2,4,6-Trinitrotoluene (and isomers in technical mixtures)

[118-96-7]	
Supplement 2008	
MAK value	**–**
Peak limitation	**–**
Absorption through the skin (1958)	**H**
Sensitization (2007)	**Sh**
Carcinogenicity (2007)	**Category 2**
Prenatal toxicity	**–**
Germ cell mutagenicity (2007)	**Category 3B**
BAT value	**–**

Since the publication of the last documentation in 1988 (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990) new data for the genotoxicity and carcinogenicity of 2,4,6-trinitrotoluene are available which make re-evaluation necessary.

On the basis of the data available in 1988, a decision could not be made regarding the carcinogenicity of 2,4,6-trinitrotoluene, as there were no investigations available of its carcinogenicity in humans or animals.

The literature published up to 1996 on epidemiology and genotoxicity has been discussed extensively in the evaluations of the IARC (1996) and ATSDR (1995). However, no adequate carcinogenicity studies in animals were available to the IARC. The ATSDR quotes two carcinogenicity studies conducted by the US Army in 1984, which are also available to the Commission. Furthermore, a review has been published by Bolt et al. (2006); this has been included in the re-evaluation by the Commission.

Mechanism of Action

The mechanism by which 2,4,6-trinitrotoluene and its metabolites produce toxic effects in a large number of organs (see also documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990), is not yet fully understood. It is, how-

The MAK-Collection Part I, MAK Value Documentations 2014
DFG, Deutsche Forschungsgemeinschaft

ever, assumed that the formation of reactive oxygen species induces lipid peroxidation in the liver and the formation of cataracts in the lens of the eye (2,4,6-trinitrotoluene cataract) (ATSDR 1995).

The mechanism for the formation of methaemoglobin by 2,4,6-trinitrotoluene is assumed to be the reduction of the nitro compounds via the nitroso compound and the hydroxylamine to form the amine after absorption into the organism. A redox cycle between the hydroxylamine and the nitroso compound takes place, which is responsible for the co-oxidation of bivalent iron in haemoglobin, that is for the formation of methaemoglobin.

In vitro investigations of the urine of workers exposed to 2,4,6-trinitrotoluene showed there to be a correlation between the mutagenic effects in bacteria and the concentration of 2,4,6-trinitrotoluene and its metabolite 4-amino-2,6-dinitrotoluene in urine (Ahlborg et al. 1985, 1988 a; see the Section "Genotoxicity").

The two main metabolites of 2,4,6-trinitrotoluene in humans, 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene, are mutagenic in Salmonella typhimurium in concentration ranges such as those occurring in the urine of workers exposed to 2,4,6-trinitrotoluene concentrations of < 0.01 to 2.53 mg/m^3 (Bolt et al. 2006; see also Table 3 in the Section "Genotoxicity").

In rat liver microsomes, ^{14}C-2,4,6-trinitrotoluene was rapidly converted in the presence of an NADPH-generating system to 4-hydroxylamino-2,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene, and further to 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene. As the formation of covalent protein adducts increased with time, the authors assumed that the reactive metabolite had to be produced from a proximal metabolite, for example 4-hydroxyl-amino-2,6-dinitrotoluene. This theory was supported by the observation that the incubation of ^{14}C-4-hydroxylamino-2,6-dinitrotoluene with liver microsomes produced an increase in protein adducts. In addition, under aerobic conditions, higher protein adduct levels were determined than under anaerobic conditions, which indicates the reactive metabolite is formed via oxidative metabolism, for example the oxidation of 4-hydroxylamino-2,6-dinitrotoluene to form 4-nitroso-2,6-dinitrotoluene. The initial reduction of the nitro groups of 2,4,6-trinitrotoluene can be catalyzed by the NADPH-cytochrome P450 reductase alone, following which cytochrome P450 enzymes are required to reduce the hydroxylamine further to the amine (Bolt et al. 2006).

Apart from this, it may be assumed that the nitroso intermediate binds to sulfhydryl groups of proteins. This bioactivation occurs in the liver presumably via oxidation of the hydroxylamine by NADPH-dependent hepatic microsomal enzymes (Leung et al. 1995).

In another in vitro study, the formation of an adduct with the prosthetic haem group of human haemoglobin under anaerobic and reductive conditions was shown; which was generated by the addition of sodium hydrogen sulfide (Bakhtiar et al. 1997).

In an animal study, after single intraperitoneal injections of ^{14}C-2,4,6-trinitrotoluene doses of up to 50 mg/kg body weight, dose-dependent covalent binding to globin, plasma protein and proteins of the liver and kidneys was demonstrated in rats (Liu et al. 1992).

The binding of 2,4,6-trinitrotoluene and its metabolites 2,4-dinitrotoluene and 2,6-dinitrotoluene, 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,4-diaminotoluene and 2,6-diaminotoluene to haemoglobin was investigated 24 hours after female Wistar rats were given single oral doses of each test substance of 0.5 mmol/kg body weight. The haemoglobin binding index (HBI = [mmol/mol Hb]/[mmol/kg]) was between < 0.02 and 6.0. The highest HBI value was observed after the administration of 2,4,6-trinitrotoluene; the hydrolysis product was 4-amino-2,6-dinitrotoluene (Zwirner-Baier et al. 1994).

In biological monitoring studies, the increased formation of haemoglobin adducts, compared with the levels in control persons, was found also in inhabitants of properties erected on ground contaminated with 2,4,6-trinitrotoluene from former munition factories. The contaminated ground in the area around one of these former factories was found to contain mainly 2,4,6-trinitrotoluene, 2,4-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene and 2,6-dinitrotoluene. The analysis of blood samples from 34 potentially exposed persons showed haemoglobin adducts to be slightly increased compared with the levels in 34 control persons only with 4-amino-2,6-dinitrotoluene (Neumann et al. 1995 a, b).

In a study with 117 workers exposed to 2,4,6-trinitrotoluene, the adduct concentration was 115 to 1106 ng per gram haemoglobin and correlated well (r = 0.88–0.89) with the external exposure to 2,4,6-trinitrotoluene (70–355 mg/worker in an 8-hour shift), which was calculated from the inhaled air and skin contamination (Liu et al. 1995).

The haemoglobin adducts of another 50 workers exposed to 2,4,6-trinitrotoluene correlated with the concentrations of 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene in urine, which were also the main metabolites of 2,4,6-trinitrotoluene eliminated in this study. The adduct levels were between 3.7 and 522 ng 4-amino-2,6-dinitrotoluene and 0 and 14.7 ng 2-amino-4,6-dinitrotoluene per gram haemoglobin. No adducts were detected in control persons (Sabbioni et al. 1996).

Subsequent investigations with workers exposed to 2,4,6-trinitrotoluene confirmed the increased formation of haemoglobin adducts with 4-amino-2,6-dinitrotoluene (Hagmann et al. 2004; Jones et al. 2005 b; Sabbioni et al. 2005, 2006).

In two workers employed in the ground storage unit of a former munitions site, and in 45 inhabitants of properties erected on ground contaminated with 2,4,6-trinitrotoluene at the same site, haemoglobin adducts were detected, although they were not significantly increased compared with the levels in 48 control persons. Widespread background contamination was concluded from this. No details were given of the level of possible exposure to 2,4,6-trinitrotoluene (Ewers et al. 2000).

Toxicokinetics and Metabolism

A comparison of the distribution of 2,4,6-trinitrotoluene in the species rat, mouse, dog and rabbit, revealed the target organs to be the same (blood, liver, spleen, kidneys and gastrointestinal tract), although there are differences between the species

as regards metabolism and elimination. These were described as primarily quantitative (ATSDR 1995).

Absorption, distribution, elimination

In humans, 2,4,6-trinitrotoluene is rapidly absorbed through the skin and from the gastrointestinal tract, but presumably also from the lungs (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990; ATSDR 1995; IARC 1996). In the rat, 2,4,6-trinitrotoluene after oral administration is found mainly in the liver and kidneys. After intravenous, intraperitoneal or percutaneous injection it is distributed throughout the whole body (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990).

In animals, 2,4,6-trinitrotoluene or its metabolites are primarily eliminated with the urine and, in smaller quantities, with the faeces (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990; ATSDR 1995). Urinary elimination takes place also in humans, as shown in the investigations with workers described in the section on metabolism.

Metabolism

The metabolism of 2,4,6-trinitrotoluene has been thoroughly investigated and described. In animal studies, 2,4,6-trinitrotoluene is subject to both oxidative and reductive metabolism, whereby the reductive pathways are predominant. Oxidation of the methyl group and the benzene ring and reduction of the three nitro groups and conjugation reactions occur (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990; ATSDR 1995; Bolt et al. 2006; IARC 1996).

The main metabolic pathways which lead to reactive metabolites are shown in Figure 1.

The main metabolites of 2,4,6-trinitrotoluene in humans are 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene. Both were found in the urine after ingestion of the substance and in workers exposed by inhalation and presumably also dermally to 2,4,6-trinitrotoluene. It is assumed that 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene are available mainly in conjugated form. Also unchanged 2,4,6-trinitrotoluene, 2,6-diamino-4-nitrotoluene and 2,4-diamino-6-nitrotoluene were eliminated in lower concentrations with the urine of workers (Bolt et al. 2006; IARC 1996).

Since the last documentation (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990), three studies with workers have been published (Bader et al. 1998; Coombs and Schillack 1998; Letzel et al. 2003; see Table 1). In all studies, 4-amino-2,6-dinitrotoluene was found to be the main metabolite, followed by 2-amino-4,6-dinitrotoluene.

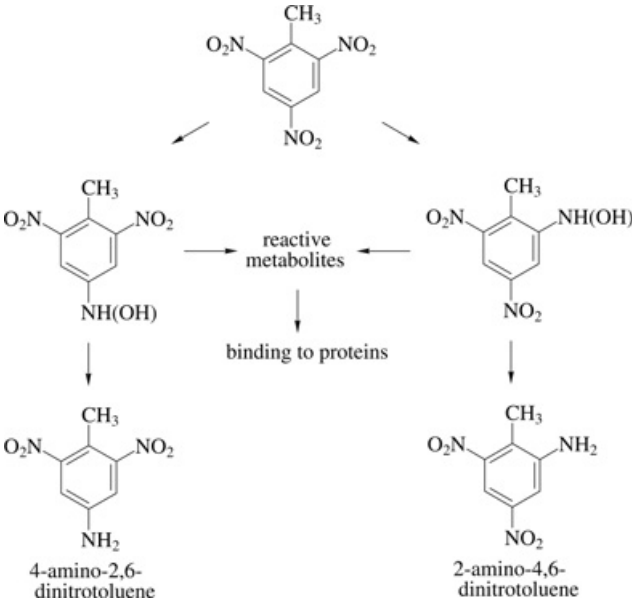

Figure 1 Main metabolic pathways of 2,4,6-trinitrotoluene resulting in reactive metabolites (Bolt et al. 2006)

In addition to the results shown in Table 1, the most recent study showed a clear difference in the concentration of the two main metabolites in the urine between workers exposed occasionally (n = 51) and those exposed regularly (n = 19). There is a significant linear correlation between the concentration in the urine and that of 2,4,6-trinitrotoluene in the air. 2,4,6-Trinitrotoluene concentrations up to 0.69 mg/m^3 and 2,4-dinitrotoluene concentrations of up to 0.02 mg/m^3 were determined by personal air sampling. The concentrations of 2-amino-4,6-dinitrotoluene in the urine of the 51 workers exposed occasionally and the 19 exposed regularly were 0 and 0–1464 µg/l, respectively, those of 4-amino-2,6-dinitrotoluene 0–44 and 0–6693 µg/l, respectively, and those of 2,4,6-trinitrotoluene 0 and 0–5 µg/l, respectively (Letzel et al. 2003). In this study, the detection limit of the metabolites in urine was one order of magnitude lower than that in the other studies shown in Table 1.

In another publication, the presence of "diazo-positive" metabolites was detected in the urine of workers exposed to 2,4,6-trinitrotoluene, that is metabolites with an amino group, in amounts dependent on the level of exposure (Ahlborg et al. 1988 b).

2,6-Dinitrobenzyl alcohol (866 nmol/g creatinine) and 4-amino-2-nitrobenzoic acid (2167 nmol/g creatinine) were detected in the urine of two workers at a Chinese chemicals factory employed in the transport and packing of 2,4,6-trinitrotoluene. The control collective consisted of a group of eleven workers not exposed who were

Table 1 Elimination of 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene in the urine of workers exposed to 2,4,6-trinitrotoluene (TNT)

TNT concentration in the air (mg/m3)	4-amino-2,6-DNT concentration in urine (mg/l)*	2-amino-4,6-DNT-concentration in urine (mg/l)*	References
< 0.01–2.53	9.7 (0.1–44) (mixture)		Woollen et al. 1986
~ 0.5	0.17 (0.03–0.32) mg/g creatinine	0.04 (0.003–0.097) mg/g creatinine	Ahlborg et al. 1988 a
0.18–0.49	day 1: 0.73 (0–2.34) day 4: 5.96 (1.2–16.8)	day 1: 0.16 (0–0.78) day 4: 1.48 (0.1–4.47)	Coombs and Schillack 1998
not stated	0.14–16.83	0.02–5.79	Bader et al. 1998
0–0.69	0.23 (0–6.69)	0.02 (0–1.46)	Letzel et al. 2003

* if not otherwise given
DNT: dinitrotoluene

employed by the same company. Neither 2,6-dinitrobenzyl alcohol nor 4-amino-2-nitrobenzoic acid could be detected in the urine of the workers in the control group (Jones et al. 2005 a).

Effects in Humans

Allergenic effects

Sensitizing effects on the skin

The skin disease after contact with 2,4,6-trinitrotoluene formerly known also as "TNT itch" was promoted by increased temperatures or perspiration; it frequently affected a large number of the workers and was observed even after contact with small amounts of 2,4,6-trinitrotoluene. Especially workers engaged in filling or unloading 2,4,6-trinitrotoluene or in filling explosive charges tended to incur dermatitis and contact allergies. There is no detailed information about allergological investigations available (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990).

In several earlier reviews, 2,4,6-trinitrotoluene is described as causing pronounced contact dermatitis (accompanied by erythema, papules and pruritis). More detailed information on the symptoms or evidence of an allergic genesis are usually not mentioned (Anderson 1944; Anonymous 1964; Bonnevie 1939; Schwartz 1944; Silver 1938). One of the authors describes the possibility that exposed persons become sensitized within "five or more days", with subsequent tolerance frequently developing later (Schwartz 1944).

In addition, a few reports of workers with contact dermatitis from 2,4,6-trinitrotoluene are available: in an earlier review of occupational skin diseases in the explosives manufacturing industry, five cases of contact dermatitis from 2,4,6-trinitrotoluene are cited. The author gives 0.5% 2,4,6-trinitrotoluene in alcohol as the test preparation (Querangal des Essarts 1955). In one worker who assembled mines filled with 2,4,6-trinitrololuol powder, generalized erythematous papular skin reactions occurred one month after starting work. The patch test produced a 1+ reaction to 5% 2,4,6-trinitrotoluene in petrolatum after 96 hours and a 2+ eczematous reaction to 10% 2,4,6-trinitrotoluene (Goh 1988). In one female worker in the ammunition industry who operated an explosive charge filling machine and had contact with 2,4,6-trinitrotoluene and tetryl among other substances (see documentation "N-Methyl-N,2,4,6-tetranitroaniline" 1998; Sh), erythematous, papular skin changes on the neck, forearm and forehead, and oedematous changes on the hands occurred three months after starting work. The patch test produced clear reactions (2+) to 5%, 10% and 25% preparations of 2,4,6-trinitrotoluene in petrolatum. Lower test concentrations produced questionable or negative results; 2,4,6-trinitrotoluene powder caused an erosive reaction. In 20 control persons, the 5% preparation of 2,4,6-trinitrotoluene in petrolatum produced no reaction (Goh and Rajan 1983).

Four months after starting work, a yellow discoloration of the hands, swelling of the lips, fingers and hands, and a rash in the neck area occurred in a worker likewise occupied in filling explosive charges with 2,4,6-trinitrotoluene and tetryl among other substances. The patch test produced a reaction to 5% 2,4,6-trinitrotoluene and, after 96 hours, to 0.05% (1+), 0.1% and 0.5% (both 2+) preparations of tetryl in petrolatum. The author interpreted the reaction to 2,4,6-trinitrotoluene as a possible cross-reaction with tetryl (Goh 1984).

Sensitizing effects on the respiratory tract

There are no data available for the sensitizing effects of 2,4,6-trinitrotoluene on the respiratory tract.

Repeated exposure

In the documentation from 1988 (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990), epidemiological studies describing increased mortality resulting from 2,4,6-trinitrotoluene in workers was discussed. The exposure concentrations were in the range of 6 to 16 mg/m^3. The clinical symptoms in the workers consisted mainly of tiredness, paleness and cyanosis, arising from methaemoglobinaemia, aplastic anaemia and changes in the blood picture, such as reduction of and damage to the erythrocytes, leukopoenia, leukocytosis, lymphocytosis and fragmented cells. Other toxic effects were irritation of the respiratory tract, digestive tract and skin as well as systemic effects on the digestive tract, liver

and gallbladder, cardiovascular system, nervous system and the formation of cataracts. The causes of death were reported to be mainly aplastic anaemia and toxic hepatitis. Further epidemiological studies not cited in the documentation of 1988 are discussed in the assessment of the IARC (1996) and confirm the previous results. These studies are described below:

In an epidemiological study conducted by the US Army in the 1970s, no significant differences in liver function were found in 626 workers–the majority of whom were exposed in four ammunition factories to 2,4,6-trinitrotoluene concentrations of ≤ 0.5 mg/m^3 and only a few (no other details) to 1.5 mg/m^3–compared with that in 865 control persons not exposed. Bilirubin, lactate dehydrogenase, alkaline phosphatase, serum alanine aminotransferase and aspartate aminotransferase were the parameters investigated (ATSDR 1995).

A case–control study in Chinese workers exposed to 2,4,6-trinitrotoluene revealed an increase in liver toxicity in the workers with increased alcohol consumption (ATSDR 1995).

In a case report published in Czech, of which only a summary is available in English, a worker developed non-alcoholic steatohepatitis after exposure to significant levels of 2,4,6-trinitrotoluene for 22 years (no other details) (Hassmanová et al. 2002).

A cross-sectional study was carried out with 82 workers involved in the disposal of waste ammunition in Germany. Of these, 51 were regularly, 19 occasionally and 12 not exposed to 2,4,6-trinitrotoluene (up to 3.25 mg/m^3) or 2,4-dinitrotoluene (up to 0.02 mg/m^3). In the regularly exposed workers, an increase in symptoms such as a bitter taste, burning eyes, and discoloration of the skin and hair were found, together with a significant increase in serum lactate dehydrogenase in clinical laboratory investigations ($p < 0.05$; Letzel et al. 1997 b, 2003).

In a case–control study with workers exposed to 2,4,6-trinitrotoluene and other chemicals in an ammunition factory for many years, the exposure conditions of 32 workers with haematological changes (neutropoenia, reduced thrombocyte count or enlarged erythrocytes) were compared with those of 322 workers without haematological changes. According to the authors, the parameters investigated could indicate a possible myelodysplastic syndrome. A slightly increased odds ratio of 2.4 (confidence interval: 0.8–7.0) for haematological changes was reported for the 25 workers exposed to 2,4,6-trinitrotoluene; the control group consisted of 205 workers not exposed to 2,4,6-trinitrotoluene without haematological changes (West and Stafford 1997).

In another cross-sectional study, four cases with cataracts were found among 23 workers exposed to 2,4,6-trinitrotoluene that could be classified as a typical 2,4,6-trinitrotoluene cataract. The blood values of the workers were within the normal range; no details were given of the exposure levels (Kruse et al. 2005).

To summarize, the effects of 2,4,6-trinitrotoluene on blood parameters and the formation of cataracts presented in the documentation from 1988 (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990) have been confirmed in further studies described in the evaluation of the IARC (1996) or in this section.

Genotoxicity

With the urine of workers exposed to 2,4,6-trinitrotoluene via inhalation and presumably also as a result of absorption of the substance through the skin, increased mutagenic activity was observed in bacterial systems.The urine samples of 14 workers exposed to 2,4,6-trinitrotoluene were found to be mutagenic in Salmonella typhimurium TA98 and in Escherichia coli WP2 uvrA without, but not with, metabolic activation. The mutagenic activity correlated with the concentration of 2,4,6-trinitrotoluene at the workplace, which reached a maximum level of 0.29 mg/m^3. Urine samples from the same workers after a four-week holiday were used as controls. The exclusion of smokers from the assessment made no change to the results. In the samples with the highest mutagenicity, unmetabolized 2,4,6-trinitrotoluene was found (Ahlborg et al. 1985).

In a subsequent study, the urine samples of 50 workers were investigated. These were classed in three groups: no exposure (2,4,6-trinitrotoluene concentration in the air below the detection limit), medium exposure (2,4,6-trinitrotoluene concentrations of 0.1–0.3 mg/m^3) and high exposure (0.2–0.5 mg/m^3). Sampling took place before and after the shift, and health status, smoking habits, alcohol consumption, diet and use of medicines were recorded. The samples were incubated in the mutagenicity test with Salmonella typhimurium TA98 and with the nitroreductase-deficient strain TA98NR without the addition of a metabolic activation system. The urine samples of the high exposure group caused clear mutagenic effects. As the reaction with the strains TA98 and TA98NR was comparable, although the nitroreductase-deficient strain was less sensitive, bacterial nitroreductase does not seem to have any significant relevance for the mutagenicity of the urine samples. Unlike in the previously described study of Ahlborg et al. (1985), no correlation was found between the 2,4,6-trinitrotoluene concentration in the urine and the mutagenicity. On the other hand, the correlation between the mutagenic activity and the 4-amino-2,6-dinitrotoluene concentration in the urine was significant (Ahlborg et al. 1988 a).

In a chromosomal aberration test with peripheral lymphocytes of 26 workers (13 men and 13 women) exposed to 2,4,6-trinitrotoluene for between 0.5 and 5 years, more irregularities were found in the conventional analysis (data not reported) and in the CISS (Color Chromosome In Situ Suppression) analysis in those persons with high internal exposure, determined as 4-amino-2,6-dinitrotoluene in blood, than in the volunteers with a low level of exposure (data for the individuals not given in the group comparison). Of these 26 persons, five were from the administrative area without direct contact with 2,4,6-trinitrotoluene, a further five were exposed irregularly and 16 regularly (no other details). The 26 workers, of whom 17 had participated in ten or more radiographic diagnoses (no other details), were found to have urinary 2,4-dinitrotoluene concentrations of 0 to 2.1 µg/l, 2,4-dinitrobenzoic acid concentrations of 0 to 50.9 µg/l, 2,6-dinitrotoluene concentrations of 0 to 3.6 µg/l, 2,4,6-trinitrotoluene concentrations of 0 to 3.8 µg/l, 2-amino-4,6-dinitrotoluene concentrations of 0 to 1147 µg/l and 4-amino-2,6-dinitrotoluene

concentrations of 6 to 3975 µg/l. Exposure-related values were not given. Compared with the incidence in the controls (22 women and seven men, of whom only one person had had a number of radiographic diagnoses), a significantly increased number of chromosomal aberrations (16.0 ± 4.0 per 1000 mitoses compared with 5.85 ± 2.9 in the controls) was found in the 26 workers (Letzel et al. 1997 a, b; Verdorfer et al. 2001). In the workers exposed to 2,4,6-trinitrotoluene, an increase in the number of stable and unstable aberrations was observed; in tumour patients there was an increase only in radiation-induced stable aberrations. Statistical evaluation of the original data using the Mann-Whitney test showed a significant increase in chromosomal aberrations for the CISS analysis ($p < 0.01$) and the conventional method ($p < 0.05$) for those persons with high internal exposure to 4-amino-2,6-dinitrotoluene of more than 800 µg/l compared with the incidence in the controls and those persons with low internal exposure to 4-amino-2,6-dinitrotoluene of less than 355 µg/l.

Overall, the investigations of the genotoxicity of the substance in humans show that inhalation exposure and presumably the simultaneous dermal exposure of workers to 2,4,6-trinitrotoluene results in a significantly increased incidence of chromosomal aberrations in the peripheral lymphocytes. In addition, the urine of workers exposed to 2,4,6-trinitrotoluene produces increased mutagenic activity in bacteria. The chromosomal aberration test in humans with positive results was carried out using a very sensitive method, which might possibly explain the discrepancy between this and the negative results in genotoxicity tests in animals.

Carcinogenicity

A 61-year-old worker who had been exposed to 2,4,6-trinitrotoluene for 35 years died of a hepatocellular carcinoma. Known risk factors for the development of a hepatocellular carcinoma, such as infectious hepatitis and alcohol-dependency, were not present. Whether the exposure to 2,4,6-trinitrotoluene played a role in the formation of the cancer is, however, not known (Garfinkel et al. 1988). A description of the route of absorption and the exposure level is not contained in this reference. Presumably, inhalation was the primary route; dermal absorption and low-level oral absorption, for example from contaminated hands, cannot, however, be excluded.

In a case-control study from the 1990s, two population groups living in the proximity of two German ammunition factories from the Second World War were investigated. Compared with control persons from the neighbouring communities, the relative risk (RR) of contracting acute myeloic leukaemia (AML) was 3.5 (confidence interval: 1.4–8.5) or 2.3 (confidence interval: 0.8–6.5) for men and 3.2 (confidence interval: 1.4–7.2) or 4.3 (confidence interval: 1.4–12.7) for women. The relative risk seemed to be particularly high in persons aged above 65 years. The case numbers in the study were very small, however, so that its usefulness is limited. In the men of the two population groups also the relative risk of contracting chronic

myeloic leukaemia (CML) was increased (RR 4.1 [confidence interval: 1.4–12.2] and RR 9.1 [confidence interval: 3.5–23.4]). In the women, on the other hand, only one case was observed (Havemann et al. 1992; Kolb et al. 1993).

Another case–control study was carried out, as the distance to the factories in which the 2,4,6-trinitrotoluene was produced, was not taken into consideration in the above-mentioned study. In addition, other factors such as possible exposure to benzene or to carcinogens at the workplace were not taken into account. As environmental pollution with toluene derivatives from former munition dumps was known in this area, it was suspected that the production of 2,4,6-trinitrotoluene and the resultant contamination of the soil and water could be responsible for the increased risks observed. However, the new case–control study did not confirm this. It merely indicated there to be a relationship between an increase in the odds ratio in a small group of persons from a specific area of one of the two communities that had been involved in the boom phase of 2,4,6-trinitrotoluene production during the 1940s. These findings are, however, spurious and cannot explain the increased frequency of leukaemia cases in the 1980s. There is no other explanation for an increased risk of contracting leukaemia in this area (Kilian et al. 2001).

A study published in Chinese, of which only an English summary is available, reports an increased incidence of malignant tumours in workers exposed to 2,4,6-trinitrotoluene compared with that in the average population. In particular, the incidence of liver tumours was related to the length of occupational exposure to 2,4,6-trinitrotoluene as well as to the level of exposure and the type of work carried out in the factory. Alcohol consumption was also discussed as a possible cause of the liver tumours (no other details; Yan et al. 2002).

Animal Experiments and in vitro Studies

Subacute, subchronic and chronic toxicity

In animal studies, methaemoglobin and Heinz bodies were found. After medium and long-term exposure, the target organs were mainly the liver, spleen, blood and testes (documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990; IARC 1996).

The oral 13-week study in male and female F344 rats (see documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990) is presented again here for the evaluation of germ cell mutagenicity. The NOAEL (no observed adverse effect level) in this study was 5 mg/kg body weight and day. The highest administered dose of 300 mg/kg body weight and day was markedly toxic. The body weights of the animals in the high dose group were about 60% of those of the control animals, and those of the animals in the middle dose group (125 mg/kg body weight and day) were about 75% of the weights of the controls. Anaemia was observed after doses of 25 mg/kg body weight and day and above, and marked

effects on the blood count at the medium dose of 125 mg/kg body weight and day and above. As regards organ weights, the increase in spleen weights correlated with the decrease in testis weights. In view of the presence of Leydig cell hyperplasia, a hormonal mechanism for the degeneration in the testes (secondary as a result of liver enzyme induction) was suggested by the authors, but oxygen deficiency resulting from anaemia is also concievable as a secondary mechanism (Levine et al. 1984).

In addition, two well-documented studies of the US Army from the 1980s are available, in which rats and mice were given 2,4,6-trinitrotoluene with the diet for two years:

Rat

Groups of 75 male and 75 female F344 rats were given 2,4,6-trinitrotoluene with the diet for 24 months in concentrations corresponding to doses of 0, 0.4, 2, 10 or 50 mg/kg body weight and day. Mortality was not increased in the treated animals compared with that in the controls. Food intake was reduced at 10 mg/kg body weight and day and above, and body weight gains were reduced by 5% to 14% in the 10 mg/kg group and by 30% to 33% in the 50 mg/kg group. Increased ocular discharge was observed in the animals of the 50 mg/kg group during the second year. Anaemia with secondary spleen damage, hepatotoxicity, urogenital lesions and tumours diagnosed as bladder papillomas and carcinomas were the main toxic effects (see the Section "Carcinogenicity", Table 5).

Anaemia occurred at doses of 10 mg/kg body weight and day and above in the rats of both sexes, though more pronounced in the males, in the form of decreased haematocrit and haemoglobin values, decreased erythrocyte counts and increased reticulocyte counts. After the administration of 2 mg/kg body weight and day, fibrosis of the bone marrow was observed in the females. In the animals given 10 mg/kg body weight and day and above, sinusoidal congestion, extramedullary haematopoiesis and haemosiderin-like pigmentation were found in the spleen. The authors concluded that 2,4,6-trinitrotoluene seems to induce anaemia by means of a haemolytic process. This was supported by the observation of Howell-Jolly and Heinz bodies as well as by the presence of methaemoglobin in the blood after doses of 10 mg/kg body weight and day and above, all of which provides evidence of the oxidizing properties of 2,4,6-trinitrotoluene or its metabolites. In addition, at 50 mg/kg body weight and day and above, thrombocytosis was observed, which mainly appeared during the second year of exposure.

The relative liver weight was increased mainly after doses of 50 mg/kg body weight and day and, to a lesser extent, after 10 mg/kg body weight and day. After doses of 10 mg/kg body weight and day and above hepatocellular hyperplasia occurred in the male animals. In addition, the hepatotoxicity was also apparent from changes in lipid metabolism, which was reflected by an increase in serum cholesterol levels (after 2 mg/kg body weight and day and above), triglycerides (after 10 mg/kg

body weight and day and above) and albumin (after 50 mg/kg body weight and day). Also, at 50 mg/kg body weight and day, increased serum protein and globulin levels were observed.

Nephrotoxicity was demonstrated by a slight increase in blood urea values in the high dose group and spots of brown pigment on the kidneys and an increase in relative organ weights after 2 mg/kg body weight and day and above in the male animals and after 10 mg/kg body weight and day and above in the female rats. After doses of 2 mg/kg body weight and day and above, hypertrophy of the proximal tubular cells of the renal cortex occurred. Inflammation with lymphocytic infiltration into the renal tissue and hyperplastic lesions in the renal pelvis, in female animals also in the bladder, affected mainly the animals of the high dose groups. The male and female animals of the 10 mg/kg and 50 mg/kg groups were found to have increased relative heart weights.

Hyperplasia, papillomas and carcinomas of the bladder occurred in female rats sporadically after doses of 10 mg/kg body weight and day and above, and were significantly increased at 50 mg/kg body weight and day (see the Section "Carcinogenicity", Table 5) (US Army 1984 a). The NOAEL for non-neoplastic effects is 0.4 mg/kg body weight and day.

Mouse

After the administration of 2,4,6-trinitrotoluene doses of 0, 1.5, 10 or 70 mg/kg body weight and day to groups of 75 male and 75 female B6C3F$_1$ mice with the diet for 24 months, mortality in the treated animals was not increased compared with that in the controls. In the males, body weight gains were reduced by 5% to 7% after doses of 10 mg/kg body weight and day and above, and in both sexes by 15% to 20% at 70 mg/kg body weight and day. Anaemia, hepatotoxicity, peripheral lymphocytosis, leukaemia and malignant lymphomas in the spleen were the main toxic effects (see the Section "Carcinogenicity", Table 5).

The slight anaemia at 70 mg/kg body weight and day was characterized by reduced haematocrit and haemoglobin values and a reduced erythrocyte count in the mice of both sexes, although this was more pronounced in the male animals. Compensatory effects, such as observed in the study with rats, presumably did not occur in view of the low degree of anaemia in the mice.

Further toxic effects were observed only at 70 mg/kg body weight and day. These were reduced serum triglyceride and globulin levels, increased relative liver and brain weights, and sporadic increases in relative kidney, spleen, heart and testis weights, all of which occurred without accompanying histopathological effects. In addition, enlarged lymph nodes were observed (US Army 1984 b). A NOAEL of 1.5 mg/kg body weight and day was obtained for non-neoplastic effects.

Allergenic effects

There are no documented studies of the allergenic effects of 2,4,6-trinitrotoluene available. In a summary of toxicological results, 2,4,6-trinitrotoluene was regarded as moderately sensitizing in an animal experiment in which positive results were obtained in four of 10 animals. No other details are given (US Army 1980).

Genotoxicity

In vitro

Bacterial test systems

2,4,6-Trinitrotoluene was mutagenic in Salmonella typhimurium, and was dependent on the one hand on enzymatic activation by the nitroreductase and on the other hand on the addition of a metabolic activation system. Nitroreductase-deficient strains were less sensitive, and also the addition of a metabolic activation system caused a reduction in the mutagenic activity (see documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990; ATSDR 1995).

The results of mutagenicity tests in bacteria correlated well (see Table 2). 2,4,6-Trinitrotoluene produced mainly frameshift mutations in the strains Salmonella typhimurium TA1537, TA1538 and TA98, but also base pair substitutions in the strains TA1535 and TA100 (ATSDR 1995).

Table 2 Genotoxicity of 2,4,6-trinitrotoluene in bacteria in vitro

Test system: Salmonella typhimurium strain	Concentration range	Effective concentration	Cytotoxicity	Result		References
				+m.A.	–m.A	
TA98	0.5–10 µg/plate	2.5 µg/plate	> 10 µg/plate	–	+	Won et al. 1976
TA98, TA100	20–200 µg/plate	20 µg/plate	no data	+a)	+a)	Tan et al. 1992
TA98, TA100	no data	no data	no data	+b)	+b)	Honeycutt et al. 1996
TA98, TA100F)	0.05–50 µM	4.8 µM	no data	+	+	Lachance et al. 1999
TA97a, TA98, TA100	2–240 µg/plate	≥ 20 µg/plate	> 240 µg/plate	+a)	+a)	Donnelly et al. 1998
TA98, TA1538	0.5–500 µg/plate	≥ 50 µg/plate	no data	+c)	+c)	Kaplan and Kaplan 1982
TA98, TA100, TA1535, TA1537, TA1538	10–5000 µg/plate	no data	no data	+; no other details		Pearson et al. 1979

Table 2 (Continued)

Test system: Salmonella typhimurium strain	Concentration range	Effective concentration	Cytotoxicity	Result		References
				+m.A.	–m.A	
TA98, TA100, TA1535, TA1537, TA1538	no data	no data	no data	+d)	+d)	ATSDR 1995
TA100, TA100NRf)	7.8–250 µg/plate	≥ 62.5 µg/plate	≥ 250 µg/plate	not tested	+e)	Spanggord et al. 1995
TA98, TA100, TA100NRf), TA1535, TA1537, TA1538	25–200 µg/plate; 50–100 µg/plate in TA100NR	50 µg/plate	no data	–g)	+h)	Whong and Edwards 1984
TA98, TA100NR3f), TA1523, TA1535, TA1537, TA1538	10–5000 µg/plate	50 µg/plate	no data	+i)	+i)	Spanggord et al. 1982
TA98, TA100, TA100NRf) TA100/1,8-DNPj)	no data	no data	no data	+k)	+k)	Karamova et al. 1994
TA98, YG1021l), YG1024m)	10–80 µg/plate in TA98; 10–120 µg/plate in YG1021/YG1024	10 µg/plate	no data	not tested	+n)	Väätänen et al. 1997

F) Fluctuation test
m.A: metabolic activation
a) greater response without m.A.; weaker response in TA98
b) negative with and without m.A. in TA100
c) not all results reported in detail
d) greater response without m.A.; greater response in TA98, TA1538, TA1537
e) negative in TA100NR
f) nitroreductase-deficient
g) investigated only with TA1538
h) greater response in TA98, TA1538, TA1537; negative in TA1535, TA100NR
i) greater response without m.A.; negative with and without m.A. in TA1535, TA100NR3
j) *O*-acetyltransferase deficient
k) negative with and without m.A. in TA100NR, TA100/1,8-DNP
l) increased nitroreductase activity
m) increased *O*-acetyltransferase activity
n) greater response in YG1021, YG1024

The spontaneous and the 2,4,6-trinitrotoluene-induced mutation spectrum at the hisD3052 allele of Salmonella typhimurium strains TA98 and YG1021 (increased nitroreductase activity) and YG1024 (increased *O*-acetyltransferase activity) was investigated, and showed that the greater enzyme activity in the strains YG1021 and YG1024 produced increased 2,4,6-trinitrotoluene mutagenicity, but had no effect on the mutation spectrum (Väätänen et al. 1997).

2,4,6-Trinitrotoluene metabolites in bacterial test systems

In a mutagenicity test with Salmonella typhimurium TA98, 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene yielded positive or weakly positive results and were dependent on activation by a nitroreductase (Spanggord et al. 1982). In another study, four possible mono and diamino metabolites of 2,4,6-trinitrotoluene likewise had weaker mutagenic effects in the strains TA98 and TA100 than 2,4,6-trinitrotoluene itself (Tan et al. 1992).

In two other investigations, 2,4,6-trinitrotoluene again produced the strongest mutagenic activity in Salmonella typhimurium TA98 and TA100, followed by 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene (Lachance et al. 1999; Spanggord et al. 1995).

The quantitative data from the study of Lachance et al. 1999 for the mutagenicity of 2,4,6-trinitrotoluene and its metabolites are shown in Table 3. 4-Amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene were mutagenic in concentration ranges (≥ 8.1 µM, about 1.8 mg/l) as occur in the urine of workers (0.02–9.7 mg/l) exposed to 2,4,6-trinitrotoluene concentrations of < 0.01 to 2.53 mg/m³ (see the Section "Metabolism", Table 1).

Table 3 Quantitative data for the mutagenicity of 2,4,6-trinitrotoluene (TNT) and its metabolites (Lachance et al. 1999)

	Salmonella typhimurium TA98				Salmonella typhimurium TA100			
+/–m. A.a)	+	+	–	–	+	+	–	–
	NOECb)	LOECb)	NOECb)	LOECb)	NOECb)	LOECb)	NOECb)	LOECb)
TNT	0.5	4.8	4.8	48.0	0.5	4.8	4.8	48.0
2-ADNT	4.1	8.1	< 8.1	8.1	3.6	6.6	6.6	13.2
4-ADNT	39.6	> 39.6	12.7	25.4	14.2	28.4	28.4	44.1
2,4-DANT	62.8	> 62.8	10.2	20.3	40.7	203.0	40.7	> 40.7
2,6-DANT	40.7	62.8	62.8	> 62.8	24.5	37.7	24.5	34.7

a) + m.A. metabolic activation with rat liver S9-Mix
b) NOEC and LOEC in µM
2-ADNT: 2-amino-4,6-dinitrotoluene; 4-ADNT: 4-amino-2,6-dinitrotoluene
2,4-DANT: 2,4-diamino-6-nitrotoluene; 2,6-DANT: 2,6-diamino-4-nitrotoluene

2,4,6-Trinitrotoluene and its urinary metabolites in bacterial test systems

The mutagenicity of the urine of Wistar rats given intraperitoneal doses of 2,4,6-trinitrotoluene of 50 mg/kg body weight, was only weak in Salmonella typhimurium TA98, but pronounced in the strains YG1021 and YG1024, which overproduce nitroreductase and *O*-acetyltransferase, respectively. In the corresponding strains TA98NR and TA98/1,8-DNP deficient in nitroreductase and *O*-acetyltransferase, the urine of rats exposed to 2,4,6-trinitrotoluene was not mutagenic (Einistö 1991).

Also another study with Salmonella typhimurium revealed mutagenic activity in the urine of rats given single oral doses of 2,4,6-trinitrotoluene of 75 mg/kg body weight.. The HPLC fractions used in the mutagenicity test were found to have some of the known mutagens such as 2,4,6-trinitrotoluene, 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene and 2,6-diamino-4-nitrotoluene, but also additional, unidentified mutagenic substances were detected. Here too, the mutagenicity was less pronounced in the nitroreductase-deficient strains TA98NR and TA100NR (Brooks et al. 1997; George et al. 1996).

2,4,6-Trinitrotoluene and its metabolites in mammalian cells

The studies of the genotoxicity of 2,4,6-trinitrotoluene in mammalian cells in vitro are summarized in Table 4.

Table 4 In vitro genotoxicity of 2,4,6-trinitrotoluene in mammalian cells

Test system	Concentration range	Effective concentration	Cytotoxicity	Results		References
				+ m.A	– m.A	
DNA repair synthesis (UDS), human fibroblasts (WI-38)	no data	no data	no data	–	+/–	ATSDR 1995
Mutagenicity test, V79 cells	440 µM	–	440 µM	–	–	Lachance et al. 1999
HPRT, CHO cells	10–100 µg/ml (soluble up to 105 µg/ml in 1% DMSO)	30 µg/plate	> 40 µg/ml, slightly cytotoxic	+	+	Kennel et al. 2000
TK+–, mouse lymphoma cells P388	8–1000 µg/ml	no data	dose-dependent; no other details	–	+?	Styles and Cross 1983

+: result questionably positive
+–: result inconclusive
m.A: metabolic activation

In a mutagenicity test with V79 cells from the Chinese hamster, not described in detail, neither 2,4,6-trinitrotoluene nor any of the metabolites 1,3,5-trinitrobenzene, 2,4,6-triaminotoluene, 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene or 2,6-diamino-4-nitrotoluene were mutagenic (Lachance et al. 1999).

In an HPRT test using CHO cells with and without the addition of a metabolic activation system, 2,4,6-trinitrotoluene was found to be mutagenic in a dose-dependent manner; this was most pronounced at 40 µg/ml, although at higher concentrations cytotoxicity was low with almost 80% survival. The ten 2,4,6-trinitrotoluene metabolites also tested had only weak or no mutagenic effects (Kennel et al. 2000).

The result of a test for DNA repair synthesis (UDS) was "inconclusive" (ATSDR 1995). As the original publication of the US Army from 1978 is not available, the test cannot be included in the evaluation.

According to the authors, 2,4,6-trinitrotoluene was genotoxic in P388 mouse lymphoma cells without the addition of a metabolic activation system, and there was a statistically significant increase in the mutation frequency at the $TK^{+/-}$ locus. In concentrations of about 100 µg/ml and above, 2,4,6-trinitrotoluene induced dose-dependent cytotoxicity with survival of 50% of the animals and below. With the addition of a metabolic activation system, 2,4,6-trinitrotoluene was not mutagenic (data not given; Styles and Cross 1983). In this publication, the results are listed logarithmically, the original data and standard deviations are not presented. It is not clear from the description whether significant genotoxic effects occurred at 40 µg/ml or not until cytotoxic concentrations were reached. In addition, no differentiation was made between small and large colonies, so that no statement can be made about possible clastogenic or mutagenic effects. Because of the lack of original data, this test result cannot be used in the evaluation of the genotoxicity of 2,4,6-trinitrotoluene as it is not possible to make any conclusive assessment.

Although the data base for the evaluation of the genotoxicity of 2,4,6-trinitrotoluene in mammalian cells in vitro is limited, the agreement within the available test systems nevertheless allows the conclusion that the substance is mutagenic in bacterial and mammalian cell cultures; the addition of a metabolic activation system is not required.

In vivo

In a study available only in summary form, the covalent DNA binding (^{32}P postlabelling) in liver cells was investigated in male rats given oral doses of 2,4,6-trinitrotoluene of 25, 50 or 70 mg/kg body weight. Three to four DNA adducts with a maximum adduct concentration of seven adducts/10^9 nucleotides were observed at 50 mg/kg body weight (no other details; Kohan et al. 1995). The adduct concentration is not to be regarded as a positive effect. As further data which would validate the study are not given in the summary, this study cannot be used in the evaluation of the genotoxicity of 2,4,6-trinitrotoluene.

2,4,6-Trinitrotoluene did not increase unscheduled DNA synthesis (UDS) in the hepatocytes of up to three male rats after single oral doses of 100, 200, 500 or 1000 mg/kg body weight (AP rats) or 200, 500 or 1000 mg/kg body weight (F344 rats). Neither cytotoxic nor genotoxic effects were observed in the hepatocytes twelve hours after treatment. The animals had increased methaemoglobin levels and red-coloured urine, which indicates systemic exposure to 2,4,6-trinitrotoluene (Ashby et al. 1985).

2,4,6-Trinitrotoluene did not increase the number of micronuclei in the bone marrow of male mice. The polychromatic erythrocytes of groups of five male (CBA×BalbC)F_1 mice were investigated 24, 48 or 72 hours after single intraperitoneal injections of 40 or 80 mg/kg body weight which, according to the authors, corresponds to 80% of the maximum tolerable dose (Ashby et al. 1985).

In a chromosomal aberration test with groups of five male Sprague Dawley rats given 0.002% or 0.25% 2,4,6-trinitrotoluene (around 2 or 250 mg/kg body weight and day) with the diet for 28 days, there was no increase in the number of chromosomal aberrations. However, no toxic effects were observed in the rats, nor cytotoxic effects in the bone marrow. In two other groups, kept without treatment for a further 28 days after exposure before subsequent investigation, no genotoxic effects in the bone marrow cells were found. Treatment was not lethal in any of the animals. The reduced body weights in the high dose group was attributed by the authors to the palatability of 2,4,6-trinitrotoluene rather than to toxic effects. The slight decrease in the mitotic indices in the high dose group was not considered to be a cytotoxic effect. The usefulness of this study is limited as the test was not carried out up to the maximum tolerable dose; in addition, no signs of toxicity were seen in any of the animals, nor were any cytotoxic effects found in the cells (ATSDR 1995).

To summarize, the in vivo studies did not reveal any genotoxic effects of 2,4,6-trinitrotoluene, although the chromosomal aberration test cannot be fully included in the evaluation as testing was not carried out up to the maximum tolerable dose.

Carcinogenicity

In a carcinogenicity study, in which only the formation of lung tumours in groups of 20 rats, mice and guinea pigs (no other details) was investigated, intratracheal instillation of 2,4,6-trinitrotoluene over a period of twelve months (no other details) did not induce tumours. This and other investigations from the 1940s and 1950s (see documentation "2,4,6-Trinitrotoluene (and isomers in technical mixtures)" 1990), in which no carcinogenicity was reported, do not meet the present-day requirements of a carcinogenicity study and can therefore not be included in the evaluation of the carcinogenicity of 2,4,6-trinitrotoluene.

Two well-documented studies by the US Army from the 1980s are available, in which rats and mice were given 2,4,6-trinitrotoluene with the diet for two years. The clinical observations presented in these studies and the determination of body

weights, food intake and organ weights, as well as haematological, clinico-chemical and ophthalmological investigations, and macroscopic and microscopic investigations of the organs are described in the Section "Subacute, Subchronic and Chronic Toxicity".

Rat

Groups of 75 male and 75 female F344 rats were given 2,4,6-trinitrotoluene with the diet for 24 months in concentrations corresponding to doses of 0, 0.4, 2, 10 or 50 mg/kg body weight and day. Mortality in the treated animals was not increased compared with that in the controls. Food intake and body weight gains were reduced after doses of 10 mg/kg body weight and day and above. Hyperplasia, papillomas and carcinomas of the bladder occurred in female rats sporadically at 10 mg/kg body weight and day and above. The increase was significant at 50 mg/kg body weight and day (see Table 5). The observed increase in carcinomas was accompanied by an increase in the incidence of hyperplastic, preneoplastic and neoplastic changes in the bladder epithelium, which occurred at 10 mg/kg body weight and day and above. In addition, hyperplastic changes in the liver and kidneys were ob-

Table 5 Studies of the carcinogenicity of 2,4,6-trinitrotoluene

Author:	US Army (1984 a)
Substance:	2,4,6-trinitrotoluene (purity: 99.05%–99.43%)
Species:	rat, F344/N, 75 ♂, 75 ♀ (interim dissection of 10 ♂ and 10 ♀ after 6 and 12 months)
Administration route:	with the diet
Dose:	0, 0.4, 2, 10, 50 mg/kg body weight and day
Duration:	2 years, continuously
Toxicity:	0.4 mg/kg body weight: NOAEL for toxicity 2 mg/kg body weight and above: relative kidney weights ↑; histopathological changes in the kidneys; serum cholesterol levels ↑; alkaline phosphatase ↓ (only after 14 days); fibrosis of the bone marrow (only ♀) 10 mg/kg body weight and above: food intake and body weight gains ↓; anaemia; histopathological changes in the spleen, Howell-Jolly and Heinz bodies ↑, methemoglobin ↑; relative liver weights ↑; hepatocellular hyperplasia (only ♂); serum triglycerides ↑; relative heart weights ↑ 50 mg/kg body weight: ocular discharge ↑; thrombocytosis, serum albumin, protein and globulin ↑; blood urea ↑ (slightly); inflammation with lymphocytic infiltration in the kidney tissue, hyperplastic lesions in the renal pelvis, hyperplastic lesions in the bladder (only ♀); relative spleen and testis weights ↑ (only recognizable at interim dissection)

Table 5 (Continued)

Tumours:

		Dose (mg/kg body weight and day)				
		0	0.4	2.0	10.0	50.0
Survival (weeks)	♂	99.4	96.8	96.1	98.4	101.3
	♀	100.1	99.2	100.8	102.8	102.9
Bladder:						
hyperplasia	♂	0/54	0/54	0/54	0/54	0/55
	♀	0/54	0/54	0/55	2/55 (3.6%)	12/55 (21.8%)**
papillomas	♂	0/54	0/54	0/54	0/54	0/55
	♀	0/54	0/54	0/55	1/55 (1.8%)	5/55 (9.1%)*
carcinomas	♂	0/54	0/54	0/54	0/54	0/55
	♀	0/54	0/54	0/55	1/55 (1.8%)	12/55 (21.8%)**

* $p < 0.05$; ** $p < 0.01$ in Fisher's Exact Test

Author:	US Army (1984 b)
Substance:	2,4,6-trinitrotoluene (purity: 98.86%–99.43%)
Species:	mouse, $B6C3F_1$, 75 ♂, 75 ♀ (interim dissection of 10 ♂ and 10 ♀ after 6 and 12 months)
Administration route:	with the diet
Dose:	0, 1.5, 10, 70 mg/kg body weight and day
Duration:	2 years, continuously
Toxicity:	1.5 mg/kg body weight: NOAEL for toxicity 10 mg/kg body weight and above: body weight gains ↓ (only ♂); hepatocellular hyperplasia (only ♂); relative heart weights ↑, lymphocytes ↑ (slightly) 70 mg/kg body weight: body weight gains ↓ (♀); slight anaemia; lymphocytosis; serum triglycerides and globulin ↓; sporadic increase in relative liver, kidney, spleen, heart, testis and brain weights

Tumoursa):

		Dose (mg/kg body weight and day)			
		0	1.5	10.0	70.0
Survival (weeks)	♂	99.6	98.9	99.2	99.8
	♀	99.4	100.3	101.3	102.2
Spleen:					
lymphatic hyperplasia	♂	19/53 (35.9%)	31/53 (58.5%)**	28/52 (53.9%)	18/54 (33.3%)
	♀	no data	no data	no data	no data
leukaemia/malignant lymphomas	♂	no data	no data	no data	no data
	♀#	9/54 (16.7%)	15/54 (27.8%)	17/54 (31.5%)	21/54 (38.9%)**

Table 5 (Continued)

Kidneysb):					
leukaemia/malig-	♂	no data	no data	no data	no data
nant lymphomas	♀	5/54 (9.2%)	11/54 (20.4%)	8/54 (14.8%)	10/54 (18.5%)
Liverb):					
extramedullary	♂	3/53 (5.7%)	7/53 (13.2%)	13/52 (25.0%)*	7/54 (13.0%)
haematopoiesis	♀	no data	no data	no data	no data
adenomas and	♂	no data	no data	no data	no data
carcinomas	♀	5/54 (9.2%)	11/54 (20.4%)	8/54 (14.8%)	10/54 (18.5%)

** $p < 0.01$ in Fisher's Exact Test
# $p = .006$ in the Cochran-Armitage trend test
a) The pathology report was not available. The results of this study were only available summarized in tabular form and are given here correspondingly.
b) Identical figures were given for the kidneys and liver; validation of the data was not possible, as the individual results were not available

served. There were no relevant findings in the bladder in the control animals. A comparison with the historical control data given in the report shows that bladder carcinomas in F344 rats are very rare: of 1794 male and 1754 female rats, six animals were found to have bladder carcinomas. No hyperplastic, preneoplastic or neoplastic changes in the bladder epithelium were found at 2 mg/kg body weight and day (US Army 1984 a).

Mouse

In groups of 75 male and 75 female B6C3F_1 mice given 2,4,6-trinitrotoluene with the diet in concentrations of 0, 1.5, 10 or 70 mg/kg body weight and day for 24 months, mortality was not increased compared with that in the controls. The body weight gains in the males were reduced after doses of 10 mg/kg body weight and day and above and in the females at 70 mg/kg body weight and day. The main effects were anaemia, hepatotoxicity, peripheral lymphocytosis and leukaemia or malignant lymphomas in the spleen. There was an increase in the incidence of leukaemia and malignant lymphomas in the spleen of female mice even after doses of 1.5 mg/kg body weight and day; the increase was dose-dependent and only statistically significant at 70 mg/kg body weight and day (US Army 1984 b). A positive trend was found also with the Cochran-Armitage trend test (see Table 6). The incidences of leukaemia and malignant lymphomas in the kidneys and adenomas and carcinomas in the liver were, in the female mice of the exposed groups, likewise higher than in the control group. However, there was no clear dose-dependent increase, and the incidences were not statistically significant. Although no historical control incidences are given in the report, a substance-related effect can be assumed on the basis of the effects of the substance on the lymphohaematopoietic system.

Manifesto (MAK value, classification)

In two-year feeding studies with rats and mice 2,4,6-trinitrotoluene was found to have carcinogenic effects. Bladder hyperplasia and bladder carcinomas were found in female rats after doses of 50 mg/kg body weight and day, and increased incidences of leukaemia and malignant lymphomas of the spleen in mice at 70 mg/kg body weight and day. Also, its structural analogy to other nitroaromatics, in particular to dinitrotoluenes, gives reason to suspect that 2,4,6-trinitrotoluene has carcinogenic potential.

2,4,6-Trinitrotolue is mutagenic in bacterial test systems and mammalian cell cultures; the addition of a metabolic activation system is not required. Also the two main metabolites of 2,4,6-trinitrotoluene in humans, 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene, cause dose-dependent mutagenic effects in bacteria in concentrations such as occur in the urine of workers exposed to 2,4,6-trinitrotoluene concentrations of < 0.01 to 2.53 mg/m^3. In vivo, 2,4,6-trinitrotoluene yielded negative results in micronucleus tests with mice after intraperitoneal administration. In male rats, it induced neither UDS in the liver nor chromosomal aberrations in the bone marrow after oral administration, although the latter investigation has only limited usefulness, as the doses tested did not reach the maximum tolerable dose.

After the inhalation of 2,4,6-trinitrotoluene and presumably also dermal exposure to the substance, a significantly increased incidence of chromosomal aberrations was found in the peripheral lymphocytes of workers, however. In addition, the urine of exposed workers had increased mutagenic effects in bacteria. Together with the observation that 2,4,6-trinitrotoluene produces methaemoglobin in humans, which presupposes the reduction of nitro groups and the formation of a reactive hydroxylamine, and the fact that reduced and mutagenic metabolites were found in the urine, genotoxic effects in humans must be assumed.

The results of studies of the genotoxicity of 2,4,6-trinitrotoluene are similar to those for other nitroaromatics. The negative results in the micronucleus test are explainable, as this test yielded negative results also for the carcinogenic dinitrotoluenes. The positive results in the chromosomal aberration test in humans was carried out using a highly sensitive method, which could explain the discrepancy between this and the negative results in in vivo genotoxicity tests in animals.

To summarize, carcinogenic effects were found in studies with the two species rat and mouse, so that genotoxic effects in humans must be assumed. Therefore 2,4,6-trinitrotoluene is classified in Carcinogen Category 2.

The effects on the testes in experimental animals occurred at clearly toxic dose levels, and Leydig cell hyperplasia has been suggested as a secondary mechanism for the death of germ cells.The data do not, therefore, unequivocally demonstrate that the test substance reaches the germ cells in active form. As a result of the genotoxicity of the substance in vitro and the clastogenicity in humans, 2,4,6-trinitrotoluene is classified in category 3B for Germ Cell Mutagens.

The good dermal penetration of 2,4,6-trinitrotoluene in humans has already been described in the documentation from 1988 (documentation “2,4,6-Trinitrotoluene

(and isomers in technical mixtures)" 1990). As 2,4,6-trinitrotoluene is a proven animal carcinogen with presumably genotoxic effects in humans, for which at present no MAK value can be given, an additional carcinogenic risk must be assumed from its demonstrated ability to penetrate the skin. The previous designation with an "H" has therefore been retained.

The data and findings for 2,4,6-trinitrotoluene are mostly from earlier literature and incomplete; its sensitizing potential cannot be unequivocally evaluated. In addition, patch tests were carried out only on rare occasions. Nevertheless, some case reports with positive patch test results indicate that 2,4,6-trinitrotoluene has sensitizing potential, so that 2,4,6-trinitrophenol has been given the designation "Sh". No findings are available for allergenic effects in the airways, so that, as before, no designation with "Sa" has been given.

References

ATSDR (Agency for Toxic Substances and Disease Registry) (1995) Toxicological profile for 2,4,6-trinitrotoluene (t-TNT). Atlanta, GA, U. S. Department of Health and Human Services, Public Health Service

Ahlborg G, Bergström B, Hogstedt C, Einistö P, Sorsa M (1985) Urinary screening for potentially genotoxic exposures in a chemical industry. *Br J Ind Med* 42: 691–699

Ahlborg G, Einistö P, Sorsa M (1988 a) Mutagenic activity and metabolites in the urine of workers exposed to trinitrotoluene (TNT). *Br J Ind Med* 45: 353–358

Ahlborg G, Ulander A, Bergström B, Oliv A (1988 b) Diazo-positive metabolites in urine from workers exposed to aromatic nitro-amino compounds. *Int Arch Occup Environ Health* 60: 51–54

Anderson NP (1944) Neutralization as a therapeutic principle in contact dermatitis. *Arch Dermatol Syphilol* 49: 176–182

Anonymous (1964) 2,4,6-Trinitrololuene (TNT). *Am Ind Hyg Assoc J* 25: 516–519

Ashby J, Burlinson B, Lefevre PA, Topham J (1985) Non-genotoxicity of 2,4,6-trinitrotoluene (TNT) to the mouse bone marrow and the rat liver: implications for its carcinogenicity. *Arch Toxicol* 58: 14–19

Bader M, Göen T, Müller J, Angerer J (1998) Analysis of nitroaromatic compounds in urine by gas chromatography-mass spectrometry for the biological monitoring of explosives. *J Chromatogr B Biomed Sci Appl* 710: 91–99

Bakhtiar R, Leung KH, Stearns RA, Hop CE (1997) Evidence for a novel heme adduct generated by the in vitro reaction of 2,4,6-trinitrotoluene with human hemoglobin using electrospray ionisation mass spectrometry. *J Inorg Biochem* 68: 273–278

Barlas N, Selmanoglu G, Koçkaya A, Songür S (2002) Effects of carbendazim on rat thyroid, parathyroid, pituitary and adrenal glands and their hormones. *Hum Exp Toxicol* 21: 217–221

Bolt HM, Degen GH, Dorn SB, Plöttner S, Harth V (2006) Genotoxicity and potential carcinogenicity of 2,4,6-trinitrotoluene: structural and toxicological considerations. *Rev Environ Health* 21: 217–228

Bonnevie P (1939) Aetiologie und Pathogenese der Ekzemkrankheiten (Aetiology and pathogenesis of eczematous diseases) (German), Johann Ambrosius Barth, Leipzig, 239

Brooks LR, Jacobson RW, Warren SH, Kohan MJ, Donnelly KC, George SE (1997) Mutagenicity of HPLC-fractionated urinary metabolites from 2,4,6-trinitrotoluene-treated Fischer 344 rats. *Environ Mol Mutagen* 30: 298–302

Coombs M, Schillack V (1998) Determination of trinitrotoluene and metabolites in urine by means of gas-chromatography with mass detection. *Int Arch Occup Environ Health* 71: 22–25

Cummings AM, Harris ST, Rehnberg GL (1990) Effects of methyl benzimidazole carbamate during early pregnancy in the rat. *Fundam Appl Toxicol* 15: 528–535

Cummings AM, Ebron-McCoy MT, Rogers JM, Barbee BD, Harris ST (1992) Developmental effects of methyl benzimidazole carbamate following exposure during early pregnancy. *Fundam Appl Toxicol* 18: 288–293

Delatour P, Besse S (1990) Benzimidazole carbamate d'éthyle: effet tératogène et présence dans le lait de vache après administration de thiophanate. (Benzimidazol ethyl carbamate: teratogenic effect and presence in cow's milk after administration of thiophanate) (French). *Ann Rech Vet* 21: 87–92

Donnelly KC, Claxton LD, Huebner HJ, Capizzi JL (1998) Mutagenic interactions of model chemical mixtures. *Chemosphere* 37: 1253–1261

Einistö P (1991) Role of bacterial nitroreductase and O-acetyltransferase in urine mutagenicity assay of rats exposed to 2,4,6-trinitrotoluene (TNT). *Mutat Res* 262: 167–169

EU (European Union) (2007) Review report on the active substance carbendazim, 5032/VI/98 final, 5 January 2007,
http://ec.europa.eu/food/plant/protection/evaluation/existactive/list_carbendazim.pdf

Ewers U, Zwirner-Baier I, Neumann HG, Zelder E, Seuren-Kronenberg K (2000) Hämoglobin-Addukt-Konzentrationen sprengstofftypischer nitroaromatischer Verbindungen im Blut von Bewohnern von Rüstungsaltstandorten (Haemoglobin adduct concentrations of nitroaromatic compounds typical of explosives in the blood of persons resident at former munitions sites) (German). Teil 2: Studie Stadtallendorf (Ehemaliges DAG- und WASAG-Gelände). *Umweltmed Forsch Prax* 5: 277–284

Fiserova-Bergerova V, Pierce JT, Droz PO (1990) Dermal absorption potential of industrial chemicals: criteria for skin notation. *Am J Ind Med* 17: 617–635

Gardiner JA, Kirkland JJ, Klopping HL, Sherman H (1974) Fate of benomyl in animals. *J Agric Food Chem* 22: 419–427

Garfinkel D, Sidi Y, Steier M (1988) Liver cirrhosis and hepatocellular carcinoma after prolonged exposure to t-TNT: Causal relationship or mere coincidence. *Med Interne* 26: 287–290

George SE, Jacobson RW, Warren SH, Brooks LR, Bae B, Kohan MJ, Donnelly KC (1996) Mutagenicity of HPLC fractionated urine metabolites from 2,4,6-trinitrotoluene-treated Fischer 344 rats. *Environ Mol Mutagen* 27: 24

Goh CL (1984) Allergic contact dermatitis from tetryl and trinitrotoluene. *Contact Dermatitis* 10: 108

Goh CL (1988) Erythema multiforme-like eruption from trinitrotoluene allergy. *Int J Dermatol* 27: 650–651

Goh CL, Rajan VS (1983) Contact sensitivity to trinitrotoluene. *Contact Dermatitis* 9: 433–434

Goldman JM, Rehnberg GL, Cooper RL, Gray LE Jr, Hein JF, McElroy WK (1989) Effects of the benomyl metabolite, carbendazim, on the hypothalamic-pituitary reproductive axis in the male rat. *Toxicology* 57: 173–182

Gray LE, Ostby J, Sigmon R, Ferrell J, Rehnberg G, Linder R, Cooper R, Goldman J, Laskey J (1988) The development of a protocol to assess reproductive effects of toxicants in the rat. *Reprod Toxicol* 2: 281–287

Gray LE, Ostby J, Linder R, Goldman J, Rehnberg G, Cooper R (1990) Carbendazim-induced alterations of reproductive development and function in the rat and hamster. *Fundam Appl Toxicol* 15: 281–297

Guo YL, Wang BJ, Lee CC, Wang JD (1996) Prevalence of dermatoses and skin sensitisation associated with use of pesticides in fruit farmers of southern Taiwan. *Occup Environ Med* 53: 427–431

Guy RH, Potts RO (1993) Penetration of industrial chemicals across the skin: a predictive model. *Am J Ind Med* 23: 711–719

Hagmann M, Weiß T, Schaller KH, Angerer J (2004) Belastung und Beanspruchung bei der Entsorgung von Explosivstoffaltlasten – Dosismonitoring und biochemisches Effektmonitoring (Exposure to explosives and the effects during the disposal of military waste – monitoring of the dose and biochemical effects) (German). *Arbeitsmed Sozialmed Umweltmed* 39: 612–620

Hassmanová V, Hulek P, Nozicka J (2002) Toxic impairment of the liver with trinitrotoluene. *Prac Lek* 54(4): 186–189

Havemann K, Kolb G, Becker N, Scheller S, Zugmaier G, Pralle H, Wahrendorf J (1992) Elevated risks for acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) in a German county contaminated with trinitrotoluene (TNT). *Ann Hematol* 65: A65

Honeycutt ME, Jarvis AS, McFarland VA (1996) Cytotoxicity and mutagenicity of 2,4,6-trinitrotoluene and its metabolites. *Ecotoxicol Environ Saf* 35: 282–287

IARC (International Agency for Research on Cancer) (1996) 2,4,6-Trinitrotoluene. IARC Monograph 65, Lyon, FR, 449–475

Janardhan A, Sattur PB, Sisodia P (1984) Teratogenicity of methyl benzimidazole carbamate in rats and rabbits. *Bull Environ Contam Toxicol* 33: 257–263

JMPR (Joint Meeting on Pesticide Residues) (1995 a) Pesticide residues in food – 1995, JMPR evaluations 1995, Part II, Carbendazim, http://www.inchem.org/documents/jmpr/jmpmono/v95pr04.htm

JMPR (1995 b) Pesticide residues in food – 1995, JMPR evaluations 1995, Part II, Benomyl, http://www.inchem.org/documents/jmpr/jmpmono/v95pr02.htm

JMPR (2005) Pesticide residues in food – 2005, JMPR evaluations 2005, Part II, Carbendazim, 87–106, http://www.inchem.org/documents/jmpr/jmpmono/v2005pr05.pdf

Jones CR, Sepai O, Liu YY, Yan H, Sabbioni G (2005 a) Urinary metabolites of workers exposed to nitrotoluenes. *Biomarkers* 10: 10–28

Jones CR, Liu Y-Y, Sepai O, Yan H, Sabbioni G (2005 b) Hemoglobin adducts in workers exposed to nitrotoluenes. *Carcinogenesis* 26: 133–143

Kadalmani B, Girija R, Faridha A, Akbarsha MA (2002) Male reproductive toxic effects of carbendazim: hitherto unreported targets in testis. *Indian J Exp Biol* 40: 40–44

Kaplan DL, Kaplan AM (1982) Mutagenicity of 2,4,6-trinitrotoluene-surfactant complexes. *Bull Environ Contam Toxicol* 28: 33–38

Karamova NS, Il'inskaia ON, Ivanchenko OB (1994) Mutagenic activity of 2,4,6-trinitrotoluene: the role of metabolizing enzymes. *Genetika* 30: 898–902

Kennel S, Foote LJ, Morris M, Vass AA, Griest WH (2000) Mutation analyses of a series of t-TNT-related compounds using the CHO-HPRT assay. *J Appl Toxicol* 20: 441–448

Kilian PH, Skrzypek S, Becker N, Havemann K (2001) Exposure to armament wastes and leukemia: a case-control study within a cluster of AML and CML in Germany. *Leuk Res* 25: 839–845

Kohan MJ, George SE, Warren SH, Jacobson RW, Brooks LR, King LC (1995) Analysis of DNA adducts and production of mutagenic urine following 2,4,6-trinitrotoluene treatment of Fischer 344 rats. *Environ Mol Mutagen* 25: 27

Kolb G, Becker N, Scheller S, Zugmaier G, Pralle H, Wahrendorf J, Havemann K (1993) Increased risk of acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) in a country of Hesse, Germany. *Soz Praeventivmed* 38: 190–195

Kruse A, Hertel M, Hindsholm M, Viskum S (2005) (TNT)-induced cataract in Danish arms factory workers. *Acta Ophthalmol Scand* 83: 26–30

Lachance B, Robidoux PY, Hawari J, Ampleman G, Thiboutout S, Sunahara GI (1999) Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro. *Mutat Res* 444: 25–39

Letzel S, Kraus T, Bader M, Angerer J, Lehnert G (1997 a) Untersuchungen zur Genotoxizität von Trinitrotoluol-haltigen Altlasten (Investigations of the genotoxicity of waste containing trinitrotoluene) (German). *Zentralbl Hyg Umweltmed* 199: 407

Letzel S, Kraus T, Bader M, Neubauer S, Gebhart E, Angerer J, Lehnert G (1997 b) Untersuchungen zur Belastung und Beanspruchung bei der Entsorgung von Trinitrotoluol-haltigen Altlasten (Investigations of the exposure to trinitrotoluene and effects after the disposal of contaminated waste) (German). in: Borsch-Galetke, E, Struwe F (Eds): Psychomentale Belastungen und Beanspruchungen im Wandel von Arbeitswelt und Umwelt – Kanzerogenese und Synkanzerogenese. Dokumentationsband über die 37. Jahrestagung der Deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin e. V. in Wiesbaden vom 12. bis 15. Mai 1997; Rindt-Druck, Fulda

Letzel S, Göen T, Bader M, Angerer J, Kraus T (2003) Exposure to nitroaromatic explosives and health effects during disposal of military waste. *Occup Environ Med* 60: 483–488

Leung KH, Yao M, Stearns R, Chiu SH (1995) Mechanism of bioactivation and covalent binding of 2,4,6-trinitrotoluene. *Chem Biol Interact* 97: 37–51

Levine BS, Furedi EM, Gordon DE, Lish PM, Barkley JJ (1984) Subchronic toxicity of trinitrotoluene in Fischer 344 rats. *Toxicology* 32: 253–265

Liu Y-Y, Lu AYH, Stearns RA, Chiu S-HL (1992) In vivo covalent binding of (14C)trinitrotoluene to proteins in the rat. *Chem Biol Interact* 82: 1–19

Liu YY, Yao M, Fang JL, Wang YW (1995) Monitoring human risk and exposure to trinitrotoluene (t-TNT) using haemoglobin adducts as biomarkers. *Toxicol Lett* 77: 281–287

Neumann HG, Albrecht O, van Dorp C, Zwirner-Baier I (1995 a) Macromolecular adducts caused by environmental chemicals. *Clin Chem* 41: 1835–1840

Neumann HG, van Dorp C, Zwirner-Baier I (1995 b) The implications for risk assessment of measuring the relative contribution to exposure from occupation, environment and lifestyle: hemoglobin adducts from amino- and nitro-arenes. *Toxicol Lett* 82/83: 771–778

Pearson JG, Glennon JP, Barkley JJ, Highfill JW (1979) An approach to the toxicological evaluation of a complex industrial wastewater. *ASTM Spec Tech Publ* 667 284–301

Querangal des Essarts J (1955) Les dermatoses professionnelles dans les arsenaux et établissements industriels des forces armées (I) (Occupational skin diseases in arsenals and industrial establishments of the armed forces) (French). *Bull Soc Fr Dermatol Syphiligr* S112–S117

Sabbioni G, Wei J, Liu YY (1996) Determination of hemoglobin adducts in workers exposed to 2,4,6-trinitrotoluene. *J Chromatogr* B 682: 243–248

Sabbioni G, Liu YY, Yan H, Sepai O (2005) Hemoglobin adducts, urinary metabolites and health effects in 2,4,6-trinitrotoluene exposed worker. *Carcinogenesis* 26: 1272–1279

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 YY (2006) Biomarkers of exposure, effect, and susceptibility in workers exposed to nitrotoluenes. *Cancer Epidemiol Biomarkers Prev* 15: 559–566

Schwartz L (1944) Dermatitis from explosives. *J Am Med Assoc* 125: 186–189

Silver ALL (1938) The treatment and prevention of industrial diseases in filling factories. *J R Army Med Corps* 71: 87–96

Spanggord RJ, Mortelmans KE, Griffin AF, Simmon VF (1982) Mutagenicity in Salmonella typhimurium and structure-activity relationships of wastewater components emanating from the manufacture of trinitrotoluene. *Environ Mutagen* 4: 163–179

Spanggord RJ, Stewart KR, Riccio ES (1995) Mutagenicity of tetranitroazoxytoluenes: a preliminary screening in Salmonella typhimurium strains TA100 and TA100NR. *Mutat Res* 335: 207–211

Styles JA, Cross MF (1983) Activity of 2,4,6-trinitrotoluene in an in vitro mammalian gene mutation assay. *Cancer Lett* 20: 103–108

Tan EL, Ho CH, Griest WH, Tyndall RL (1992) Mutagenicity of trinitrotouluene and its metabolites formed during composting. *J Toxicol Environ Health* 36: 163–175

US-Army (1980) US-Army Medical Research and Development Command, MRI Project Number 3900-B. Mammalian toxicity of munitions compounds. Summary of toxicity of Nitrotoluenes. Progress report no. 11. U. S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD 21701

US-Army (1984 a) US-Army Medical Research and Development Command, IITRI Project Number L6116-Study 9. Determination of the chronic mammalian toxicological effects of TNT; twenty-four month chronic toxicity/carcinogenicity study of trinitrotoluene (TNT) in the Fischer 344 rat. Volume 1. NTIS ADA168637, NTIS, Springfield, VA

US-Army (1984 b) US-Army Medical Research and Development Command, IITRI Project Number L6116-Study 11. Determination of the chronic mammalian toxicological effects of TNT; twenty-four month chronic toxicity/carcinogenicity study of trinitrotoluene (TNT) in the B6C3F1 hybrid mouse. Volume 1. NTIS ADA168754, NTIS, Springfield, VA

Väätänen AK, Ridanpaa M, Norppa H, Kociba P (1997) Spectrum of spontaneous and 2,4,6-trinitrotoluene (TNT)-induced mutations in Salmonella typhimurium strains with different nitroreductase and O-acetyltransferase activities. *Mutat Res* 379: 185–190

Verdorfer I, Neubauer S, Letzel S, Angerer J, Arutyunyan R, Martus P, Wucherer M, Gebhart E (2001) Chromosome painting for cytogenetic monitoring of occupationally exposed and non-exposed groups of human individuals. *Mutat Res* 491: 97–109

West RR, Stafford DA (1997) Occupational exposures and haematological abnormalities among ordnance factory workers: A case control study. *Leuk Res* 21: 675–680

Whong WZ, Edwards GS (1984) Genotoxic activity of nitroaromatic explosives and related compounds in Salmonella typhimurium. *Mutat Res* 136: 209–215

Won WD, DiSalvo LH, Ng J (1976) Toxicity and mutagenicity of 2,4,6-trinitrotoluene and its microbial metabolites. *Appl Environ Microbiol* 31: 576–580

Woollen BH, Hall MG, Craig R, Steel GT (1986) Trinitrotoluene: assessment of occupational absorption during manufacture of explosives. *Br J Ind Med* 43: 465–473

Yan C, Wang Y, Xia B, Li L, Zhang Y, Liu Y (2002) The retrospective survey of malignant tumor in weapon workers exposed to 2,4,6-trinitrotoluene. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 20: 184–188

Zwirner-Baier I, Kordowich F-J, Neumann H-G (1994) Hydrolyzable hemoglobin adducts of polyfunctional monocyclic N-substituted arenes as dosimeters of exposure and markers of metabolism. *Environ Health Perspect* 102: 43–45

completed 28.03.2007