hepatocellular carcinomas	♂	1/25 (4%)	0/28 (0%)	1/19 (5%)	6/29 (21%)
	♀	0/23 (0%)	0/35 (0%)	1/27 (4%)	18/34 (53%)*
mammary glands:					
tumour	♂	0/25 (0%)	0/28 (0%)	0/19 (0%)	2/30 (7%)
	♀	11/23 (48%)	12/35 (34%)	17/27 (63%)	33/35 (94%)*
skin:					
subcutaneous	♂	2/25 (8%)	4/28 (14%)	3/19 (16%)	17/30 (57%)*
	♀	1/22 (5%)	3/35 (9%)	0/27 (0%)	6/35 (17%)

*p ≤ 0.05; subsequently calculated using the one-sided Fisher's exact test

Tab. 6 Carcinogenicity study in mice with 2,4-dinitrotoluene

Author:	Ellis et al. 1979				
Substance:	2,4-dinitrotoluene				
Species:	mouse, CD1, 58 ♂, 58 ♀				
Administration route:	with the feed				
Dose:	0, 13.5, 95, 900 mg/kg body weight and day				
Duration:	104 weeks, daily				
Toxicity:	13.5 mg/kg body weight and above: body weight gains ↓				
		Dose [mg/kg body weight and day]			
		0	13.5	95	900
surviving animals	♂	33/58	33/58	28/58	as of month 10 mortality > 60%, up to month 18 100% mortality
	♀	31/58	29/58	31/58	as of month 10 mortality > 60%, up to month 21 100% mortality
kidneys:					
tumours	♂	0/33 (0%)	5/33 (15%)	16/28 (57%)*	3/40 (7%)
	♀	0/31 (0%)	0/29 (0%)	0/31 (0%)	1/32 (3%)
toxic nephropathy	♂	0/33 (0%)	3/33 (9%)	3/28 (11%)	32/40 (80%)*
	♀	0/31 (0%)	3/29 (10%)	2/31 (6%)	10/32 (31%)*
liver:					
liver cell tumours	♂	7/33 (21%)	9/33 (27%)	8/28 (28%)	5/40 (12%)
	♀	2/31 (6%)	1/29 (3%)	3/31 (10%)	1/33 (3%)
dysplasia	♂	2/33 (6%)	14/33 (42%)*	12/28 (43%)*	40/40 (100%)*
	♀	5/31 (16%)	3/29 (10%)	5/31 (16%)	29/33 (88%)*

*p ≤ 0.05; subsequently calculated using the one-sided Fisher's exact test

6 Manifesto (MAK value/classification)

The critical effect induced by the (technical) dinitrotoluenes is a carcinogenic effect in the liver of rats and in the kidneys of mice.

Carcinogenicity. The epidemiological studies, and particularly the case-cohort study (Seidler et al. 2014 a, b), are reliable. Their findings indicate that the carcinogenic effects induced by technical dinitrotoluene mixtures in animal studies may be relevant also for humans. However, no determined values are available for the external or internal exposure of the workers who were exposed between 1940 and 1990 (Brüning et al. 1999; Seidler et al. 2014 a, b). The exposure levels were assessed about 20 years after the end of exposure based on a job exposure matrix prepared by an expert, the former technical director of the copper mines in Mansfeld. Varying data are available for the composition of the explosive sticks (Section 4.7). Therefore, the observed effects cannot be attributed with confidence to technical dinitrotoluene. Furthermore, the increased risk was statistically significant only in a small number of cases (cases with medium/high inhalation/dermal exposure). Overall, the data support the classification of the dinitrotoluenes in Carcinogen Category 2 (carcinogenic in animal studies with relevance to human health). The data available are not considered sufficient for re-classification in Category 1 mainly because the exposure concentrations are not based on determined values.

Germ cell mutagenicity. 2,4-Dinitrotoluene and 2,6-dinitrotoluene and several of the metabolites that were tested were mutagenic in the Salmonella mutagenicity test. However, the mutagenicity tests in mammalian cells yielded mainly

negative results. In vivo, no mutations were observed in the reticulocytes and erythrocytes of rats (Pig-a assay, 2,6-dinitrotoluene) or in the melanocytes of mice (coat colour spot test, 2,4-dinitrotoluene).

The dinitrotoluenes were clastogenic in the liver of rodents, the target organ of the carcinogenic effects. Clastogenicity tests in the bone marrow, the peripheral blood and in the germ cells of rodents produced negative results. A dominant lethal test was carried out only with the 2,4-isomer; therefore, there are no data for the 2,6-isomer. The end point mutagenicity has not been tested in germ cells. Although the germ cells may be reached, no evidence of the active form has been found in germ cells. Therefore, the dinitrotoluenes have been classified in Category 3B for germ cell mutagens.

Similar data are available for 2,4-toluenediamine, which is formed during metabolism, leading to its classification in Category 3B for germ cell mutagens (Hartwig and MAK Commission 2023).

MAK value and peak limitation. The derivation of a MAK value is not possible because of the classification in Category 2 for carcinogens and the genotoxic effects. Peak limitation does not apply.

Prenatal toxicity. In a prenatal developmental toxicity study in rats given gavage doses of technical dinitrotoluene from gestation days 7 to 20, no developmentally toxic or teratogenic effects were observed up to the high dose of 150 mg/kg body weight and day. Maternal toxicity in the form of methaemoglobin formation occurred at doses of 100 mg/kg body weight and day and above (Henschler 1986; Research Triangle Institute 1982).

As a MAK value cannot be derived, assignment to one of the pregnancy risk groups is not applicable.

Absorption through the skin. The dinitrotoluenes are genotoxic in vitro and in vivo and have been classified in Carcinogen Category 2. No systematic studies have been carried out in vivo or in vitro to investigate the dermal absorption of the dinitrotoluenes.

Absorption through the skin is assumed based on the results of model calculations (Section 3.1) and the findings of the human biomonitoring study of Woollen et al. (1985). The substance is a known genotoxic carcinogen and there is no non-hazardous level of exposure. As a result, even slight amounts absorbed through the skin are assumed to increase the carcinogenic risk. The dinitrotoluenes therefore remain designated with an “H” (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. No reliable data are available for the evaluation of the skin sensitizing potential of 2,3-dinitrotoluene, 2,4-dinitrotoluene, 2,5-dinitrotoluene and 3,4-dinitrotoluene. An animal study found evidence that 2,6-dinitrotoluene has skin sensitizing potential. Overall, the mixture of dinitrotoluenes has not been designated with “Sh” (for substances which cause sensitization of the skin).

Only one well-documented case report is available that shows that 2,6-dinitrotoluene causes photosensitizing effects; however, in view of its widespread use in explosives, this finding is not sufficient to apply the “SP” designation (for substances which cause photocontact sensitization of the skin).

There are no meaningful data for the sensitizing effects of the dinitrotoluenes on the airways. Therefore, they have not been designated with “Sa” (for substances which cause sensitization of the airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (https://www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

The views expressed in these publications are those of the individual authors acting in their personal capacity as experts and do not represent the positions of their respective institutions or employers.

References

- ATSDR (Agency for Toxic Substances and Disease Registry) (2016) Toxicological profile for dinitrotoluenes. Atlanta, GA: ATSDR. <https://www.atsdr.cdc.gov/ToxProfiles/tp109.pdf>, accessed 21 Jul 2022
- Brüning T, Chronz C, Thier R, Havelka J, Ko Y, Bolt HM (1999) Occurrence of urinary tract tumors in miners highly exposed to dinitrotoluene. *J Occup Environ Med* 41(3): 144–149. <https://doi.org/10.1097/00043764-199903000-00003>
- Brüning T, Thier R, Mann H, Melzer H, Bröde P, Dallner G, Bolt HM (2001) Pathological excretion patterns of urinary proteins in miners highly exposed to dinitrotoluene. *J Occup Environ Med* 43(7): 610–615. <https://doi.org/10.1097/00043764-200107000-00007>
- Dertinger SD, Avlasevich SL, Torous DK, Singh P, Khanal S, Kirby C, Drake A, MacGregor JT, Bemis JC (2019) 3Rs friendly study designs facilitate rat liver and blood micronucleus assays and Pig-a gene mutation assessments: proof-of-concept with 13 reference chemicals. *Environ Mol Mutagen* 60(8): 704–739. <https://doi.org/10.1002/em.22312>
- ECHA (European Chemicals Agency) (2020) Dinitrotoluene (CAS Number 25321-14-6). Registration dossier. Joint submission, first publication 27 Apr 2020, last modification 02 Apr 2020. <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/30782>, accessed 13 Sep 2022
- Ellis HV III, Hagensen JH, Hodgson JR, Minor JL, Hong C-B, Ellis ER, Girvin JD, Helton DO, Herndon BL, Lee C-C (1979) Mammalian toxicity of munition compounds. Phase III. Effects of lifetime exposure. Part I. 2,4-Dinitrotoluene. MRI Project No 3900-B. Fort Belvoir, VA: Defense Technical Information Center. <https://apps.dtic.mil/sti/pdfs/ADA077692.pdf>, accessed 17 Nov 2022
- Emtestam L, Forsbeck M (1985) Occupational photosensitivity to dinitrotoluene. *Photodermatol* 2(2): 120–121
- Fiserova-Bergerova V, Pierce JT, Droz PO (1990) Dermal absorption potential of industrial chemicals: criteria for skin notation. *Am J Ind Med* 17(5): 617–635. <https://doi.org/10.1002/ajim.4700170507>
- Haas R, Thieme J (2023) Lexikon der deutschen Explosivstoffmischungen. <http://www.r-haas.de/v11.html>, accessed 05 Jul 2023
- Harth V, Bolt HM, Brüning T (2005) Cancer of the urinary bladder in highly exposed workers in the production of dinitrotoluenes: a case report. *Int Arch Occup Environ Health* 78(8): 677–680. <https://doi.org/10.1007/s00420-005-0012-4>
- Hartwig A, MAK Commission (2023) 2,4-Toluenediamine. MAK Value Documentation, addendum – Translation of the German version from 2021. MAK Collect Occup Health Saf 8(4): Doc080. https://doi.org/10.34865/mb9580e8_4ad
- Henschler D, editor (1986) Dinitrotoluole (alle Isomeren in technischen Gemischen). In: *Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten*. 11th issue. Weinheim: VCH. Also available from <https://doi.org/10.1002/3527600418.mb2532114xisd0011>
- Huang Q, Wang L, Han S (1995) The genotoxicity of substituted nitrobenzenes and the quantitative structure-activity relationship studies. *Chemosphere* 30(5): 915–923. [https://doi.org/10.1016/0045-6535\(94\)00450-9](https://doi.org/10.1016/0045-6535(94)00450-9)
- IARC (International Agency for Research on Cancer) (1996) 2,4-Dinitrotoluene, 2,6-dinitrotoluene and 3,5-dinitrotoluene. In: *Printing processes and printing inks, carbon black and some nitro compounds*. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 65. Lyon: IARC Press. p. 309–368. <https://publications.iarc.who.int/download/mono65.pdf>, accessed 24 Jul 2023
- Imamura T, Koeda A, Morimoto K, Hatakeyama H, Suzuki H, Wako Y, Kawasaki K, Otabe K, Sato S-I (2015) Evaluation of a repeated-dose liver micronucleus assay with 2,6-dinitrotoluene using young adult rats. *Mutat Res Genet Toxicol Environ Mutagen* 780–781: 46–50. <https://doi.org/10.1016/j.mrgentox.2014.11.004>
- Kanerva L, Laine R, Jolanki R, Tarvainen K, Estlander T, Helander I (1991) Occupational allergic contact dermatitis caused by nitroglycerin. *Contact Dermatitis* 24(5): 356–362. <https://doi.org/10.1111/j.1600-0536.1991.tb01750.x>
- Lane RW, Simon GS, Dougherty RW, Egle JL, Borzelleca JF (1985) Reproductive toxicity and lack of dominant lethal effects of 2,4-dinitrotoluene in the male rat. *Drug Chem Toxicol* 8(4): 265–280. <https://doi.org/10.3109/01480548509038650>
- Lee C-C, Dilley JV, Hodgson JR, Helton DO, Wiegand WJ, Roberts DN, Andersen BS, Halfpap LM, Kurtz LD, West N (1975) Mammalian toxicity of munition compounds: phase I: acute oral toxicity, primary skin and eye irritation, dermal sensitization, and disposition and metabolism. Report No. 1. Contract No. DAMD-17-74-C-4073. MRI Project No. 3900. Fort Belvoir, VA: Defense Technical Information Center. <https://apps.dtic.mil/sti/tr/pdf/ADB011150.pdf>, accessed 27 Jul 2023
- Lee CC, Hong CB, Ellis HV III, Dacre JC, Glennon JP (1985) Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part II. CD® rats. *Int J Toxicol* 4(4): 243–256. <https://doi.org/10.3109/10915818509078677>
- Leonard TB, Graichen ME, Popp JA (1987) Dinitrotoluene isomer-specific hepatocarcinogenesis in F344 rats. *J Natl Cancer Inst* 79(6): 1313–1319
- Maeda A, Tsuchiyama H, Asaoka Y, Hirakata M, Miyoshi T, Oshida K, Miyamoto Y (2015) Evaluation of the repeated-dose liver micronucleus assay using 2,4-dinitrotoluene: a report of a collaborative study by CSGMT/JEMS.MMS. *Mutat Res Genet Toxicol Environ Mutagen* 780–781: 41–45. <https://doi.org/10.1016/j.mrgentox.2014.05.002>
- Magnusson B, Kligman AM (1969) The identification of contact allergens by animal assay. The guinea pig maximization test. *J Invest Dermatol* 52(3): 268–276. <https://doi.org/10.1038/jid.1969.42>
- NIOSH (The National Institute for Occupational Safety and Health) (2011) NIOSH skin notation (SK) profiles; dinitrotoluene [CAS No. 25321–14–6]; 2,4-dinitrotoluene (2,4-DNT) [CAS No. 121–14–2]; 2,6-dinitrotoluene (2,6-DNT) [CAS No. 606–20–2]. Cincinnati, OH: NIOSH. <https://www.cdc.gov/niosh/docs/2011-138/pdfs/2011-138.pdf?id=10.26616/NIOSH-PUB2011138>, accessed 27 Jul 2023

- OECD (Organisation for Economic Co-operation and Development) (2004) Dinitrotoluene (isomers mixture) CAS No: 25321-14-6. OECD SIDS Initial Assessment Report for SIAM 18. Geneva: OECD. <https://hpvchemicals.oecd.org/UI/handler.axd?id=02042b6f-37d6-4939-8731-952192affca4>, accessed 15 Jun 2022
- Research Triangle Institute (1982) Teratological and postnatal evaluation of dinitrotoluene in Fischer 344 rats with cover letter outlining current study of tetrahydrofuran toxicity dated 05/10/94 (sanitized). OTS0557140. Alexandria, VA: NTIS. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0557140.xhtml>, accessed 07 Jul 2023
- RKI (Robert Koch Institut) (2022) Nierenkrebs. Stand 30. Sep 2022. Zentrum für Krebsregisterdaten. https://www.krebsdaten.de/Krebs/DE/Content/Krebsarten/Nierenkrebs/nierenkrebs_node.html, accessed 20 Feb 2023
- Roschlau H, Bergmann S, Klose G, Kluge E (1983) Sprengstoffe, die im Bergbau der DDR verwendet werden. In: Der Sprengberechtigte im Bergbau und in der Natursteinindustrie., 4th ed. Leipzig: VEB Deutscher Verlag für Grundstoffindustrie
- Sabbioni G, Jones CR, Sepai O, Hirvonen A, Norppa H, Järventaus H, Glatt H, Pomplun D, Yan H, Brooks LR, Warren SH, DeMarini DM, Liu Y-Y (2006) Biomarkers of exposure, effect, and susceptibility in workers exposed to nitrotoluenes. *Cancer Epidemiol Biomarkers Prev* 15(3): 559–566. <https://doi.org/10.1158/1055-9965.EPI-05-0677>
- Sayama M, Mori M-A, Shirokawa T, Inoue M, Miyahara T, Kozuka H (1989) Mutagenicity of 2,6-dinitrotoluene and its metabolites, and their related compounds in *Salmonella typhimurium*. *Mutat Res* 226(3): 181–184. [https://doi.org/10.1016/0165-7992\(89\)90017-1](https://doi.org/10.1016/0165-7992(89)90017-1)
- Sayama M, Mori M-A, Shoji M, Uda S-I, Kakikawa M, Kondo T, Kodaira KI (1998) Mutagenicities of 2,4- and 2,6-dinitrotoluenes and their reduced products in *Salmonella typhimurium* nitroreductase- and O-acetyltransferase-overproducing Ames test strains. *Mutat Res* 420(1–3): 27–32. [https://doi.org/10.1016/s1383-5718\(98\)00139-9](https://doi.org/10.1016/s1383-5718(98)00139-9)
- Seidler A (2023) Angaben zur Zusammensetzung der Sprengstoffstäbe in Kupfermine in Mansfeld. Email, 04 Jul 2023
- Seidler A, Brüning T, Taeger D, Möhner M, Gawrych K, Bergmann A, Haerting J, Bolt HM, Straif K, Harth V (2014 a) Cancer incidence among workers occupationally exposed to dinitrotoluene in the copper mining industry. *Int Arch Occup Environ Health* 87(2): 117–124. <https://doi.org/10.1007/s00420-012-0842-9>
- Seidler A, Harth V, Taeger D, Möhner M, Gawrych K, Bergmann A, Haerting J, Kahmann H-J, Bolt HM, Straif K, Brüning T (2014 b) Dinitrotoluene exposure in the copper mining industry and renal cancer: a case-cohort study. *Occup Environ Med* 71(4): 259–265. <https://doi.org/10.1136/oemed-2013-101850>
- Soares ER, Lock LF (1980) Lack of an indication of mutagenic effects of dinitrotoluenes and diaminitoluenes in mice. *Environ Mutagen* 2(2): 111–124. <https://doi.org/10.1002/em.2860020203>
- Stayner LT, Dannenberg AL, Bloom T, Thun M (1993) Excess hepatobiliary cancer mortality among munitions workers exposed to dinitrotoluene. *J Occup Med* 35(3): 291–296
- Suzuki H, Imamura T, Koeda A, Morimoto K, Wakasa Y, Takei Y, Amemiya R, Hatakeyama H, Satoh H, Sato S-I (2011) Genotoxicity studies of 2,6-dinitrotoluene (2,6-DNT). *J Toxicol Sci* 36(4): 499–505. <https://doi.org/10.2131/jts.36.499>
- Tibaldi R, ten Berge W, Drolet D (2014) Dermal absorption of chemicals: estimation by IH SkinPerm. *J Occup Environ Hyg* 11(1): 19–31. <https://doi.org/10.1080/15459624.2013.831983>
- Turner MJ Jr, Levine RJ, Nystrom DD, Crume YS, Rickert DE (1985) Identification and quantification of urinary metabolites of dinitrotoluenes in occupationally exposed humans. *Toxicol Appl Pharmacol* 80(1): 166–174. [https://doi.org/10.1016/0041-008x\(85\)90111-5](https://doi.org/10.1016/0041-008x(85)90111-5)
- US EPA (US Environmental Protection Agency) (2013) Provisional peer-reviewed toxicity values for 2,6-dinitrotoluene (CASRN 606-20-2). EPA/690/R13/009F. Cincinnati, OH: US EPA. <https://cfpub.epa.gov/ncea/pprtv/documents/Dinitrotoluene26.pdf>, accessed 27 Jul 2023
- Woollen BH, Hall MG, Craig R, Steel GT (1985) Dinitrotoluene: an assessment of occupational absorption during the manufacture of blasting explosives. *Int Arch Occup Environ Health* 55(4): 319–330. <https://doi.org/10.1007/BF00377690>
- Yang L, Xu J-B, Zhen L, Sun Z-W, Shi L, Jin M-H, Liu X-M (2005) DNA damage of germ cell of rat induced by nitrotoluene chemicals. *J Environ Sci (China)* 17(1): 84–90