3-Nitrotoluene

(CAS No: 99-08-1)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

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1 Introduction

The present document contains the assessment of the health hazard of 3-nitrotoluene by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by AAE Wibowo, Ph.D. (Coronel Institute of the Academic Medical Centre, University of Amsterdam, the Netherlands).

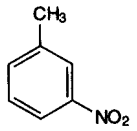
In February 1998, literature was searched in the databases Medline, Embase, and Chemical Abstracts, starting from 1966, 1988 and 1970, respectively, using the following key words: m-nitrotoluene, nitrotoluene, methylnitrobenzene, nitrotoluol, and 99-08-1. HSELINE, CISDOC, MHIDAS, and NIOSHTIC (covering the period from 1985/87 up to 1997) and Poltox (Toxline, Cambridge Scient Abstr, FSTA) (from 1990 up to and including 1994), databases available from CD-ROM, were consulted as well.

In February 2001, the President of the Health Council released a draft of the document for public review. No comments were received.

An additional search in Toxline and Medline in September 2004 did not result in information changing the committee's conclusions.

2 Identity

name	:	3-nitrotoluene
synonyms	:	*m*-nitrotoluene; 1-methyl-3-nitrobenzene; 3-methylnitrobenzene; 3-nitrotoluol
molecular formula	:	$CH_3C_6H_4NO_2$
structural formula	:	



CAS number	:	99-08-1

3 Physical and chemical properties

molecular weight	:	137.14
boiling point	:	232.6°C
melting point	:	16°C
flash point	:	106°C (closed cup)
vapour pressure	:	at 20°C: 16 Pa
solubility in water	:	not soluble (at 20°C: 0.05 g/100 mL)
log $P_{octanol/water}$	:	2.45 (experimental); 2.36 (estimated)
conversion factors	:	at 20°C, 101.3 kPa: 1 mg/m^3 = 0.18 ppm 1 ppm = 5.7 mg/m^3

Data from ACG02, Lun92, NLM04, http://www.syrres.com/esc/est_kowdemo.htm.

3-Nitrotoluene is a yellowish liquid at room temperature (ACG02).

Odour thresholds of 0.045 and 1.74 ppm (0.26 and 9.9 mg/m^3) have been reported (ACG02, Amo83).

4 Uses

Like the two other isomers, 3-nitrotoluene is used in the manufacture of other substances such as pigments and explosives (Lun92).

5 Biotransformation and kinetics

The committee did not find quantitative data on the uptake, biotransformation, and excretion of 3-nitrotoluene in humans.

The metabolism and kinetics of nitroaromatic compounds, including 3-nitrotoluene, have been investigated in male and female Fischer 344 rats at the Chemical Industry Institute of Toxicology (CIIT, USA) (Chi84, Chi85, deB84, Ric84; see also review in Ric87). A metabolism scheme for 3-nitrotoluene is presented in Figure 1 (see Annex I).

Seventy-two hours after oral administration of 200 mg/kg ^{14}C-ring-labelled 3-nitrotoluene, 68, 12.5, and 1% of the dose were recovered in the urine, faeces, and expired air, respectively. 3-Acetamidobenzoic acid (12%), 3-nitrobenzoic acid (21%), and 3-nitrohippuric acid (24%) were the main metabolites identified in the urine. About 1.5% of the dose was excreted as *S*-(3-nitrobenzyl)glutathione, presumably formed via the *O*-sulphate of the 3-nitrobenzyl alcohol. This indicates a major role of oxidation of the 3-nitrobenzyl

alcohol (Chi84). In bile-cannulated male rats, 11% of the dose was recovered in 12-hour bile, 27% (i.e., 2.8% of the dose) of which consisted of the glucuronide of 3-nitrobenzyl alcohol; in bile-cannulated females, 4.3% was found in 12-hour bile, 16% (i.e., 0.7% of the dose) of which was this glucuronide. During the same period, about 45% and 0.5% of the dose were excreted in the urine and faeces of sham-operated male and female controls, respectively. This indicates almost complete intestinal absorption of 3-nitrotoluene and a minor extent of biliary excretion of the nitrobenzylic glucuronide (Chi85). This conclusion is confirmed by metabolic studies using freshly isolated hepatocytes, where 3-nitrotoluene was also predominantly oxidised into the corresponding nittrobenzoic acid as compared with glucuronidation of the nitrobenzylic OH-group (deB84). Hepatic covalent macromolecular binding of 3-nitrotoluene was 50% inhibited by bile cannulation. DNA binding was negligible as compared with 2-nitrotoluene. Both parameters were not modified by inhibitors of sulphation, indicating that bioactivation via the pathways described above is of negligible importance for 3-nitrotoluene (Chi84, Ric84). On the other hand, for 2-nitrotoluene, these pathways proved to be important determinants for its binding to DNA. This is in agreement with the observation that 2-nitrotoluene, but not 3-nitrotoluene, induced unscheduled DNA excision repair in the *in vivo-in vitro* Fischer 344 rat hepatocyte assay, an effect that depends on the intestinal microflora (Doo83).

3-Nitrotoluene was found to bound to haemoglobin, forming haemoglobin adducts, in female rats 24 hours after oral (gavage) administration (doses not presented). The haemoglobin index (i.e., [mmmol compound/mol Hb]/[mmol compound/kg bw]) was calculated to be 1.0 (Sab95).

6 Effects and mechanism of action

Human data

The committee did not find human data on the effects of (occupational) exposure to 3-nitrotoluene.

Animal data

Irritation and sensitisation

Application of 0.5 mL of undiluted 3-nitrotoluene to the clipped intact and abraded skin of rabbits was not irritating (mean Draize score 0.37; maximum possible score: 8.0).

No irritation (all scores: 0) was seen following instillation of 0.1 mL of 3-nitrotoluene into the eyes of rabbits (Cis81).

It is not known whether the compound produced any sensitisation to the skin.

Acute toxicity

LC_{50} values of 693 and 425 mg/m^3 were listed for rats and mice, respectively (duration not indicated) (NIO04). Oral LD_{50} values for rats were 1072 (NIO04) and 2200 (males) and 2000 (females) mg/kg bw (Cis80a). Ciss et al. reported that agitation, increased respiration rate, prostration, brief convulsions, weakness, and atony occurred immediately after administration. Animals died between 18 and 48 hours post-administration (Cis80a). For mice, rabbits, and guinea pigs, oral LD_{50} values of 330, 1750, and 3600 mg/kg bw, respectively, were listed (NIO04).

3-Nitrotoluene, like most other aromatic nitro compounds, may cause elevation of blood methaemoglobin levels in cases of acute poisoning (Lun92). However, the increase produced by 3-nitrotoluene is relatively small compared to that produced by other methaemoglobin-forming agents. French et al. conducted *in vitro* tests, using Dorset sheep erythrocytes, to compare the methaemoglobin-forming capacity of various agents. In the absence of bioactivation, 3-nitrotoluene at concentrations of 2.5, 5.0, 7.5 and 10.0 mM, produced methaemoglobin levels of 2.5, 3.7, 4.5, and 5.0%, respectively. With NADP bioactivation, the corresponding methaemoglobin levels were statistically significantly increased when compared to control values (2.5, 4.7, 5.6, and 6.4%, respectively, vs. <3% in controls) (Fre95).

Repeated-dose toxicity

Rats (n=10/sex/group) were given oral doses of 0, 500, or 1000 mg/kg bw/day, 5 days/week, for 4 weeks. In the animals of the low-dose group, no compound-related mortality occurred. Irregular breathing, convulsions, weakness, and atony were observed in the first treatment week. Thereafter, the behaviour of the treated animals was generally comparable with that of controls. Apart from the first days, there was no difference in food intake and body weight gain between low-dose and control animals. At post-mortem macroscopic and microscopic examinations, there were increased spleen weights and sizes and decreased testicular weights and sizes, which were accompanied by histological changes such as splenic haemosiderosis and seminiferous tubular atrophy with reduction or absence of mature spermatids. Treatment of the high-dose animals was

terminated after the first week, because of severe toxic effects including increased respiratory rate, convulsions, prostration, body weight loss, and mortality in 6 males and 6 females. Post-mortem examinations showed similar effects as found in the low-dose animals, but more prominently present (Cis80a). In a subsequent experiment, oral (gavage) doses of 3-nitrotoluene (vehicle: olive oil) of 300 mg/kg bw/day administered to male and female rats, 5 days/week, for 6 months, did not affect survival, behaviour, or body weights. Symptoms observed were limited to alopecia in females. When compared to controls, there were no effects on red and whit blood cell counts, but total haemoglobin levels were decreased by ca. 15 and 7% in males and females, respectively. Clinical chemistry parameters changed included increases in serum alkaline phosphatase, serum cholinesterase, and creatine phosphokinase activities. Following post-mortem macroscopic and microscopic examinations, only lesions of the spleen were seen (increased weight and size) (Cis80b).

In a range-finding study, rats (F344/N; n=5/sex/group) were given 3-nitrotoluene in the diet at concentrations of 625-10,000 ppm, resulting in daily doses of 61-881 and 58-754 mg/kg bw for males and females, respectively, for 14 consecutive days. There were no clear compound-related clinical signs in any of the treated groups, other than decreased body weights at doses of 259, 431, and 881 mg/kg bw in males (by 9, 12, and 15%, respectively, compared to controls) and at doses of 420 and 754 mg/kg bw in females (by 7 and 14%, respectively). Feed consumption was decreased at these levels. Post-mortem findings were limited to effects on testes and uterus in the animals given 10,000 ppm (see ‘Reproduction toxicity’) (Dun92). In the subsequent 13-week study, rats (F344/N; n=10/sex/group) were given 3-nitrotoluene in the diet at concentrations of 0, 625, 1250, 2500, 5000, and 10,000 ppm resulting in daily doses of 46-661 and 48-638 mg/kg bw for males and females, respectively. Animals were observed twice daily for mortality/moribundity. Body weights and clinical observations were recorded weekly and at necrospy; feed consumption was measured weekly. Blood and serum samples were taken and analysed at week 1, 3, and 13. At study termination, complete necropsy was performed on all animals and reproductive system evaluation on all animals of the 3 higher dose groups. All animals survived treatment. Decreased final body weights and feed consumption were seen in males given 661 mg/kg bw/day (by 19%, compare to controls) and in females at 336 and 638 mg/kg bw/day (by 9 and 14%, respectively). No clinical signs were observed in any of the treated groups. Clinical chemistry/haematology evaluations at week 1 showed only changes in the high-dose animals, including mild increases in erythrocyte counts and haemoglobin concentrations in males and in haematocrit in males and females,

and further mild decreases in reticulocyte and platelet counts, and alkaline phosphatase activity and mild increases in urea nitrogen, creatinine, and albumine. At week 3, erythrocyte counts, haemoglobin concentrations, and haematocrit were decreased in most male dose groups and in the high-dose female group. Increased reticulocyte, nucleated erythrocyte, and platelet (males only) counts and methaemoglobin levels were seen in the male and female animals of the highest dose groups, as well as increased lymphocyte counts in high-dose males and females and increased leukocyte counts in high-dose females. Clinical chemistry changes included minimal decreased urea nitrogen levels in male rats and increased creatinine levels in male and female rats, as well as mild increased alanine aminotransferase activity in females of the 3 highest dose groups. At study termination, decreased erythrocyte counts, haemoglobin levels, and haematocrit were reported for females of the highest 1 or 2 dose groups and decreased erythrocyte counts in males of the highest dose group. In males and females given 5000 and 10,000 ppm, mean corpuscular volume, mean corpuscular haemoglobin, reticulocyte and platelet counts, and methaemoglobin concentrations were increased. Clinical chemistry changes were limited to mild to moderate increases bile acid concentrations in male and female animals dosed with 5000 and/or 10,000 ppm. Post-mortem evaluations showed moderately increased relative liver weights in 10,000-ppm males and females, increased relative kidney weights in males and females given 5000 and 10,000 ppm, and decreased absolute and relative testis weights in 10,000-ppm males. Gross examination only revealed smaller testes and epididymis in 4/10 rats of the 10,000-ppm group. Upon microscopic examination (see Table 1), there were increases in the incidences of haemosiderin pigment and congestion of the spleen - mostly of minimal severity - in male and female animals when compared to controls. Kidney lesions were seen in males only and included a hyaline-droplet nephropathy in almost all treated animals, characterised by the presence of eosinophilic protein droplets in the renal tubular epithelium and tubule lumen. The droplets were irregularly shaped and increased in size and number as compared to the protein ‘resorption droplets’ typically present in the kidney of the male controls. The nephropathy was of minimal severity but the number of protein droplets increased with dose. The amount of $\alpha_{2\mu}$-globulin was not measured. However, in concomitant studies with 2- and 4-nitrotoluene, similar hyaline-droplet nephropathy appeared to be associated with increased $\alpha_{2\mu}$-globulin levels. There was no evidence of renal necrosis or proliferative lesions. Examination of the reproductive system (see ‘Reproduction toxicity’) revealed reduced testis sizes and testicular degeneration in all male animals given 661

mg/kg bw/day and effects on the oestrus cycle in female animals given 420 and 754 mg/kg bw/day (Dun92, Dun94).

Table 1 Incidence and severity[a] of lesions in rats orally dosed with 3-nitrotoluene for 13 weeks (Dun92, Dun94).

	0 ppm	625 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm
				males		
kidney						
- hyaline-droplet nephropathy	0	9 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)
spleen						
- haemosiderin pigment	0	1 (1.0)	0	2 (1.0)	5 (1.0)	10 (1.4)
- congestion	1 (1.0)	0	0	1 (1.0)	0	9 (1.0)
testis						
- degeneration	0	0	0	0	0	9 (2.2)
				females		
spleen						
- haemosiderin pigment	1 (1.0)	9 (1.1)	10 (1.1)	10 (1.2)	8 (1.5)	10 (1.2)
- congestion	0	0	0	0	2 (1.0)	9 (1.0)

[a] everity scores (between brackets) are based on a scale of 1 to 4 (1=minimal, 2=mild, 3=moderate, 4=marked) and averages from the number of animals with lesions from groups of 10.

The committee considers the kidney lesions found in this study in male rats as a $\alpha_{2\mu}$-globulin-induced, typical male rat event and, therefore, not relevant for human risk assessment. The committee could not establish a no-adverse-effect level, since there was an increased incidence of spleen lesions (haemosiderosis of minimal severity) in female animals at 48 mg/kg bw/day, the lowest dose tested.

Mice ($B6C3F_1$; n=5/sex/group) were given dietary concentrations of 3-nitrotoluene of 388-5000 ppm for 14 days, resulting in daily doses of 66-779 and 92-901 mg/kg bw for males and females, respectively. Treatment did not affect survival, body weight (gain), or feed consumption, and no clinical signs were observed. At necropsy, there were increased relative liver weights in females at doses of 164 mg/kg bw (i.e., 675 ppm) and more and in males at doses of 409 and 779 mg/kg bw (i.e., 2500 and 5000 ppm) (Dun92).

In the subsequent 13-week study, mice ($B6C3F_1$;n=10/sex/group) were treated with dietary concentrations of 0, 625, 1250, 2500, 5000, and 10,000 ppm, resulting in doses of 114-1422 and 139-1550 mg/kg bw/day. All animals survived treatment. Body weight decreases, accompanied by decreased feed consumption, were observed in the animals given 5000 and 10,000 ppm (by 12 and ca. 24-28%, respectively, when compared to controls). There were no clinical signs of toxicity in any of the treated groups. At post-mortem examinations, dose-related increased relative liver weights were seen in male and

female mice and increased relative lung weights in male and females of the 10,000-ppm group. However, there were no macroscopic or microscopic changes in these or other organs, including the reproductive system (Dun92, Dun94).

Burns et al. conducted extensive studies of 3-nitrotoluene immunotoxicity in female $B6C3F_1$ mice. Mice were exposed to 3-nitrotoluene by gavage at doses of 200, 400 and 600 mg/kg bw/day for 14 consecutive days. The liver and kidney weights of high-dose animals were increased whereas thymus weights were decreased at the mid and high doses. The spleen, lungs, thymus, kidneys, and mesenteric lymph nodes were histologically normal at all doses. In the livers, hepatocytes adjacent to central veins showed mild cellular swelling at all doses. There was no evidence of necrosis and the swellings seemed to be reversible. The haematological parameters (erythrocytes, leukocytes, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, differential counts) and serum chemistry (alanine aminotransferase, urea nitrogen, glucose, albumin and total protein levels) were unaffected although there was a modest decrease in the percentage of polymorphonuclear leukocytes and eosinophils in the differential counts. Bone marrow cellularity and the colony-forming ability were unaffected. However, 3-nitrotoluene suppressed the IgM response to sheep red blood cells and the delayed hypersensitivity response to keyhole-limpet haemocyanin at the two higher doses. There was a slight (8%) decrease in the percentage of B lymphocytes in the spleen. The response to the T cell mitogens was suppressed by as much as 39%. Fluorescent covasphere-mediated adherence and phagocytosis of chicken erythrocytes, as well as natural killer cell activity showed dose-dependent increases. Several immune parameters were unaffected by exposure to 3-nitrotoluene, including the IgG response to sheep reed blood cells, responses to B-cell mitogen lipopolysaccharides and to allogeneic cells, and serum interferon levels. Resistance to *Streptococcus pneumoniae* and *Plasmodium yoelii* were also unaffected. Resistance to the tumour model PYB6 was increased. Exposure of mice to 3-nitrotoluene decreased resistance to *Listeria monocytogenes*, which might be related to an effect on T cells, evidenced by a decrease in T cell numbers and in the delayed hypersensitivity response (Bur94). The committee concludes that the liver and immune system may be target organs in mice, 200 mg/kg bw, the lowest dose tested, being an effect level.

Carcinogenicity

The committee did not find data from long-term studies on 3-nitrotoluene. However, no neoplasia or pre-neoplastic lesions were observed in rats and mice of both sexes in the 13-week oral NTP studies (Dun92, Dun94). Although this exposure period is too short to assess the carcinogenic potential, 2-nitrotoluene induced 2 - rare - mesotheliomas of the *tunica vaginalis* of the epididymis of male rats exposed to 353 mg/kg bw/day and - preneoplastic - hyperplasia (but no mesotheliomas) at the same location in males at 696 mg/kg bw/day (Dun92, Dun94).

Mutagenicity and genotoxicity

IARC (IARC96) summarised studies on the mutagenicity and genotoxicity of 3-nitrotoluene:

- 3-Nitrotoluene was negative in several mutation assays in *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535, TA1537, and TA1538, both in the presence and absence of metabolic activating systems.
  The compound was negative in a differential killing-rec assay in *B. subtilis* strains H17 (rec^+) and M45 (rec^-), an indicator for DNA damage.
- In mammalian cell systems, 3-nitrotoluene weakly induced sister chromatid exchanges (SCE) in Chinese hamster ovary cells (positive in the absence, negative in the presence of a metabolic activating system). It did not cause an increase in chromosomal aberrations in Chinese hamster ovary cells (tested with and without metabolic activation) or Chinese hamster lung fibroblasts (tested without metabolic activation only).
  3-Nitrotoluene did not induce unscheduled DNA synthesis (UDS) in rat hepatocytes and rat spermatogonia (both tests without metabolic activation only).
- *In vivo*, 3-nitrotoluene was negative in a micronucleus test in mice.
  It did not induce UDS in hepatocytes obtained from male rats after a single oral dose of 500 mg/kg bw. It did not covalently bind to male rat liver DNA at a single oral dose of 200 mg/kg bw.

Additional information included a positive result in an *in vitro* chromosomal aberration test in human peripheral lymphocytes *in vitro* (Hua95, Qin96) and a negative result in *in vitro* UDS test in primary rat hepatocytes following culture in serum-free defined media (Par95).

Reproduction toxicity

Ciss et al. studied the reproduction toxicity of 3-nitrotoluene (see 'Repeated-dose toxicity'). Doses of 3-nitrotoluene in olive oil of 300 mg/kg bw were orally (gavage) given to groups of male and female Wistar rats, 5 days/week, for 3 months. Thereafter, the rats (n=5/sex/group) were mated in 4 groups: (1) exposed males and exposed females, (2) exposed males and unexposed females, (3) unexposed males and unexposed females, and (4) unexposed males and exposed females, and treated for another 3 months. The treatment did not affect the number of pups born or pup survival or behaviour. No organ damage was seen in the pups at post-mortem examinations, apart from spleen lesions, which were less severe than in the parents, in pups from 3-nitrotoluene-treated females (Cis80b). Because of the small sizes of the mated groups, the committee considers this study of limited importance.

In the NTP studies (see 'Repeated-dose toxicity'), 3-nitrotoluene was found to induce reduced testis sizes and testicular degeneration characterised by mild to moderate degeneration with loss of germinal epithelium and the presence of abnormal spermatids in the lumen of the seminiferous tubules and ducts of the epididymis in rats orally (diet) dosed with 881 mg/kg bw/day for 14 days. No such effects were seen at 431 mg/kg bw/day. Daily administration of 661 mg/kg bw for 13 weeks caused smaller testes and epididymis in 4/10 rats. Histological examination showed mild to moderate degeneration of the testis in all 10 animals with reduction of germ cells and mature spermatids in the seminiferous tubules and cellular debris in the epidydimal ducts. This was accompanied by reduced epidydimal sperm count and concentration. These abnormalities were not seen at 342 mg/kg bw/day. In female rats, administration of oral (diet) doses of 754 mg/kg bw for 14 days resulted in smaller uteri with thinner muscular walls and less development of the endometrium compared to controls and groups treated with doses of 58-420 mg/kg bw/day. Thirteen-week treatment with doses of 336 and 638 mg/kg bw/day caused changes in the length (increase) and stages (decreased oestrus and increased dioestrus as percentage of cycle) of the oestrus cycle while the number of cycling animals diminished. The no-effect level was 172 mg/kg bw/day. There were no macroscopic or microscopic effects on uterus or ovaries. In male and female mice, no changes were found in the reproductive system evaluations at doses of 1422 and 1550 mg/kg bw/day, respectively (Dun92, Dun94).

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for 3-nitrotoluene in the Netherlands is 6 mg/m^3 (1 ppm), 8-hour TWA, with a skin notation.

Existing occupational exposure limits for 3-nitrotoluene in some European countries and the USA are summarised in Annex II.

8 Assessment of health hazard

The committee did not find data on the biotransformation and kinetics of 3-nitrotoluene following inhalation or skin exposure. Data from oral adminstration to male rats and from studies in isolated rat hepatocytes indicated almost complete intestinal absorption of 3-nitrotoluene and minor biliary excretion (of the nitrobenzylic glucuronide) and the major pathway for metabolism through oxidation of the methyl group into nitrobenzyl alcohol which is converted further. Following administration of 200 mg/kg bw, 68% of the dose was excreted in the urine within 72 hours with 3-nitrobenzoic acid (21%), 3-nitrohippuric acid (24%), and 3-acetamidobenzoic acid (12%) as major metabolites.

The committee did not find data on the toxic effects of 3-nitrotoluene in humans.

Experimental animal data showed that 3-nitrotoluene is not irritating to the skin and eyes. LC_{50} values were 693 and 425 mg/m^3 for rats and mice, respectively (duration not indicated); oral LD_{50} values 330 mg/kg bw for mice, 1072 and ca. 2100 mg/kg bw for rats, 1750 mg/kg bw for rabbits, and 3600 mg/kg bw for guinea pigs. The compound is a methaemoglobin-forming agent with a low potency.

The committee did not find data from repeated inhalation studies. In 13-week oral toxicity studies 3-nitrotoluene affected body weights (decreases), liver (increased relative weights; changes in serum bile acid levels and liver-specific enzyme activities), kidneys (increased relative weights; male rat-specific, $\alpha_{2\mu}$-globulin-induced hyaline-droplet nephropathy), spleen (haemosiderosis, congestion, haematological effects), and reproductive system (decreased testis sizes; testicular degeneration; oestrus cycle) in male and/or female rats. Apart from the kidney and spleen lesions, these effects were generally observed at doses of ca. 340 and 650 or ca. 650 mg/kg bw/day. The male rat-specific nephropathy occurred at all doses tested, i.e., as low as 42 mg/kg bw/day. The spleen lesions, which were mostly of minimal severity, were found in all female

dose groups, i.e., as low as 48 mg/kg bw/day. In mice, the only effects observed were decreased body weights at daily doses of 779 (males) and 901 (females) mg/kg bw and increased relative liver weights, which were not accompanied by macroscopic or microscopic changes, at all doses tested, i.e., as low as 66 (males) and 92 (females) mg/kg bw/day. In separate studies, 3-nitrotoluene affected the humoral and cell-mediated immunity in mice at 200 mg/kg bw, the lowest dose tested, and more.

The committee did not find data on long-term toxicity and carcinogenicity of 3-nitrotoluene.

The genotoxicity data showed that the compound is a weak inducer of sister chromatid exchanges, but did not cause mutations or DNA damage in bacteria or chromosome or DNA damage in mammalian cell systems. *In vivo*, it did not induce micronuclei in mice; it did not bind to male rat liver DNA.

The committee considers the spleen to be the critical organ and takes the lowest-observed-adverse-effect level (LOAEL) of 48 mg/kg bw/day from the 13-week oral rat study (Dun92, Dun94) as a starting point in deriving a health-based recommended occupational exposure limit (HBROEL). Since workers are exposed for 5 days/week, this LOAEL from a continuous feeding study (i.e., 7 days/week) is adjusted by multiplying with a factor of 7/5, resulting in a lowest adverse effect level (LAEL) of 63 mg/kg bw. For the extrapolation to a HBROEL, a factor of 4 for allometric scaling from rats to humans, based on caloric demand, and an overall factor of 36, covering the absence of a NOAEL, inter- and intraspecies variation, and differences between experimental conditions and the exposure pattern of the worker, are applied. This results in a NAEL for humans of 0.44 mg/kg bw/day. From this, a health-based occupational exposure limit of 2 mg/m^3 is recommended for 3-nitrotoluene, assuming a 70-kg worker inhales 10 m^3 of air during an 8-hour working day and a retention of 100%, and applying the preferred-value approach.

The committee recommends a health-based occupational exposure limit for 3-nitrotoluene of 2 mg/m^3 (0.4 ppm), as an 8-hour time weighted average (TWA).

Because of lack of quantitative data on skin absorption, the committee could not assess the need for a skin notation.

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Annex I

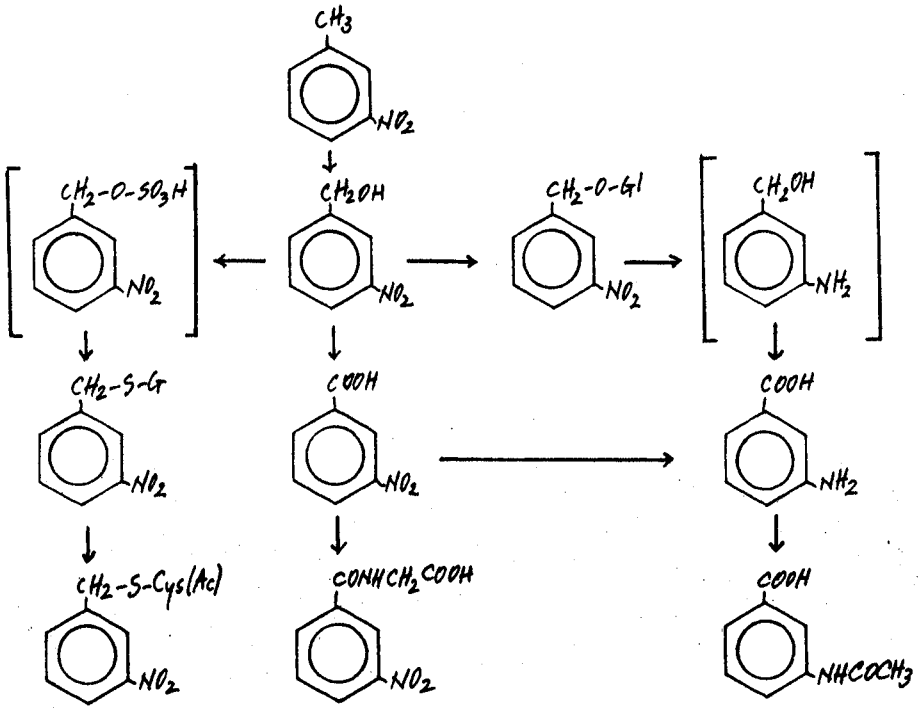

Figure 1 Metabolic pathway of 3-nitrotoluene (adapted from Cis84).
Structures between brackets are postulated intermediates or inconclusively identified metabolites.
S-G=glutathione; S-Cys(Ac)=*N*-acetylcysteine; Gl=glucuronide

Annex II

Occupational exposure limits for 3-nitrotoluene in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	1	6	8 h	administrative	S	SZW04
Germany						
- AGS	5	28	8 h		S	TRG04
	20	112	15 min			
- DFG MAK-Kommission	5	28	8 h		S	DFG04
	10	56	15 min[c]			
Great Britain						
- HSE	5	29	8 h	OES	S	HSE02
	10	57	15 min			
Sweden	1	6	8 h		S	Swe00
	2	11	15 min			
Denmark	2	12	8 h		S	Arb02
USA						
- ACGIH	2	-	8 h	TLV	S	ACG04b
- OSHA	5	30	8 h	PEL	S	ACG04a
- NIOSH	2	11	10 h	REL	S	ACG04a
European Union						
- SCOEL	-	-				EC04

[a] S = skin notation; which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Maximum number per shift: 4, with a minimum interval between peaks of 1 hour.