4-Nitrotoluene
CAS N°: 99-99-0
1. **Chemical Name:** 4-Nitrotoluene
2. **CAS Number:** 99-99-0
3. **Sponsor Country:** Germany
   Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit)
   Contact person: Prof. Dr. Ulrich Schlottmann
   Postfach 12 06 29
   D- 53048 Bonn-Bad Godesberg
4. **Shared Partnership with:**
5. **Roles/Responsibilities of the Partners:**
   - Name of industry sponsor /consortium: Bayer AG, Germany
     Contact person: Dr. Burkhardt Stock
     D-51368 Leverkusen
     Gebäude 9115
   - Process used: see next page
6. **Sponsorship History**
   - How was the chemical or category brought into the OECD HPV Chemicals Programme? by ICCA-Initiative
7. **Review Process Prior to the SIAM:**
   - last literature search (update):
     April 01, 2003 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms
     March 25, 2003 (Environment/Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms
8. **Quality check process:**
   - As basis for the SIDS-Dossier the IUCLID was used. All data have been checked and validated by BUA.
9. **Date of Submission:** August 12, 2003
10. **Date of last Update:**
11. Comments:

**OECD/ICCA - The BUA* Peer Review Process**

Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:

- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
- Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications (if original reports are missing: reliability (4), i.e. reliability not assignable)
- Review of validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
- In case of data gaps, review of testing plan or rationale for not testing

* BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)
SIDOS INITIAL ASSESSMENT PROFILE

<table>
<thead>
<tr>
<th>CAS No.</th>
<th>99-99-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Name</td>
<td>4-Nitrotoluene</td>
</tr>
<tr>
<td>Structural Formula</td>
<td><img src="attachment" alt="O2N(CH3)" /></td>
</tr>
</tbody>
</table>

SUMMARY CONCLUSIONS OF THE SIAR

**Human Health**

4-Nitrotoluene is rapidly absorbed via skin, gastrointestinal or respiratory tract, and distributed throughout the body. The primary metabolic pathway is side-chain or ring oxidation and conjugation with glucuronic acid and inorganic sulfates with subsequent renal excretion. In rats, the involvement of enterohepatic circulation was also observed.

4-Nitrotoluene is a methemoglobin forming chemical. Tachypnea, wheezing, somnolence and cyanosis were the predominant clinical signs following oral doses near to or exceeding the LD50 values. Methemoglobinemia was reported in rats after dermal exposure to high dose levels (LD50, oral, rat: 2144 - 4700 mg/kg bw; LD50, dermal, rats: > 750 mg/kg bw, LD50, dermal, rabbits: > 20,000 mg/kg bw; LC50, inhalation, rat: > 851 mg/m³/4h; no information on particle size available).

4-Nitrotoluene is not irritating to the skin and eyes of rabbits (OECD TG 404, 405). It was not sensitizing to the skin of guinea pigs in the Single Injection Adjuvant Test (SIAT) and in the Buehler test (OECD TG 406).

In 13 week and 2 year feeding studies with rats, 4-nitrotoluene caused hematopoiesis and hemosiderin pigment accumulation in the spleen of both sexes at all dose levels tested. Methemoglobinemia was noted at study end in the 13 week study at 10,000 ppm (male: approximately 723 mg/kg bw/day, female: approximately 680 mg/kg bw/day). At high and systemically toxic exposure levels, testicular degeneration was found in the males, and lengthened estrous cycles in the females. In male rats, ß2-globulin nephropathy was observed in all dosed groups. This effect is species specific and therefore of no relevance for humans (LOAEL: 625 ppm, corresponding to approximately 42 mg/kg bw/day, based on splenic toxicity). No relevant chemical related lesions were seen in mice in 13 week feeding studies. The NOAEL based on body weight reduction was 2500 ppm (approximately 439 mg/kg bw/day). In 2-year feeding studies, male and female mice showed an increase in alveolar bronchiolar epithelialization, and syncytial alterations in hepatocytes were found in males (LOAEL 1250 ppm = approximately 155 - 170 mg/kg bw/day). Immunological dysfunction has been reported in mice. The toxicological significance of the effects is not certain.

In vitro, 4-nitrotoluene showed no mutagenic effect in good quality Ames tests with *Salmonella typhimurium* and *Escherichia coli*, with and without metabolic activation. In cultured mammalian cells, 4-nitrotoluene has demonstrated the potential to cause mutagenicity in the presence of metabolic activation. The chemical did not induce unscheduled DNA synthesis in hepatocytes. In vivo, 4-nitrotoluene had no genotoxic activity. The substance did not induce micronuclei in rat and mice bone marrow cells in studies performed according to the current standard (OECD TG 474), and it did not induce unscheduled DNA synthesis in rat ex vivo hepatocytes.

Under the conditions of the two year feed studies, there was equivocal evidence of carcinogenic activity of 4-nitrotoluene in male rats based on the increased incidences of subcutaneous skin neoplasms. There was some evidence of carcinogenic activity in female rats based on increased incidences of clitoral gland neoplasms. There was equivocal evidence of carcinogenic activity in male mice based on increased incidences of alveolar/bronchiolar neoplasms. There was no evidence of carcinogenic activity in female mice exposed to 1250, 2500, or 5000 ppm (approximately 155, 315, or 660 mg/kg bw/day).

4-Nitrotoluene had no adverse effects on most reproductive endpoints (insemination index, fertility index, time to insemination, gestation length, number of corpora lutea and number of implantation sites, live birth index) in a rat oral Reproductive/Developmental Toxicity Screening Test (OECD TG 421), even under conditions where overt
systemic toxicity was observed. A reduction in the gestation index, increased prenatal loss and reduced litter size and pup weights were reported at parentally toxic doses. Testicular degeneration was found in subchronic studies at systemically toxic dose levels characterized by reduced body weights and toxicity to the spleen subsequent to the erythrocyte damaging effect of 4-nitrotoluene (NOAEL reproductive toxicity: 25 mg/kg bw/day; NOAEL developmental toxicity: 25 mg/kg bw/day, NOAEL (male) general toxicity: 25 mg/kg bw/day; LOAEL (female) general toxicity: 25 mg/kg bw/day).

Based on the available data, there was no evidence of a relevant hormonal activity of 4-nitrotoluene from various in vitro and in vivo screening tests.

Cases of poisoning from nitrotoluene are uncommon. They are reported only from early production units and relate to mixed exposures. The signs of intoxication included cyanosis, difficulties in breathing and tachycardia. In the recent open literature reports of human poisoning could not be identified.

**Environment**

4-Nitrotoluene has a melting point of 51.3 °C, a boiling point of 238 °C and a density of 1.29 g/ml at 20 °C. It has a vapour pressure of 13 Pa at 20°C. The log Kow is 2.37. The solubility in water is 345 mg/l at 20 °C. The flash point is ca. 103 °C, the auto flammability (ignition temperature) 450 °C. With regard to its chemical structure 4-nitrotoluene is not expected to hydrolyse under environmental conditions. During 8 days of a stability experiment at pH 8 and 25 °C about 6 % of 4-nitrotoluene (purity of 99.5 %) were lost in water.

According to Mackay level I fugacity model the main target compartments for 4-nitrotoluene are air (63.6 %) and water (35 %). A measured Henry’s law constant of 0.57 Pa·m³·mol⁻¹ indicates a moderate potential for volatilization of 4-nitrotoluene from aqueous solution. In the atmosphere 4-nitrotoluene is degraded due to indirect photolysis (t₁/₂air: 20.8 days) and direct photolysis. In surface waters the half life is estimated to be 6 hours due to photodegradation.

Since in the MITI-test, only 0.8 % of 4-nitrotoluene were mineralised within 14 days, 4-nitrotoluene is not readily biodegradable. Nevertheless studies on inherent biodegradation show 4-nitrotoluene to be biodegradable under aerobic conditions with adapted bacteria (degradation 100 % after 21 d including 10 d adaptation). Bioconcentration factors determined for fish were in the range of 3.7 – 27 and thus indicate no significant bioaccumulation potential of 4-nitrotoluene. Binding to soil organic matter has been calculated with Koc = 309. 4-Nitrotoluene can be regarded as a substance with medium geoaccumulation properties. The adsorption constants of 4-nitrotoluene were 5 - 45 l/kg on three clay minerals indicating a low adsorption by clays.

Concerning the acute toxicity of 4-nitrotoluene towards aquatic species reliable experimental results of tests with fish, daphnids, and algae are available.

The acute fish toxicity was 10.5 mg/l for Carassius auratus (48 h-LC₅₀), ca. 40 mg/l for Cyprinus carpio (96 h-LC₅₀), 50 mg/l for Pimephales promelas (96 h-LC₅₀), and 74 mg/l (48 h-LC₅₀) for Oryzias latipes. For Daphnia magna 48 h-EC₅₀-values of 4.2, 7.5, and 11.8 mg/l were found. In the algae growth inhibition tests with Chlorella pyrenoidosa the 96 h-EC₅₀ was 22.2 mg/l, and with Scenedesmus obliquus the 48 h-EC₅₀ was 25 mg/l.

The long-term toxicity to fish (Oryzias latipes, Poecilia reticulata) for the endpoints mortality and swimming behaviour, was evaluated by two 28 days tests. The NOEC values were 0.8 mg/l and 10 mg/l. A chronic toxicity test for the endpoint hatching rate of Oryzias latipes yielded a 40 d-NOEC of 32 mg/l. For the endpoints mortality, growth, and swimming behaviour of Oryzias latipes, a 40 d-NOEC of 1 mg/l were determined. Two chronic tests with Daphnia magna are available. The 21 d-NOECs were 0.7 mg/l and 1 mg/l, respectively, both for the endpoint reproduction rate. In a non-guideline study with the non-standard test species, the mollusc Lymnaea stagnalis, a 40 d-NOEC of 0.32 mg/l was determined for the endpoint reproduction. In the growth inhibition test with algae (Scenedesmus pannonicus) no effect on biomass was observed at 10 mg/l 4-nitrotoluene after 4 days.

Based on the chronic aquatic toxicity data on three trophic levels (fish, invertebrate, algae), a Predicted No Effect Concentration (PNEC) can be calculated with an assessment factor of 10. Using a 40 d-NOEC of 0.32 mg/l of Lymnaea stagnalis, a PNEC of 32 µg/l was determined.

**Exposure**

About 77,000 tonnes of 4-nitrotoluene were produced worldwide in 2000; Western Europe 30,000 t/a, China 26,000 t/a, US 9,000 t/a, Eastern Europe 5,000 t/a, India 4,000 t/a, and South Korea 3,200 t/a. The total manufacturing capacity of the lead company amounts to 28,000 t/a in 2000.

4-Nitrotoluene is a basic chemical for the synthesis of intermediates which are further processed to optical brighteners, coloring agents, pharmaceuticals, and agrochemicals, and others within the chemical industry. A direct use is not known.
From the production and processing site of the lead company virtually no 4-nitrotoluene was emitted into the environment in 2001. Taking into account the detection limit (2 µg/l), the 10 percentile of the river flow (1050 m³/s), and the dilution factor (700), for the receiving water a Predicted Environmental Concentration (PEC) of < 2.8 ng/l is calculated. In Germany in 1999, the 90-percentile of the 4-nitrotoluene concentrations in the River Rhine was < 0.5 µg/l and in the River Danube < 0.02 µg/l. For the River Elbe the maximum was 0.05 µg/l. During manufacturing and processing of 4-nitrotoluene workers may be exposed through the inhalational, dermal and oral routes. At the lead company the exposure of workers is well below the German Occupational Exposure Limit of 5 ppm (28 mg/m³). The levels of metabolic products of 4-nitrotoluene in workers are not higher than in the unexposed population. 4-Nitrotoluene is formed during tobacco smoking. At former munition manufacturing sites or at historic landfills 4-nitrotoluene might occur in groundwater and leachate. A significant indirect exposure of the general public via the environment is however not expected.

**RECOMMENDATION**

The chemical is currently of low priority for further work.

**RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED**

**Human Health:**
The chemical possesses properties indicating a hazard for human health. Based on data presented by the sponsor country, exposure is controlled in occupational settings, and is negligible for consumers. Any exposure scenario not presented by the Sponsor country will have to be investigated, however.

**Environment:**
The chemical possesses properties indicating a hazard for the environment. Based on data presented by the Sponsor country, exposure to the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country, e.g. exposure from munitions dumps or former munitions sites.
SIDS Initial Assessment Report

1  IDENTIFY

1.1 Identification of the Substance

CAS Number: 99-99-0
IUPAC Name: 4-Nitrotoluene
Molecular Formula: \( \text{C}_7\text{H}_7\text{NO}_2 \)

Structural Formula:

\[
\text{O}_2\text{N} - \text{CH}_3
\]

Synonyms:
- 1-Methyl-4-nitrobenzene
- 4-Methylnitrobenzene
- 4-Nitro-1-methylbenzene
- 4-Nitrotoluene
- 4-Nitrotoluol
- Benzene, 1-methyl-4-nitro
- p-Methylnitrobenzene
- p-Nitrotoluene
- p-Nitrotoluol
- PNT
- Toluene, p-Nitro

1.2 Purity/Impurities/Additives

Substance type: organic compound
Physical status: colourless to light yellow crystalline substance
Purity: > 99.5 % w/w (industrial grade pure substance)

Impurities:
- 3-nitrotoluene
- 2-nitrotoluene*
- dinitrotoluenes*
- water

*Industrial product manufactured in the sponsor country is virtually free of these byproducts
1.3 Physico-Chemical properties

4-Nitrotoluene has the following properties:

Table 1 Summary of physico-chemical properties

<table>
<thead>
<tr>
<th>IUCLID</th>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Molar mass</td>
<td>137.13 Dalton</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>Melting point</td>
<td>51.3 °C</td>
<td>Verschueren, 1996</td>
</tr>
<tr>
<td>2.2</td>
<td>Boiling point at 1013 hPa</td>
<td>238 °C</td>
<td>Verschueren, 1996</td>
</tr>
<tr>
<td>2.3</td>
<td>Density at 20 °C</td>
<td>1.29 g/cm³</td>
<td>Verschueren, 1996</td>
</tr>
<tr>
<td>2.4</td>
<td>Vapour pressure at 20 °C</td>
<td>13 Pa</td>
<td>Auergesellschaft, 1988</td>
</tr>
<tr>
<td>2.5</td>
<td>Octanol/water partition coefficient (log (K_{ow})) at 25 °C</td>
<td>2.37 (measured)</td>
<td>Fujita et al., 1964</td>
</tr>
<tr>
<td>2.6.1</td>
<td>Water solubility at 20 °C</td>
<td>345 mg/l (measured)</td>
<td>Bayer AG, 1987</td>
</tr>
<tr>
<td></td>
<td>Solubility in organic solvents</td>
<td>Soluble in most organic solvents</td>
<td>Booth, 2002</td>
</tr>
<tr>
<td>2.7</td>
<td>Flash point</td>
<td>103 °C (DIN 51758)</td>
<td>Bayer AG, 2001</td>
</tr>
<tr>
<td>2.8</td>
<td>Auto flammability (ignition temperature)</td>
<td>450 °C</td>
<td>Hommel, 1997</td>
</tr>
<tr>
<td>2.14</td>
<td>Conversion factors for the vapour phase</td>
<td>1 mg/m³ = 0.18 ppm</td>
<td>Verschueren, 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 ppm = 5.70 mg/m³</td>
<td></td>
</tr>
<tr>
<td>2.14</td>
<td>Vapour density in relation to air ((=1))</td>
<td>4.72</td>
<td>Verschueren, 1996</td>
</tr>
<tr>
<td>2.14</td>
<td>Decomposition</td>
<td>Decomposes on heating producing toxic fumes (nitrogen oxides)</td>
<td>CEC/IPCS, 2002</td>
</tr>
</tbody>
</table>

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

Production

In Germany 4-nitrotoluene is manufactured in an industrial scale only at the Bayer AG Leverkusen plant (Bayer AG, 2002a).

4-Nitrotoluene is produced continuously by nitrination of toluene with a mixture of sulfuric acid, nitric acid, and water. This gives a crude product of nitrotoluenes with the isomeric ratio of about 60 % 2-nitrotoluene, 35 % 4-nitrotoluene and 5 % 3-nitrotoluene. After separating the organic phase with the nitrotoluenes from the aqueous phase, the washed and dried mixture is separated into the isomers by fractionated distillation. The nitrotoluenes are produced and used in closed systems (Bayer AG, 2002a).

According to Srour (2002), the worldwide output can be estimated to about 77,000 t/a in 2000: Western Europe 30,000 t/a, China 26,000 t/a, US 9,000 t/a, Eastern Europe 5,000 t/a, India
4,000 t/a, and South Korea 3,200 t/a. At Bayer AG, the total production capacity of the nitrotoluene isomers amounts to 80,000 t/a, with 4-nitrotoluene amounting to 28,000 t/a (Bayer AG, 2002a).

**Processing and use**

About 50% of Bayer’s 4-nitrotoluene is processed by Bayer and the rest is sold to a limited number of customers (chemical companies in Europe and Asia; Bayer AG, 2002a).

4-Nitrotoluene is used as a basic chemical in the chemical industry for the manufacturing of intermediates. The Western European market of 4-nitrotoluene breaks down to intermediates such as 4-nitrotoluene-2-sulfonic acid (about 52%), p-toluidine (about 33%), 2-chloro-4-nitrotoluene (about 11%), and 4-nitrobenzoic acid (about 4%) (Srour, 2002). These intermediates are further used in the production of optical brighteners, coloring agents, pharmaceuticals, and agrochemicals (Bayer AG 2002a). 4-Nitrotoluene occurs as an intermediate in the production of di- and trinitrotoluenes but in general isolated 4-nitrotoluene is not used for the synthesis of these products (e.g. 2,4,6-trinitrotoluene: Boileau et al., 2002). Although 4-nitrotoluene was repeatedly mentioned as an intermediate for the production of fragrances, this application is neither mentioned in the latest Ullmann encyclopedia (Booth, 2002) nor is it mentioned in the product review of Srour (2002). No direct use of 4-nitrotoluene is known (Bayer AG, 2002a).

In Sweden 4-nitrotoluene is listed as a raw material (Swedish Product Register, 2002). There are no data on 4-nitrotoluene in the Danish product Register (2002) and in the Norwegian Product Register (2003). 4-Nitrotoluene is not mentioned in the Swiss product register (2001).

### 2.2 Environmental Exposure and Fate

Releases of 4-nitrotoluene into the environment may occur during its manufacturing and processing.

Information on exposure from manufacturing and processing of the chemical is available for the Bayer AG production plant at Leverkusen, Germany (Bayer AG, 2002a).

The Bayer plant in Leverkusen is a dedicated system in which the three nitrotoluene isomers are manufactured and separated. Bayer sells about half of its production as a chemical intermediate (see above; Bayer AG, 2002a).

Manufacturing, processing, filling, and transport of 4-nitrotoluene are executed in closed systems (e.g. transport via pipings, ISO-container [20 feet container] and rolling channel drums; sampling without dead volume, gas-shuttle pipe for filling processes). Cleaning of the reactors takes place only in the case of maintenance (see also 2.2 Human exposure; Bayer AG, 2002a). Concerning transport, the European chemical industry established the cooperative program ICE (International Chemical Environment) within the framework of Responsible Care. In the event of a distribution incident, the chemical industry will provide information, practical help, and - if necessary and possible - appropriate equipment to the competent emergency authorities in order to minimize any adverse effects (CEFIC, 2003). The exhausts from manufacturing (with the exception of the distillation) and processing of 4-nitrotoluene are connected to air washing units and thermal exhaust purification plants. Exhausts of the distillation are led into air washing units followed by activated carbon filters. Thus, at the Bayer AG production and processing site virtually no 4-nitrotoluene was emitted into the atmosphere in the year 2001 (Bayer AG, 2002a).

Waste from the manufacturing and processing of 4-nitrotoluene is incinerated in an incinerator for hazardous wastes (Bayer AG, 2002a).

At the Bayer AG production plant, wastewater with significant organic load is separated from wastewater with minor load. The significantly loaded wastewater is extracted and the extract is recycled to recover 4-nitrotoluene and other valued substances. The extracted wastewater is stripped
and the remainder is lead to the Leverkusen industrial and municipal wastewater treatment plant, together with the wastewater with minor load (Bayer AG, 2002a).

The concentrated sewage sludge is incinerated in a hazardous waste incinerator especially dedicated to this sludge (Bayer AG, 2002a).

24 h/d, 365 d/a, the air and water emissions of the integrated production site at Leverkusen are monitored by an Environmental Surveillance Group which operates independently of any manufacturing unit. This group is equipped with mobile detectors for various potential emissions. It also operates stations with measuring and sampling devices for air and water (Bayer AG, 2002a).

In 2001 in the effluent of the Leverkusen wastewater treatment plant, 4-nitrotoluene was neither detectable by the daily monitoring with a detection limit of 20 µg/l nor by the weekly fine monitoring with a detection limit of 2 µg/l (Bayer AG, 2002a).

The effluent of the Bayer Leverkusen plant passes into the Rhine. For the receiving water a PEC of < 2.8 ng/l is calculated taking into account the 10 percentile of the river flow (1050 m³/s), the dilution factor (700), and the detection limit (2 µg/l; Bayer AG, 2002a).

No information on environmental releases from other production and processing sites is available.

With a minimum limit of detection of 10 ng/l, 4-nitrotoluene was found neither in the influents nor in the effluents of any of 27 Japanese wastewater treatment plants (Nasu et al., 2001).

4-Nitrotoluene has repeatedly been reported to occur in wastewater of munitions factories (Liu et al., 1983; Toze and Zappia, 1999), and in leachates and groundwater from decommissioned munition sites (Rügge et al., 1999; Toze et al., 1999; Weissmahr et al., 1999). At these sites 4-nitrotoluene might be a byproduct of munition manufacturing (e.g. unreacted intermediate) or a degradation intermediate of higher nitrated compounds.

**2.2.1 Sources of Environmental Exposure**

**2.2.2 Stability and Abiotic Degradation**

With regard to its chemical structure 4-nitrotoluene is not expected to hydrolyse under environmental conditions.

The results of a stability experiment in non-aerated open vessels carried out by Canton et al. (1985) show only a decay of the test compound (4-nitrotoluene, purity of 99.5 %) in the test medium of 6 % after 8 days at pH 8 and 25 °C. The experiment was performed as pre-test to ecotoxicity studies with the intention to examine possible disappearance of 4-nitrotoluene from test solutions.

The indirect photochemical degradation in air by hydroxyl radicals is calculated via AOPWIN v. 1.90 with a half-life of 20.8 days (500,000 OH radicals/cm³ as a 24 h average; Bayer AG, 2002b).

Since 4-nitrotoluene significantly absorbs UV-B radiation [Molar absorptivity epsilon is 14,900 M⁻¹ cm⁻¹ at 284 nm (Takahashi et al., 2001)], it is expected that 4-nitrotoluene will undergo direct photolysis due to absorbance of environmental UV light, however, the respective half-life is not known.

The photodegradation in the compartment water was investigated by Simmons and Zepp (1986), a half-life of 5.9 hours can be derived under the conditions of latitude 40°N in surface waters. It has to be kept in mind that this half-life cannot be transferred directly to environmental conditions because the photolytical active zone is only close to the surface of surface waters due to turbidity and absorption.
2.2.3 Biodegradation

Based on the available experimental data 4-nitrotoluene is not readily biodegradable but inherently biodegradable. In a modified MITI I test according to OECD guideline 301 C a non adapted mixed microbial inoculum mineralised 0.8 % of the initial test substance concentration within 14 days (MITI, 1992).

With adapted activated sludge from an industrial sewage treatment plant a test on inherent biodegradation was conducted. The procedure followed the OECD guideline 302 B. After 21 days (10 days adaptation) 100 % of the initial concentration were removed (Wellens, 1990).

A test on inherent biodegradability was conducted by Pitter (1976). The test design is comparable to the Zahn-Wellens-test. The test substance 4-nitrotoluene in a concentration of 200 mg/l COD was the sole source of carbon. Activated sludge from a sewage treatment plant adapted for 20 days to 4-nitrotoluene was used as inoculum in a concentration of 100 mg/l dry matter. Based on COD measurement a removal of 95 % within 5 days was obtained in an open system.

The distribution and elimination of 4-nitrotoluene in a sewage treatment plant (microorganisms adapted to 4-nitrotoluene) with primary sedimentation and a sludge loading rate of 0.15 kg BOD/kg dry matter/d was estimated according to the model Simple Treat 3.0 (Struijs, 1996). With a degradation rate constant of 0.1 h⁻¹ (derived from the test by Pitter according to the EU Technical Guidance Document), a Henry constant of 0.57 Pa m³ mol⁻¹ and a log Kow of 2.37 the following results were obtained (Bayer AG, 2003a):

<p>| | |</p>
<table>
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</thead>
<tbody>
<tr>
<td>% to air</td>
<td>0.8</td>
</tr>
<tr>
<td>% to water</td>
<td>56.2</td>
</tr>
<tr>
<td>% to sludge</td>
<td>4.3</td>
</tr>
<tr>
<td>% degraded</td>
<td>38.6</td>
</tr>
<tr>
<td>% removal (sum of losses to air, removal with sludge and degradation)</td>
<td>43.8</td>
</tr>
</tbody>
</table>

Thus under the conditions of an industrial sewage treatment plant the substance will be eliminated mainly by biodegradation. Elimination by adsorption to sewage (see below) and volatilization seem to be less important.

In the Leverkusen industrial and municipal wastewater treatment plant the one-time maximum influent concentration was 0.6 mg/l (24 h sample), whereas in the corresponding effluent the 4-nitrotoluene concentration was below the detection limit of 2 µg/l (Bayer AG, 2002a). The elimination of the Leverkusen industrial and municipal wastewater treatment plant exceeds 99 %. This removal cannot be transferred to other sewage treatment plants due to possible different wastewater composition and adaptation processes.

In several experiments with activated sludge 4-nitrotoluene was degraded under conditions which mimicked these of sewage treatment plants (Hallas and Alexander, 1983; Struijs and Stoltenkamp, 1986). Various examinations of former ammunition sites indicate that 4-nitrotoluene can be degraded by microorganisms in the environment. (Best et al., 2001; Spain et al., 1999; Wikström et al., 2000)

2.2.4 Environmental Distribution

According to the Mackay Fugacity Model Level I (input parameter: vapour pressure 13 Pa, water solubility 345 mg/l, log Kow 2.37), the main target compartments for 4-nitrotoluene are the air with
63.7%, and the hydrosphere with 35.0%, followed by the soil and sediment with each 0.65% (Bayer AG 2002b). The measured Henry constant is 0.57 Pa m³ mol⁻¹ (Altschuh et al., 1999) and indicates a moderate potential for volatilization from surface waters according to the scheme of Thomas (1990).

No test result on geoaccumulation is available. Binding to soil organic matter has been calculated with Koc = 309 (Bayer AG, 2002b). Thus it is supposed that 4-nitrotoluene would adsorb slightly to sewage sludge, suspended solids, and sediment in water. According to Litz (1990) 4-nitrotoluene can be regarded as a substance with medium geoaccumulation properties. Haderlein et al. (1996) report adsorption constants of 5 - 45 l/kg of 4-nitrotoluene on three monoionic K⁺ clay minerals indicating a low adsorption by clays.

Measured bioconcentration factors (BCF) determined for fish (Cyprinus carpio) according to OECD guideline 305 C, were in the range of 3.7 – 8.0. 4-Nitrotoluene concentrations of 0.01 and 0.1 mg/l were tested (MITI, 1992). Another experimental BCF value of 27 is available by Canton et al. (1985) (no information on test concentration). Thus no significant potential for bioaccumulation of 4-nitrotoluene in aquatic organisms is indicated.

2.2.5 Environmental Monitoring

Throughout Germany a comprehensive monitoring program on several chemicals in surface waters has been realised to check whether the limit values are not exceeded. For 1999 the following values were obtained:

- River Danube: < 0.02 µg/l (90-percentile)
- River Rhine: < 0.5 µg/l (90-percentile)
- River Elbe: 0.05 µg/l (maximum)

For 4-nitrotoluene the limit values in surface waters have been set at 70 µg/l to protect aquatic life and at 10 µg/l to protect drinking water. These values have not been exceeded in the years 1996 - 2000 (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, 2001; update by Umweltbundesamt, 2002).

At former munition manufacturing sites or at historic landfills nitrocompounds (including 4-nitrotoluene from munition) might occur in groundwater and leachate at considerable levels (Toze et al., 1999; Weissmahr et al., 1999; Rügge et al., 1999). From the aforementioned data it is concluded that these contaminations are important only on a local scale. However, munition sites and munition manufacturing are not in the focus of this study.

There is no information on the release of 4-nitrotoluene into the environment from other products.

Minor amounts of 4-nitrotoluene are formed photochemically in the atmosphere (for 2-nitrotoluene see Calvert et al., 2002).

2.3 Human Exposure

2.3.1 Occupational Exposure

Workplaces
Workers may be exposed to 4-nitrotoluene during manufacture and processing of the chemical with the dermal and the respiratory routes being the main routes of potential exposure. At the Bayer manufacturing site, workplaces where 4-nitrotoluene is manufactured or processed include manufacturing processes: Nitrification of toluene to nitrotoluenes, phase separation and distillation. Processing: The reduction to p-toluidine, the sulfonation to 4-nitrotoluene-2-sulfonic acid, and the chlorination yielding 2-chloro-4-nitrotoluene.

For on-site processing, 4-nitrotoluene is transported in pipelines. About half of the 4-nitrotoluene is sold as a commercial product to professional (industrial) customers for further chemical processing. To these customers 4-nitrotoluene is transported in ISO tank containers or metal drums. Bulk volumes of 4-nitrotoluene are transported in a molten state at about 100 °C (Bayer AG, 2002a).

A leakage in the production and processing units would probably be recognized due to the strong odour of the nitrotoluenes and - in the nitration unit - due to the odour and highly visibility of nitrous gases (Bayer AG, 2002a).

Workplace surveys are regularly performed according to the German Technical Guidance TRGS 402 (AGS, 1997). This includes regular inspections of the working areas for any potential exposures to dangerous substances at different work situations and appropriate control measures.

To protect workers from exposure, several precautionary and protective measures are taken. These measures include technical equipment like suction devices at the filling and sampling stations as well as appropriate personal protection equipment which is prescribed in detail for different work situations (e.g. during sampling, maintenance, and repair work). For sampling, devices without dead volume are used, and the persons involved have to wear goggles and gloves. Depending on the work to be done during maintenance, gas filter masks or a respirator with independent air supply have to be used as well as full protective clothing.

Down stream (industrial) users of 4-nitrotoluene are informed by way of a material safety data sheet on the recommended safety measures (Bayer AG, 2002a).

**Biological monitoring**

The levels of 4-toluidine-adducts in blood and of 4-toluidine in urine are measured at least once a year in each worker of the 4-nitrotoluene manufacturing plant of the Bayer AG as part of the Bayer health surveillance program. The measured values for hemoglobin-adducts were not higher than in the unexposed population (Bayer AG, 2003b). It is noted here that the internal 4-toluidine-level is associated with smoking habits (Richter, 1996).

Average levels of 4-toluidine-adducts in blood and of 4-toluidine in urine were:

<table>
<thead>
<tr>
<th></th>
<th>Worker Nitration 2002</th>
<th>Worker Distillation 2002</th>
<th>General Population Non-Smoker</th>
<th>General Population Smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Toluidine-adducts in blood (ng/l)</td>
<td>11 (max. 40)</td>
<td>&lt; 20</td>
<td>26</td>
<td>70</td>
</tr>
<tr>
<td>4-Toluidine in urine (µg/l)</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Exposure of users of final products

In the sponsor country, 4-nitrotoluene is exclusively used as an intermediate for chemical synthesis (cf Chapter 2 / Processing and use). No direct use is known (Bayer AG, 2002a). Residual levels of 4-nitrotoluene in Bayer products, e.g. 2-chloro-4-toluidine and 4-toluidine are below the detection limit of 100 ppm (Bayer AG, 2002a).

Exposure of the general public

The only known use of 4-nitrotoluene is that as an industrial intermediate (Bayer AG, 2002a). Since residues of 4-nitrotoluene will be reduced in the production chain, e.g. during hydrogenations, distillations, and phase separations, final products are generally free of 4-nitrotoluene.

However, 4-nitrotoluene is formed during tobacco smoking (Hoffmann and Rathkamp, 1970).

In the USA, in 7 air and 6 dust samples taken in residential homes and commercial areas like offices and a plastics melting and gluing workplace, 4-nitrotoluene was not detected by gas chromatography/mass spectrometry at a detection level of 0.0127 µg/sample (Rudel et al., 2001).

At former munition manufacturing sites or at historic landfills nitrocompounds (including 4-nitrotoluene from munition) may occur in groundwater and leachate at considerable levels (Toze et al., 1999; Weissmahr et al., 1999; Rügge et al., 1999). However, from the aforementioned data it is concluded that these contaminations are important only on a local scale.

Based on the very low emissions of 4-nitrotoluene into air and water by the manufacturing and processing plants in the sponsor country (cf Chapter 2.1), and the low potential for bioaccumulation, a significant indirect exposure of the general public via the environment is not expected.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Nitrotoluenes are readily absorbed via the gastrointestinal tract, the lungs and, to a lesser extent, via the skin (BUA, 1989). 4-Nitrotoluene is rapidly distributed throughout the body (Sipes and Carter, undated). Excretion takes place principally via the urine (> 80 % within 72 hours), and only small amounts are eliminated in the feces (2 - 5 %). In rats, enterohepatic circulation was also observed. Excretion via exhaled air, however, does not seem to be a relevant route of elimination (Chism et al., 1984; Chism and Rickert, 1985; U.S. Department for Health and Human Services, 2002).

In the urine of rats, the major metabolites were identified as 4-nitrobenzoic acid (30 - 45 % of the administered dose), 4-acetamidobenzoic acid, and 4-nitrohippuric acid, whereas in mice the main metabolic pathway was ring hydroxylation and conjugation with glucuronide and sulfate. p-Nitrobenzyl mercapturic acid was found only in rats, indicating that a potentially reactive benzylating agent is formed during metabolism of 4-nitrotoluene in rats (U.S. Department for Health and Human Services, 2002).

No relevant differences were found in the urinary metabolite profiles after nine daily doses of 200 mg/kg bw to that seen after a single dose (U.S. Department for Health and Human Services, 2002).
The ratios of urinary 4-nitrobenzoic acid to creatinine and urinary 4-acetamidobenzoic acid to creatinine were followed as biomarkers of exposure in a 2 year carcinogenicity study, and were found to be linearly related to dietary doses of 4-nitrotoluene in rats (U.S. Department for Health and Human Services, 2002).

Involvement of enterohepatic circulation of 4-nitrotoluene metabolites (mainly nitrobenzylglucuronides) was observed in rats. The nitro groups of the metabolites secreted with the bile are reduced by intestinal bacteria and subsequently reabsorbed while generating reactive products in the liver (BUA, 1989).

Conclusion

4-Nitrotoluene is rapidly absorbed via skin, gastrointestinal or respiratory tract, and distributed throughout the body. The primary metabolic pathway is side-chain or ring oxidation and conjugation with glucuronic acid and inorganic sulfates with subsequent renal excretion. In rats, the involvement of enterohepatic circulation was also observed.

3.1.2 Acute Toxicity

There is no study according to the current guidelines, but there are studies, which are adequately documented and are considered of sufficient quality to allow an evaluation of this endpoint:

Oral

Groups of rats received different doses of 4-nitrotoluene ranging from 100 to 2,250 mg/kg bw in polyethylene glycol 400. The LD50 was determined as > 2,250 mg/kg bw (Bayer AG, 1976). In other rat studies, in which 4-nitrotoluene was administered in 1% aqueous methylcellulose, an LD50 value of 3,200 mg/kg bw was found in females, and a value of 4,700 mg/kg bw in males (Ciss, 1978; Ciss et al., 1980b). Clinical signs included poor condition, tachypnea, somnolence, atony, convulsions and wheezing for up to 24 hours. Survivors appeared normal one week after administration of the test substance. In a further study, an LD50 of 2,144 mg/kg bw was determined in male rats, with cyanogenic effects reported at 3,400 mg/kg bw and above (DuPont Chem, 1972).

Dermal

Neat 4-nitrotoluene (up to 20,000 mg/kg bw) was applied to the clipped back of 3 rabbits and kept in place by occlusive dressing for 24 hours. No rabbit died. No local or systemic effects were reported during treatment or after removal of the dressing and the following 14-day period (Kinkead, 1977). When applied as an emulsion in polyethylene glycol 400 at a dose level of 750 mg/kg bw to the back of 5 rats/sex/group, no deaths during the 24 hour treatment period and during the one week observation period were noted, but the rats showed poor general condition from 18 hours post application up to 4 days after application (Bayer AG, 1976). In a poorly documented study with rats, application of up to 16,000 mg/kg bw caused methemoglobinemia of up to 25% within 72 hours which was reported to be reversible; no deaths occurred. No further details were described (Sisa et al., 1959).

Inhalation

Five male rats and 10 male mice were exposed to 4-nitrotoluene dust for one hour and then observed for 7 days to determine LC50-values. At the highest exposure level of 4,167 mg/m³, no animal died and no signs of intoxication were noted during or post exposure (Bayer AG, 1976). In other studies 10 rats and 10 mice were exposed to an atmosphere essentially saturated with 4-nitrotoluene for four hours (rat: 152 ppm = 851 mg/m³; mouse: 228 ppm = 1,277 mg/m³). No death occurred during exposure or during the subsequent 14 day post exposure observation period. No
lesions attributable to exposure could be discovered during gross pathological evaluation, neither in rats nor in mice (Kinkead, 1977). For none of the studies was information on particle size available.

Conclusion

4-Nitrotoluene is a methemoglobin forming chemical. Tachypnea, wheezing, somnolence and cyanosis were the predominant clinical signs following oral doses near to or exceeding the LD50 value. Methemoglobinemia was reported in rats after dermal exposure to high dose levels (LD50, oral, rat: 2,144 – 4,700 mg/kg bw; LD50, dermal, rat: > 750 mg/kg bw; LD50, dermal, rabbit: > 20,000 mg/kg bw; LC50, inhalation, rat: > 851 mg/m³/4h; no information on particle size available).

3.1.3 Irritation

Skin Irritation

4-Nitrotoluene, moistened with polyethylene glycol 400, was not irritating to the skin of rabbits when applied under semi-occlusive condition for four hours as described in OECD TG 404. The mean Draize scores for edema and erythema were each “0” (Hoechst, 1986a).

Conclusion

4-Nitrotoluene is not irritating to the skin of rabbits (OECD TG 404).

Eye Irritation

In a test according to OECD TG 405, 100 mg of neat 4-nitrotoluene was applied into the conjunctival sac of the left eye of each of three rabbits. 24 hours later the eyes were rinsed. There were no effects on cornea and iris, and only a slight redness (Draize scores between 1 and 2) was noted at 1 and 24 hours after instillation, which was completely reversible within 48 hours (Hoechst, 1986b)

Conclusion

4-Nitrotoluene is not irritating to the eyes of rabbits (OECD TG 405).

3.1.4 Sensitisation

4-Nitrotoluene was not sensitizing to the skin in a single injection adjuvant test (SIAT) performed on 10 guinea pigs using intradermal injection of 0.001 µg/ml in complete Freund's adjuvants as induction. After 13 days an occlusive patch soaked with 0.0026 µg 4-nitrotoluene / ml as challenge was applied to the skin of the guinea pigs for 6 hours and the results were recorded 18 and 24 hours after the removal of the patch (Roberts et al., 1983).

A Buehler test performed with 20 guinea pigs according to OECD TG 406 did not reveal any skin sensitization (Chemfirst Inc., 1998). Induction was performed by dermal application of a 50 % solution in acetone and a 10 % solution was used for challenge. Concurrent control guinea pigs were treated with acetone alone. Animals treated with 1-chloro-2,4-dinitrobenzene (10/10 positive at 24 and 48 hrs), or Ûhexylcinnamaldehyde (10/10 positive at 24 hrs, 7/10 at 48 hrs) served as positive controls.

Conclusion

4-Nitrotoluene was not sensitizing to the skin of guinea pigs in the Single Injection Adjuvant Test (SIAT) and in the Buehler test (OECD TG 406).
3.1.5 Repeated Dose Toxicity

4-Nitrotoluene has been tested in a variety of studies. Significant findings of the most relevant studies are summarized in the following table:
Table 2: Summary of repeated dose toxicity studies

<table>
<thead>
<tr>
<th>Type</th>
<th>Species</th>
<th>Dose levels</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-week</td>
<td>Rat F344/N - dietary</td>
<td>0; 625; 1,250; 2,500; 5,000; 10,000 ppm (m: 0; 42; 82; 165; 342, 723 mg/kg bw/day)</td>
<td>10,000 ppm: bile acids Ŷ m: testis degeneration, liver weight (abs) Z 8.1% Met-Hb f: lengthened estrous cycle, liver weight (rel) Ŷ, 9.0% Met-Hb Ŷ5,000 ppm: m: body weight Ŗ, liver weight (rel) Ŷ Ŷ1,250 ppm: f: body weight Ŗ Ŷ625 ppm (LOAEL): splenic hematopoiesis Ŷ hemosiderin deposition, congestion Ŷ m: Ū2u nephropathy</td>
<td>U.S. Department of Health and Human Services, 1992. Dunnick et al., 1994</td>
</tr>
<tr>
<td>13-week</td>
<td>Mouse B6C3F1 - dietary</td>
<td>0; 625; 1,250; 2,500; 5,000; 10,000 ppm (m: 0; 131; 212; 439; 813, 1491 mg/kg bw/day)</td>
<td>10,000 ppm: f: body weight Ŗ Ŷ5,000 ppm: m: body weight Ŗ 2,500 ppm: NOAEL</td>
<td>U.S. Department of Health and Human Services, 1992 Dunnick et al., 1994</td>
</tr>
<tr>
<td>13-week</td>
<td>Rat F33/N - gavage</td>
<td>0; 90, 180, 360 mg/kg bw/day (in corn oil)</td>
<td>360 mg/kg bw/day: m: terminal body weight Ŗ absolute cauda epididymis, absolute epididymis, absolute testis weights Ŗ relative testis weight Ŗ sperm parameters: not affected f: estrous length not affected 180 mg/kg bw/day: NOAEL</td>
<td>Morrissey et al., 1983</td>
</tr>
<tr>
<td>13-week</td>
<td>Mouse / gavage</td>
<td>0; 40, 80, 160 mg/kg bw/day (in corn oil)</td>
<td>160 mg/kg bw/day: NOAEL (no adverse effects on male reproductive organ weights, sperm parameters, estrous cycle)</td>
<td>Morrissey et al., 1983</td>
</tr>
<tr>
<td>26-week</td>
<td>Rat Wistar - gavage</td>
<td>0; 400 mg/kg bw/day (susp. in 1% methylcellulose) mated in week 13</td>
<td>400 mg/kg bw/day: LOAEL m: reduced body weight gain, testicular atrophy, necroses of seminiferous tubules f, offspring: no apparent effects</td>
<td>Ciss et al., 1980a</td>
</tr>
<tr>
<td>Type</td>
<td>Species</td>
<td>Dose levels</td>
<td>Effects</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
</tbody>
</table>
| 2-year        | Rat F344/N - dietary  | 0; 1,250; 2,500; 5,000 ppm (m: 0; 55; 110; 240 mg/kg bw/day) (f: 0; 60; 125; 265 mg/kg bw/day) | 5,000 ppm: body weight $\bar{Z}$  
|               |                       |                                                                             | m: eosinophilic foci $\bar{Y}$  
|               |                       |                                                                             | m: testis degeneration, interstitial cell adenoma $\bar{Y}$  
|               |                       |                                                                             | f: oncocytic renal tubule hyperplasia  
|               |                       |                                                                             | $\bar{Y}$2,500 ppm m: liver basophilic and clear cell foci  
|               |                       |                                                                             | f: eosinophilic liver foci  
|               |                       |                                                                             | at 2,500 ppm only:  
|               |                       |                                                                             | f: clitoris gland adenomas/carcinomas $\bar{Y}$  
|               |                       |                                                                             | m: subcutaneous fibromas/fibrosarcomas $\bar{Y}$  
|               |                       |                                                                             | $\bar{Y}$1,250 ppm: LOAEL  
|               |                       |                                                                             | m: f: splenic hematopoiesis $\bar{Y}$, hemosiderin deposition, congestion; nasal and eye discharge  
|               |                       |                                                                             | m: $\bar{Y}$2u nephropathy  
|               |                       |                                                                             | f: body weight $\bar{Z}$  
| 2-year        | Mouse B6C3Fa – dietary| 0; 1,250; 2,500; 5,000 ppm (m: 0, 170, 345, 690 mg/kg bw/day) (f: 0;155; 315; 660 mg/kg bw/day) | 5,000 ppm: body weight $\bar{Z}$  
|               |                       |                                                                             | m: hepatocyte syncytial alterations  
|               |                       |                                                                             | f: body weight $\bar{Z}$  
|               |                       |                                                                             | $\bar{Y}$1,250 ppm: LOAEL  
|               |                       |                                                                             | m: body weight $\bar{Z}$  
|               |                       |                                                                             | m,f: alveolar bronchiolar epithelialization $\bar{Y}$ (no evidence of viral infection)  
| 14-day,       | Mouse B6C3F1 - gavage | 0; 200; 400; 600 mg/kg bw/day (females only)                                | $\bar{Y}$400 mg/kg bw/day: swelling of hepatocytes adjacent to the central veins, no necroses  
| immunotoxicity|                       |                                                                             | eosinophils $\bar{Y}$, macrophage activity $\bar{Y}$  
| study         |                       |                                                                             | $\bar{Y}$200 mg/kg bw/day: Antibody response to sRBC $\bar{Z}$  
|               |                       |                                                                             | CD4+ splenic T cells $\bar{Z}$  
|               |                       |                                                                             | delayed antigen response | Burns et al., 1994 |

$m = \text{male, } f = \text{female; rel = relative, abs = absolute; } \bar{Y} = \text{increase, } \bar{Z} = \text{decrease;}$

Met-Hb = methemoglobin; sRBC = sheep red blood cells

In the 13-week feeding studies with rats (U.S. Department of Health and Human Services, 2002; Dunnick et al., 1994), there were no effects on survival and decreased body weights were only observed at the higher dose levels ($\bar{O}5,000$ ppm, corresponding to 342 mg/kg bw/day). In male rats, $\bar{U}$2u-globulin nephropathy was seen in all dosed groups, i.e. at $\bar{O}625$ ppm, corresponding to
42 mg/kg bw/day. This type of nephropathy is species and gender specific and therefore not of relevance for humans.

In the spleen, most of exposed male and female rats had increases in the incidences of hematopoiesis, hemosiderin deposition and/or congestions at 625 ppm, corresponding to 42 mg/kg bw/day in males and 44 mg/kg bw/day in females. High, systemically toxic doses induced degeneration of the testis in males (at 10,000 ppm, corresponding to 723 mg/kg bw/day), and an increase in the length of the estrous cycle in females (at 10,000 ppm, corresponding to 680 mg/kg bw/day). Thus a NOAEL for systemic toxicity could not be derived. The LOAEL is 625 ppm (corresponding to 42 mg/kg bw/day in males and 44 mg/kg bw/day in females).

No relevant substance related lesions were seen in mice in 13 week feeding studies (U.S. Department of Health and Human Services, 2002; Dunnick et al. 1994). The NOAEL based on body weight reduction was 2500 ppm (approximately 439 mg/kg bw/day).

In the 2-year combined chronic toxicity and carcinogenicity studies with rats (U.S. Department of Health and Human Services 2002), hematological data and clinical chemistry data were not reported and could therefore not be considered for the NOAEL or LOAEL. Î gamma-Globulin nephropathy was found in all exposed male dose groups, and there were increased incidences of mild hematopoietic cell proliferation and pigmentation in the spleen of exposed male and female animals at 1,250 ppm, corresponding to 55 mg/kg bw/day in males, and to 60 mg/kg bw/day in females. In livers of males and females, increased incidences of various types of altered cell foci were found at 2,500 ppm (corresponding to 110 mg/kg bw/day in males, and to 125 mg/kg bw/day in females). Testicular degeneration was seen in high-dose male rats (at 5,000 ppm, corresponding to 240 mg/kg bw/day). In the corresponding study with mice, hematological data and clinical chemistry data were also not reported and could therefore not be considered for the NOAEL or LOAEL. Male and female mice showed an increase in the incidence of alveolar bronchiolar epithelialization (without evidence of a viral infection) at 1,250 ppm (corresponding to 170 mg/kg bw/day in males and 155 mg/kg bw/day in females), and males an increase in the incidence of hepatocyte syncytial alterations at 5,000 ppm (690 mg/kg bw/day). Thus a NOAEL for systemic toxicity could not be derived neither for rats nor for mice. The LOAEL for both species is 1,250 ppm (corresponding to 55 mg/kg bw/day in male and 60 mg/kg bw/day in female rats and 170 mg/kg bw/day in male and 155 mg/kg bw/day in female mice, respectively).

4-Nitrotoluene has an influence on the immune system at a high dose level (400 mg/kg bw/day). It has been demonstrated to suppress the antibody response to SRBC, to decrease the number of CD4+ splenic T cells, and to inhibit the DHR to keyhole limpet hemocyanin (KLH). In addition, host resistance to L. monocytogenes was impaired, suggesting the T cell as a primary target. The significance of these findings are difficult to interpret because the toxic effects of 4-nitrotoluene on mice in the above reported experiments were not reported in detail. For example, there was evidence for unspecific inflammatory reactions by the increase of eosinophils as well as the pronounced increase in macrophage activity starting at the mid dose. In addition, some findings point to an enhanced activity of the immune function (host resistance to S. pneumoniae and tumor cells) which do conflict with the judgement that the T cells are a primary target of the compound (Burns et al., 1994).

Conclusion

In 13 week and 2 year feeding studies with rats, 4-nitrotoluene caused hematopoiesis and hemosiderin pigment accumulation in the spleen of both sexes at all dose levels tested. Methemoglobinemia was noted at study end in the 13 week study at 10,000 ppm (male: approximately 723 mg/kg bw/day, female: approximately 680 mg/kg bw/day). At high and systemically toxic exposure levels, testicular degeneration was found in the males, and lengthened
estrous cycles in the females. In male rats, ÚHu-globulin nephropathy was observed in all dosed groups. This effect is species specific and therefore of no relevance for humans (LOAEL: 625 ppm, corresponding to approximately 42 mg/kg bw/day, based on splenic toxicity). No relevant chemical related lesions were seen in mice in 13 week feeding studies. The NOAEL based on body weight reduction was 2,500 ppm (approximately 439 mg/kg bw/day). In 2-year feeding studies, male and female mice showed an increase in alveolar bronchial epithelialization, and syncytial alterations in hepatocytes were found in males (LOAEL 1250 ppm = approximately 155 - 170 mg/kg bw/day). Immunological dysfunction has been reported in mice. The toxicological significance is not certain.

3.1.6 Mutagenicity

In vitro Studies

In tests performed according to current standards, 4-nitrotoluene was not mutagenic in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA102, TA104 and in Escherichia coli WP2uvra (dose-range, with and without metabolic activation: 0.0763 - 5000 µg/plate (JETOC 1996); 3.3 - 1000 µg/plate (U.S. Department of Health and Human Services, 2002)). The studies gave no indication of gene mutation with and without metabolic activation. The positive controls were functional.

In earlier studies also some positive responses were obtained, mainly in Salmonella typhimurium TA100. These results, however, were either not well documented, or were obtained with non-specified test material or in non-standard test systems (e.g. Shimizu and Yano, 1986; Nohmi et al., 1984; Melnikov et al., 1981). These positive findings were therefore considered as of less relevance as compared to the data from tests performed in accordance with current standards. Overall, based on the studies of best quality, 4-nitrotoluene is considered as non-mutagenic in the Ames test system.

4-Nitrotoluene was tested positive in a L5178Y mouse lymphoma assay in the presence of rat liver S9 mix. As the colony size is not reported, it is unclear whether this response can be attributed to gene mutations or cytogenetic effects (U.S. Department of Health and Human Services, 1992; 2002).

Effects on chromosomes were studied in Chinese hamster ovary (CHO) cells in tests corresponding to the current guideline (Galloway et al., 1983; 1987; US Department of Health and Human Services, 1992; 2002). In the presence, but not in the absence of metabolic activation, 4-nitrotoluene induced chromosome damage at cytotoxic dose levels.

4-Nitrotoluene induced increases in sister chromatid exchange rates in Chinese Hamster Ovary (CHO) cells with and without S9-mix at doses that induced cell cycle delay, which is an indication of cytotoxicity (Galloway et al., 1987; US Department of Health and Services, 1992; 2002).

Indicator test

4-nitrotoluene caused no unscheduled DNA synthesis (UDS) in cultured primary hepatocytes of rats (Doolittle et al., 1983).

Conclusion

In vitro, 4-nitrotoluene showed no mutagenic effect in good quality Ames tests with Salmonella typhimurium and Escherichia coli, with and without metabolic activation. In cultured mammalian cells, 4-nitrotoluene has demonstrated the potential to cause mutagenicity in the presence of metabolic activation. The chemical did not induced unscheduled DNA synthesis in hepatocytes.
In vivo Studies

There are bone marrow micronucleus tests available, that were performed with male rats and male mice according to OECD TG 474.

4-Nitrotoluene caused no increases in micronucleated polychromatic erythrocytes (PCEs) in the bone marrow of male rats given 0, 150, 300, or 600 mg/kg bw by intraperitoneal injection three times at 24 hour intervals.

In male mice, treated with 0, 150, 300, or 600 mg/kg bw by intraperitoneal injection three times at 24 hour intervals, results of a first trial were considered positive, based on the responses of the two lowest doses; the trend test was not significant due to a downturn at the highest dose level. A second trial failed to induce a significant increase in micronucleated PCEs over the same dose range. Therefore the authors concluded the overall results as negative.

Neither in the study with rats nor in the studies with mice were any signs of toxicity reported (U.S. Department of Health and Human Services, 2002).

Indicator test

4-Nitrotoluene did not induce unscheduled DNA synthesis (UDS) in hepatocytes of rats after single oral treatment by gavage with 0, 100, 200, 500 mg/kg bw dissolved in corn oil or 0, 50, 200, 1000 mg/kg bw in corn oil (US Department of Health and Human Services, 1992; Mirsalis, 1989).

Conclusion

In vivo, 4-Nitrotoluene had no genotoxic activity. The substance did not induce micronuclei in rat and mice bone marrow cells in studies performed according to the current standard (OECD TG 474) and it did not induce unscheduled DNA synthesis in rat ex vivo hepatocytes.

3.1.7 Carcinogenicity

The carcinogenic potential of 4-nitrotoluene (purity > 99 %) was examined in a combined chronic toxicity and carcinogenicity feeding study with F344/N rats and B6C3F1 mice, that was essentially performed in accordance with OECD guideline 453 (U.S. Department of Health and Human Services, 2002).

For the description of non-neoplastic lesions observed in this study, cf. section “Repeated dose toxicity” in this document.

Studies in rats

Groups of 50 male and female rats were fed diets containing 0, 1250, 2500, or 5000 ppm 4-nitrotoluene (equivalent to average daily doses of approximately 0, 55, 110, or 240 mg/kg bw/day for males and 0, 60, 125, or 265 mg/kg bw/day for females) for 105 to 106 weeks. No interim kill was performed.

Survival in all groups (including control groups) was similar. Mortality was between 10 and 20 % in all substance treated groups, and was 38 % in male controls. At the end of the study, there was no difference in mean body weights of males except for the 5000 ppm group: 366 g versus 402 g of the controls. In females, mean body weights of the exposed groups were less than in the control group: 294 g (control), and 272 g, 262 g, 210 g, for the low, mid and high dose group, respectively. As clinical signs of toxicity only nasal and eye discharge were observed in all exposed male and female rats. Hematology data or clinical chemistry data were not reported.
In female rats, 4-nitrotoluene caused an increased incidence of clitoral gland neoplasms (adenomas, carcinomas or adenomas and carcinomas combined). The overall rate was 8/50 in controls, and 12/50, 20/50, and 8/49 for the low, mid and high dose groups, respectively. The incidence in the mid-dose group was statistically significant and exceeded also the historical control range. The study authors note that in a previous study of 4-nitrobenzoic acid, a major metabolite of 4-nitrotoluene, an increased incidence of clitoral gland neoplasms also occurred. The absence of an increased incidence of clitoral gland neoplasms in 5,000 ppm females may have been related to lower body weights in this group (the final body weight in this group was only 71 % of the controls).

In male rats, 4-nitrotoluene may have induced subcutaneous tumors (fibromas, fibrosarcomas). Incidences for fibromas were reported as 1/50, 2/50, 7/50, and 1/50 in controls, low, mid, and high dose groups, and for fibrosarcomas as 1/50, 2/50, 9/50, 1/50, respectively. The incidences of fibroma and fibroma or fibrosarcoma (combined) were significantly greater than those in controls and exceeded the ranges observed in historical controls. As no supporting increased incidence was found at 5,000 ppm males, the increased incidences were considered as “uncertain finding” (although body weights of 5,000 ppm males were slightly less than those of the controls, subcutaneous neoplasms are not known to be sensitive to body weight reduction).

No increased incidences of renal neoplasms were observed, which might be explained by the comparatively weak Ú2u-globulin inducing effect of 4-nitrotoluene.

There were increased incidences of hematopoietic cell proliferation and pigmentation in the spleen of all exposed males and females, and decreased incidences of mononuclear cell leukemia.

Significantly increased incidences of various types of altered cell foci in the liver of males and females of mid- and high dose groups were possibly associated with exposure to 4-nitrotoluene, but could have been also related to the decreased incidences of mononuclear cell leukemia, since leukemic infiltration of the liver often obscures the detection of altered liver cell foci.

In male rats of the high dose group, incidences of germinal epithelial atrophy of the testes were significantly increased as compared to the controls (7/50 (control), 11/50, 8/50, 30/50 in the low, mid and high dose groups, respectively). The incidence of interstitial cell adenoma of the testis, however, was significantly decreased in the high dose males (49/50 (controls), 34/50 high dose males). From the presence of moderate to severe atrophy at 2 years, and the presence of degeneration in the 13-week study, the study authors surmise a direct toxic effect.

In summary, there was equivocal evidence of carcinogenic activity of 4-nitrotoluene in male rats and some evidence of carcinogenic activity in female rats.

Studies in mice

Groups of 50 male and female mice were fed diets containing 0, 1,250, 2,500 or 5,000 ppm 4-nitrotoluene (equivalent to average daily doses of approximately 0, 170, 345, or 690 mg/kg bw/day for males and 0, 155, 315, or 660 mg/kg bw/day for females) for 105 to 106 weeks. No interim kill was performed.

Survival in all groups (including control groups) was similar, with mortality levels being between 10 and 20 %. No clinical signs of toxicity were observed in male and female mice. In male mice of the highest dose group, the incidence of alveolar/bronchiolar adenomas or carcinomas was significantly increased: 8/50 (controls), and 14/50, 12/50, and 19/50 for the low, mid and high dose groups, respectively. While the increase was still within the historical control range of studies using the same diet, it exceeded the range in untreated controls from the larger historical NIH database. The study authors therefore concluded that the increased incidences of lung neoplasms in male mice
may have been related to the exposure to 4-nitrotoluene. Incidences of lung neoplasms were not increased in female mice.

In the liver, the incidences of hepatocyte syncytial alterations were increased in all exposed groups of males. This change was not observed in female mice, and it was not considered to be preneoplastic by the study authors.

In summary, there was equivocal evidence of carcinogenic activity in male mice (based on increased incidences of alveolar/bronchiolar neoplasms) and no evidence of carcinogenic activity in female mice.

In a tumor initiation-promotion test different dose levels of 4-nitrotoluene dissolved in acetone were dermally applied once (initiator: 50, 250, 400 mg/kg bw) and TPA (12-O-tetradecanoylphorbol-13-acetate) in acetone served as promoter (4 µg/kg bw once per week for 30 weeks). 4-Nitrotoluene was found to have no activity as a tumor initiator (Slaga et al., 1985).

**Conclusion**

Under the conditions of 2-year feed studies, there was equivocal evidence of carcinogenic activity of 4-nitrotoluene in male rats based on the increased incidences of subcutaneous skin neoplasms. There was some evidence of carcinogenic activity in female rats based on increased incidences of clitoral gland neoplasms. There was equivocal evidence of carcinogenic activity in male mice based on increased incidences of alveolar/bronchiolar neoplasms. There was no evidence of carcinogenic activity in female mice exposed to 1,250, 2,500, or 5,000 ppm (approximately 155, 315, or 660 mg/kg bw/day).

### 3.1.8 Toxicity for Reproduction

**Studies in Animals**

**Effects on Fertility**

12 male and 12 female rats per group received 0, 25, 100, or 400 mg 4-nitrotoluene/kg bw/day by gavage during a Reproduction/Developmental Toxicity Screening Test in compliance with OECD TG 421. Additional investigations included histopathological examinations of liver, spleen, kidney, pituitary gland, uterus, uterine cervix and vagina, mammary gland, epididymides and prostate (Bayer, 2002c).

All rats, including control rats, showed marked salivation that was attributed to the vehicle used in this study, i.e. polyethylene glycol 400. In the high dose group, signs of severe toxicity were observed in males and females including piloerection, respiratory sounds, increased water intake and urination, sunken flanks, reduced amount of feces and in females in addition hypoactivity, alteration of gaits, and increased incidence of soft and light colored feces. Distinctly decreased feed intake and severe body weight loss resulted in the death of 1 male; 2 further males and 5 females of this dose group had to be sacrificed in a moribund condition. Another female of the high dose group had to be sacrificed in moribund condition on day 22 p.c. (day of delivery). Signs of intoxication were ventral posture, hypoactivity, piloerection, ptosis and cold skin. Gross pathologic examination showed that all fetuses of its litters were dead.

Reduced feed intake and reduced body weight gain during lactation was seen in females at 25 and 100 mg/kg bw/day; these effects were significant at 400 mg/kg bw/day. There were no measurable effects on food consumption and body weight gain of dams of the low- and mid-dose groups before parturition. Clinical signs observed in the low-dose female group were ventral posture,
hypoactivity, high stepping gait, and piloerection. No clinical signs were observed in males of the low- and mid-dose groups.

Effects on organ weights were recorded in the mid-dose group (males: increased absolute and relative liver weight) and in the high-dose group (males: increased absolute and relative liver and spleen weights; females: increased absolute and relative spleen weights). Histopathological findings included increased amounts of iron pigment in the spleen in the mid and high dose groups, an increased hematopoiesis and congestion in the spleen, and iron pigment in the liver at 400 mg/kg bw/day. In a single male of the high-dose group, debris was observed in the epididymides together with exfoliation of spermatids. No histological changes in male accessory sexual glands, ovaries, female mammae with mamillae and pituitary gland were observed.

Insemination index, fertility index and time to insemination were not affected by treatment up to and including 400 mg/kg bw/day. The gestation index was not affected up to and including 100 mg/kg bw/day. In the high-dose group the gestational index was marginally reduced (85.7 versus 100 % in controls), because dead litter was found in 1 female together with an increased prenatal loss of the remaining litters in this dose group (3.17 versus 0.89 % in controls). Gestation length was comparable in all groups including the control group. Except the female which had to be sacrificed in moribund condition on the day of delivery (death of the total litter, for which a treatment related effect could not be excluded) no other observations which could indicate a substance related effect on the course of birth were made. Due to the female with death of the total litter mean number of the pups delivered was reduced in the high-dose group (9.17 versus 12.11 of controls).

Live birth index of pups was not affected by treatment, the viability index was reduced in the low-dose group (88.89 versus 98.99 %) because 1 female of this group lost the complete litter on day of birth, but due to the lack of a dose-response relationship, this effect was not considered as substance related. The sex ratio of pups was not affected by treatment. On the day of birth mean body weight of the pups was slightly, but significantly (p < 0.05) reduced in the mid-dose group (5.36 g versus 6.12 g: minus 14 % as compared to the controls), and clearly reduced in the high-dose group (4.80 g versus 6.12 g: minus 30 % as compared to the controls). Although the toxicological significance of the value at 100 mg/kg bw/day is not clear, the dose-response characteristics of the substance appear to indicate that adverse effects may be predicted to actually occur at this dose-level (LOAEL).

On day 4 post partum mean body weight of pups were only slightly reduced in the mid-dose group (8.26 g versus 9.43 g), but significantly in the high-dose group (6.68 g versus 9.43 g). The following No or Lowest Observed Adverse Effect Levels were derived from this study:

NOAEL general toxicity, males: 25 mg/kg bw/day;
LOAEL general toxicity, females: 25 mg/kg bw/day;
NOAEL reproduction toxicity: 25 mg/kg bw/day;
Mice and rats were evaluated for reproductive parameters (male reproductive organ weights, sperm number, morphology and motility, estrous length) at the end of 13 week feeding studies (US Department of Health and Human Services 1992). No adverse effects on reproductive parameters were found in mice up to and including the highest tested dose level of 10,000 ppm in the diet (corresponding to approximately 1491 - 1634 mg/kg bw/day), and a reproductive toxicity NOAEL or LOAEL for mice could therefore not be determined. In female rats of the 10,000 ppm group (680 mg/kg bw/day), the portion of animals in diestrus was increased. In males of the same dose group (10,000 ppm, 723 mg/kg bw/day), degeneration of testes, and a decrease in the number and motility of sperm was noted. At 5000 ppm (342 mg/kg bw/day) there was a reduction in absolute
testis weight, but without changes in sperm parameters. Clear signs of systemic toxicity were observed at 10,000 ppm and hematology showed typical effects secondary to methemoglobinemia (i.e. 8.1 % in males and 9.0 % in females of the high dose group), such as a decrease in erythrocyte count, and significant decreases in hematocrir and hemoglobin content. A systemic LOAEL of 42 mg/kg bw/day was derived in this study.

Decreases in terminal body weight, and reduced absolute cauda epididymis, epididymis and testes weights and relative epididymis weights, but no changes in sperm parameters were evident in rats treated for 13 weeks with 360 mg/kg bw/day by gavage. The same treatment had no effects on estrous cycle length in females. No adverse effects on male reproductive organ weights, sperm parameters or estrous cycle length were noted in mice dosed up to and including 160 mg/kg bw/day by gavage for 13 weeks (Morrissey et al. 1988).

In a study by Ciss et al. (1980a) the effects of 4-nitrotoluene on Wistar rats were investigated by exposing groups of males and females to 400 mg/kg bw/day by oral gavage daily for 3 months. The rats were paired with exposed animals of the other sex and the treatment was continued for another 3 months. The males showed testicular atrophy, necrosis of the seminiferous tubules, and an increase in spleen weight. No significant effect on the reproduction or on the offspring were observed.

Conclusion

4-Nitrotoluene had no adverse effects on most reproductive endpoints (insemination index, fertility index, time to insemination, gestation length, number of corpora lutea and number of implantation sites, live birth index) in a rat oral Reproductive/Developmental Toxicity Screening Test (OECD TG 421), even under conditions where overt systemic toxicity was observed. A reduction in the gestation index, increased prenatal loss and reduced litter size and pup weights were reported at parentally toxic doses. Testicular degeneration was found in subchronic studies at systemically toxic dose levels characterized by reduced body weights and toxicity to the spleen subsequent to the erythrocyte damaging effect of 4-nitrotoluene (NOAELreproductive toxicity: 25 mg/kg bw/day; NOAELmale)general toxicity: 25 mg/kg bw/day (male), LOAELfemale)general toxicity: 25 mg/kg bw/day (female)).

Developmental Toxicity

12 male and 12 female rats per group received 0, 25, 100 or 400 mg/kg bw/day in polyethylene glycol 400 by gavage during a reproduction/developmental toxicity Screening test according OECD TG 421 (Bayer, 2002c). Pups were observed until day 4 after birth for postnatal development.

Clear signs of maternal toxicity were observed in the 400 mg/kg bw/day group and included reduced feed intake, severe body weight loss, hypoactivity, alteration of gaits, piloerection, respiratory sounds, increased water intake and urination, sunken flanks, and reduced amount of feces with an increased incidence of soft and light colored feces. Six females had to be sacrificed in a moribund condition. Spleen weights were increased. At 100 and 25 mg/kg bw/day, feed intake and body weight gain during lactation were marginally reduced. Thus a NOAEL for maternal toxicity could not be derived; the LOAEL was 25 mg/kg bw/day.

Live birth index was not affected by treatment with 4-nitrotoluene. The viability index was lowered in the 25 mg/kg bw/day group (88.89 versus 98.99 %), because one female of this group lost the complete litter on the day of birth. Due to the lack of a dose-response relationship, this finding was not considered as substance-related. Sex ratio of pups was not affected by treatment. On the day of birth, mean body weights of pups were significantly reduced in the mid-dose and high-dose groups (5.36 g and 4.80 g versus 6.12 g in the controls). On day 4 post partum mean body weight of pups were only slightly reduced in the 100 mg group, but still significantly reduced in the 400 mg group.
(6.68 g versus 9.43 g). Reduced pup viability and reduced milk consumption by pups in the high dose group may have been substance-related. In the high dose group, hematomas at different localizations were observed. No other substance-related clinical findings were reported. Based on the reduced body weight which occurred in the presence of clear maternal toxicity, the NOAEL for developmental toxicity was set at 25 mg/kg bw/day.

Conclusion:

In a Reproductive/Developmental Toxicity Screening Test (OECD TG 421), 4-nitrotoluene caused significantly reduced pup body weights at dose levels at which maternal toxicity was observed (NOAELdevelopmental toxicity: 25 mg/kg bw/day; LOAELdevelopmental toxicity: 100 mg/kg bw/day; LOAELmaternal toxicity: 25 mg/kg bw/day).

3.1.9 Experience with human exposure

Cases of poisoning from nitrotoluene are uncommon. There is some evidence that the different isomers vary somewhat in toxicity, and that these chemicals are capable of forming methemoglobin (ACGIH, 2001). A 1898 survey of poisoning in an aniline factory mentions 10 cases involving 2- and 4-nitrotoluene mixtures ("red oil"). 4-Nitrotoluene was characterized as relatively non-poisonous, while 2-nitrotoluene was characterized as comparable to that of nitrobenzene (Bachfeld, 1898). A 1930 communication describes a case of poisoning by nitrotoluene and nitrochlorobenzene mixtures (ortho- and para-isomers) including cyanosis of the lips, gingiva, nose, paleness, difficulties in breathing and tachycardia. The observed effects, however, cannot definitely be attributed to nitrotoluene because of the co-exposure to nitrochlorobenzene (Schwanke, 1930).

Nowadays, nitrotoluenes are produced in closed systems and used as a basic chemical in the chemical industry for the manufacturing of intermediates (see Chapter 2). The annually occupational medical performed surveillance of workers handling 4-nitrotoluene has shown that there were no health effects (Met-Hb was always below 5 %; Bayer AG, 2003b)

In the recent open literature, reports of cases of occupational poisoning could not be identified.

Conclusion:

Cases of poisoning from nitrotoluene are uncommon. They are reported only from early production units and relate to mixed exposures. The signs of intoxication include cyanosis, difficulties in breathing and tachycardia. In the recent open literature reports of human poisoning could not be identified.

3.1.10 Other Relevant Information

The in vitro and in vivo screening data on estrogen-like activity are either inconclusive (Smith and Quinn, 1992) or do not demonstrate an estrogen-like activity of 4-nitrotoluene (Jobling et al., 1995; Nishihara et al., 2000).

Smith and Quinn (1992) found no dose-response in an uterotrophic screening assay using SD rats, and only the doses of 30 and 100 mg/kg bw/day caused increased uterine weights whereas doses of 300 and 1000 mg/kg bw/day were without effect. Due to a marked variability in the control uterine weight data, the interpretation of these data is difficult.

Kondo (2000) found a weak binding capacity to the human estrogen receptor at concentrations that were about 120,000 times higher than those of 17-β-estradiol and diethylstilbestrol. The same author did not find a significant increase in mouse uterine weights in the uterotrophic screening assay (Kondo, 2000).
None of the repeated dose toxicity studies in mammals revealed results that are indicative of a biologically relevant adverse effect caused by endocrine activity in mammalian species (US Department of Health and Services, 1992; 2002).

4-Nitrotoluene is used in the production of 4,4’-diaminostilbene-2,2’-disulfonic acid (DAS), a stilbene intermediate in the manufacture of fluorescent whitening agents. Occupational exposure to DAS has been associated with alterations in male reproductive hormone levels and effects on male sexual function (Whelan, 1996). These effects, however, cannot be attributed to 4-nitrotoluene, which is used in the process, but are more likely the effect of the stilbene compound.

Conclusion:

Based on the available data, there is no evidence of a relevant hormonal activity from various in vitro and in vivo screening tests.

3.2 Initial Assessment for Human Health

4-Nitrotoluene is rapidly absorbed via skin, gastrointestinal or respiratory tract, and distributed throughout the body. The primary metabolic pathway is side-chain or ring oxidation and conjugation with glucuronic acid and inorganic sulfates with subsequent renal excretion. In rats, the involvement of enterohepatic circulation was also observed.

4-Nitrotoluene is a methemoglobin forming chemical. Tachypnea, wheezing, somnolence and cyanosis were the predominant clinical signs following oral doses near to or exceeding the LD50 values. Methemoglobinemia was reported in rats after dermal exposure to high dose levels (LD50, oral, rat: 2,144 - 4,700 mg/kg bw; LD50, dermal, rat: > 750 mg/kg bw, LD50, dermal, rabbit: > 20,000 mg/kg bw; LC50, inhalation, rat: > 851 mg/m³/4 h; no information on particle size available).

4-Nitrotoluene is not irritating to the skin and eyes of rabbits (OECD TG 404, 405). It was not sensitizing to the skin of guinea pigs in the Single Injection Adjuvant Test (SIAT) and in the Buehler test (OECD TG 406).

In 13 week and 2 year feeding studies with rats, 4-nitrotoluene caused hematopoiesis and hemosiderin pigment accumulation in the spleen of both sexes at all dose levels tested. Methemoglobinemia was noted at study end in the 13 week study at 10,000 ppm (male: approximately 723 mg/kg bw/day; female: approximately 680 mg/kg bw/day). At high and systemically toxic exposure levels, testicular degeneration was found in the males, and lengthened estrous cycles in the females. In male rats, Êu-globulin nephropathy was observed in all dosed groups. This effect is species specific and therefore of no relevance for humans (LOAEL: 625 ppm, corresponding to approximately 42 mg/kg bw/day, based on splenic toxicity). No relevant chemical related lesions were seen in mice in 13 week feeding studies. The NOAEL based on body weight reduction was 2,500 ppm (approximately 439 mg/kg bw/day). In 2-year feeding studies, male and female mice showed an increase in alveolar bronchiolar epithelialization, and syncytial alterations in hepatocytes were found in males (LOAEL 1,250 ppm = approximately 155 - 170 mg/kg bw/day). Immunological dysfunction has been reported in mice. The toxicological significance of the effects is not certain.

In vitro, 4-nitrotoluene showed no mutagenic effect in good quality Ames tests with Salmonella typhimurium and Escherichia coli, with and without metabolic activation. In cultured mammalian cells, 4-nitrotoluene has demonstrated the potential to cause mutagenicity in the presence of metabolic activation. The chemical did not induce It unscheduled DNA synthesis in hepatocytes. In vivo, 4-Nitrotoluene had no genotoxic activity. The substance did not induce micronuclei in rat and
mice bone marrow cells in studies performed according to the current standard (OECD TG 474) and it did not induce unscheduled DNA synthesis in rat ex vivo hepatocytes.

Under the conditions of the two year feed studies, there was equivocal evidence of carcinogenic activity of 4-nitrotoluene in male rats based on the increased incidences of subcutaneous skin neoplasms. There was some evidence of carcinogenic activity in female rats based on increased incidences of clitoral gland neoplasms. There was equivocal evidence of carcinogenic activity in male mice based on increased incidences of alveolar/bronchiolar neoplasms. There was no evidence of carcinogenic activity in female mice exposed to 1,250, 2,500, or 5,000 ppm (approximately 155, 315, or 660 mg/kg bw/day).

4-Nitrotoluene had no adverse effects on most reproductive endpoints (insemination index, fertility index, time to insemination, gestation length, number of corpora lutea and number of implantation sites, live birth index) in a rat oral Reproductive/Developmental Toxicity Screening Test (OECD TG 421), even under conditions where overt systemic toxicity was observed. A reduction in the gestation index, increased prenatal loss and reduced litter size and pup weights were reported at parentally toxic doses. Testicular degeneration was found in subchronic studies at systemically toxic dose levels characterized by reduced body weights and toxicity to the spleen subsequent to the erythrocyte damaging effect of 4-nitrotoluene (NOAEL<sub>reproductive</sub> toxicity: 25 mg/kg bw/day; NOAEL<sub>developmental</sub> toxicity: 100 mg/kg bw/day; NOAEL<sub>(male)general</sub> toxicity: 25 mg/kg bw/day; LOAEL<sub>(female)general</sub> toxicity: 25 mg/kg bw/day).

Based on the available data, there was no evidence of a relevant hormonal activity of 4-nitrotoluene from various in vitro and in vivo screening tests.

Cases of poisoning from nitrotoluene are uncommon. They are reported only from early production units and relate to mixed exposures. The signs of intoxication included cyanosis, difficulties in breathing and tachycardia. In the recent open literature reports of human poisoning could not be identified.

## 4 HAZARDS TO THE ENVIRONMENT

### 4.1 Aquatic Effects

The lowest valid test concentrations of acute and chronic testing are presented in the following.

The lowest acute ecotoxicological effect concentration of a fish test was a 48 h-LC<sub>50</sub> of 10.5 mg/l for the goldfish Carassius auratus obtained in a study according to ISO/DIS 7346/1.2.3 from 1982. For this study no quality criteria on the performance of the test were reported (Liu et al., 1997), however, the study can be regarded as valid. The second lowest acute toxicity of about 40 mg/l resulted from two semistatic 96 h studies similar to OECD Guideline 203 with the carp, Cyprinus carpio (Lang, 1996; Zhao et al., 1997). In another test with Cyprinus carpio Yen and coworkers (2002) reported a 96 h-LC<sub>50</sub> of 68 mg/l. All effect values are based on nominal concentrations. In a test performed in a flow through system with Pimephales promelas a 96 h-LC<sub>50</sub> of 49.7 mg/l was measured. This test was done according to the US-EPA method described in EPA-660/375-009 in 1975. Analytical monitoring was conducted and the recovery was averaged to 101 % (Bailey and Spanggord, 1984). With the species Oryzias latipes a LC<sub>50</sub> of 74 mg/l after 48 h was obtained in a semistatic test in accordance to the Japanese Industrial standard method JIS K 0102-1986-71 (MITI 1982). In this test no information is available on analytical monitoring of the test concentration.

The lowest acute ecotoxicological effect concentration in a test with Daphnia magna was a 48 h-EC<sub>50</sub> of 4.2 mg/l obtained in a study according to ISO 6341. For this study no quality criteria on the performance of the test were reported (Liu et al. 1997). An acute toxicity (24 h-EC<sub>50</sub>) of 6.4 mg/l
(nominal concentration) in *Daphnia magna* tests was obtained in a test according to OECD Guideline 202 part I (Zhao and Wang, 1995; Zhao et al., 1995). In a test conducted in analogy to the OECD 202 proposal of 1979 a 48 h-EC50 of 7.5 mg/l was determined. The results of the stability experiment performed before testing Daphnia toxicity showed a decay of the test compound in the test medium of only 6% after 8 days. Therefore nominal concentrations can be used (see Chapter 2.1) (Canton et al., 1985). A test according to the US-EPA method described in EPA-660/375-009 in 1975 showed a 48 h-EC50 of 11.8 mg/l (Liu et al., 1983). With *Daphnia pulex* Yen and coworkers (2002) obtained an 4h-EC50 of 41 mg/l. Endpoint in all these studies was immobilisation.

For the algae *Chlorella pyrenoidosa* a 96 h-EC50 on a decrease of 50% in the maximum density (yield) is reported with a nominal concentration of 22.2 mg/l (Deneer et al., 1988; Deneer et al., 1989). The test was conducted according to the modified OECD Guideline 201. Studies according to OECD Guideline 201 (except for the shorter exposure duration) on *Scenedesmus obliquus* yielded a 48 h-EC50 for growth rate inhibition of ca. 25 mg/l (nominal value) (Liu and Lang, 1995; Liu and Lang, 2000; Zhao et al., 1997; Lu et al., 2001). The lowest valid acute test results of aquatic testing determined for fish, *Daphnia*, algae are:

<table>
<thead>
<tr>
<th>Fish</th>
<th>Carassius auratus</th>
<th>48 h-LC50</th>
<th>10.5 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Daphnia</em></td>
<td><em>Daphnia magna</em></td>
<td>48 h-EC50</td>
<td>4.2 mg/l</td>
</tr>
<tr>
<td>Algae</td>
<td><em>Chlorella pyrenoidosa</em></td>
<td>96 h-EC50</td>
<td>22.2 mg/l</td>
</tr>
</tbody>
</table>

Long-term studies are available for fish, *Daphnia*, some other invertebrates, and algae.

In a semi-chronic test with *Oryzias latipes* a 28 d-NOEC of 0.8 mg/l for the endpoint effects mortality and swimming behaviour was determined. The results of the stability experiment performed before testing fish toxicity showed a decay of the test compound in the test medium of only 6% after 8 days. Therefore nominal concentrations can be used (see Chapter 2.1) (Canton et al., 1985). In another long-term study with *Poecilia reticulata* (semistatic exposure) a 28 d-NOEC of 10 mg/l concerning the endpoints mortality and swimming behaviour was found (Slooff and Canton, 1983). The same authors found in a chronic toxicity test to *Oryzias latipes* (semistatic exposure) a 40 d-NOEC of 32 mg/l concerning the endpoint hatching rate and a 40 d-NOEC of 1 mg/l concerning the endpoints mortality, growth and swimming behaviour. The reported values in this study are all nominal (Slooff and Canton, 1983). No explanation is given by the authors for the higher sensitivity of the endpoint mortality compared to hatching.

In a reproduction test with *Daphnia magna* a 21 d-NOEC of 0.7 mg/l was obtained. In this study only nominal concentrations are given, as it was taken into account that 4-nitrotoluene was stable in a preliminary test (see above) (Canton et al., 1985). The second available test shows with *Daphnia magna* a 21 d-NOEC (reproduction) nominal of 1 mg/l (Slooff and Canton, 1983).

In a non-guideline study with the non-standard test species, the freshwater snail *Lymnaea stagnalis*, a 40 d-NOEC of 0.32 mg/l was determined for the endpoint reproduction (Slooff and Canton, 1983). The publication does not provide many details of the test, but states the basic test parameters and thus is considered valid.

Chronic toxicities have also been measured for 2 other aquatic invertebrates: for the aquatic insect larvae of *Culex pipiens* a 25 d-NOEC of 3.2 mg/l (development), for the Hydrozoan *Hydra oligactis* a 21 d-NOEC of 3.2 mg/l (specific growth rate; Slooff and Canton, 1983).
In a toxicity test with the algae *Scenedesmus pannonicus* a 96 h-NOEC of 10 mg/l was observed for the endpoint biomass (nominal concentration) (Slooff and Canton, 1983).

The lowest valid chronic NOECs of aquatic testing are:

<table>
<thead>
<tr>
<th>Species</th>
<th>Incubation period</th>
<th>Endpoint</th>
<th>NOEC (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish <em>Oryzias latipes</em></td>
<td>28 d (prolonged fish test)</td>
<td>Mortality and swimming behaviour</td>
<td>0.8*</td>
</tr>
<tr>
<td>Fish <em>Oryzias latipes</em></td>
<td>40 d</td>
<td>Growth and swimming behaviour</td>
<td>1</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>21 d</td>
<td>Reproduction</td>
<td>0.7*</td>
</tr>
<tr>
<td>Mollusc <em>Lymnaea stagnalis</em></td>
<td>40 d</td>
<td>Reproduction</td>
<td>0.32*</td>
</tr>
<tr>
<td>Insect <em>Culex pipiens</em></td>
<td>25 d</td>
<td>Development</td>
<td>3.2</td>
</tr>
<tr>
<td>Hydrozoan <em>Hydra oligactis</em></td>
<td>21 d</td>
<td>Specific growth rate</td>
<td>3.2</td>
</tr>
<tr>
<td>Algae <em>Scenedesmus pannonicus</em></td>
<td>96 h</td>
<td>Growth (biomass)</td>
<td>10*</td>
</tr>
</tbody>
</table>

* study used for assessment

For the derivation of the Predicted No Effect Concentration (PNEC), long-term tests with species from three trophic levels are available. According to the EU Technical Guidance Document, an assessment factor of 10 is to be applied. Using the long-term NOEC of 0.32 mg/l of a non-guideline study with the non-standard test species, *Lymnaea stagnalis*, a PNECaqua of 32 µg/l is calculated.

The following effect values for microorganisms were obtained with 4-nitrotoluene:

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Endpoint</th>
<th>Result (mg/l)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge</td>
<td>3 h-EC₅₀</td>
<td>100</td>
<td>Yoshioka et al., 1986</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>16 h-EC₃</td>
<td>26</td>
<td>Bringmann and Kühn, 1976; Bringmann and Kühn, 1977; Bringmann and Kühn, 1980a</td>
</tr>
<tr>
<td><em>Entosiphon sulcatum</em></td>
<td>72 h-NOEC</td>
<td>8.6</td>
<td>Bringmann and Kühn, 1980a ; Bringmann and Kühn, 1981</td>
</tr>
<tr>
<td><em>Uronema parduzci</em></td>
<td>20 h-NOEC</td>
<td>0.89</td>
<td>Bringmann and Kühn, 1980b</td>
</tr>
</tbody>
</table>

All values are related to nominal concentrations.

### 4.2 Terrestrial Effects

No guideline study with terrestrial organisms is available that was performed with 4-nitrotoluene. In humid sand, the 6d-EC₅₀ of 4-nitrotoluene for terrestrial plants was about 200 mg/l for *Phaseolus aureus* and about 300 mg/l for *Cucumis sativus* (Eckert, 1962).

The 14 d EC₅₀ of *Lactuca sativa* was measured for various chloro(nitro)benzenes and other compounds including e.g. toluene, but not 4-nitrotoluene. An equation for the calculation of the EC was derived (Hulzebos et al., 1993), which was used to calculate the EC₅₀ of 4-nitrotoluene to about 15 mg/l.

### 4.3 Other Environmental Effects

No data available.
4.4 Initial Assessment for the Environment

With regard to its chemical structure 4-nitrotoluene is not expected to hydrolyse under environmental conditions. According to a Mackay calculation level I the favourite target compartments of 4-nitrotoluene are air with 63.6% and water with 35%. In air, the substance is indirectly photodegradable with $t_{1/2} = 20.8$ days. In surface waters the half life is estimated to be 6 hours due to photodegradation. Since in the MITI-test, only 0.8% of 4-nitrotoluene were mineralised within 14 days, 4-nitrotoluene is not readily biodegradable. A test according to OECD guideline 302 B showed complete removal of 4-nitrotoluene (100%) after 21 days (10 days adaptation) thus indicating that 4-nitrotoluene is inherently biodegradable. In the adapted industrial and municipal wastewater treatment plant in Leverkusen 4-nitrotoluene elimination is more than 99%.

Measured bioconcentration factors in fish are in the range of 3.7 – 27, which indicates no significant bioaccumulation potential of 4-nitrotoluene. The measured Henry constant 0.57 Pa m$^3$ mol$^{-1}$ indicates a moderate potential for volatilization from surface waters. A calculated Koc of 309 suggests the substance to have a medium geoaccumulation potential in soil. The adsorption constants of 4-nitrotoluene were 5 - 45 l/kg on three clay minerals indicating a low adsorption by clays.

The lowest measured 6d-EC$_{50}$ was about 200 mg/l for the plant Phaseolus aureus and about 300 mg/l for Cucumis sativus. For Lactuca sativa a 14d EC$_{50}$ of 4-nitrotoluene was calculated to be about 15 mg/l.

Concerning the acute toxicity of 4-nitrotoluene towards aquatic species reliable experimental results of tests with fish, Daphnia, and algae are available. The acute fish toxicity was 10.5 mg/l for Carassius auratus (48 h-LC$_{50}$), ca. 40 mg/l for Cyprinus carpio (96 h-LC$_{50}$), 50 mg/l for Pimephales promelas (96 h-LC$_{50}$), and 74 mg/l (48 h-LC$_{50}$) for Oryzias latipes.

For Daphnia magna 48 h-EC$_{50}$-values of 4.2, 7.5, and 11.8 mg/l, and a 24 h-EC$_{50}$ of 6.4 mg/l were measured.

In the algae growth inhibition tests with Chlorella pyrenoidosa the 96 h-EC$_{50}$ was 22.2 mg/l, and with Scenedesmus obliquus the 48 h-E$_{50}$ was 25 mg/l.

For microorganisms tests with activated sludge, Pseudomonas putida and protozoans are available. The lowest effect value was the 20h-NOEC of 0.89 mg/l for Uronema parduzci.

The long-term toxicity to fish (Oryzias latipes, Poecilia reticulata) for the endpoints mortality and swimming behaviour, was evaluated by two 28 days tests. The NOEC values were 0.8 mg/l and 10 mg/l. A chronic toxicity test for the endpoint hatching rate of Oryzias latipes yielded a 40 d-NOEC of 32 mg/l. For the endpoints mortality, growth, and swimming behaviour of Oryzias latipes, a 40 d-NOEC of 1 mg/l were determined.

Two chronic tests with Daphnia magna are available. The 21 d-NOECs were 0.7 mg/l and 1 mg/l, respectively, both for the end point reproduction rate. In a non-guideline study with the non-standard test species, the mollusc Lymnaea stagnalis, a 40 d-NOEC of 0.32 mg/l was determined for the endpoint reproduction. With the aquatic insect larvae of Culex pipiens a 25 d-NOEC of 3.2 mg/l was obtained for the endpoint development. For the Hydrozoan Hydra oligactis a 21 d-NOEC of 3.2 mg/l was measured for the endpoint growth.

In the growth inhibition test with algae (Scenedesmus pannonicus) no effect on biomass was observed at 10 mg/l 4-nitrotoluene after 4 days.

Following the EU Technical Guidance Document, for the derivation of the PNECaqua an assessment factor of 10 is appropriate in the case of 3 chronic endpoints from different trophic
levels. Using the NOEC of 0.32 mg/l of a study with the non-standard test species, *Lymnaea stagnalis*, a PNECaqua of 32 µg/l is derived.

5 RECOMMENDATIONS

Environment:

The chemical possesses properties indicating a hazard for the environment. Based on data presented by the sponsor country, exposure to the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the sponsor country, e.g. exposure from munitions dumps or former munitions sites.

Human Health:

The chemical is currently of low priority for further work. The chemical possesses properties indicating a hazard for human health. Based on data presented by the sponsor country, exposure is controlled in occupational settings, and is negligible for consumers. Any exposure scenario not presented by the sponsor country will have to be investigated, however.
6 REFERENCES


Bayer AG (2002a). Internal data on production volume, processing and use, and environmental emissions: 4-Nitrotoluene.

Bayer AG (2002b). Calculation of:
- Mackay-Distribution Level I according to Mackay D, 1991.


Chemfirst Inc. (1998). Delayed contact hypersensitivity study in guinea pigs (Buehler Technique) of Paranitrotoluene with cover letter (at the request of First Mississippi Corporation). EPA-OTS0559506.


Danish Product Register (2002). Quantity and use of a specific substance: 4-nitrotoluene (excerpt 2002-02-26).


Sipes IG, Carter DE (no date). Pharmacokinetics of xenobiotics: p-nitrotoluene, NIEHS-Contract-No. NO1-ES-8-2130.


Swedish Product Register (2002). Communication to BUA.

Swiss Product Register (2001). Communication to BUA.


IUCID

Data Set

Existing Chemical
ID: 99-99-0
CAS No.: 99-99-0
EINECS Name: 4-nitrotoluene
EC No.: 202-808-0
TSCA Name: Benzene, 1-methyl-4-nitro-
Molecular Formula: C7H7NO2

Producer related part
Company: Bayer AG
Creation date: 07.03.1994

Substance related part
Company: Bayer AG
Creation date: 07.03.1994

Status:
Memo: X AKTUELL EG Update 1998 / ICCA

Printing date: 25.11.2004
Revision date: 04.06.1994
Date of last update: 03.11.2004
Number of pages: 176

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
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1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name : 1-Methyl-4-nitrobenzene
Smiles Code :
Molecular formula : C7H7NO2
Molecular weight : 137.13
Petrol class :

Flag : Critical study for SIDS endpoint
29.11.2002

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type :
Substance type : organic
Physical status : solid
Purity : >= 99.5 % w/w
Colour : colourless to light yellow
Odour :


Flag : Critical study for SIDS endpoint
23.01.2003

22.11.1999

1.1.2 SPECTRA

Type of spectra : UV
Result : Molar absorptivity epsilon (M exp-1 cm exp-1) at 252 nm is 2933
26.01.2003

Type of spectra : UV
Result : Molar absorptivity epsilon (M exp-1 cm exp-1) at 284 nm is 14,900

(1) (2) (3) (4)
1. GENERAL INFORMATION

1.2 SYNONYMS AND TRADENAMES

**1-Methyl-4-nitrobenzene**

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**4-Nitro-1-methylbenzene**

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**4-Nitrotoluene**

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**Benzene, 1-methyl-4-nitro-**

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**p-Methylnitrobenzene**

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**p-Nitrotoluene**

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**p-Nitrotoluol**

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**PNT**

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1.3 IMPURITIES

Flag: Critical study for SIDS endpoint
29.11.2002

Purity: typical for marketed substance
CAS-No: 99-08-1
EC-No: 202-728-6
EINECS-Name: 3-nitrotoluene
Molecular formula: C7H7NO2
Value: <= .5 % w/w

Remark: Industrial product manufactured in the Sponsor country is virtually free of other byproducts
Flag: Critical study for SIDS endpoint
04.02.2003 (7)

Purity: 88-72-2
CAS-No: 99-08-1
EC-No: 202-728-6
EINECS-Name: 2-nitrotoluene
Molecular formula: C7H7NO2
Value: <= .5 % w/w

04.02.2003 (7)

Purity: 7732-18-5
CAS-No: 99-08-1
EC-No: 202-728-6
EINECS-Name: Water
Molecular formula: H2O
Value: <= .1 % w/w

23.01.2003 (7)

Purity: Dinitrotoluenes
CAS-No: 99-08-1
EC-No: 202-728-6
EINECS-Name: Dinitrotoluenes
Molecular formula: C7H6N2O4
Value: <= .1 % w/w

24.01.2003 (7)

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity: ca. 50000 - 100000 tonnes produced in 2000

Remark: worldwide manufacturing volume
Flag: Critical study for SIDS endpoint
30.01.2003
### 1.6.1 LABELLING

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<tr>
<td>Symbols</td>
<td>T, N, ,</td>
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<tr>
<td>Nota</td>
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<td>R-Phrases</td>
<td>(23/24/25) Toxic by inhalation, in contact with skin and if swallowed</td>
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<tr>
<td></td>
<td>(33) Danger of cumulative effects</td>
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<td></td>
<td>(51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment</td>
</tr>
<tr>
<td>S-Phrases</td>
<td>(28) After contact with skin, wash immediately with plenty of water and soap, if possible with Polyethylene glycol 400, too</td>
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<tr>
<td></td>
<td>(37) Wear suitable gloves</td>
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### 1.6.2 CLASSIFICATION

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<td>(23/24/25) Toxic by inhalation, in contact with skin and if swallowed</td>
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### 1.6.3 PACKAGING
1.7 USE PATTERN

Type of use : type
Category : Use in closed system
Flag : Critical study for SIDS endpoint

Type of use : industrial
Category : Chemical industry: used in synthesis
Flag : Critical study for SIDS endpoint

Type of use : use
Category : Intermediates
Flag : Critical study for SIDS endpoint

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : MAK (DE)
Limit value : 30 mg/m³
Short term exposure limit value
Limit value : 10 other: ppm
Time schedule : 30 minute(s)
Frequency : 4 times
Remark : (TRGS 900 (DE))
Cat. II, 1
risk of cutaneous absorption
26.08.1999

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

Classified by : KBwS (DE)
Labelled by : KBwS (DE)
Class of danger : 2 (water polluting)
Remark : Kenn-Nummer 644
30.01.2003
1.8.4 MAJOR ACCIDENT HAZARDS

Legislation : Stoerfallverordnung (DE)
Substance listed :
No. in Seveso directive :

Remark : App. I, No. 2
23.01.2003

1.8.5 AIR POLLUTION

Classified by : TA-Luft (DE)
Labelled by : TA-Luft (DE)
Number : 3.1.7 (organic substances)
Class of danger : I

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

Memo : Substance identification

Remark : As long as no substance is reported under the item "Test substance", the substance corresponding to the CAS No. mentioned on every page of the IUCLID is meant. Purities are reported as far as available. If another isomer has been tested, this is in every case indicated in the "Test substance" item.

04.12.2003

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered : 1
Date of search : 20.09.2002

Flag : Critical study for SIDS endpoint
27.01.2003

Type of search : Internal and External
Chapters covered : 2
Date of search : 20.09.2002
1. GENERAL INFORMATION

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### 1.13 REVIEWS

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<td>Year</td>
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<td>GLP</td>
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**Remark**: The handbook data of Verschueren was selected as the key value because the majority of the melting point values is in the range of 51 - 52 °C. Two print publications report 51.3 °C and some other sources very similar values. The latest value, the 2002 data (54 °C) differs from most other values.

**Reliability**: (2) valid with restrictions

**Flag**: Critical study for SIDS endpoint

06.08.2003 (4)

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<tr>
<td>Sublimation</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>1992</td>
</tr>
<tr>
<td>GLP</td>
<td></td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
</tbody>
</table>

**Reliability**: (2) valid with restrictions

06.08.2003 (9)

<table>
<thead>
<tr>
<th>Value</th>
<th>51 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sublimation</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>1997</td>
</tr>
<tr>
<td>GLP</td>
<td></td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
</tbody>
</table>

**Reliability**: (2) valid with restrictions

06.08.2003 (10)

<table>
<thead>
<tr>
<th>Value</th>
<th>51.6 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sublimation</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>2000</td>
</tr>
<tr>
<td>GLP</td>
<td></td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
</tbody>
</table>

**Reliability**: (2) valid with restrictions

06.08.2003 (11)

<table>
<thead>
<tr>
<th>Value</th>
<th>51.9 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sublimation</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>1968</td>
</tr>
<tr>
<td>GLP</td>
<td></td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
</tbody>
</table>

**Reliability**: (2) valid with restrictions

06.08.2003 (10)

Reliability : (2) valid with restrictions  
Data from handbook or collection of data

06.08.2003

Value : 54 °C
Sublimation :
Method :
Year : 2002
GLP :
Test substance :

Reliability : (2) valid with restrictions  
Data from handbook or collection of data

06.08.2003

Value : 55 °C
Sublimation :
Method :
Year : 1998
GLP :
Test substance :

Reliability : (2) valid with restrictions  
Data from handbook or collection of data

06.08.2003

2.2 BOILING POINT

Value : = 238 °C at 1013 hPa
Reliability : (2) valid with restrictions  
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint

06.08.2003

Value : 238 °C at 1013 hPa
Reliability : (2) valid with restrictions  
Data from handbook or collection of data

06.08.2003

Value : 238.3 °C at 1013 hPa
Reliability : (2) valid with restrictions  
Data from handbook or collection of data

06.08.2003

Value : 237.7 - 239 °C at 1013 hPa
Reliability : (2) valid with restrictions  
Data from handbook or collection of data

06.08.2003

Value : 105 °C at 12 hPa
2. PHYSICAL-CHEMICAL DATA

2.3 DENSITY

<table>
<thead>
<tr>
<th>Type</th>
<th>Value</th>
<th>Reliability</th>
<th>Flag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>density</td>
<td>1.29 g/cm³ at 20 °C</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Data from handbook or collection of data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.286 g/cm³ at 20 °C</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Data from handbook or collection of data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.14 g/cm³ at 55 °C</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Data from handbook or collection of data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1038 g/cm³ at 75 °C</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Data from handbook or collection of data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1 g/cm³ at 80 °C</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Data from handbook or collection of data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1 g/cm³ at 100 °C</td>
<td>(4) not assignable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not assignable/manufacturer data without proof</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.104 g/cm³ at °C</td>
<td>(4) not assignable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not assignable/manufacturer data without proof</td>
</tr>
</tbody>
</table>
2. PHYSICAL-CHEMICAL DATA

Reliability : (2) valid with restrictions
06.08.2003

Type : density
Value : 1.286 g/cm³ at °C

Reliability : (2) valid with restrictions
06.08.2003

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : 0.13 hPa at 20 °C
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.08.2003

Value : 0.055 hPa at 25 °C
Reliability : (2) valid with restrictions
06.08.2003

Value : 0.27 hPa at 30 °C
Reliability : (2) valid with restrictions
06.08.2003

Value : 1 hPa at 50 °C
Reliability : (2) valid with restrictions
06.08.2003

Value : = 1.3 hPa at 53.7 °C
Remark : Reference not available
Reliability : (4) not assignable
06.08.2003

Value : = 13.3 hPa at 100 °C
Remark : Reference not available
Reliability : (4) not assignable
06.08.2003

2.5 PARTITION COEFFICIENT
<table>
<thead>
<tr>
<th>Date</th>
<th>ID</th>
<th>Partition coefficient</th>
<th>Log pow</th>
<th>pH value</th>
<th>Method</th>
<th>Year</th>
<th>GLP</th>
<th>Test substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.09.2004</td>
<td>99-99-0</td>
<td>octanol-water</td>
<td>2.37</td>
<td></td>
<td>other (measured)</td>
<td>1964</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.08.2003</td>
<td>(18) (19)</td>
<td>octanol-water</td>
<td>2.42</td>
<td></td>
<td>other (measured)</td>
<td>1989</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.08.2003</td>
<td></td>
<td>octanol-water</td>
<td>2.4</td>
<td></td>
<td>other (measured)</td>
<td>2002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.08.2003</td>
<td></td>
<td>octanol-water</td>
<td>2.36</td>
<td></td>
<td>other (calculated): with SRC-KOWWIN v.1.66, 2000</td>
<td>2002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.08.2003</td>
<td></td>
<td>octanol-water</td>
<td>2.42</td>
<td></td>
<td>other (measured)</td>
<td>2002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Test condition:
- Before test, octanol was washed with sulfuric acid, treated with sodium hydroxide, followed by distillation
- Octanol-saturated water and water-saturated octanol used
- Photometric absorbance measurements in the water phase with Cary Model 14 spectrometer
- Duplicate measurements at at least 2 volume ratios

Reliability: (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

Flag: Critical study for SIDS endpoint

Reliability: (2) valid with restrictions
Data from handbook or collection of data

Method: Calculated according to Leo et al. 1971
Accepted calculation method

Reliability: (2) valid with restrictions
Accepted calculation method
<table>
<thead>
<tr>
<th>Method</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1971</td>
</tr>
<tr>
<td>GLP</td>
<td></td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
<tr>
<td>Reliability</td>
<td>(2)</td>
</tr>
<tr>
<td>Data from handbook or collection of data</td>
<td></td>
</tr>
<tr>
<td>06.08.2003</td>
<td>(23)</td>
</tr>
</tbody>
</table>

| Partition coefficient  | octanol-water |
| Log pow                | 2.53 at °C    |
| pH value               |               |
| Method                 | other (calculated) |
| Year                   | 2002          |
| GLP                    |               |
| Test substance         |               |
| Method                 | Calculated according to Hansch and Leo with SRC-WSKOW, version 1.26 (1996) |
| Reliability            | (2)  valid with restrictions |
| Accepted calculation method |      |
| 06.08.2003             | (24) |

| Partition coefficient  | octanol-water |
| Log pow                | 2.34 at °C    |
| pH value               |               |
| Method                 | other (calculated) |
| Year                   | 1987          |
| GLP                    |               |
| Test substance         |               |
| Method                 | Calculated from capacity factors |
| Remark                 | Deneer et al. compare their calculated data with data of Hansch and Leo (1979) [Hansch C, Leo A (1979) Substituent Constants for Correlation Analysis in Chemistry and Biology. John Wiley & Sons, New York-Chichester-Brisbane-Toronto, p 218], but they state that Hansch and Leo reported 2.39 (as the average of two results: 2.37 and 2.42) instead of 2.40. They also state that 2.39 was measured although no information is given whether both original data were measured. |
| Reliability            | (2)  valid with restrictions |
| Study acceptable for assessment |      |
| 06.08.2003             | (25) |

| Partition coefficient  | octanol-water |
| Log pow                | 2.37 at °C    |
| pH value               |               |
| Method                 |               |
| Year                   | 2001          |
| GLP                    |               |
| Test substance         |               |
| Reliability            | (4) not assignable |
| Secondary literature   |                 |
| 06.08.2003             | (26) |

<p>| Partition coefficient  | octanol-water |
| Log pow                | 2.38 at °C    |
| pH value               |               |
| Test substance         |               |</p>
<table>
<thead>
<tr>
<th>Method</th>
<th>other (calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1998</td>
</tr>
<tr>
<td>GLP</td>
<td></td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>log Pow was calculated with CLOGP ver 3.55 software</td>
</tr>
<tr>
<td>Reliability</td>
<td>(2) valid with restrictions Accepted calculation method</td>
</tr>
<tr>
<td>06.08.2003</td>
<td>(27)</td>
</tr>
</tbody>
</table>

| Partition coefficient | octanol-water       |
| Log pow               | 2.52 at °C          |
| pH value              |                    |
| Method                |                    |
| Year                  | 1997               |
| GLP                   |                    |
| Test substance        |                    |
| Result                |                    |
| Reliability           | (4) not assignable  |
| 06.08.2003            | (28)               |

| Partition coefficient | octanol-water       |
| Log pow               | 2.52 at °C          |
| pH value              |                    |
| Method                | not specified, whether measured or calculated |
| Year                  | 2002               |
| GLP                   |                    |
| Test substance        |                    |
| Remark                | Only short abstract in English available |
| Reliability           | (4) not assignable  |
| Original reference    | Not assignable      |
| 06.08.2003            | (29)               |

| Partition coefficient | octanol-water       |
| Log pow               | 2.4 at °C           |
| pH value              |                    |
| Method                |                    |
| Year                  | 1985               |
| GLP                   |                    |
| Test substance        |                    |
| Remark                | Authors do not indicate whether octanol-water partition coefficient was taken from literature or experimentally determined. |
| Reliability           | (4) not assignable  |
| 06.08.2003            | (30)               |

| Partition coefficient | octanol-water       |
| Log pow               | 2.43 at °C          |
| pH value              |                    |
| Method                |                    |
| Year                  | 2001               |
| GLP                   |                    |
| Test substance        |                    |
| Remark                | The authors cite the Japanese Environmental Agency (1988) Summary Data on the Environmental Chemicals, Maruzen, Tokyo |
| Result                | Authors do not indicate whether octanol-water partition coefficient was |

06.08.2003
### 2. PHYSICAL-CHEMICAL DATA

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(4) not assignable</th>
<th>Secondary literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>06.08.2003</td>
<td></td>
</tr>
</tbody>
</table>

**Partition coefficient**: octanol-water

**Log pow**: 2.34 at °C

**pH value**: (not assignable)

**Method**: (not assignable)

**Year**: 1998

**GLP**: (not assignable)

**Test substance**: (not assignable)

**Remark**: Value taken from "Wood CA (1994), 2nd Ann.SPMD Workshop, Missouri"

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(4) not assignable</th>
<th>Secondary literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>06.08.2003</td>
<td></td>
</tr>
</tbody>
</table>

**Partition coefficient**: octanol-water

**Log pow**: 2.53 at °C

**pH value**: (not assignable)

**Method**: (not assignable)

**Year**: 1993

**GLP**: (not assignable)

**Test substance**: (not assignable)

**Remark**: The source of the data is reported to be "from Yalkowsky et al [1, 17] and calculated according to Hansch and Leo [14]" (The first reference of this citation does not refer to a Yalkowsky paper)

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(4) not assignable</th>
<th>Not assignable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>06.08.2003</td>
<td></td>
</tr>
</tbody>
</table>

**2.6.1 SOLUBILITY IN DIFFERENT MEDIA**

**Solubility in**: Water

**Value**: .345 g/l at 20 °C

**pH value**: (not assignable)

**Temperature effects**: (not assignable)

**Examine different pol. concentration**: at °C

**pKa**: (not assignable)

**Description**: (not assignable)

**Stable**: (not assignable)

**Deg. product**: (not assignable)

**Method**: other: measured via HPLC

**Year**: 1987

**GLP**: (not assignable)

**Test substance**: other TS: 99.7 % purity

**Method**: - stirring 3 - 4 days

- measured via HPLC

- mean value of 4 measurements

**Test substance**: 99.7 % purity, measured via HPLC, mean value of 4 measurements

**Reliability**: (2) valid with restrictions

**Flag**: Critical study for SIDS endpoint

| Date              | 16.10.2003         |                |

**Solubility in**: Organic Solvents
**Value** : at °C
**pH value** :  
**concentration** : at °C

**Temperature effects** :  
**Examine different pol.** :  

**pKa** : At 25 °C
**Description** :  
**Stable** :  
**Deg. product** :  
**Method** :  
**Year** : 1991
**GLP** :  
**Test substance** :  

**Result** : Soluble in most organic solvents
**Reliability** : (2) valid with restrictions  
Data from handbook or collection of data
**Flag** : Critical study for SIDS endpoint

06.08.2003

**Solubility in** : Water
**Value** : 442 mg/l at 30 °C
**pH value** :  
**concentration** : at °C

**Temperature effects** :  
**Examine different pol.** :  

**pKa** : At 25 °C
**Description** :  
**Stable** :  
**Deg. product** :  
**Method** :  
**Year** : 1989
**GLP** :  
**Test substance** :  

**Reliability** : (2) valid with restrictions  
Data from handbook or collection of data

06.08.2003

**Solubility in** : Organic Solvents
**Value** : at °C
**pH value** :  
**concentration** : at °C

**Temperature effects** :  
**Examine different pol.** :  

**pKa** : At 25 °C
**Description** :  
**Stable** :  
**Deg. product** :  
**Method** :  
**Year** : 1989
**GLP** :  
**Test substance** :  

**Result** : Soluble in ethanol, hexane and heptane
**Reliability** : (2) valid with restrictions
Reliable source

06.08.2003

**Solubility in** : Organic Solvents
**Value** : at °C
**pH value** :  
**concentration** : at °C

**Temperature effects** :  
**Examine different pol.** :  

**pKa** : at 25 °C
Description:
Stable
Deg. product
Method
Year
GLP
Test substance

Result: Soluble in alcohol, benzene, ether, chloroform, and acetone
Reliability: (2) valid with restrictions
Data from handbook or collection of data

Solubility in Organic Solvents
Value at °C
pH value
concentration at °C
Temperature effects
Examine different pol.
pKa Description
Stable
Deg. product
Method
Year
GLP
Test substance

Result: Soluble in ethanol, and acetone
Reliability: (2) valid with restrictions
Data from handbook or collection of data

Solubility in Water
Value .419 g/l at 20 °C
pH value
concentration at °C
Temperature effects
Examine different pol.
pKa Description
Stable
Deg. product
Method
Year
GLP
Test substance

Reliability: (2) valid with restrictions
Data from handbook or collection of data

Solubility in Water
Value .44 g/l at 30 °C
pH value
concentration at °C
Temperature effects
Examine different pol.
pKa Description
Stable
### 2.6.2 SURFACE TENSION

<table>
<thead>
<tr>
<th>Method</th>
<th>Year</th>
<th>GLP</th>
<th>Test substance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2002</td>
<td></td>
<td>other TS: presumably molten substance</td>
<td>Beilstein's reported values for surface tension are: 37.41 - 20.91 g/s² at 56 - 220 °C</td>
</tr>
</tbody>
</table>

**Reliability**: (2) valid with restrictions  
Data from handbook or collection of data  
06.08.2003  
(1)

### 2.7 FLASH POINT

<table>
<thead>
<tr>
<th>Value</th>
<th>Type</th>
<th>Method</th>
<th>Year</th>
<th>GLP</th>
<th>Test substance</th>
<th>Reliability</th>
<th>Flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>103 °C</td>
<td>closed cup</td>
<td>other: DIN 51758</td>
<td>1978</td>
<td></td>
<td></td>
<td>(2) valid with restrictions</td>
<td>Critical study for SIDS endpoint</td>
</tr>
<tr>
<td>106 °C</td>
<td>closed cup</td>
<td></td>
<td>1989</td>
<td></td>
<td></td>
<td>(2) valid with restrictions</td>
<td>Data from handbook or collection of data</td>
</tr>
</tbody>
</table>

06.08.2003  
(10)  
(16)  
(35)

<table>
<thead>
<tr>
<th>Value</th>
<th>Type</th>
<th>Method</th>
<th>Year</th>
<th>GLP</th>
<th>Test substance</th>
<th>Reliability</th>
<th>Flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>103 °C</td>
<td>closed cup</td>
<td></td>
<td>1997</td>
<td></td>
<td></td>
<td>(2) valid with restrictions</td>
<td>Data from handbook or collection of data</td>
</tr>
<tr>
<td>106 °C</td>
<td>closed cup</td>
<td></td>
<td>1997</td>
<td></td>
<td></td>
<td>(2) valid with restrictions</td>
<td>Data from handbook or collection of data</td>
</tr>
</tbody>
</table>

06.08.2003  
(2)  
(10)
2.8 AUTO FLAMMABILITY

Value : 450 °C at
Method : 
Year : 1997
GLP : 
Test substance : 

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint

06.08.2003 (10)

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

Method : 
Year : 2002
GLP : 
Test substance : 

Result : Beilstein's reported values for dynamic viscosity are in the range of 0.01 - 0.008 g/cm*s (at 60 - 100 °C)
Reliability : (2) valid with restrictions

06.08.2003 (15)

2.14 ADDITIONAL REMARKS

Memo : Beilstein Reference No. 4-05-00-00848
Reliability : (2) valid with restrictions

06.08.2003 (15)
Memo : Merck No. 6572

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003

Memo : Conversion factors volume /weight concentration

Remark : Conversion factor for the vapour phase
1 mg/m3 = 0.18 ppm
1 ppm = 5.70 mg/m3

Reliability : (2) valid with restrictions
Data from handbook or collection of data

Flag : Critical study for SIDS endpoint
06.08.2003

Memo : Odour treshold concentration

Remark : odour treshold concentration for detection: 0.003 mg/kg in water

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003

Memo : Some chemical hazards

Remark : The substance decomposes on heating producing toxic fumes (nitrogen oxides).
Reacts violently with strong oxidizers or sulfuric acid. Fire and explosion hazard.

Reliability : (2) valid with restrictions
Data from handbook or collection of data

Flag : Critical study for SIDS endpoint
06.08.2003

Memo : Vapour density

Remark : vapour density in relation to air ( = 1): 4.72

Reliability : (2) valid with restrictions
Data from handbook or collection of data

Flag : Critical study for SIDS endpoint
06.08.2003

(1) (12) (4)
### 3.1.1 PHOTODEGRADATION

<table>
<thead>
<tr>
<th>Type</th>
<th>other: (calculated) with SRC-AOPWIN v. 1.90 (2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light source</td>
<td></td>
</tr>
<tr>
<td>Light spectrum</td>
<td>nm</td>
</tr>
<tr>
<td>Relative intensity</td>
<td>based on intensity of sunlight</td>
</tr>
<tr>
<td>INDIRECT PHOTOLOYSIS</td>
<td></td>
</tr>
<tr>
<td>Sensitizer</td>
<td>OH</td>
</tr>
<tr>
<td>Conc. of sensitizer</td>
<td>500000 molecule/cm³</td>
</tr>
<tr>
<td>Rate constant</td>
<td>.00000000000007722 cm³/(molecule*sec)</td>
</tr>
<tr>
<td>Degradation</td>
<td>50 % after 20.8 day(s)</td>
</tr>
<tr>
<td>Deg. product</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>2002</td>
</tr>
<tr>
<td>GLP</td>
<td></td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
</tbody>
</table>

**Remark**: In deviation from the U.S. EPA AOPWIN calculation program the calculated half-life is based on a mean OH radical concentration of 500,000 OH radicals/cm³ as a 24 h average.

**Reliability**: (2) valid with restrictions

**Flag**: Critical study for SIDS endpoint

**06.02.2003**

<table>
<thead>
<tr>
<th>Type</th>
<th>water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light source</td>
<td></td>
</tr>
<tr>
<td>Light spectrum</td>
<td>nm</td>
</tr>
<tr>
<td>Relative intensity</td>
<td>based on intensity of sunlight</td>
</tr>
<tr>
<td>Deg. product</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>other (measured): see Method</td>
</tr>
<tr>
<td>Year</td>
<td>1986</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Method**: Saturated solutions in distilled water were centrifuged at 15,000 rpm for 30 min. The supernatant was removed and diluted to concs of 10-6 to 10-5 M in distilled water, natural waters and aqueous solutions of extracted natural humic materials. Triplicate solutions were exposed to mid-day sunlight and monochromatic light (366 nm) in a merry-go-round-photoreactor. The pH was 5.5. Exposure times were varied, achieving approx. 30 % reaction for each exposure. Dark controls were used in each run. The solutions were then analyzed by reverse phase HPLC. Dark controls showed no transformation during the periods required for the experiments, which in most cases were less than 1 day.

**Remark**: It was observed that the photodegradation in pure water is slower than in natural water. The photodegradation depends on the content in humic acid and nitrates, which is higher in natural water.

**Result**: The quantum yield was measured to 0.0052. Taking into account the averaged annual values that pertain to near-surface conditions at latitude 40°N and based on the obtained quantum yield a half-life can be derived: \( t_{1/2} = 5.9 \) hours

**Reliability**: (2) valid with restrictions

**Flag**: Critical study for SIDS endpoint

**26.01.2003**

<table>
<thead>
<tr>
<th>Type</th>
<th>water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light source</td>
<td></td>
</tr>
</tbody>
</table>
### Test substance 1: Photocatalysis and Photo-Fenton reaction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light spectrum</td>
<td>nm</td>
</tr>
<tr>
<td>Relative intensity</td>
<td>based on intensity of sunlight</td>
</tr>
<tr>
<td>Conc. of substance</td>
<td>0.1 mmol/l at 30 °C</td>
</tr>
<tr>
<td>Deg. product</td>
<td>no</td>
</tr>
<tr>
<td>Method</td>
<td>other (measured): see TC</td>
</tr>
<tr>
<td>Year</td>
<td>1997</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: purity not stated</td>
</tr>
</tbody>
</table>

**Result:**
- 0.49 µmol/l x min
- 1.01 µmol/l x min in the presence of 20 mmol/l H2O2 (photooxidation with hydrogenperoxide)
- 1.45 µmol/l x min in the presence of 100 µmol/l Fe2(SO4)3
- 14.5 µmol/l x min in the presence of both 20 mmol/l H2O2 and 100 µmol/l Fe2(SO4)3 (photo-Fenton-reaction)

**Test condition:**
- Start concentration of the test substance was 100 µmol/l (checked by HPLC and UV)
- Wavelength 300 - 400 nm
- for homogenous solutions photon flux density 0.8 µmol photons/min
- for photooxidation with hydrogenperoxide, Fe2(SO4)3, or both H2O2 and Fe2(SO4)3 (photo-Fenton-reaction) photon flux density 0.16 µmol photons/l x min
- Incubation solution (test volume 5 ml) was air-saturated, pH 3, temperature 30 °C

**Reliability:**
(2) valid with restrictions
Study well documented and meets acceptable scientific principles

### Test substance 2: Photocatalysis and Photo-Fenton reaction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light spectrum</td>
<td>nm</td>
</tr>
<tr>
<td>Relative intensity</td>
<td>based on intensity of sunlight</td>
</tr>
<tr>
<td>Conc. of substance</td>
<td>0.1 mmol/l at 30 °C</td>
</tr>
<tr>
<td>Deg. product</td>
<td>not measured</td>
</tr>
<tr>
<td>Method</td>
<td>other (measured): see TC</td>
</tr>
<tr>
<td>Year</td>
<td>1999</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: purity not stated</td>
</tr>
</tbody>
</table>

**Result:**
- 6.9 µmol/l x min in the presence of TiO2 (photocatalysis)
- 14.5 µmol/l x min in the presence of 100 µmol/l Fe2(SO4)3, without oxalate (photo-Fenton reaction)
- 60 µmol/l x min in the presence of both 100 µmol/l Fe2(SO4)3 and 150 µmol/l oxalate (photo-Fenton reaction)

**Test condition:**
- Start concentration of the test substance was 100 µmol/l (checked by HPLC and UV)
- Wavelength 300 - 400 nm
- Photon flux density 0.8 µmol photons/min
- Incubation solution/suspension (test volume 5 ml) was air-saturated, pH 3, temperature 30 °C
- for photocatalysis 1 g/l TiO2 was added, light intensity about 725 µmoles/l x min
- for photo-Fenton-reaction 100 µmol/l Fe2(SO4)3 and 20 mmol/l H2O2, light intensity 160 µmol/l x min

**Reliability:**
(2) valid with restrictions
Study well documented and meets acceptable scientific principles

**Type:** water
### 3.1.2 STABILITY IN WATER

| Light source | : | Light spectrum | : | nm |
| Relative intensity | : | based on intensity of sunlight |
| Deg. product | : | yes |
| Method | : | other (measured): see TC |
| Year | : | 2002 |
| GLP | : | no data |
| Test substance | : | other TS: reagent grade (Wako Pure Chemical Inc.) |
| Deg. products | : | 106-44-5 203-398-6 p-cresol |
| | : | 106-49-0 203-403-1 p-toluidine |
| | : | 50-00-0 200-001-8 formaldehyde |
| | : | 5428-54-6 226-580-7 5-nitro-o-cresol |
| | : | 608-25-3 210-155-8 2-methylresorcinol |
| | : | 64-18-6 200-579-1 formic acid |
| | : | 64-19-7 200-580-7 acetic acid |

**Remark**: 5-Nitro-o-cresol, p-cresol, 2-methylresorcinol and p-toluidine are intermediates which can be detected for some hours during the degradation of 4-nitrotoluene. Ammonia, nitrate and CO₂ were formed in different ratios depending on the test conditions employed. Acetic acid, formic acid and trace amounts of formaldehyde were also formed.

**Result**: Authors conclude that 2 independent routes for initial degradation exist. Pseudo-first order photolytic degradation rate constant $k_1$ is 0.045 1/min (concentration of test substance 0.0001 mol/l) which equals about 60 min half life for the removal of TOC. 

$$k = 0.00000962 \text{ mol/l } \times \text{ min}$$

**Test condition**: - TiO₂, anastase, specific surface 17.3 m²/g
- Test substance and most degradation products were analyzed by HPLC
- Acetic acid, formic acid, nitrate and ammonium were determined by ion chromatography

**Reliability**: (3) invalid

Important data not supplied e.g. temperature, experimental design.

24.01.2003 (39)

### 3.1.3 STABILITY IN SOIL

| Degradation | : | 6 % after 8 day(s) at pH 8 and 25 °C |
| Deg. product | : | not measured |
| Method | : | other: according to Canton and Slooff (1982) |
| Year | : | 1985 |
| GLP | : | no data |
| Test substance | : | other TS: > 99.5 % Purity |

**Method**: Canton JH, Slooff W (1982) Toxicity and accumulation studies of cadmium (Cd²⁺) with freshwater organisms of different trophic levels. Ecotoxicol Environ Safety 6: 113 - 128

**Remark**: - The decline of the concentration in non-aerated standardized medium (Canton and Slooff 1982) was studied at room temperature (25 °C)
- The analytical analysis was performed using gas chromatography or high-pressure liquid chromatography

**Reliability**: (2) valid with restrictions

Basic data given

**Flag**: Critical study for SIDS endpoint

30.01.2003 (30)
### 3.2.1 MONITORING DATA

<table>
<thead>
<tr>
<th>Type of measurement</th>
<th>background concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>surface water</td>
</tr>
<tr>
<td>Concentration</td>
<td>&lt;= .05 µg/l</td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
</tbody>
</table>

**Remark**
Throughout Germany a comprehensive monitoring program on several chemicals in surface waters has been realised to check whether the limit values are not exceeded:
For 1999 the following values were obtained:
- River Danube: < 0.02 µg/l (90-percentile)
- River Rhine: < 0.5 µg/l (90-percentile)
- River Elbe: 0.05 µg/l (Maximum)

For 4-nitrotoluene the limit values have been set at 70 µg/l to protect aquatic life and at 10 µg/l to protect drinking water. These values have not been exceeded in the years 1996 - 1998.

**Reliability**
(2) valid with restrictions
Basic data given

**Flag**
Critical study for SIDS endpoint
03.07.2003 (40)

---

<table>
<thead>
<tr>
<th>Type of measurement</th>
<th>background concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>surface water</td>
</tr>
<tr>
<td>Concentration</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
</tbody>
</table>

**Remark**

**Result**
Throughout Germany a comprehensive monitoring program on several chemicals in surface waters has been realised to check whether the limit values are not exceeded:
For 4-nitrotoluene limit values have been set at 70 µg/l to protect aquatic life and at 10 µg/l to protect drinking water. These values have also not been exceeded in the years 1998 - 2000.

**Reliability**
(2) valid with restrictions
Basic data given

**Flag**
Critical study for SIDS endpoint
03.07.2003 (41)

---

<table>
<thead>
<tr>
<th>Type of measurement</th>
<th>concentration at contaminated site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>other: sewage treatment plant</td>
</tr>
<tr>
<td>Concentration</td>
<td>&lt; .01 µg/l</td>
</tr>
<tr>
<td>Method</td>
<td>GC/MS</td>
</tr>
</tbody>
</table>

**Method**
- Sampling points in every sewage treatment plant were
  -- Wastewater influent into the wastewater treatment plant
  -- Influent in the primary settling tank
  -- Effluent from the primary settling tank
  -- Effluent from the final sedimentation tank
  -- Effluent from the wastewater treatment plant
  - Study season was autumn 1998
  - Solvent GC/MS Analysis according to methods proposed by the Japanese Environmental Agency (The authors announce that they will publish details of their method in another paper)
  - Target minimum limit of detection 0.01 µg/l

**Remark**
A group of substances assumed to be contained in domestic and industrial wastewater were monitored in sewage influent and effluent of 27 sewage
4-Nitrotoluene was only monitored in autumn 1998. The occurrence of 4-nitrotoluene was below limit of detection (0.01 µg/l) in sewage.

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(2) valid with restrictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flag</td>
<td>Critical study for SIDS endpoint</td>
</tr>
</tbody>
</table>

**Type of measurement**: concentration at contaminated site  
**Media**: ground water  
**Concentration**: 14 mg/l  
**Method**: Groundwater screening:  
- Extraction with CH2Cl2 for 10 min  
- o-Terphenyl used as internal standard  
- Organic phase analyzed by GC/MS (Hewlett Packard 6890/5973) using PE-5MS column (Perkin Elmer, Norwalk, CT)

**Remark**: The groundwater originated from a Swedish location where ammunition destruction by open burning has been performed for more than 40 years.

**Reliability**: (2) valid with restrictions  
**Flag**: Critical study for SIDS endpoint  
03.07.2003 (42)

**Type of measurement**: other: tobacco smoke  
**Media**: other: smoke from tobacco product  
**Concentration**:  
**Method**: - For isolation of nitrobenzenes 30-channel automatic smoker with vibrating liquid trap to collect mainstream smoke used  
- For quantitative analysis twenty-port automated Phipps and Bird machine used  
- GC analysis: Perkin Elmer Model 800  
- ECD quantification: Varian Aerograph Model 1200  
- Scintillation counting from 14C internal standard: Nuclear Chicago Scintillation System 720  
- MS: Hitachi-Perkin-Elmer RMU-6D  
- Internal standard: Nitrobenzene-U-14C (3.2 mCi/mM from Amersham)  
- For isolation of nitrobenzenes 4100 cigarettes without filter tips used  
- Residues (154 g) collected in acetone  
- Clean up including e.g. extraction with ether  
- For quantification 50 cigarettes (85 mm) without filter tips smoked individually in Phipps and Bird machine  
- Smoke filtered and washed  
- Clean up and quantification  
- To check influence of nitrate cigarettes enriched in nitrate were manufactured and the nitrobenzenes content quantified

**Result**: 4-Nitrotoluene is present in any type of cigarette smoke. Although the authors do not report the exact amount of 4-nitrotoluene, an estimate can be derived: 7 ng/cigarette. Cigarettes with very low nitrate levels contained less nitrobenzenes. In smoke from cigarettes with added nitrate the contents of nitrobenzenes were up to 8-fold increased. The authors conclude that nitrate (e.g. from the treatment of tobacco products) is the precursor of nitrocompounds in cigarette smoke.

**Reliability**: (2) valid with restrictions  
**Flag**: Critical study for SIDS endpoint  
03.07.2003 (43)

**Type of measurement**: concentration at contaminated site  
**Media**: ground water  
**Concentration**: 14 mg/l  
**Method**: - Extraction with CH2Cl2 for 10 min  
- o-Terphenyl used as internal standard  
- Organic phase analyzed by GC/MS (Hewlett Packard 6890/5973) using PE-5MS column (Perkin Elmer, Norwalk, CT)

**Remark**: The groundwater originated from a Swedish location where ammunition destruction by open burning has been performed for more than 40 years.

**Reliability**: (2) valid with restrictions  
**Flag**: Critical study for SIDS endpoint  
03.07.2003 (44)
Type of measurement: concentration at contaminated site
Media: ground water
Concentration: 4-Nitrotoluene is present in groundwater from a decommissioned munitions production facility near Melbourne, Australia. No numeric data given.
Method: reverse phase HPLC/UV detection

Result: 4-Nitrotoluene is present in ground water from decommissioned munitions site
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

Type of measurement: other: wastewater
Media: wastewater
Concentration: ca. .295 mg/l
Method: HPLC and GC
Remark: Condensate wastewater from US munitions production site

Result: Untreated wastewater from ADIMulwala Munition Production Facility (Australia) contains about 20 mg/l 4-nitrotoluene and other nitroaromatic compounds
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
### 3.2.2 FIELD STUDIES

#### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

<table>
<thead>
<tr>
<th>Type</th>
<th>Media</th>
<th>Air</th>
<th>Water</th>
<th>Soil</th>
<th>Biota</th>
<th>Soil</th>
<th>Method</th>
<th>Year</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>adsorption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adsorption of 4-nitrotoluene (and other nitroaromatic compounds) to 3 homoionic kalium ion clay minerals was determined:</td>
</tr>
<tr>
<td>Media</td>
<td>water - soil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1. Kaolinite</td>
</tr>
<tr>
<td>Air</td>
<td>% (Fugacity Model Level I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Distribution coefficient Kd (l/kg dry matter) 4.9</td>
</tr>
<tr>
<td>Water</td>
<td>% (Fugacity Model Level I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Illite</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Distribution coefficient Kd (l/kg dry matter) 24</td>
</tr>
<tr>
<td>Biota</td>
<td>% (Fugacity Model Level II/III)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. Montmorillonite</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level II/III)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Distribution coefficient Kd (l/kg dry matter) 45</td>
</tr>
<tr>
<td>Method</td>
<td>other: see Test condition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Further results were:</td>
</tr>
<tr>
<td>Year</td>
<td>1996</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Adsorption of nitroaromatic compounds is high when the exchangeable cations at the clays include K+ or NH4+ but much smaller for homoionic clays containing Na+, Ca2+, Mg2+, and Al3+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Highest adsorption coefficients are found for polynitroaromatic compounds</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Ionic strength (in the range of 0.0001 - 0.1 M) had no measurable effect on the adsorption</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>It is rationalized that electron donor-acceptor complex formation occurs with oxygen at the external siloxane surface of clay minerals (which increases in the aforementioned order of the three minerals). The mobility of nitroaromatic compounds decreases with increasing degree of nitration. Bulky alkyl groups decrease the adsorption although p-nitrotoluene is more strongly adsorbed than nitrobenzene</td>
</tr>
</tbody>
</table>

**Test condition**

- Solutions of test substances were prepared in methanol (or acetonitrile if not soluble in methanol), final concentration of organic solvent <= 0.5 %
- Solutions were spiked with known quantities of air-dried clay minerals (5 - 200 g/l)
- Equilibrium was reached after about 30 - 60 min on rotary shaker in the dark at 21 ± 1.5 °C
- Phase separation by centrifugation at 12,000 rpm for 1 min
- HPLC-UV analysis of solutes in the supernatant
- Cation analysis with ion chromatograph Metrohm Model 690, Herisau, CH, using Metrohm Super-Sep cation column

**Test substance**

- Minimum purity 97 % (obtained from Fluka AG, Buchs, CH)

**Reliability**

- (2) valid with restrictions
- Basic data given

**Flag**

- Critical study for SIDS endpoint

07.02.2003
Method: other: measured (thermodynamic method)
Year: 1999

Method:
- Aqueous solution of the TS produced in a generator column
- Solution is passed through gas liquid desorption column where it contacts a gas stream and the partition equilibrium is reached
- Gas and water are separated: water flows to the receiver dosing funnel, the gas is conducted into an absorption vessel where the TS is absorbed in organic solvent

Result:
Unitless Henry’s Law Constant: \( H = 0.00023 \) at 25 °C
\[
H = 0.00023 \times 8.314 \text{ Pa m}^3/\text{mol K} \times 298 \text{ K} = 0.57 \text{ Pa m}^3 \text{ mol}^{-1} \text{ at 25°C}
\]

Test condition:
Temperature 25 °C
Gas phase: Nitrogen
Liquid phase: Demineralized, distilled water
Analysis: GC/ECD

Reliability:
(2) valid with restrictions
Study meets generally accepted scientific principles
Flag:
06.08.2003 (50)

Type: volatility
Media: water - air

Air:
% (Fugacity Model Level I)

Water:
% (Fugacity Model Level I)

Soil:
% (Fugacity Model Level I)

Biota:
% (Fugacity Model Level II/III)

Soil:
% (Fugacity Model Level II/III)

Method: other: Estimation of the Henry Constant
Year: 2002

Result:
2.38 Pa x m3/mol (Bond method)
4.83 Pa x m3/mol (Group method)
(both results at 25 °C)

Reliability:
(2) valid with restrictions
accepted calculation method
27.01.2003 (22)

Type: adsorption
Media: water - soil

Air:
% (Fugacity Model Level I)

Water:
% (Fugacity Model Level I)

Soil:
% (Fugacity Model Level I)

Biota:
% (Fugacity Model Level II/III)

Soil:
% (Fugacity Model Level II/III)

Method: other: as described by Patterson (1996)
Year: 1999

Remark:
Stainless steel columns containing weathered basalt were used for sorption studies to estimate the mobility of munition residues (e.g. p-nitrotoluene) in the aquifer material according to Patterson (1996).

Result:
Kd = 2.4 l/kg (relative to bromide); Kd = 1.4 l/kg (relative to 2-nitrotoluene)

Reliability:
(2) valid with restrictions
Basic data given
27.01.2003 (45)

Type: adsorption
### Environmental Fate and Pathways

#### 4-nitrotoluene

**Date:** 09.09.2004

<table>
<thead>
<tr>
<th>Media</th>
<th>Water - Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>% (Fugacity Model Level I)</td>
</tr>
<tr>
<td>Water</td>
<td>% (Fugacity Model Level I)</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level I)</td>
</tr>
<tr>
<td>Biota</td>
<td>% (Fugacity Model Level II/III)</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level II/III)</td>
</tr>
</tbody>
</table>

**Method:** other: (calculated) SRC-PCKOCWIN v1.66 (2000)
**Year:** 2002

**Result:** $K_{oc} = 309$

**Reliability:** (2) valid with restrictions
**Flag:** Critical study for SIDS endpoint

**Type:** adsorption

---

**Media:** water - soil

<table>
<thead>
<tr>
<th>Air</th>
<th>% (Fugacity Model Level I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>% (Fugacity Model Level I)</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level I)</td>
</tr>
<tr>
<td>Biota</td>
<td>% (Fugacity Model Level II/III)</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level II/III)</td>
</tr>
</tbody>
</table>

**Method:** other: (calculation) Kenaga & Goring 1978
**Year:** 1990

**Result:**
- $K_{oc} = 494$ (based on log Kow)
- $K_{oc} = 175$ (based on log S)

**Reliability:** (2) valid with restrictions
**Flag:** Critical study for SIDS endpoint

---

**Media:** water - soil

<table>
<thead>
<tr>
<th>Air</th>
<th>% (Fugacity Model Level I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>% (Fugacity Model Level I)</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level I)</td>
</tr>
<tr>
<td>Biota</td>
<td>% (Fugacity Model Level II/III)</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level II/III)</td>
</tr>
</tbody>
</table>

**Method:**
- Calculation of soil adsorption coefficient from Kow (octanol/water partition coefficient):
  \[
  \log K_{oc} = 0.544 \log K_{ow} + 1.377
  \]
- Calculation of soil adsorption coefficient from $S$ (water solubility):
  \[
  \log K_{oc} = -0.55 \log S + 3.64 \quad (S \text{ in mg/l})
  \]

Both equations see Kenaga EE, Goring CAI (1978) Relationship Between Water Solubility, Soil sorption, Octanol-Water Partitioning, and Bioconcentration of Chemicals in Biota, Special Technical Publication 707, ASTM, Philadelphia, PA

**Result:**
- $K_{oc} = 494$ (based on log Kow)
- $K_{oc} = 175$ (based on log S)

**Reliability:** (2) valid with restrictions
**Flag:** Critical study for SIDS endpoint

---

**Media:** water - soil

<table>
<thead>
<tr>
<th>Air</th>
<th>% (Fugacity Model Level I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>% (Fugacity Model Level I)</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level I)</td>
</tr>
<tr>
<td>Biota</td>
<td>% (Fugacity Model Level II/III)</td>
</tr>
<tr>
<td>Soil</td>
<td>% (Fugacity Model Level II/III)</td>
</tr>
</tbody>
</table>

**Method:** other: see TC
**Year:** 1998

**Result:** Sorption to three sediments of 4-nitrotoluene in the vicinity of a former ammunition site:
1. clay mineral containing medium-grained sand
   - Effective grain size (mm) 0.143
   - Organic content (g/kg dry matter) 0.5
   - Clay content (g/kg dry matter) 7.1
   - Distribution coefficient $K_d$ (l/kg dry matter) 0.75
2. clay mineral containing fine sand
   - Effective grain size (mm) 0.037
   - Organic content (g/kg dry matter) 0.9
   - Clay content (g/kg dry matter) 32
Phyllosilicates of clay are strong and specific sorbents for aromatic nitro compounds. Sorption of 4-nitrotoluene to 2 homoionic Kalium ion clays was determined:

1. Kaolinite
   - Distribution coefficient Kd (l/kg dry matter) 1
2. Montmorillonite
   - Distribution coefficient Kd (l/kg dry matter) 1.7

For 2- and 3-layer clays at varying equivalent fractions of exchangeable K+ and at various ionic strengths:
- Sorption was very low for homoionic Ca2+ or Na+-clays
- For Ca2+/K+ or Na+/K+ clays sorption increases with the degree of K+ saturation of the clay minerals (montmorillonite, smectit and kaolinite) with exchangeable kalium ions
- Sorption decreases with the concentration of the kalium ions in the solution around the clay minerals.

Some tests were done to desorb soil contaminants from clay by exchange of K+ with Ca2+, which succeeded in a remobilization of 4-nitrotoluene.

Test condition: Sorption of test substance to clay:
- 3 replicates
- equilibration period 2 hours to focus on fast transport reactions and to minimize formation of reduction products (no hydroxylamino or amino transformation was detected)
- 1.8 ml screw glass vials
- centrifugation for phase separation
- HPLC-UV analysis of solutes in the supernatant

Reliability: (2) valid with restrictions

Basic data given

30.01.2003 (46)
Result: For the material used in the study the following results were obtained in single batch experiments for 4-nitrotoluene:
- Langmuir affinity constant $K(L) = 0.0108 \text{ l/µmol}$
- Maximum sorbed-phase solute concentration 980 µmol/kg

Mobility was influenced by the presence of other test substances since they compete for binding sites of the mineral (competitive sorption)

Test condition:
- Columns filled with quartz sand coated with aggregated montmorillonite clay minerals which mimics the typical clay distribution pattern in natural matrices
  - Temperature 22 °C
  - Solute concentration 10 mM KCl
  - Test substance concentration at start of experiment 10 µmol/l

Reliability: (2) valid with restrictions

Basic data given

3.3.2 DISTRIBUTION

Media: air - biota - sediment(s) - soil - water
Method: Calculation according Mackay, Level I
Year: 2002

Method:
- Data used in the calculation:
  - Temperature (°C): 20
  - Molar Mass (g/mol): 137.14
  - Vapour pressure (Pa): 13
  - Water solubility (g/m³): 345
  - log Pow: 2.37
- Air: $6 \times 10^9 \text{ m}^3$
- Water: $7 \times 10^6 \text{ m}^3$
- Soil: $4.5 \times 10^4 \text{ m}^3$ 1500 kg/m³ 2 % org. C
- Sediment: $2.1 \times 10^4 \text{ m}^3$ 1300 kg/m³ 5 % org. C
- Suspended sediment: 35 m³ 1500 kg/m³ 16.7 % org. C
- Aerosols: 0.12 m³ 1500 kg/m³
- Aquatic biota: 7 m³ 1000 kg/m³ 5 % fat

Result: The main target compartments for 4-nitrotoluene are the air with 63.7 %, and the hydrosphere with 35.0 %, followed by the soil and sediment with each 0.65 %

Reliability: (2) valid with restrictions
- accepted calculation method

Flag: Critical study for SIDS endpoint

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type: aerobic
Inoculum: other: sludge samplings from different sewage plants, rivers, bays and a lake
Concentration: 100 mg/l related to Test substance related to
### Environmental Fate and Pathways

**ID:** 99-99-0  
**DATE:** 09.09.2004

<table>
<thead>
<tr>
<th>Contact time</th>
<th>Degradation</th>
<th>Result</th>
<th>Deg. product</th>
<th>Method</th>
<th>Year</th>
<th>GLP</th>
<th>Test substance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.8 (±) % after 14 day(s)</td>
<td>under test conditions no biodegradation observed</td>
<td></td>
<td>other: Japanese Guideline by MITI of 1974; corresponds to OECD 301C Modified MITI Test I</td>
<td>1992</td>
<td>no data</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Remark:** "Biodegradation test of chemical substance by microorganisms etc." stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974, Order of the Prime Minister, Minister of Health and Welfare, the MITI No. 1). This guideline corresponds to "301C, Ready Biodegradability: Modified MITI Test I", stipulated in the OECD Guidelines for Testing of Chemicals (May 12, 1981).

<table>
<thead>
<tr>
<th>Sludge conc.</th>
<th>30 mg/l</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Reliability</th>
<th>Flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) valid without restriction Guideline study</td>
<td>Critical study for SIDS endpoint</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>Inoculum</th>
<th>Contact time</th>
<th>Degradation</th>
<th>Result</th>
<th>Deg. product</th>
<th>Method</th>
<th>Year</th>
<th>GLP</th>
<th>Test substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>aerobic</td>
<td>activated sludge, industrial, adapted</td>
<td>21 day(s)</td>
<td>100 (±) % after 21 day(s)</td>
<td>other: ultimate biodegradation</td>
<td>not measured</td>
<td>OECD Guide-line 302 B &quot;Inherent biodegradability: Modified Zahn-Wellens Test&quot;</td>
<td>1990</td>
<td>no</td>
<td>no data</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reliability</th>
<th>Flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2) valid with restrictions Guideline study without detailed documentation</td>
<td>Critical study for SIDS endpoint</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test condition</th>
<th>Result</th>
<th>Test condition</th>
<th>Result</th>
</tr>
</thead>
</table>
| - Activated sludge 1.1 g/l dry weight  
- Test substance concentration 50-400 mg/l DOC, 200-1000 mg/l COD  
- Acclimatization phase 10 days | Rate of biodegradation: 32.5 mg COD/g x h | - Duration of the test: 120 h  
- Concentration tested of the test substance: 200 mg COD/l  
- Inoculum was adapted during 20 days. Inoculum concentration applied: 100 mg/l dry matter |
### 3. ENVIRONMENTAL FATE AND PATHWAYS

**ID:** 99-99-0  
**DATE:** 09.09.2004

- The tested substance was the sole carbon source
- Temperature 20 °C
- pH 7.2

**Reliability:** (2) valid with restrictions

Study meets generally accepted scientific principles. Basic data given.

**Flag:** 26.01.2003  
Critical study for SIDS endpoint (55)

<table>
<thead>
<tr>
<th>Type</th>
<th>aerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>activated sludge, domestic, adapted</td>
</tr>
<tr>
<td>Concentration</td>
<td>40.8 mg/l related to Test substance related to</td>
</tr>
<tr>
<td>Contact time</td>
<td></td>
</tr>
<tr>
<td>Degradation</td>
<td>&gt; 90 (±) % after 21 day(s)</td>
</tr>
<tr>
<td>Result</td>
<td></td>
</tr>
<tr>
<td>Deg. product</td>
<td>not measured</td>
</tr>
<tr>
<td>Method</td>
<td>other: modified test method described by Pitter in 1976 (similar to OECD302B)</td>
</tr>
<tr>
<td>Year</td>
<td>1986</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: analytical grade, not further specified</td>
</tr>
</tbody>
</table>

**Result:**  
> 90 % degradation after 2 weeks in the test with the composite sludge (adapted 3 weeks)  
> 90 % degradation after 3 weeks in the test with the activated sludge from a municipal sewage treatment plant (adapted 3 weeks)  
> 90 % degradation after about 5 days in the test with the activated sludge from a municipal sewage treatment plant (adapted 4 weeks)  

Authors conclude that degradation time depends on the time it takes to increase the number of bacteria with degradation potential

**Test condition:**  
- Two test systems were used with two different types of activated sludge: one used activated sludge from a municipal sewage treatment plant, and the other test used a composite sludge consisting of the aforementioned activated sludge and an extract of river mud (ratio 1:1)  
- Both inocula were adapted to the test compound during 3 weeks  
- 2-Chloraniline and 2-Chloro-4-nitroaniline were used as reference substances  
- Analytical-monitoring: DOC  
- Concentrations applied: 25 mgC/l test substance and 10 and 100 mg/l inoculum  
- Incubation in the dark at 25 °C

**Reliability:** (2) valid with restrictions

Study meets generally accepted scientific principles. Basic data given.

30.01.2003  
(56)

<table>
<thead>
<tr>
<th>Type</th>
<th>aerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>other: soil population proceeding from groundwater of a well located at the border of a TNT manufactury valley</td>
</tr>
<tr>
<td>Contact time</td>
<td></td>
</tr>
<tr>
<td>Degradation</td>
<td>&gt; 85 (±) % after 7 day(s)</td>
</tr>
<tr>
<td>Result</td>
<td></td>
</tr>
<tr>
<td>Deg. product</td>
<td>not measured</td>
</tr>
<tr>
<td>Method</td>
<td>other: see Test condition</td>
</tr>
<tr>
<td>Year</td>
<td>2001</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Result:**  
The initial concentration of nitrocompounds in effluent decreased exponentially with time. Concentrations were far lower in the effluents of the planted than of the non-planted lagoons.
The contribution of photodegradation to the removal rates was less than 10% in the planted and in the non-planted treatments. In the sum nitrotoluenes (among others 4-nitrotoluene) were eliminated to at least 85%. 

**Test condition**: Removal of p-Nitrotoluene was investigated in wetland mesocosms under field conditions in small-scale 4-months field study as a surface-flow, modular system. 

The groundwater of a well located at the border of a TNT manufactury valley was used as influent. The influent contained 30 mg/l of the test substance. 

The effect of 3 treatments were compared: notably planted, non planted and UV-shielded in three different lagoons. Explosives-contaminated groundwater was continuously pumped into the lagoons and a 7-day hydraulic retention time was maintained.

**Reliability**: (2) valid with restrictions 
Study well documented, meets generally accepted scientific principles 

26.01.2003 (57) 

<table>
<thead>
<tr>
<th>Type</th>
<th>Anaerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>domestic sewage, non-adapted</td>
</tr>
<tr>
<td>Concentration</td>
<td>10 mg/l related to Test substance related to</td>
</tr>
<tr>
<td>Contact time</td>
<td>14 day(s)</td>
</tr>
<tr>
<td>Degradation</td>
<td>75 - 100 (±) % after 14 day(s)</td>
</tr>
<tr>
<td>Result</td>
<td>Yes</td>
</tr>
<tr>
<td>Deg. product</td>
<td>Yes</td>
</tr>
<tr>
<td>Method</td>
<td>other: see below Test conditions</td>
</tr>
<tr>
<td>Year</td>
<td>1998</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: substance of the highest purity available</td>
</tr>
<tr>
<td>Deg. products</td>
<td>106-49-0 203-403-1 p-toluidine</td>
</tr>
</tbody>
</table>

**Result**: After 14 days in the aerobic system the UV-Absorption of the test substance was reduced to 0% of that measured at the beginning of the test, whereas in the anaerobic system UV-absorption was reduced to 25%. Under anaerobic conditions the degradation product toluidine was detected. Under aerobic conditions no aromatic amines were observed.

**Test condition**: The biodegradation was tested under aerobic and under anaerobic conditions. 

The test medium and solution was from a primary effluent of a municipal sewage treatment plant in Ithaca, N.Y. 

Each sample was amended with 10 mg/l test substance. Degradation rate was obtained by monitoring the UV-absorption.

**Reliability**: (2) valid with restrictions 
Study well documented, meets generally accepted scientific principles 

26.01.2003 (58) 

<table>
<thead>
<tr>
<th>Type</th>
<th>Anaerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>domestic sewage, non-adapted</td>
</tr>
<tr>
<td>Contact time</td>
<td>14 day(s)</td>
</tr>
<tr>
<td>Degradation</td>
<td>&gt; 90 (±) % after 150 day(s)</td>
</tr>
<tr>
<td>Result</td>
<td>Yes</td>
</tr>
<tr>
<td>Deg. product</td>
<td>Yes</td>
</tr>
<tr>
<td>Method</td>
<td>other: see remarks</td>
</tr>
<tr>
<td>Year</td>
<td>1998</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Remark**: 3 test systems: Reactors installed in a leachate well at an actual landfill, reactors submerged in an artificial leachate well in the laboratory and
<table>
<thead>
<tr>
<th>Type</th>
<th>Aerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>other: mixed culture of bacteria acclimated to mono- and dinitrotoluenes</td>
</tr>
<tr>
<td>Contact time</td>
<td></td>
</tr>
<tr>
<td>Degradation</td>
<td>100 (±) % after 90 minute(s)</td>
</tr>
<tr>
<td>Result</td>
<td></td>
</tr>
<tr>
<td>Deg. product</td>
<td>not measured</td>
</tr>
<tr>
<td>Method</td>
<td>other: see Test condition</td>
</tr>
<tr>
<td>Year</td>
<td>1999</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
<tr>
<td>Result</td>
<td>The removal of 4-nitrotoluene was 100 % in all phases independent from hydraulic retention time (10 - 60 min), nitrotoluene load (0.45 - 3.5 kg/m3 x d), and COD load (0.9 - 7.6 kg COD/m3 x d).</td>
</tr>
<tr>
<td>Test condition</td>
<td>A pilot-scale field demonstration was conducted with a continuously operating aerobic, biological fluidized bed reactor (FED) system that treats groundwater contaminated with nitrotoluenes. This demonstration consisted of seven evaluation periods (phases) for which the conditions were varied. The seven phases included five different feed flow rates and two different feed water compositions.</td>
</tr>
<tr>
<td>Reliability</td>
<td>(2) valid with restrictions</td>
</tr>
</tbody>
</table>

30.01.2003

<table>
<thead>
<tr>
<th>Type</th>
<th>Aerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>other: Mycobacterium strain HL 4-NT-1</td>
</tr>
<tr>
<td>Deg. product</td>
<td>Yes</td>
</tr>
<tr>
<td>Method</td>
<td>other: e.g. enzyme tests</td>
</tr>
<tr>
<td>Year</td>
<td>2000</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

| Remark     | A Mycobacterium strain was used which was able to grow on 4-nitrotoluene as the sole source of nitrogen, carbon and energy. |
| Result     | Pathway for degradation of 4-nitrotoluene via 2-amino-4-methylphenol elucidated (extradiol-like ring cleavage) |
| Reliability | (2) valid with restrictions |

26.01.2003

<table>
<thead>
<tr>
<th>Type</th>
<th>Aerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>other: aquatic microcosms</td>
</tr>
<tr>
<td>Concentration</td>
<td>20 mg/l related to Test substance</td>
</tr>
<tr>
<td>Contact time</td>
<td>36 day(s)</td>
</tr>
<tr>
<td>Degradation</td>
<td>(±) % after</td>
</tr>
<tr>
<td>Result</td>
<td>other: Calculated half-life in aquatic microcosm: 12 days (non-sterile)</td>
</tr>
<tr>
<td>Deg. product</td>
<td>not measured</td>
</tr>
<tr>
<td>Method</td>
<td>other: see test conditions</td>
</tr>
<tr>
<td>Year</td>
<td>1999</td>
</tr>
<tr>
<td>Test substance</td>
<td></td>
</tr>
</tbody>
</table>
**GLP** : No  
**Test substance** : other TS: proceeding from a wastewater sample  

<table>
<thead>
<tr>
<th>Result</th>
<th>Percentage decrease of the TS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- Sterile: 25 %</td>
</tr>
<tr>
<td></td>
<td>- Nonsterile (microbial consortium): 38 %</td>
</tr>
<tr>
<td></td>
<td>- Nonsterile half-strength: 32 %</td>
</tr>
<tr>
<td></td>
<td>Calculated half-life in the nonsterile microcosm: 12 days</td>
</tr>
</tbody>
</table>

| Test condition | Samples from wastewater were collected. They were incubated in 3 different microcosms systems during 36 days in an sterile and nonsterile and half-strength system.  
A combination of aerobic and anaerobic conditions was employed.  
| Reliability | (2) valid with restrictions  
Study meets generally accepted scientific principles. Basic data given.  
| Date | 06.08.2003 (48)  

| Type | Aerobic  
| Inoculum | other: microbial community from contaminated groundwater  
| Contact time |  
| Degradation | > 99 (±) % after 11 day(s)  
| Result |  
| Deg. product | No  
| Method | other: see remarks  
| Year | 1999  
| GLP | No  
| Test substance | no data  

| Result | Initial microbial community was able to degrade 4-nitrotoluene. After the second addition of substrate, 4-nitrotoluene remained undegraded for 3 days. This observation might depend on an extended lag period due to an increase in nitrate concentration or depletion of background concentration originated from the first round of degradation of 4-nitrotoluene. At day 63 of the experiment p-nitrotoluene was degraded to > 99 %.  
| Test condition | For the test sealed flasks, shaken and incubated at 25°C, containing contaminated groundwater and salts were amended with p-nitrotoluene or other nitroaromatics. Groundwater originated from a Swedish location where ammunition destruction by open burning has been performed for more than 40 years.  
| Reliability | (2) valid with restrictions  
Study meets generally accepted scientific principles. Basic data given.  
| Date | 30.01.2003 (43)  

| Type | Anaerobic  
| Inoculum | other: Geobacter metallireducens  
| Deg. product | not measured  
| Method | other: see remarks  
| Year | 1999  
| GLP | No  
| Test substance | other TS: minimum purity analytical grade, not further specified  

| Remark | Reduction of nitroaromatic compounds by Fe (II) or by hydroquinone moieties was tested. The reduction kinetics were investigated in sterile batch systems as well as in columns containing either FeOOH-coated sand and a pure culture of the iron-reducing bacterium Geobacter metallireducens or ferrogenic consortia in aquifer sediments.  
| Result | The Qc (competition coefficient) was determined using the measured zero-order rate constant for the nitroaromatic compounds and for the reference compound 4-chloronitrobenzene. The zero-order rate constant was defined by the authors to be the difference between the concentration of the test substance in influent
minus its concentration in the effluent. It is concluded that even under abiotic conditions (poly)nitroaromatic compounds are reduced by Fe(II) present at the surface of Fe(III)(hydr)oxides or by hydroquinone moieties of (natural) organic matter.

**Reliability**

(2) valid with restrictions

Study meets generally accepted scientific principles. Basic data given.

26.01.2003

**Type**

Aerobic

**Inoculum**

other: Azetobacter agilis

**Concentration**

132 mg/l related to Test substance

related to

**Contact time**

**Degradation**

100 (±) % after 36 hour(s)

**Result**

Deg. product: No

Method: other: simulation of a munition industrial sewage treatment plant

Year: 1971

GLP: No

Test substance: no data

**Remark**

Degradation products not detectable because concentration of degradation products were smaller than limit of detection

**Result**

p-Nitrotoluene concentration in effluent of first treatment step was less than 1 mg/l (duration: 36 h)

**Test condition**

- The bacteria (Azetobacter agilis) were isolated from a compost soil sample suspended in nutrient solution containing 130 mg/l 2,4,6-trinitrotoluene
- Incubations were done in a model 2 step wastewater treatment plant (both steps aerobic) although the inoculum does not represent the activated sludge of an ordinary wastewater treatment plant
- For incubation 4-nitrotoluene was dissolved in bidestilled water, filtered, and 1g/l K2HPO4, 5 g/l Glucose, and 5 mg/l Na2MoO4 x 2 H2O were added
- Additional nutrients were supplied daily directly into the model wastewater treatment plant
- Temperature 25 °C
- Spectrometric analysis after reduction and azo coupling at 490 nm

**Reliability**

(2) valid with restrictions

Study meets generally accepted scientific principles. Basic data given.

06.08.2003

**Type**

Aerobic

**Inoculum**

predominantly domestic sewage

**Concentration**

1.8 mg/l related to Test substance

related to

**Contact time**

**Degradation**

0 (±) % after 20 day(s)

**Result**

Deg. product: not measured

Method: other: Test corresponds to a Closed Bottle Test (OECD 301 D)

Year: 1973

GLP: No

Test substance: no data

**Result**

At 24 ml of the test substance, corresponding to 5.3 mg/l, 47 %, 55 %, and 61 % degradation were achieved during 5, 10 and 20 days, respectively. All results are listed below:

<table>
<thead>
<tr>
<th>Conc. (mg/l)</th>
<th>Degradation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>47</td>
</tr>
<tr>
<td>10 days</td>
<td>55</td>
</tr>
<tr>
<td>20 days</td>
<td>61</td>
</tr>
</tbody>
</table>
Test condition:
- Tested concentrations: 1.8, 5.3, 17.8, 53.3 mg/l
- Inoculum concentration: 1ml/l
- Reference substance: phenol
- Analytical-monitoring: BOD

Reliability:
(2) valid with restrictions
Test procedure according to guideline study. Basic data given
06.08.2003 (64)

Type: Aerobic
Inoculum: other: activated sludge adapted and non-adapted
Contact time:
Degradation: < 50 (±) % after 28 day(s)
Result:
Deg. product:
Method:
other: 3 Procedures (see below)
Year: 1985
GLP: no data
Test substance:
other TS: > 99.5 % Purity

Method:
3 methods were applied:
1) Revised OECD test, 1971 (Determination of the Biodegradability of Anionic Surface Active Agents)

Result:
When the inoculum was not adapted, the half-life was greater than 4 weeks; with adapted inoculum the half-life was 2 to 3 weeks. Author’s remark: "Not-readily biodegradable"

Reliability:
(3) invalid
Insufficient documentation: no details on origin and density of inoculum, and on tested concentrations and test conditions

06.08.2003 (30)

Type: Aerobic
Inoculum: other: suspension of Niagara silt loam
Concentration: 8 mg/l related to Test substance
related to
Contact time: 64 day(s)
Degradation: (±) % after
Result: other: under test conditions no significant ring cleavage detected
Deg. product: not measured
Method: other: Test on biodegradation in soil
Year: 1966
GLP: No
Test substance: no data
Remark:
- Possible unsuitability of the test conditions for active microorganisms
- Small inoculum selected to avoid problems during measuring and due to release of aromatics from soil
- Study actually measured cleavage of the aromatic ring and is hampered when the aromatic ring is incorporated in biomolecules e.g. amino acids which might have been accumulated by the microorganisms

Result: The test substance was still detectable after 64 days with UV-spectometry.
The nitro compounds were quite difficult to degrade under the test conditions.

**Test condition**
- Nutrient solution contained inorganic nutrients and the test substance as the sole carbon source.
- 1 ml 1% suspension of Niagara silt loam was added to closed bottle containing 40 ml of nutrient solution
- Bottles were incubated in the dark at 25 °C
- Contact time was up to 64 days including adaptation period
- Ring cleavage was checked by decrease of absorbance at 285 nm, measured after centrifugation in the supernatant. Precipitates and supernatants were returned to the appropriate reaction bottles
- Control tests were performed with identical samples except that 8 mg of HgCl2 and 5E-7 M Tween 80 were added into each bottle
- Tests for toxicity of test substances to microorganisms were done on identical samples but using glucose as an additional source of carbon

**Reliability**
(3) invalid
Design of study chosen to derive some general conclusions on biodegradability but not to examine the biogradability of individual compounds in detail. Some important data not supplied, see Remarks

<table>
<thead>
<tr>
<th>06.08.2003</th>
<th>(65)</th>
</tr>
</thead>
</table>

**Type**
Anaerobic

**Inoculum**
other: OSU-G, 8

**Concentration**
90 mmol/l related to Test substance related to

**Contact time**

**Degradation**
ca. 0 (±) % after 24 hour(s)

**Result**

**Deg. product**
other: see test conditions

**Method**

**Year**
1997

**GLP**
No

**Test substance**
other TS: no purity given (origin: (Aldrich Chemicals Co.)

**Result**
Biodegradation rate: a normalized concentration to the initial concentration (C/C0) after 24 h was calculated to approx. 1 indicating no biodegradation under test conditions.

**Test condition**
The reduction rate by isolated bacteria, thermodynamic data and molecular electrostatic potential values for each test compound were measured. For testing biodegradation the following conditions were applied:
- Test system: anaerob batch bioassay, 37 °C
- Escherichia coli from goat rumen

**Reliability**
(3) invalid
Unsuitable test system. The test conditions are not representative for the environment

<table>
<thead>
<tr>
<th>06.08.2003</th>
<th>(66)</th>
</tr>
</thead>
</table>

**Type**

**Inoculum**
other: specific isolated bacteria from different soils

**Contact time**

**Degradation**
(±) % after

**Result**

**Deg. product**
other: biodegradation influenced by several soil-dependent factors

**Method**

**Year**
2000

**GLP**
No

**Test substance**

**Reliability**
(4) not assignable
Literature not available

| 06.08.2003 | (67) |
Type: Aerobic
Inoculum: other: contained in river and sea water samples
Deg. product: 
Method: 
Year: 1995
GLP: 
Test substance: 

Result: Results with light
pH=5 16 mg/l residual after 1h: 104%
residual after 5d: ----
pH=7 16 mg/l residual after 1h: 99%
residual after 5d: 89%
pH=9 16 mg/l residual after 1h: 93%
residual after 5d: ----

Results without light
pH=5 16 mg/l residual after 5d: 93%
pH=7 16 mg/l residual after 5d: 88%
pH=9 16 mg/l residual after 5d: 89%

Test condition: Degradation was observed at pH 5,7,9 after 1 hour and 5 days under different conditions: degradation with sunlight and degradation in a cool and dark place.
Derivates of the nitrobenzenes were contained in the river and sea sample with 1.0 ng/ml. 4-Nitrotoluene was not the sole source of organic carbon.

Reliability: (3) invalid
Insufficient documentation
06.08.2003

3.6 BOD5, COD OR BODS/COD RATIO

3.7 BIOACCUMULATION

Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 42 day(s) at °C
Concentration: .1 mg/l
BCF: 3.7 - 7.2
Elimination: 
Method: other: see below
Year: 1992
GLP: no data
Test substance: no data

Method: Method: "Bioaccumulation test of chemical substance in fish and shellfish" stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974, Order of the Prime Minister, the Minister of Health and Welfare, the MITI No. 1). This guideline corresponds to "305C, Bioaccumulation: Degree of Bioconcentration in Fish" stipulated in the OECD Guidelines for Testing of Chemicals (May 12, 1981).

Remark: conc. 0.01 mg/l... BCF 4.5 - 8.0
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
04.12.2002

Species: Poecilia reticulata (Fish, fresh water)
### OECD SIDS 4-NITROTOLUENE

#### 3. ENVIRONMENTAL FATE AND PATHWAYS

**ID:** 99-99-0  
**DATE:** 09.09.2004

**Exposure period:** at °C

**Concentration:** no data

**BCF:** 27

**Elimination Method:** other: method described by Canton et al. (1975)

**Year:** 1985

**GLP:** No

**Test substance:** no data

**Remark:** BCF: 27 (experimentally determined)

### Result:

BCF: 39 (theoretical value, calculated from log Pow-value=2.4)

**Reliability:** (2) valid with restrictions

Acceptable calculation method. To the experimental value, the method used is given without further experimental details

**Flag:** Critical study for SIDS endpoint

09.12.2002

**Species:** Poecilia reticulata (Fish, fresh water)

**Exposure period:** 3 day(s) at °C

**Concentration:** 7.4 mg/l

**BCF:** ca. 234

**Elimination Method:** other: see Test condition

**Year:** 1987

**GLP:** no data

**Test substance:** other TS: >98% purity

**Result:** BCF-Value is related to the weight

Results are given in the original reference as log BCF: log BCF = 2.37 +/- 0.05

**Test condition:** Tested concentration corresponds to 1:5 of the LC50 measured value with the same fish species (see chapter 4.1). The experiment was carried out in glass jars with 1 l test solution and 9 females guppies. The test solution was renewed every day. Exposure time: in a preliminary experiment it was established that the uptake of the test substances proceeded very fast, thus enabling a short-term assay of max. 3 days. The test concentrations were monitored with gaschromatography.

**Reliability:** (3) invalid

Invalid: Not a guideline study. Accumulation factors (no steady state reported) were calculated for fat content. Concentrations used were 1/5 dilution of the LC50 concentration and thus too high for BCF determination.

30.01.2003

**BCF:** 39.26

**Elimination Method:** other: calculated

**Year:** 1983

**GLP:** no data

**Test substance:** no data

**Reliability:** (2) valid with restrictions

Acceptable calculation method

17.12.2002

**Species:** Carassius auratus (Fish, fresh water)

**Exposure period:** at °C

**Concentration:** no data

**BCF:** < 1

**Elimination:** no data
### 3. ENVIRONMENTAL FATE AND PATHWAYS

**Method**: Bioaccumulation to *Carassius auratus* in continuous-flow

**Year**: 2002

**GLP**: no data

**Test substance**: no data

**Reliability**: (4) not assignable

- Yi et al. (1998): Literature not available

**Species**: *Carassius auratus* (Fish, fresh water)

**Exposure period**: 20 day(s) at 15 °C

**Concentration**:

**BCF**: 234

**Elimination Method**:

**Year**: 1999

**GLP**: no data

**Test substance**: no data

**Remark**: Scope of the study was to compare Semipermeable membrane devices (SPMD) filled with triolein with goldfish to check whether SPMD is suited to simulate Goldfish in bioaccumulation experiments.

**Test condition**:

- sterilized gold fish reared under laboratory conditions 2 weeks before experiment
- Flow through
- 40 Fish in 70 l exposure chamber
- Controls 15 fish in 70 l exposure chamber
- Incubation at pH 7.5
- During the incubation, samples were taken frequently and extracted

**Reliability**: (4) not assignable


### 3.8 ADDITIONAL REMARKS

**Memo**: Degradation of nitroaromatics by superoxide radicals

**Remark**: Study elucidated the role of the manganese peroxidase (MnP) from white-rot fungi.

50 µM of the test substance were incubated for 96 hours at 20 °C in a system containing oxalate and Mn(III), under aerobic conditions. The reaction mixture was sterilized. Quantitative determination of nitroaromatic compounds was performed by reversed phase HPLC.

**Result**: 42.8% (+/- 5.2) of the initial concentration of 4-nitrotoluene were transformed presumably by superoxide radicals formed from the reaction of cleavage of oxalate to .COO- radicals. These radicals reacted with oxygen to yield superoxide radicals which in water become protonated to effective .HOO radicals.

**03.02.2003**

(32)
### 4.1 ACUTE/PROLONGED TOXICITY TO FISH

**Type**: Semistatic  
**Species**: Oryzias latipes (Fish, fresh water)  
**Exposure period**: 48 hour(s)  
**Unit**: mg/l  
**LC50**: 74  
**Limit test**: No  
**Analytical monitoring**: no data  
**Method**: other: Japanese Industrial Standard (JIS K 0102-1986-71) "Testing methods for industrial waste water"  
**Year**: 1992  
**GLP**: no data  
**Test substance**: no data  
**Test condition**:  
- Orange-red killifish (Oryzias latipes) was obtained from Nakashima fish farm, Daimyojin Nagasu-cho Tamana-gun Kumamoto 869-01 Japan  
- After external desinfection, the fish were reared in a flow through system for 3 - 5 weeks  
- Fish were reared in an acclimatization tank for 28 d at 25 +/- 2 °C  
- Water was groundwater from the Kurume Research Laboratories  
- Water temperature, pH, dissolved oxygen were continuously measured  
- Total hardness, COD, chloride, and other parameters were measured every 6 months  
- Incubation of each 10 fish in round glass vessels containing 4 l of liquid each  
- Incubation temperature 25 +/- 2 °C  
- 48 h LC50 was estimated by Doudoroff method or Probit method  

**Reliability**: (2) valid with restrictions  
**Guideline study**  
**Flag**: Critical study for SIDS endpoint  
**22.10.2003 (9)**

**Type**: Static  
**Species**: Carassius auratus (Fish, fresh water)  
**Exposure period**: 48 hour(s)  
**Unit**: mg/l  
**LC50**: ca. 10.5  
**Limit test**:  
**Analytical monitoring**: no data  
**Method**: other: ISO/DIS 7346/1.2.3 (1982)  
**Year**: 1997  
**GLP**: no data  
**Test substance**: other TS: >=98%  
**Test condition**:  
- Standard dilution water with Ca hardness of 250 mg/l obtained by addition of 294 mg/l CaCl2*2H2O, 123.3 mg/l MgSO4*7H2O, 63 mg/l NaHCO3, 5.5 mg/l KCl; pH 7.8 +/- 0.2, oxygen saturation > 90 %  
- Fish length 30 +/- 5 mm, acclimated at least 7 d before start of incubation  
- No food during incubation  
- 23 +/- 1 °C  
- Each incubation vessel 10 l, 7 fish  
- Daily check of oxygen concentration, pH, temperature  
- Quality criteria:  
  - oxygen concentration > 60 % of saturation  
  - TS concentration not significantly changed  
  - Mortality or number of fish with abnormal behaviour does not exceed 10 % in controls  
  - toxicity reference K2Cr2O7
Reliability : (2) valid with restrictions
Guideline study without detailed documentation. Experimental details missing

Flag : Critical study for SIDS endpoint
01.08.2003

Type : flow through
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : 49.7
Limit test : No
Analytical monitoring : Yes
Year : 1983
GLP : no data
Test substance : other TS: 95 to 99% purity


Remark : The test concentrations were analytically monitored with the modified "automated colorimetric micro determination" method described by Gales (1975).
The substance recovery was ca. 101%.

Test condition : The test was performed at 20 °C.
Several glass pickle jars containing 15 l of test solution and 10 fish per jar were used at each concentration level.
At least five concentrations plus a control were tested.
The parameters of the test system: pH, dissolved oxygen and temperature were monitored during the test.

Reliability : (2) valid with restrictions
Test procedure according to national standards. Basic data given.
Flag : Critical study for SIDS endpoint
02.12.2003

Type : Semistatic
Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : 40.5
Limit test : No
Analytical monitoring : no data
Method : other: see test conditions
Year : 1996
GLP : no data
Test substance :

Test condition : pH = 7 - 7.5
Temperature: 15 - 18 °C
20 l test water (tap water dechlorinated) was used with 10 fishes in each tank
Solvent: aceton 0.05 - 0.1 %
At least 5 concentrations levels were tested

Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
04.12.2003
Type : other: semistatic, renewal at 12 hours
Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : ca. 40
Limit test : No
Analytical monitoring : No
Method : other: see test conditions
Year : 1997
GLP : No
Test substance : no data

Test condition : - Fish were purchased and were kept under laboratory conditions for more than 2 weeks
- Fish: about 5 g, about 5 cm
- 10 fish in 16 l of test water, water renewal every 12 h
- Water temperature 20 +/- 1 °C
- 6 replicates for each concentration

Reliability : (2) valid with restrictions
Basic data given

Flag : Critical study for SIDS endpoint
04.12.2003

Type : other: not specified
Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 
Unit : mg/l
LC50 : 68
Limit test : No
Analytical monitoring : No
Method : other: see test conditions
Year : 2002
GLP : no data
Test substance : other TS: > 98 % (Purchased from E. Merck, Darmstadt, Germany)

Result : In Table 4 of their publication, Yen et al. report the LC50 to be 0.684 mg/l. After request, one of the authors (Wang 2003) communicated that the reported values are below the observed values by a factor of 100. Thus, the correct LC50 is 68 mg/l

Test condition : - Carp were fed with yeast
- Acclimatization in aquaria for 2 weeks under conditions similar to those under which the test performed;
- Temperature 25 +/- 1 °C, water (pH 6.6, DO 4.9 mg/l, hardness 215 mg/l as CaCO3);
- 4 Carp, 2 - 6 cm, were introduced in each 10 l beaker containing 5 l of test chemicals with 5 different concentrations (10, 50, 100, 200, 500 µg/ml)
- Each concentration was tested in duplicate
- Test period > 48 h
- Calculations according to Spearmann-Karber

Reliability : (2) valid with restrictions
Basic data given. Restrictions of the method are:
- Test period not given (Personal communication Wang 2003: 96 h)
- 4 Fish used instead of at least 7 fish as recommended by the OECD guideline 203
- Temperature during the test was higher (25 °C) than the one suggested by OECD and other current guidelines (20 - 24 °C)
- Yeast is not a standard food for carp
- Length of fish (2- 6 cm) varied more than recommended in OECD guideline

Flag : Critical study for SIDS endpoint
04.12.2003
**OECD SIDS**

**4-NITROTOLUENE**

**4. ECOTOXICITY**

**DATE: 09.09.2004**

| Type: Static | Species: Poecilia reticulata (Fish, fresh water) |
| Exposure period: 96 hour(s) | Unit: mg/l |
| LC50: 49 | EC50: 21 |
| Limit test: No | Analytical monitoring: Yes |
| Method: other: Analogy with the OECD proposal to short-term toxicity tests performed on fish (Poecilia reticulata) (1979) |
| Year: 1985 | GLP: no data |
| Test substance: other TS: > 99.5 % Purity |

**Remark**

- EC50 is the measured behaviour. It is not specified which endpoint was observed.
- The stability of the compound was analysed before testing.

| Result: Nominal concentrations. |

| Test condition: |
| Age of fish 3 - 4 weeks |
| 10 Organisms per group |
| 1 l Testvolume per group |
| No food during incubation |
| Temperature 23+/- 2 °C |
| Lighting circadic |
| Culturing media (1 l bidest. water containing 100 mg/l NaHCO3, 200 mg/l CaCl2*2H2O, 20 mg/l KHCO3, 180 mg/l MgSO4*7H2O) |
| Endpoints motality and immobility |

**Reliability**: (2) valid with restrictions

Comparable to guideline study, without detailed documentation.

**06.08.2003**

| Type: Static | Species: Pimephales promelas (Fish, fresh water) |
| Exposure period: 96 hour(s) | Unit: mg/l |
| LC50: 49.9 | Limit test: No |
| Analytical monitoring: no data |
| Year: 1979 | GLP: no data |
| Test substance: |

**Method**

"Methods for Acute Toxicity Testing with Fish, Macroinvertebrates, and Amphibians," Ecological Research Series, EPA-66013-75-009, National Environmental Research Center, Office of Research and Development, U.S. Environmental Protection Agency, Corvallis, OR, 1975

**Remark**

The test was performed according to the procedure as described in the guideline method (s.above) with the exception that the temperature was maintained at 20°C

**Result**

Effect values were calculated with the probit analysis

**Reliability**

(2) valid with restrictions

Test procedure according to national standards with some restrictions.

**20.02.2003**
<table>
<thead>
<tr>
<th>Type</th>
<th>other: not specified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Poecilia reticulata (Fish, fresh water)</td>
</tr>
<tr>
<td>Exposure period</td>
<td>14 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>LC50</td>
<td>36.9</td>
</tr>
<tr>
<td>Limit test</td>
<td>No</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td>other: according to the method described by Könemann (1981)</td>
</tr>
<tr>
<td>Year</td>
<td>1986</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: &gt;98% purity</td>
</tr>
</tbody>
</table>

Remark: Verhaar et al. (1992), Verhaar et al. (1996), Katritzky et al. (2001), and Ivanciuc (2002) cite apparently the work of Maas-Diepeveen and van Leeuwen (1986)

Result: Results are given in the original reference of Deneer et al. (1987) as log LC50: log LC50 = 2.43 (LC50 µmol/l)

Test condition:
- Temperature: 21 - 23 °C
- pH = 6.8 - 7.2
- Endpoint: mortality

Reliability: (2) valid with restrictions

<table>
<thead>
<tr>
<th>Type</th>
<th>flow through</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Pimephales promelas (Fish, fresh water)</td>
</tr>
<tr>
<td>Exposure period</td>
<td>96 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>LC50</td>
<td>23.8</td>
</tr>
<tr>
<td>Limit test</td>
<td>No</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>Yes</td>
</tr>
<tr>
<td>Method</td>
<td>other: Method by US-EPA 1975 (EPA-660/3-75-009)</td>
</tr>
<tr>
<td>Year</td>
<td>1984</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: &gt;95% purity</td>
</tr>
</tbody>
</table>

Remark: In some of the studies the method is described but not used to test the effects of 4-nitrotoluene on Pimephales promelas

Result: The first study in this row of citations (Hall, Kier and Phipps 1984) reports a -log(LC50) of 3.76 which equals 23.8 mg/l. However, they (Hall, Kier and Phipps 1984) cite a paper of Bailey and Spanggord (1984), which reports a LC50 of 49.7 mg/l [equals -log(LC50) of 3.44]. In the same year, one of the above mentioned authors reports 49.7 mg/l in another study [Phipps GL et al. (1984) J Water Pollut Control Fed 56 (6): 725 - 758]. Thus it is assumed that the 23.8 mg/l stem from a citation error.

Reliability: (3) invalid

<table>
<thead>
<tr>
<th>Type</th>
<th>Static</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Brachydanio rerio (Fish, fresh water)</td>
</tr>
<tr>
<td>Exposure period</td>
<td>96 hour(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>LC0</td>
<td>75</td>
</tr>
<tr>
<td>LC50</td>
<td>87</td>
</tr>
<tr>
<td>LC100</td>
<td>100</td>
</tr>
<tr>
<td>Limit test</td>
<td>No</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td>other: Letale Wirkung beim Zebrabaerbling, UBA-Verfahrensvorschlag, Mai 1984, Letale Wirkung beim Zebrabaerbling Brachydanio rerio LC0, LC50, LC100, 48-96h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>other: not specified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Poecilia reticulata (Fish, fresh water)</td>
</tr>
<tr>
<td>Exposure period</td>
<td>14 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>LC50</td>
<td>36.9</td>
</tr>
<tr>
<td>Limit test</td>
<td>No</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td>other: according to the method described by Könemann (1981)</td>
</tr>
<tr>
<td>Year</td>
<td>1986</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: &gt;98% purity</td>
</tr>
</tbody>
</table>

Remark: Verhaar et al. (1992), Verhaar et al. (1996), Katritzky et al. (2001), and Ivanciuc (2002) cite apparently the work of Maas-Diepeveen and van Leeuwen (1986)

Result: Results are given in the original reference of Deneer et al. (1987) as log LC50: log LC50 = 2.43 (LC50 µmol/l)

Test condition:
- Temperature: 21 - 23 °C
- pH = 6.8 - 7.2
- Endpoint: mortality

Reliability: (2) valid with restrictions

Basic data given
<table>
<thead>
<tr>
<th>Year</th>
<th>1985</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>as prescribed by 1.1 - 1.4</td>
</tr>
</tbody>
</table>

**Result**
- LC50 value is the geometric mean
- 10 fish per test concentration in 5 l water were applied
- Solvent for preparation of test substance in water: acetone 1:1
- Concentrations tested: 1, 10, 17.8, 56.2, 75 und 100 mg/l

**Reliability**
- (4) not assignable
- Only raw data available

**Test condition**
- 10 fish per test concentration in 5 l water were applied
- Solvent: acetone 1:1
- Concentrations tested: 1, 10 und 100 mg/L

**Species**
- Leuciscus idus (Fish, fresh water)

**Exposure period**
- 96 hour(s)

**Unit**
- mg/l

**NOEC**
- > 10

**Limit test**
- No

**Analytical monitoring**
- No

**Method**
- other: see below test conditions

**Year**
- 1985

**GLP**
- No

**Test substance**
- as prescribed by 1.1 - 1.4

<table>
<thead>
<tr>
<th>Type</th>
<th>other: not specified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Oryzias latipes (Fish, fresh water)</td>
</tr>
<tr>
<td>Exposure period</td>
<td>48 hour(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>LC50</td>
<td>69</td>
</tr>
<tr>
<td>Limit test</td>
<td>No</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>no data</td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>1986</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Remark**
- Yoshioka Y, Ose Y, Sato T (1986) [Testing and evaluation of chemical toxicity on Tubifex. Elsei-Kagaku 32: 308 - 311]: Only abstract and tables in English, the rest in Japanese

**Reliability**
- (4) not assignable
- Not assignable/Original reference not translated

**Type**
- other: not specified

**Species**
- Pimephales promelas (Fish, fresh water)

**Exposure period**
- 96 hour(s)

**Unit**
- mg/l
<table>
<thead>
<tr>
<th>Result</th>
<th>The experimental results are cited from unspecified source. They are given as log LC50 (unit of LC50 not given): log LC50 = 3.44. The calculated value is slightly lower: 3.62.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>The publication of Ramos et al. (1998) is not available</td>
</tr>
<tr>
<td>Reliability</td>
<td>(4) not assignable</td>
</tr>
<tr>
<td>Secondary literature not specified</td>
<td></td>
</tr>
</tbody>
</table>

| Type | other: not specified |
| Species | Poecilia reticulata (Fish, fresh water) |
| Exposure period | 96 hour(s) |
| Unit | mg/l |
| LC50 | 29.32 |
| Limit test | |
| Analytical monitoring | no data |
| Method | other: not specified |
| Year | 1998 |
| GLP | no data |
| Test substance | no data |

| Result | Gunatilleka et al. (1999) give the results as \(-\log \text{LC50} = 3.67\) and report that this value is taken from Ramos et al. (1998). |
| Source | The publication of Ramos et al. (1998) is not available |
| Reliability | (4) not assignable |
| Secondary literature | |

| Type | other: not specified |
| Species | Pimephales promelas (Fish, fresh water) |
| Exposure period | 96 hour(s) |
| Unit | mg/l |
| LC50 | 33 calculated |
| Limit test | |
| Analytical monitoring | no data |
| Method | other: calculation |
| Year | 2001 |
| GLP | no data |
| Test substance | no data |

| Method | Using a cytosolic extract from the liver of rainbow trout, it was quantified the binding of the test substance to the fish estrogen receptor |
4. ECOTOXICITY

ID: 99-99-0
DATE: 09.09.2004

Reliability : (4) not assignable
Unsuitable test system for the hazard assessment of chemicals. Test with liver of rainbow trout

03.02.2003

Type
Species : other: see below
Exposure period
Unit : mg/l
LC50 : 19 - 49.9
Method
Year : 1990
GLP
Test substance :

Result : Measured LC50 concentrations were obtained from Aquire Database. They were compared with the predicted LC50 by using QSAR-models. The duration of the test to determine LC50 and other details about the test system are not given.

For Fathead minnow (Pimephales promelas):
LC50 measured: 19.0, 49.7, 49.9 mg/l
LC50 calculated:80.36 mg/l

For Rainbow trout (Oncorhynchus mykiss):
LC50 measured: not reported
LC50 calculated:73.02 mg/l

For Bluegill (Lepomis macrochirus):
LC50 measured: not reported
LC50 calculated:75.47 mg/l

Reliability : (4) not assignable
Secondary literature

03.02.2003

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : 11.8
Analytical monitoring : Yes
Year : 1983
GLP : no data
Test substance :

Method :
"Methods for Acute Toxicity Testing with Fish, Macroinvertebrates, and Amphibians," Ecological Research Series, EPA-66013-75-009, National Environmental Research Center, Office of Research and Development, U.S.

Remark : The invertebrate species were from stocks reared in the laboratory at SRI International where the study was performed.

Test condition :
- Test water:
  Dechlorinated tap water was used to prepare stock and test solutions of the test substance and to rear and maintain the test animals. The water treatment system comprised several 75 µm particle filters and several 0.042 m3 activated carbon columns. The means of hardness, pH, alkalinity, conductivity, and residual chlorine of water samples were collected monthly during a significant portion of the study.
  Test temperature:
The temperature was 20 °C
- Food:
  During the static test no food was provided to the organisms.
- Stock solution:
  The stock solution was prepared by dissolving a measured amount of chemical in a known volume of water. No carrier was used. The mixing time was about 24 h. The stock solution was filtered through a 5 µm filter and analyzed for the chemical.

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Source: Matheson Chemical Co.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reliability</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td>Flag</td>
<td>Critical study for SIDS endpoint</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date: 30.07.2003</th>
</tr>
</thead>
</table>

**Test condition:**
- Daphnias were 1 day old
- Culturing and test medium: NaHCO₃ 100 mg/l, CaCl₂·2H₂O 200 mg/l, KHCO₃ 20 mg/l, MgSO₄·7H₂O 180 mg/l
- No food during incubation
- 25 organisms per 1 litre of test medium
- Incubation temperature 19 +/- 1 °C
- Circadian lighting
- Endpoint: Mortality/immobility

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(2) valid with restrictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flag</td>
<td>Comparable to guideline study, without detailed documentation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date: 06.08.2003</th>
</tr>
</thead>
</table>

**Test condition:**
- Test organisms sieved to obtain animals less than 24 h old
- Standard dilution water with Ca hardness of 250 +/- 25 mg/l obtained by addition of 294 mg/l CaCl₂·2H₂O, 123.3 mg/l MgSO₄·7H₂O, 65 mg/l NaHCO₃, 5.8 mg/l KCl; pH 7.8 +/- 0.2, oxygen saturation > 90 %
- No food during incubation
- 20 +/- 2 °C
- Each incubation vessel up to 20 Daphnias, at least 2 ml incubation medium per animal
- Daily check of oxygen concentration, pH, temperature
- Quality criteria:
  - Oxygen concentration at least 2 mg/l
- Immobilization does not exceed 10 % in controls
- Toxicity reference K2Cr2O7 24 h EC50 0.6 - 1.7 mg/l
- Endpoint immobilization
- Probit analysis of data

Reliability:
(2) valid with restrictions
Guideline study without detailed documentation. Experimental details missing

Flag:
01.08.2003
Critical study for SIDS endpoint

Type:
Static

Species:
Daphnia magna (Crustacea)

Exposure period:
24 hour(s)

Unit:
mg/l

EC50:
ca. 6.4

IC50:
ca. 11 calculated

Limit Test:
No

Analytical monitoring:
No

Method:
other: see Test condition

Year:
1995

GLP:
no data

Test substance:
no data

Remark:
QSAR results compared with experimental result. Zhao, Cronin, and Dearden (1998), and Zhao and Wang (1995) cite the work of Zhao, He, and Wang (1995)

Result:
Measured result was reported to be log 1/IC50 = 4.33, calculated result was reported to be log 1/IC50 = 4.08.

Test condition:
- Daphnids were cultured parthenogenetically in an environmental chamber at 22 +/- 1 °C
- Photoperiod 14 hours, dark 10 hours
- For culturing a green algae diet was fed
- 6 to 24 hours old daphnids were used for toxicity test
- Incubation 24 hours at 22 +/- 1 °C, algae are not fed
- 5 replicates for every concentration
- Results were considered valid, when oxygen concentration was > 60 % of saturation, and if immobilization in controls was zero at the end of experiment
- Endpoint: immobilization

Reliability:
(2) valid with restrictions
Basic data given

Flag:
16.10.2003
Critical study for SIDS endpoint

Type:
Static

Species:
Daphnia pulex (Crustacea)

Exposure period:
4 hour(s)

Unit:
mg/l

LC 50:
41

Analytical monitoring:
no data

Method:

Year:
2002

GLP:
no data

Test substance:
other TS: > 98 % (Purchased from E. Merck, Darmstadt, Germany)

Result:
In Table 4 of their publication, Yen et al. report the LC50 to be 0.407 mg/l. After request, one of the authors (Wang 2003) communicated that the reported values are below the observed values by a factor of 100. Thus, the correct LC50 is 41 mg/l.

Test condition:
- Daphnia were fed with yeast in a 224 (diameter)x 46 (height)-cm circular plastic pool;
- acclimatization in aquaria for 2 weeks under conditions similar to those under which the test performed;
- temperature 25 ± 1 °C, water (pH 6.6, DO 4.9 mg/l, hardness 215 mg/l as CaCO3);
- 20 daphnia (24 hr after hatching) introduced in each 250 ml beaker containing 100 ml of test chemicals with 6 different concentrations (0, 50, 100, 200, 500, 1000 µg/ml) were prepared in duplicate
- mortality was observed after an incubation time of 4 h

Reliability: (2) valid with restrictions
Basic data given. Restrictions of the method are:
- Test period was only of 4 hours (represents only 8 % of the in the OECD and other current guidelines suggested test period)
- Temperature during the test was higher (25 °C) than the one suggested by OECD and other current guidelines (22 °C)
- Yeast is not a standard food for Daphnia


Flag: Critical study for SIDS endpoint
04.12.2003

Type: Static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l
EC50: 12.1
Limit Test: No
Year: 1979
GLP: No
Test substance: no data


The test was performed according to the procedure as described in the guideline method; with the exception that the temperature was maintained at 20 °C

Result: Effect values were calculated with the probit analysis
Reliability: (2) valid with restrictions
Test procedure according to national standards with some restrictions
03.02.2003

Type: Static
Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l
EC0: 7
EC50: 11
EC100: 20
Analytical monitoring: No
Method: other: Immobilization test
Year: 1977
GLP: No
Test substance: no data

---

UNEP PUBLICATIONS
| Test condition                          | - The fastest proliferating clone of Daphnias was selected from about 30 clones isolated from a pond  
- The Daphnias were fed cultured Chlorella vulgaris daily  
- Daphnias were filtered to select young animals up to 24 hours old  
- For incubation 10 Daphnias per concentration were used  
- Duplicate samples  
- Test vessels loosely capped with filter paper  
- Temperature 20°C  
- Initial pH 7.6 - 7.7 (not adjusted during exposure);  
- Chlorine free tap water, hardness 16 German degrees: 286 mg CaCO3/l |
| Reliability                            | (2) valid with restrictions  
Test procedure comparable to standard test method and in accordance with generally accepted scientific standards; sufficient documentation |
| Date                                   | 16.10.2003 |
| Type                                    | Static |
| Species                                 | Daphnia magna (Crustacea) |
| Exposure period                         | 24 hour(s) |
| Unit                                    | mg/l |
| EC0                                     | 5.6 |
| EC50                                    | 9 |
| EC100                                   | 12 |
| Analytical monitoring                   | No |
| Method                                  | other: Immobilization test according to Bringmann G, Kuehn R (1977) Z Wasser Abwasser Forsch 10, 162 - 166 |
| Year                                    | 1982 |
| GLP                                     | No |
| Test substance                          | no data |
| Method                                  | 10 daphnia (strain: IRCHA; <=24 h old) per concentration; duplicate samples; test vessels loosely capped with filter paper |
| Remark                                  | Effect values refer to nominal TS concentrations |
| Test condition                          | - Temperature 20 °C  
- Initial pH 8.0 +/- 0.2 (not adjusted during exposure)  
- Water hardness: 286 mg CaCO3/l |
| Reliability                            | (2) valid with restrictions  
Test procedure comparable to standard test method and in accordance with generally accepted scientific standards; sufficient documentation |
| Date                                   | 04.12.2003 |
| Type                                    | Static |
| Species                                 | Daphnia magna (Crustacea) |
| Exposure period                         | 48 hour(s) |
| Unit                                    | mg/l |
| EC50                                    | 19 |
| Analytical monitoring                   | No |
| Method                                  | other: NEN 6501: Determination of acute toxicity with Daphnia magna (1980) with slight modifications (Van Leeuwen et al. 1985b) |
| Year                                    | 1986 |
| GLP                                     | no data |
| Test substance                          | other TS: >98 % Purity |
| Result                                  | The daphnias were feed on 1.0E+8 cells/l Chlorella pyrenoidosa. |
| Result                                  | Results are given in the original reference as log IC50: log IC50 = 2.14 |
OECD SIDS  4-NITROTOLUENE

date: 09.09.2004

UNEP PUBLICATIONS

Maas-Diepeveen and van Leeuwen (1986) calculated LC-50 48-h values and their 95% confidence intervals according to Litchfield and Wilcoxon (1949).

Test condition:
- During the tests daphnids were fed with Chlorella pyrenoidosa, which at the start of the experiments were present at a concentration of 1.0E+8 cells/l
- IC50 values were calculated according to Litchfield and Wilcoxon (1948)
- The oxygen content of all solutions did not decrease below 7.9 mg/l (85%)
- Mortality in the controls never exceeded 10%

Test substance:
Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO; Merck, Purity > 98%)

Reliability:
(2) valid with restrictions
Test procedure in accordance with national standard methods, without detailed documentation.

Test condition:
- Snails from laboratory culture, fed with lettuce, 5 month old animals
- 0.05 l Testvolume per group
- Temperature 20 +/- 1 °C
- Illumination circadic
- Culturing medium contains per 1 l bidestilled water: 100 mg/l NaHCO3, 200 mg/l CaCl2*2H2O, 20 mg/l KHCO3, 180 mg/l MgSO4*7H2O

Reliability:
(2) valid with restrictions
Basic data given
Analytical monitoring: Yes
Method: other
Year: 1985
GLP: no data
Test substance: other TS: > 99.5 % Purity

Test condition:
- 3-4 days old larvae were used
- Each 10 larvae were tested in 1 l of test medium
- Larvae were not fed during incubation
- Incubation temperature 23 +/- 2 °C
- Circadic illumination
- Endpoint: mortality

Reliability: (3) invalid
Method not clearly indicated, without detailed documentation

03.02.2003 (30)

Type: Semistatic
Species: other aquatic mollusc: Lymnaea stagnalis
Exposure period: 24 hour(s)
Unit: mg/l
LC50: 21
Limit Test: No
Analytical monitoring: Yes
Method: other: not specified
Year: 1985
GLP: no data
Test substance: other TS: > 99.5 % Purity

Reliability: (3) invalid
Method not clearly indicated, without detailed documentation

06.08.2003 (30)

Type: other aquatic worm: Tubifex
Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l
LC50: ca. 110
Analytical monitoring: no data
Method: other: see TC
Year: 1986
GLP: no data
Test substance: no data

Result: The sensitivity of the Tubifex test was found to lie between the Activated Sludge Inhibition Test and the Oryzias latipes Acute Toxicity Test
Test condition:
- Tubifex 30 - 50 mm length
- Incubation 24 or 48 hours at 20 °C
- Test results were compared with three other tests: Activated Sludge Inhibition Test, Oryzias latipes Acute Toxicity Test, and Tetrahymena pyriformis proliferation inhibition test

Reliability: (4) not assignable
For review only a short English abstract was available

16.10.2003 (92)
### Test substance

- **Result**: Measured EC50 concentrations were obtained from Aquire Database. They were compared with the predicted LC50 by using QSAR-models. The duration of the test to determine LC50 and other details about the test system are not given.
  - LC50 measured: 11.0 mg/l
  - LC50 calculated: 66.09 mg/l

- **Reliability**: (4) not assignable

- **Secondary literature**
  - 03.02.2003 (98)

### Type
- **Species**: Daphnia magna (Crustacea)
- **Exposure period**: 24 hour(s)
- **Unit**: mg/l
- **EC0**: = 3 - 4
- **EC50**: = 7 - 11
- **Analytical monitoring**: No
- **Year**: 1982
- **GLP**: No

- **Test substance**: as prescribed by 1.1 - 1.4

- **Reliability**: (4) not assignable

- **Literature not available**
  - 03.02.2003 (105)

### 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

- **Species**: Chlorella pyrenoidosa (Algae)
- **Endpoint**: other: reduction of the maximum growth
- **Exposure period**: 96 hour(s)
- **Unit**: mg/l
- **EC50**: 22.2
- **Limit test**: no data
- **Analytical monitoring**: no data
- **Method**: other: OECD Guideline 201 (1984)
- **Year**: 1986
- **GLP**: Test substance
- **Test substance**: other TS: p-Nitrotoluene, purity 98%

- **Method**: The experiments were carried out according to the OECD guideline 201 (1984), with slight modifications according to NEN 6506 C.3. ALGAL INHIBITION TEST (http://europa.eu.int/comm/enterprise/chemicals/chempol/reach/volume5_final.pdf) (test species Chlorella pyrenoidosa instead of Chlorella vulgaris), duration of incubation [96 h instead of 72 h]).

- **Result**: Results are given in the original reference as log EC50: log EC50 = 2.21 (EC50 µmol/l);

  - The concentrations causing 50 % reduction of the maximum density (yield) of Chlorella pyrenoidosa during a 96 h period of exposure were calculated according to Kooijman et al. (1983). Parametric analysis of population growth in bio-assays. Water Research 17: 527 - 538

- **Test condition**: - Endpoint: growth inhibition
  - Stock solutions of the test compound were prepared in dimethylsulfoxide (DMSO; Merck, purity 99%)

- **Reliability**: (2) valid with restrictions

  - Guideline study without detailed documentation.
<table>
<thead>
<tr>
<th>Flag</th>
<th>Critical study for SIDS endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>05.12.2003</td>
<td>(106) (103) (79)</td>
</tr>
<tr>
<td>Species</td>
<td>Scenedesmus pannonicus (Algae)</td>
</tr>
<tr>
<td>Endpoint</td>
<td>Biomass</td>
</tr>
<tr>
<td>Exposure period</td>
<td>4 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>NOEC</td>
<td>10</td>
</tr>
<tr>
<td>Limit test</td>
<td></td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>1983</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Test condition**
- Static conditions
- ca. 1.5 * 10E+6 organisms (in 3-fold) per group
- 0.15 l Testvolume (per group)
- Temperature (23 +/- 2 °C)
- Lighting (13 W/m²)
- Culturing media (1 l bidest. containing 35 mg/l CaCl₂*2H₂O, 75 MgSO₄*7H₂O, 52 mg/l K₂HPO₄, 6 mg/l citric acid, 500 mg/l NaNO₃, 54 mg/l Na₂CO₃*10H₂O, 6 mg/l Ferricitrate, 330 mg/l NH₄NO₃, 1 ml (0.0006 g Na₂MoO₄*2H₂O, 2.9 g H₃BO₃, 0.11 g ZnCl₂, 0.08 g CuSO₄*5H₂O, 0.018 g (NH₄)₆Mo₇O₂₄))

**Reliability**
(2) valid with restrictions
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

<table>
<thead>
<tr>
<th>Flag</th>
<th>Critical study for SIDS endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>04.12.2003</td>
<td>(104)</td>
</tr>
<tr>
<td>Species</td>
<td>other aquatic plant: Lemna minor</td>
</tr>
<tr>
<td>Endpoint</td>
<td>growth rate</td>
</tr>
<tr>
<td>Exposure period</td>
<td>7 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>NOEC</td>
<td>10</td>
</tr>
<tr>
<td>Limit test</td>
<td></td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>1983</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Test condition**
- Static conditions
- 2 fronts (in 2-fold)
- 0.2 l Testvolume (per group)
- Temperature (25 +/- 1 °C)
- Lighting (35 W/m²)
- Culturing medium (1 l bidest. containing 207 mg/l NaHCO₃, 276 mg/l CaCl₂*2H₂O, 198 mg/l MgSO₄*7H₂O, 15 mg/l K₂HPO₄, 16 mg/l NH₄Cl, 111 mg/l NaCl, 30 mg/l KNO₃, 5 ml solution with different minerals)

**Reliability**
(2) valid with restrictions
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

| Species | other algae: Scenedesmus obliquus |
| Endpoint | growth rate |
| Exposure period | 48 hour(s) |
| Unit | mg/l |
| EC₅₀ | 24.9 |
### Method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test substance</td>
<td>other TS: no purity given</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Year</td>
<td>1995</td>
</tr>
<tr>
<td>Method</td>
<td>other: OECD-Guideline 201 (algae, Growth inhibition test, 1981)</td>
</tr>
</tbody>
</table>

### Test condition

- Stock solution prepared in aceton (1 ml/l)
- Initial cell concentration was approx. 10000 cells/ml
- Temperature 20 °C +/- 1 °C, pH 7.2 +/- 0.2, continuous light provided by white Neon lamps (3,600 lux)
- Stock solution prepared in aceton (1 ml/l)
- Initial cell concentration was approx. 10000 cells/ml

### Reliability

(2) valid with restrictions

Guideline study without detailed documentation

### Flag

Critical study for SIDS endpoint

04.12.2003

### Species

other algae: Scenedesmus obliquus

### Endpoint

growth rate

### Exposure period

96 hour(s)

### Unit

mg/l

### EC50

ca. 25

### Limit test

No

### Analytical monitoring

No

### Method

other: OECD-Guideline 201 (algae, Growth inhibition test, 1981)

### Year

1995

### Test substance

no data

### Test condition

- Algae were cultured in an unspecified growth medium at 24 +/- 1 °C. The photoperiod was 12 hours under cool white fluorescent light of 4000 +/- 400 Lux, followed by 12 hours darkness
- 50 ml incubation solution in 100 ml sterile-closed flasks
- Initial algae density 10000 cells
- 5 different concentrations, each 3 replicates
- Cell density determination after 0, 24, 48, 72, and 96 hours, optical density after 96 hours at 650 nm
- Endpoint: growth inhibition

### Reliability

(2) valid with restrictions

Basic data given

16.10.2003

### Species

other algae: Scenedesmus obliquus

### Endpoint

growth rate

### Exposure period

48 hour(s)

### Unit

mg/l

### EC50

25

### Limit test

No

### Analytical monitoring

no data

### Method


### Year

2000

### GLP

no data

### Test substance

no data

### Method

Continuous light was applied.

During the test, temperature was of 20 +/- 1°C.

Aqueous medium was prepared according to Lang (1994).

5 concentrations were tested in the range of 1.17*E-5 to 7.32*10E-5 mol/L.

There were 4 replicates for each concentration and a control.
Result: Results are reported in mol/l
EC50 = 1.82*10E-4 mol/l = EC50 = 25.0 mg/l
Reliability: (2) valid with restrictions
Guideline study. No analytical monitoring is mentioned
Flag: Critical study for SIDS endpoint

Species: Scenedesmus quadricauda (Algae)
Endpoint: Biomass
Exposure period: 8 day(s)
Unit: mg/l
TT: 15
Limit test: No
Analytical monitoring: No
Method: other: Cell multiplication inhibition test
Year: 1977
GLP: No
Test substance: no data

Method: Static incubation
- 100 ml test solution (neutralized) of certain dilutions of the test substance
  prepared in replicate
- Test solution contains nutrient medium (all media for test and culture are
  described in detail in the reference)
- Incubation of 10 ml aliquots for 8 d at 27 °C in Kapsenberg-culture vials in
  artificial continuous light
- Cell multiplication measured turbidimetrically at 578 nm with an optical
  path length of 10 mm

Remark: TT (Toxicity Threshold) comparable to EC3; value refers to
nominal TS concentration
Reliability: (3) invalid
It is unclear whether the algae are within the exponential growth throughout
the whole exposure period of 8 days

16.10.2003

Species: other algae: Scenedesmus vacuolatus
Endpoint: growth rate
Exposure period: 24 hour(s)
Unit: mg/l
EC50: 17.2
Limit test: No
Analytical monitoring: no data
Method: other: Method according to Altenburger (1990)
Year: 2000
GLP: no data
Test substance: other TS: purity > 97 %

Remark: Scenedesmus vacuolatus was formerly referred to as Chlorella fusca.
Result given as logEC50 (mol/l)
Test condition: The test was performed under the following conditions:
- Temperature 28 °C +/- 0.5 °C, pH 6.7
- Initial cell concentration was approx. 1E5 cells/ml
Reliability: (3) invalid
Study without detailed documentation

16.10.2003

Species: other algae: Scenedesmus subspicatus or Chlorella fusca
Endpoint: other: fluorescence
Exposure period: 90 minute(s)
Unit: mg/l
EC10: 5
| **Limit test** | : |  |
| **Analytical monitoring** | : | No |
| **Method** | : | other: see TC |
| **Year** | : | 1986 |
| **GLP** | : | No |
| **Test substance** | : | no data |

**Test condition**
- Principle: low fluorescence emission after illumination indicates impaired electron transport in plant photosynthesis
- An algae fluorescence autometer is described in detail, containing a measuring and a controlling unit
- Two algae were used for measurements of fluorescence: Scenedesmus subspicatus and (or?) Chlorella fusca
- After incubation for 90 minutes algae were pumped into a flow-through cuvette
- Algae were illuminated for 30 seconds with filtered light with a spectral maximum of 450 nm
- Fluorescence is measured at 685 nm

**Reliability**: (3) invalid
Not described
- how incubation was performed
- which algae species was actually used to obtain the reported results
- how algae were cultured and prepared before the incubation

**Species**: Scenedesmus pannonicus (Algae)
**Endpoint**: growth rate
**Exposure period**: Unit: mg/l
**TGK**: 15
**Limit test**: No
**Analytical monitoring**: No
**Method**: other: Analogy with the OECD proposal to short-term toxicity tests performed on algae (Scenedesmus pannonicus) (1979)
**Year**: 1985
**GLP**: no data
**Test substance**: other TS: > 99.5 % Purity

**Remark**: The period of exposure is not specified.
**Reliability**: (4) not assignable
Secondary literature. Although it is stated that the cited result was obtained from Scenedesmus pannonicus, the literature cited contained data only on Scenedesmus quadricauda

**Species**: other aquatic plant: Lemna minor
**Endpoint**: 
**Exposure period**: 
**Unit**: mg/l
**EC50**: 51
**Method**: 
**Year**: 1990
**GLP**: 
**Test substance**: 
**Remark**: Data taken from unpublished RIVM data
**Test condition**: Test period is not given
**Reliability**: (4) not assignable
Secondary literature. Original literature is not available
### 4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
<td>Aquatic</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>activated sludge of a predominantly domestic sewage</td>
</tr>
<tr>
<td><strong>Exposure period</strong></td>
<td>3 hour(s)</td>
</tr>
<tr>
<td><strong>Unit</strong></td>
<td>mg/l</td>
</tr>
<tr>
<td><strong>EC50</strong></td>
<td>100</td>
</tr>
<tr>
<td><strong>Analytical monitoring</strong></td>
<td>No</td>
</tr>
<tr>
<td><strong>Method</strong></td>
<td>OECD Guide-line 209 &quot;Activated Sludge, Respiration Inhibition Test&quot;</td>
</tr>
<tr>
<td><strong>Year</strong></td>
<td>1986</td>
</tr>
<tr>
<td><strong>GLP</strong></td>
<td>No</td>
</tr>
<tr>
<td><strong>Test substance</strong></td>
<td>no data</td>
</tr>
</tbody>
</table>

**Test condition**
- Activated sludge was obtained from a municipal wastewater treatment plant
- When the sludge was not used on the day of collection, then 50 ml of sewage were added to every liter of sludge and the sludge was aerated overnight
- At least 3 times the sludge was washed and resuspended in distilled water
- The inoculum contained 4 g/l of mixed liquor suspended solids
- Test substance was dissolved in a mixture of dimethyl sulfoxide to HCO-40 (a surfactant) at the ratio of 4:1, final concentration 2000 mg/l which had only a negligible effect on sludge respiration
- Further procedure see OECD Guideline 209
- Test substance in synthetic sewage feed (16 ml) was added to TS stock solution and made up to 300 ml with distilled water
- 200 ml activated sludge were added
- Aerated and stirred for 3 h at 20 °C
- Filled in gas tight vessel and oxygen consumption recorded for over 10 min

**Reliability**
(1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

**Flag**
16.10.2003
Critical study for SIDS endpoint

---

**Type**
- Aquatic

**Species**
- Pseudomonas putida (Bacteria)

**Exposure period**
- 16 hour(s)

**Unit**
- mg/l

**TT**
- 26

**Analytical monitoring**
- No

**Method**
- other: Basis for later German DIN 38412-8 (cell multiplication inhibition test)

**Year**
- 1976

**GLP**
- No

**Test substance**
- no data

**Method**
- Static incubation
  - 100 ml test solution (neutralized) of certain dilutions of the test substance
  - Test solution contains nutrient medium (all media for test and culture are described in detail in the reference)
  - Incubation for 16 h at 25 °C
  - Cell multiplication measured turbidimetrically at 436 nm with an optical path length of 10 mm (in some rare cases of strongly coloured test substances measurement is made at 578 nm)

**Result**
- TT (Toxicity Threshold) comparable to EC3; value refers to nominal TS concentration

**Reliability**
(2) valid with restrictions
### Test procedure according to national standards, well documented

<table>
<thead>
<tr>
<th>Flag</th>
<th>Critical study for SIDS endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.10.2003</td>
<td>(115) (110) (111)</td>
</tr>
</tbody>
</table>

#### Type
- Aquatic

#### Species
- Entosiphon sulcatum (Protozoa)

#### Exposure period
- 72 hour(s)

#### Unit
- mg/l

#### TT
- 8.6

#### Analytical monitoring
- No

#### Method
- other: cell multiplication inhibition test

#### Year
- 1980

#### GLP
- no data

#### Test substance
- no data

#### Result
- TT (Toxicity Threshold) comparable to EC5; value refers to nominal TS concentration

#### Test condition
- 25°C; initial pH 6.9 (adjusted)

#### Reliability
- (2) valid with restrictions
  - Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

<table>
<thead>
<tr>
<th>Flag</th>
<th>Critical study for SIDS endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.10.2003</td>
<td>(111) (116)</td>
</tr>
</tbody>
</table>

#### Type
- Aquatic

#### Species
- Uronema parduzci (Protozoa)

#### Exposure period
- 20 hour(s)

#### Unit
- mg/l

#### TGK (EC5)
- .89

#### Analytical monitoring
- No

#### Method
- other: see TC

#### Year
- 1980

#### GLP
- no data

#### Test substance
- no data

#### Remark
- Description of new assay, applied on 169 substances
  - Test substance was dissolved in distilled water pH 6.9
  - Up to 11 different dilutions prepared for each substance
  - Uronema is cultured in a mineral nutrient medium (all media are described in detail in the reference)
  - Uronema is fed specially cultured life E. coli
  - During the 20 hours incubation period Uronema (about 15000 cells per ml) is fed inactivated bacteria to avoid degradation of test substance
  - Uronema cells are counted in a Coulter Counter
  - Decrease of 5% of the cell number in test medium as compared to controls is defined as the TGK = toxicity threshold

#### Reliability
- (2) valid with restrictions
  - Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

<table>
<thead>
<tr>
<th>Flag</th>
<th>Critical study for SIDS endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>04.12.2003</td>
<td>(117)</td>
</tr>
</tbody>
</table>

#### Type
- Aquatic

#### Species
- Tetrahymena pyriformis (Protozoa)

#### Exposure period
- 24 hour(s)

#### Unit
- mg/l

#### EC50
- 82

#### Analytical monitoring
- No

#### Method
- other: cell multiplication inhibition test

#### Year
- 1985

#### GLP
- no data
<table>
<thead>
<tr>
<th>Test substance</th>
<th>:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
<td>Cell counting by microscope and Coulter counter</td>
</tr>
<tr>
<td><strong>Test condition</strong></td>
<td>Incubation at 30°C without agitation</td>
</tr>
<tr>
<td><strong>Reliability</strong></td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td><strong>Analytical monitoring</strong></td>
<td>No</td>
</tr>
<tr>
<td><strong>Type</strong></td>
<td>Aquatic</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Uronema parduzci (Protozoa)</td>
</tr>
<tr>
<td><strong>Exposure period</strong></td>
<td>20 hour(s)</td>
</tr>
<tr>
<td><strong>Unit</strong></td>
<td>mg/l</td>
</tr>
<tr>
<td><strong>TT</strong></td>
<td>46</td>
</tr>
<tr>
<td><strong>Year</strong></td>
<td>1980</td>
</tr>
<tr>
<td><strong>GLP</strong></td>
<td>No</td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td>TT (Toxicity Threshold) comparable to EC5; value refers to nominal TS concentration</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test substance</th>
<th>:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
<td>Aquatic</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Pseudomonas fluorescens (Bacteria)</td>
</tr>
<tr>
<td><strong>Exposure period</strong></td>
<td>7 hour(s)</td>
</tr>
<tr>
<td><strong>Unit</strong></td>
<td>mg/l</td>
</tr>
<tr>
<td><strong>NOEC</strong></td>
<td>10</td>
</tr>
<tr>
<td><strong>Analytical monitoring</strong></td>
<td>No</td>
</tr>
<tr>
<td><strong>Method</strong></td>
<td>other: cell multiplication inhibition test</td>
</tr>
<tr>
<td><strong>Year</strong></td>
<td>1983</td>
</tr>
<tr>
<td><strong>GLP</strong></td>
<td>No</td>
</tr>
<tr>
<td><strong>Test substance</strong></td>
<td>no data</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test substance</th>
<th>:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
<td>Aquatic</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Microcystis aeruginosa (Bacteria)</td>
</tr>
<tr>
<td><strong>Exposure period</strong></td>
<td>20 hour(s)</td>
</tr>
<tr>
<td><strong>Unit</strong></td>
<td>mg/l</td>
</tr>
<tr>
<td><strong>TGK (EC1)</strong></td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Analytical monitoring</strong></td>
<td>no data</td>
</tr>
<tr>
<td><strong>Method</strong></td>
<td>other: see TC</td>
</tr>
<tr>
<td><strong>Year</strong></td>
<td>1976</td>
</tr>
<tr>
<td><strong>GLP</strong></td>
<td>no data</td>
</tr>
<tr>
<td><strong>Test substance</strong></td>
<td>no data</td>
</tr>
</tbody>
</table>
### Remark

- TGK (toxic threshold) equals 1% effect. However, no standard deviations are given.
- The second reference does cite only the results but not the details of the method.

It is unclear whether the algae are within the exponential growth throughout the whole exposure period of 8 days.

### Test condition

- Microcystis aeruginosa is cultured in 100 ml Erlenmeyer vessels in 20 ml nutrient solution in continuous artificial light at 27 °C.
- Every 10 days new cultures are cultivated by transferring 2 ml cell suspension into sterilized vessels containing nutrient solution.
- Before incubation, bacteria are collected on a filter and washed, resuspended, measured at 578 nm, and diluted to obtain a transmission of 0.37.
- Test substance is dissolved in distilled water, pH 7 (adjusted).
- 11 dilutions.
- 40 ml test substance solution, 5 ml bacteria suspension, 5 ml nutrient solution were mixed and three 10 ml aliquots transferred into Kaysenberg-culture vials.
- Incubation for 8 days at continuous light at 27 °C.
- Endpoint growth, measured by transmission at 578 nm.

### Reliability

(2) valid with restrictions

Unsuitable test system

16.10.2003

### Type

Aquatic

### Species

Chilomonas paramecium (Protozoa)

### Exposure period

48 hour(s)

### Unit

mg/l

### TT

16

### Analytical monitoring

No

### Method

other: cell multiplication inhibition test

### Year

1980

### GLP

No

### Test substance

TT (Toxicity Threshold) comparable to EC5; value refers to

### Result

20 °C; pH 6.9 (adjusted)

### Reliability

(2) valid with restrictions

Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

16.10.2003

### Type

other: plant colonizing fungus

### Species

other fungi: Phytium ultimum Trow.

### Exposure period

88 hour(s)

### Unit

mg/l

### EC50

ca. 30

### Method

other

### Year

1962

### GLP

No

### Test substance

other TS: recrystallized

### Result

30 mg/l equals about 0.2 mmol/l

### Test condition

- Tests were performed on an autoclaved nutrient agar at pH 6.4 - 6.6.
- Test substance was dissolved in 1 % Triton X-100 in diethyl ether (1 ml) which was made up with sterile water to 50 ml of a relatively stable milky suspension.
- Aliquots of this suspension were added to sterile melted agar to give the
desired stock agar concentration
- Aliquots of this agar were diluted with fresh agar to yield 4 final test concentrations
- After the agars solidified in growth tubes, each tube was inoculated with a 8 mm plug of the fungus and incubated at 24 °C
- Linear growth measurements were taken after 40 and 88 hours

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(2) valid with restrictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study with acceptable restrictions: up to date method by the time the study was undertaken</td>
<td></td>
</tr>
</tbody>
</table>

16.10.2003 (120)

<table>
<thead>
<tr>
<th>Type</th>
<th>Aquatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Microcystis aeruginosa (Bacteria)</td>
</tr>
<tr>
<td>Exposure period</td>
<td>4 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>NOEC</td>
<td>3.2</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td>other: see TC</td>
</tr>
<tr>
<td>Year</td>
<td>1983</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

| Test condition | - Microcystis cyanobacteria were in the log-phase |
|               | - Complex culturing media: 1 l bidestilled water containing 100 mg/l NaHCO3, 5 mg/l CaCl2*2H2O, 50 mg/l MgSO4*7H2O, 25 mg/l K2HPO4, 38 mg/l NH4Cl, mixture of trace metals |
|               | - About 15 000 organisms in each assay |
|               | - Test volume 150 ml, static |
|               | - Temperature 23 +/- 2 °C |
|               | - Incubation in the light, 13 W/m2 |
|               | - Endpoint: specific growth rate |
| Reliability   | (2) valid with restrictions |
|               | Test procedure in accordance with generally accepted scientific standards and described in sufficient detail |

16.10.2003 (104)

<table>
<thead>
<tr>
<th>Type</th>
<th>other: plant-colonizing fungus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>other fungi: Rhizoctonia solani Kühn</td>
</tr>
<tr>
<td>Exposure period</td>
<td>88 hour(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>EC50</td>
<td>ca. 100</td>
</tr>
<tr>
<td>Method</td>
<td>other</td>
</tr>
<tr>
<td>Year</td>
<td>1962</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: recrystallized</td>
</tr>
</tbody>
</table>

| Remark | Although author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in μmoles / liter. |
|        | Log graphic shows about 0.9 mmol/l, which equals about 0.1 g/l |
| Test condition | - Tests were performed on an autoclaved nutrient agar at pH 6.4 - 6.6 |
|               | - Test substance was dissolved in 1 % Triton X-100 in diethyl ether (1 ml) which was made up with sterile water to 50 ml of a relatively stable milky suspension |
|               | - Aliquots of this suspension were added to sterile melted agar to give the desired stock agar concentration |
|               | - Aliquots of this agar were diluted with fresh agar to yield 4 final test concentrations |
|               | - After the agars solidified in growth tubes, each tube was inoculated with a 8 mm plug of the fungus and incubated at 24 °C |
|               | - Linear growth measurements were taken after 40 and 88 hours |
| Reliability   | (2) valid with restrictions |
16.10.2003

Type: Aquatic
Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 15 minute(s)
Unit: mg/l
EC50: 10.9
Method: other: Microtox toxicity analyzer
Year: 1989
GLP: 
Test substance: 

Method: The test and calculation of the concentration causing 50% reduction of bioluminescence after 15 min. of exposure were carried out as described in the Beckman Intruments Manual (1982).
Remark: The concentration values causing 50% reduction of bioluminescence after 15 min of exposure were determined. Photobacterium phosphoreum is now referred to as Vibrio fischeri.
Result: Results are given in the original reference as log EC50: log EC50 = 1.90 (EC50 µmol/l)
Reliability: (3) invalid
Unsuitable test system

19.12.2002

Type: Aquatic
Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 15 minute(s)
Unit: mg/l
EC50: 11
Analytical monitoring: No
Method: other: see TC
Year: 1986
GLP: No
Test substance: other TS: >98% Purity

Remark: Photobacterium phosphoreum is now referred to as Vibrio fischeri
Test condition: - Microtox test applied and results calculated according to manual of analyzer (Model 2055 Beckman 1982)
- Incubation 15 min
- Endpoint: 50% inhibition of bioluminescence
Reliability: (3) invalid
Unsuitable test system

16.10.2003

Type: other: microplate
Species: Vibrio fisheri (Bacteria)
Exposure period: 6 hour(s)
Unit: mg/l
EC50: 99.1
Analytical monitoring: Yes
Method: other: DIN 38412 L37
Year: 1999
GLP: no data
Test substance: no data

Remark: Vibrio fischeri was formerly referred to as Photobacterium phosphoreum
Result: In quartz glass EC50 was 99.1 +/- 27.8 mg/l. Due to effects of the plastic material of the microplates, EC50 was 157 +/- 19.2 mg/l in polystyrene
Test condition: - Microplate (96 wells) materials were quartz glass or polystyrene plastic
OECD SIDS 4-NITROTOLUENE

4. ECOTOXICITY

DATE: 09.09.2004

Id: 99-99-0

(Selected due to different binding abilities of the material), sterilized
- Each used well (370 µl) contained 140 µl testing material, 40 µl nutrient medium (5 x times concentrated) and 20 µl inoculum, 12 replicates
- Solutions were prepared in sterile distilled water by agitation for 24 hours followed by a 12 hours sedimentation. TOC was checked before start of incubation
- pH 7, 2% NaCl
- Cultures were incubated for 6 hours at 20 °C during constant agitation of 115 rpm
- Growth rate was measured as turbidity at a wavelength of 450 nm, inhibition was calculated in comparison to control cultures

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(3) invalid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>16.10.2003</strong></td>
<td>Unsuitable test system</td>
</tr>
</tbody>
</table>

**Type** : other: in vitro  
**Species** : Vibrio fisheri (Bacteria)  
**Exposure period** : 15 minute(s)  
**Unit** : mg/l  
**EC50** : ca. 14  
**Method** : other: bioluminescence (Microtox)  
**Year** : 2002  
**GLP** : no data  
**Test substance** : no data  

**Remark** : Toxicity is given in mol/l. Authors also checked toxicities of mixture with 2,4-dinitrotoluene. Vibrio fisheri was formerly referred to as Photobacterium phosphoreum

**Test condition** : - Bacterial cultures were maintained in nutrient medium, pH 7 +/- 0.5, containing 30 g/l NaCl
- For each compound 5 concentration gradients were prepared, 3 replicates
- 0.5 ml of bacterial culture were added to 2 ml of test solution
- Toxicities were determined from the reduction of bioluminescence (Microtox test)

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(3) invalid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>16.10.2003</strong></td>
<td>Unsuitable test system</td>
</tr>
</tbody>
</table>

**Type** : Aquatic  
**Species** : Vibrio fisheri (Bacteria)  
**Exposure period** : 15 minute(s)  
**Unit** : mg/l  
**EC10** : 17.26  
**Analytical monitoring** : no data  
**Method** : other: see test conditions  
**Year** : 1998  
**GLP** : no data  
**Test substance** : other TS: >95% purity  

**Test condition** : Bioluminescence was measured after 15 minutes exposure.

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(3) invalid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>03.02.2003</strong></td>
<td>Insufficient documentation for assessment</td>
</tr>
</tbody>
</table>

**Type** : Aquatic  
**Species** : Photobacterium phosphoreum (Bacteria)  
**Exposure period** : 15 minute(s)  
**Unit** : mg/l  
**EC50** : ca. 18  
**Analytical monitoring** : No
Method: other: Microtox
Year: 1993
GLP: no data
Test substance: no data

Remark: The work of Zhao et al. (1995) is cited by Gunatilleka and Poole (1999)
Result: - Results are given in mol/l
- It is reported that also tests with an incubation period of 30 min had been performed, and that the results were similar to those performed with a 15 min incubation period
- Unfortunately method only partly described

Test condition: - Microtox test applied according to manual of analyzer (Model Toxicity Analyzer DXY-2 of the Institute of Soil Science, Academia Sinica, Nanjing)
- Incubation 15 min at 20 °C
- Endpoint: 50 % inhibition of bioluminescence

Reliability: (4) not assignable
Unsuitable test system, important information missing (e.g. quality criteria)

16.10.2003

Type: other: in vitro
Species: Tetrahymena pyriformis (Protozoa)
Exposure period: 40 hour(s)
Unit: mg/l
EC50: ca. 30
Analytical monitoring: No
Method: other: Population Growth Impairment Assay
Year: 1999
GLP: no data
Test substance: other TS: > 95 % purity

Remark: 200 substances tested to derive QSAR. Result (log IGC50) given in mmol/l.
Test condition: - The ciliate Tetrahymena pyriformis (strain GL-C) was used
- Stock solutions were prepared in dimethylsulfoxide
- Test was performed according to Schultz TW (1997) Tetraox: The Tetrahymena pyriformis population growth impairment endpoint - A surrogate for fish lethality. Toxicol Methods 7: 289 - 309
- The endpoint population density was measured spectrophotometrically at 540 nm

Reliability: (4) not assignable
Secondary literature in regard to experimental work

16.10.2003

Type: other: in vitro
Species: Tetrahymena pyriformis (Protozoa)
Exposure period: 40 hour(s)
Unit: mg/l
EC50: ca. 93
Analytical monitoring: No
Method: other: Population Growth Impairment Assay
Year: 2001
GLP: no data
Test substance: other TS: > 95 % purity

Remark: 203 substances tested to derive QSAR. Result (log IGC50) given in mmol/l.
Test condition: - The ciliate Tetrahymena pyriformis (strain GL-C) was used
- Stock solutions were prepared in dimethylsulfoxide
- Test was performed according to Schultz TW (1997) Tetraox: The Tetrahymena pyriformis population growth impairment endpoint - A surrogate for fish lethality. Toxicol Methods 7: 289 - 309
- The endpoint population density was measured spectrophotometrically at 540 nm
4. ECOTOXICITY

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(4) not assignable</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.10.2003</td>
<td>Secondary literature in regard to experimental work</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>Aquatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>other bacteria: not specified</td>
</tr>
<tr>
<td>Exposure period</td>
<td></td>
</tr>
<tr>
<td>Unit</td>
<td></td>
</tr>
<tr>
<td>EC0</td>
<td>= 7</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td>other: Gähhörchentest</td>
</tr>
<tr>
<td>Year</td>
<td>1984</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>as prescribed by 1.1 - 1.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(4) not assignable</th>
</tr>
</thead>
<tbody>
<tr>
<td>03.02.2003</td>
<td>Insufficient documentation for risk assessment. No unit for the effect concentration is given</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>other: in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Vibrio fisheri (Bacteria)</td>
</tr>
<tr>
<td>Exposure period</td>
<td>15 minute(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>EC50</td>
<td>ca. 5 calculated</td>
</tr>
<tr>
<td>Method</td>
<td>other: QSAR</td>
</tr>
<tr>
<td>Year</td>
<td>1999</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Remark</th>
<th>Vibrio fisheri was formerly referred to as Photobacterium phosphoreum. Result given in mmol/l. Various equations are used to derive prediction on toxicity which is compared to data of Schultz TW, Sinks GD, Bearden AP (1998) QSAR in aquatic toxicology: A mechanism of action approach comparing toxic potency to Pimephales promelas, Tetrahymena pyriformis, and Vibrio fisheri. In: Devillers (ed) Comparative QSAR. Taylor and Francis, Philadelphia, pp 51 - 109. This observed result was about 11 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reliability</td>
<td>(4) not assignable</td>
</tr>
<tr>
<td>03.02.2003</td>
<td>Calculation which yields result far below the observed toxicity.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>Aquatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Pseudomonas fluorescens (Bacteria)</td>
</tr>
<tr>
<td>Exposure period</td>
<td>16 hour(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>EC0</td>
<td>1000</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td>other: Bestimmung der biologischen Schadwirkung toxischer Abwaesser gegen Bakterien. DEV, L 8 (1968) modified</td>
</tr>
<tr>
<td>Year</td>
<td>1973</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(4) not assignable</th>
</tr>
</thead>
<tbody>
<tr>
<td>03.02.2003</td>
<td>Original data not available</td>
</tr>
</tbody>
</table>

4.5.1 CHRONIC TOXICITY TO FISH
### Species
- Oryzias latipes (Fish, fresh water)

### Endpoint
- other: mortality, swimming behaviour

### Exposure period
- 40 day(s)

### Unit
- mg/l

### NOEC
- 1

### Analytical monitoring
- No

### Method
- other: (semi)chronic toxicity test

### Year
- 1983

### GLP
- no data

### Test substance
- no data

#### Result
- 40 d NOEC (hatching growth) 32 mg/l

#### Test condition
- - Semistatic conditions
- - Stage: eggs
- - 35 organisms per group
- - 1 l test volume per group
- - Food: Paramecium, Artemia, Micromin
- - Temperature 23 +/- 2 °C
- - Lighting circadic
- - Culturing medium (1 l bidest. water containing 100 mg/l NaHCO3, 200 mg/l CaCl2*2H2O, 20 mg/l KHCO3, 180 mg/l MgSO4*7H2O)
- - Test concentrations differed by a factor of square root 10

#### Reliability
- (2) valid with restrictions
- Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

#### Flag
- Critical study for SIDS endpoint

---

### Species
- Oryzias latipes (Fish, fresh water)

### Endpoint
- other: mortality and swimming behaviour

### Exposure period
- 28 day(s)

### Unit
- mg/l

### NOEC
- .8

### LC50
- 3.5

### Analytical monitoring
- Yes

### Method
- other: see below test conditions

### Year
- 1985

### GLP
- No

### Test substance
- other TS: > 99.5 % Purity (Origin: Fluka)

#### Remark
- 28d EC50 (behaviour): 2.8 mg/l

#### Test condition
- - Semistatic conditions
- - 50 animals (3 - 4 weeks old) each test
- - Fish were fed with Tetramine, Tetraphyll
- - Temperature 23 +/- 2 °C
- - Photoperiod (day-/night rhythm)
- - 3-times a week adjustment of the concentration
- - Culturing and test medium: NaHCO3 100 mg/l, CaCl2*2H2O 200 mg/l, KHCO3 20 mg/l, MgSO4*7H2O 180 mg/l

#### Reliability
- (2) valid with restrictions
- Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

#### Flag
- Critical study for SIDS endpoint

---

### Species
- Poecilia reticulata (Fish, fresh water)

### Endpoint
- other: mortality, swimming behaviour, and growth
**OECD SIDS**  
**4-NITROTOLUENE**  
**ID: 99-99-0**  
**DATE: 09.09.2004**

### 4. ECOTOXICITY

<table>
<thead>
<tr>
<th>Exposure period</th>
<th>28 day(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>NOEC</td>
<td>10</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td>other: (semi)chronic toxicity test</td>
</tr>
<tr>
<td>Year</td>
<td>1983</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Result**: For all three endpoints, the same NOEC was determined

**Test condition**:
- Semistatic conditions
- Age of fish 3-4 weeks old
- 25 Organisms per group
- 1 l Testvolume per group
- Food (Micromin, Tetramin)
- Temperature (23+/- 2 °C)
- Lighting circadic
- Culturing media (1 l bidest. water containing 100 mg/l NaHCO3, 200 mg/l CaCl2*2H2O, 20 mg/l KHCO3, 180 mg/l MgSO4*7H2O)

**Reliability**: (2) valid with restrictions

**Flag**: Critical study for SIDS endpoint

### 4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

<table>
<thead>
<tr>
<th>Species</th>
<th>Daphnia magna (Crustacea)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endpoint</td>
<td>reproduction rate</td>
</tr>
<tr>
<td>Exposure period</td>
<td>21 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>NOEC</td>
<td>1</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td>other: see below test conditions</td>
</tr>
<tr>
<td>Year</td>
<td>1983</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Result**: 21 d NOEC (mortality) 3.2 mg/l

**Test condition**:
- Semistatic conditions
- Age: 1 day
- 25 organisms (in 2-fold)
- 1 l testvolume (per group)
- Food: Chlorella
- Temperature 19 +/- 1 °C
- Illumination circadic
- Culturing medium contains per 1 l bidistilled water: 100 mg/l NaHCO3, 200 mg/l CaCl2*2H2O, 20 mg/l KHCO3, 180 mg/l MgSO4*7H2O

**Reliability**: (2) valid with restrictions

**Flag**: Critical study for SIDS endpoint

### Other aquatic mollusc: Lymnaea stagnalis

<table>
<thead>
<tr>
<th>Species</th>
<th>other aquatic mollusc: Lymnaea stagnalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endpoint</td>
<td>reproduction rate</td>
</tr>
<tr>
<td>Exposure period</td>
<td>40 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>NOEC</td>
<td>.32</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
</tbody>
</table>

**Result**: 40 d NOEC (mortality) 0.32 mg/l

**Test condition**:
- Semistatic conditions
- Age: 4 weeks
- 25 Organisms per group
- 1 l Testvolume per group
- Food: Tetramin
- Temperature 23°C
- Lighting circadic
- Culturing media (1 l bidest. water containing 100 mg/l NaHCO3, 200 mg/l CaCl2*2H2O, 20 mg/l KHCO3, 180 mg/l MgSO4*7H2O)

**Reliability**: (2) valid with restrictions

**Flag**: Critical study for SIDS endpoint
### OECD SIDS

#### 4. ECOTOXICITY

**ID:** 99-99-0

**DATE:** 09.09.2004

<table>
<thead>
<tr>
<th>Method</th>
<th>other: see TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1983</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Result:** For the endpoint mortality, the NOEC was 10 mg/l

<table>
<thead>
<tr>
<th>Test condition</th>
<th>- Semistatic conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 5 Month old snails from laboratory culture</td>
<td></td>
</tr>
<tr>
<td>- 20 Snails per group</td>
<td></td>
</tr>
<tr>
<td>- 20 l Test volume per group</td>
<td></td>
</tr>
<tr>
<td>- Food: Lettuce</td>
<td></td>
</tr>
<tr>
<td>- Temperature 20 +/- 1 °C</td>
<td></td>
</tr>
<tr>
<td>- Circadic illumination</td>
<td></td>
</tr>
<tr>
<td>- Culturing medium contains per 1 l bidistilled water: 100 mg/l NaHCO₃, 200 mg/l CaCl₂<em>2H₂O, 20 mg/l KHCO₃, 180 mg/l MgSO₄</em>7H₂O</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(2) valid with restrictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic data given. Since this is a non-guideline study with non-standard test organisms, further information on the test, its quality criteria, and the performance of controls, is advisable.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flag</th>
<th>Critical study for SIDS endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>04.12.2003</td>
<td>(104)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Daphnia magna (Crustacea)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endpoint</td>
<td>reproduction rate</td>
</tr>
<tr>
<td>Exposure period</td>
<td>21 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>NOEC</td>
<td>.7</td>
</tr>
<tr>
<td>EC50</td>
<td>1.8</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>Yes</td>
</tr>
<tr>
<td>Method</td>
<td>other: in analogy with the OECD 202 proposal 1979</td>
</tr>
<tr>
<td>Year</td>
<td>1985</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: &gt; 99.5 % Purity (Origin: Fluka)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Remark</th>
<th>21d-LC50: 3.2 mg/l</th>
<th>21d-EC50 (mortality and behaviour): 3.2 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test condition</td>
<td>- Daphnias were 1 day old at start of incubation</td>
<td></td>
</tr>
<tr>
<td>- Culturing and test medium: NaHCO₃ 100 mg/l, CaCl₂<em>2H₂O 200 mg/l, KHCO₃ 20 mg/l, MgSO₄</em>7H₂O 180 mg/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Food during incubation: Chlorella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 25 organisms per 1 litre of test medium, 2 replicates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Incubation temperature 19 +/- 1 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Circadic lighting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Endpoint: Mortality/reproduction</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reliability</th>
<th>(2) valid with restrictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparable to guideline study, without detailed documentation</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flag</th>
<th>Critical study for SIDS endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.07.2003</td>
<td>(30)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>other aquatic arthropod: aquatic larvae of Culex pipiens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endpoint</td>
<td>other: development, mortality</td>
</tr>
<tr>
<td>Exposure period</td>
<td>25 day(s)</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/l</td>
</tr>
<tr>
<td>NOEC</td>
<td>3.2</td>
</tr>
<tr>
<td>Analytical monitoring</td>
<td>No</td>
</tr>
<tr>
<td>Method</td>
<td>other: see TC</td>
</tr>
<tr>
<td>Year</td>
<td>1983</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

| Remark | For both endpoints, the same NOEC were obtained |

**UEP PUBLICATIONS**

115
| Test condition | - Semistatic conditions  
|                | - 1st instar larvae  
|                | - 30 organisms (in 2-fold)  
|                | - 0.05 l testvolume (per group)  
|                | - Food: Puppy food, milk powder, blood  
|                | - Temperature 27 +/- 1 °C  
|                | - Illumination circadic  
|                | - Culturing medium contains per 1 l bidestilled water: 100 mg/l NaHCO3, 200 mg/l CaCl2*2H2O, 20 mg/l KHCO3, 180 mg/l MgSO4*7H2O  
| Reliability    | (2) valid with restrictions  
|                | Test procedure in accordance with generally accepted scientific standards and described in sufficient detail  
| Flag           | Critical study for SIDS endpoint  
| Species        | other: Hydrozoan Hydra oligactis  
| Endpoint       | other: specific growth rate  
| Exposure period| 21 day(s)  
| Unit           | mg/l  
| NOEC           | 10  
| Analytical monitoring | No  
| Method         | other: see TC  
| Year           | 1983  
| GLP            | no data  
| Test substance | no data  

| Test condition | - Semistatic conditions  
|                | - Budless hydrozoans  
|                | - 2 organisms (in 5-fold)  
|                | - 0.05 l testvolume (per group)  
|                | - Food: Daphnia, Artemia  
|                | - Temperature 18 +/- 1 °C  
|                | - Illumination circadic  
|                | - Culturing medium contains per 1 l bidestilled water: 100 mg/l NaHCO3, 200 mg/l CaCl2*2H2O, 20 mg/l KHCO3, 180 mg/l MgSO4*7H2O  
| Reliability    | (2) valid with restrictions  
|                | Test procedure in accordance with generally accepted scientific standards and described in sufficient detail  
| Flag           | Critical study for SIDS endpoint  
| Species        | Daphnia magna (Crustacea)  
| Endpoint       | reproduction rate  
| Exposure period| 21 day(s)  
| Unit           | mg/l  
| LOEC           | 5.6  
| EC50           | 7.1  
| Analytical monitoring | no data  
| Method         | other: NEN 6502: Determination of chronic toxicity to Daphnia magna (1980)  
| Year           | 1989  
| GLP            | no data  
| Test substance | no data  

| Method         | Method of the Dutch Standardization Organization, Rijswijk, The Netherlands  
| Result         | Three chronic effects presented.  
|                | 1. Population growth:  
|                | LRCT(Rm) = Lowest rejected concentration tested that significantly lowered the population growth constant (Rm) after 21 days of exposure.  
|                | log LRCT(Rm) = 1.71  

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2. Body length:
LRCT(L) = Lowest rejected concentration tested that significantly lowered
the mean length (L) of animals after 21 days of exposure.
log LRCT(L) = 1.61
3. Immobilization:
IC50 = 21 d immobilization concentration
log IC50 = 1.61

Test condition:
- The population growth constant (Rm) of D.magna was determined in a
  semi-static test over a 21-day period, using 10 daphnids per concentration,
  and one animal per jar containing 10 ml medium.
- The test was carried out in a room at 20°C illuminated 12 h/day. A
  synthetic test medium was used with a hardness of 200 mg/l as CaCO3
  and a pH of 8.4.

Reliability:
(2) valid with restrictions
Test procedure in accordance with national standard methods. No
information about an analytical monitoring

Species:
other aquatic mollusc: Lymnaea stagnalis

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

Species:
Tubifex

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species:
Phaseolus aureus (Dicotyledon)
### Test Condition
- The test solution was prepared by dissolving 4-nitrotoluene in Hoagland nutrient solution.
- A definite amount of test solution was added to sand.
- 15 seeds were placed in beakers after 160 g of sand had been added.
- The seeds were covered with another 60 g of sand containing about 36 ml of test solution.
- Three concentrations were tested (20, 50, and 100 ppm by weight).
- Seedling cultures were incubated in the dark at 25 °C and the relative humidity approaching 100 % in a Mangelsdorf seed-germinator.
- After 6 days the seedlings were washed and weighed.
- Ungerminated seeds/smallest seedlings (up to five) were discarded.

### Reliability
(2) valid with restrictions
Study with acceptable restrictions: up to date method by the time the study was undertaken.

### Flag
- Critical study for SIDS endpoint

---

### Species
- other terrestrial plant: Cucumis sativus var. National Pickling

### Endpoint
- Growth

### Exposure period
- 6 day(s)

### Unit
- mg/l

### EC50
- ca. 300

### Method
- other: germination and growth of seedlings in sand

---

### Test Condition
- The test solution was prepared by dissolving 4-nitrotoluene in Hoagland nutrient solution.
- A definite amount of test solution was added to sand.
- 15 seeds were placed in beakers after 160 g of sand had been added.
- The seeds were covered with another 60 g of sand containing about 36 ml of test solution.
- Three concentrations were tested (20, 50, and 100 ppm by weight).
- Seedling cultures were incubated in the dark at 25 °C and the relative humidity approaching 100 % in a Mangelsdorf seed-germinator.
- After 6 days the seedlings were washed and weighed.
- Ungerminated seeds/smallest seedlings (up to five) were discarded.

### Reliability
(2) valid with restrictions
Study with acceptable restrictions: up to date method by the time the study was undertaken.

### Flag
- Critical study for SIDS endpoint

---

### Species
- Lactuca sativa (Dicotyledon)

### Endpoint
- Growth

### Exposure period
- 14 day(s)

### Unit
- mg/l

### EC50
- ca. 15 calculated

### Method
- OECD Guide-line 208 "Terrestrial Plants, Growth Test"
GLP
Test substance: other TS: toluene and various nitro- and chlorocompounds but not 4-nitrotoluene

Remark: Lactuca sativa Ravel R2

Test condition: - 10 Seeds per tray. Trays covered with glass plates. Temperature 21 °C, photoperiod 16 h light / 8 h dark, light intensity 6500 lux, humidity 40 - 80 %
- 4-Nitrotoluene was not tested but wide range of other chloro- and nitrocompounds
- The authors derived an equation for the QSAR for the relationship between log EC50 (y, in µmol/l) and the log Kow (x) for miscellaneous compounds:
  \[ y = -0.33x + 2.83 \]
  Using log kow = 2.37, log EC50 = 2.048 and EC50 = 15 mg/l

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

Memo: Chronic Toxicities on Xenopus laevis (African Clawed Frog)

Result: The following results were obtained:
- Endpoint mortality NOLC 10 mg/l
- Endpoint development NOEC 3.2 mg/l
- Endpoint growth NOEC 32 mg/l

Test condition: - Semistatic conditions
- 75 frogs younger than 2 days
- 50 l testvolume
- Food: nettle powder, heart
- Temperature 20 +/- 1 °C
- Illumination circadic
- Culturing medium contains per 1 l bidestilled water: 100 mg/l NaHCO3, 200 mg/l CaCl2*2H2O, 20 mg/l KHCO3, 180 mg/l MgSO4*7H2O
- Incubation period 100 days

Reliability: (2) valid with restrictions

Memo: Review on the effects of chemicals on microorganisms

Remark: Cites the following toxicities of 4-nitrotoluene to
- Activated sludge respiration EC50 >100000 mg/l
- Tubifex (worm) LC50 64 - 36 mg/l
- Activated sludge EC50 >100 mg/l
- Oryzias latipes (fish) LC50 69 mg/l
- Tetrahymanena pyriformis EC50 82 mg/l

17.12.2002

Memo : Toxicities on Xenopus laevis (African Clawed Frog)

Method : Method not clearly indicated, without detailed documentation

Result : LC50 = 15 mg/l
EC50 = 15 mg/l

Test condition : 3 - 4 weeks old larvae were used
- 96 h exposure to test medium
- each 10 animals were incubated in 1 l of test solution
- animals were not fed during incubation
- temperature was 21 +/- 2 °C
- circadian illumination
- dosing was semistatic, once per day
- endpoint mortality

10.12.2002
5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo : In vivo
Type : Metabolism
Species : Rat
Number of animals
   Males : 5
   Females : 5
Doses
   Males : 0, 1250, 2500, 5000 ppm
            (approx. 0, 55, 110, 240 mg/kg bw/day)
   Females : 0, 1250, 2500, 5000 ppm
            (approx. 0, 60, 125, 265 mg/kg bw/day)
Vehicle : other: none
Route of administration : oral feed
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
           2nd:
           3rd:
Toxic behaviour :
Deg. product :
Method : other: see freetext Method
Year : 2001
GLP : Yes
Test substance : other TS: 99 %

Method :
   SIZE OF STUDY GROUPS: 5 males and 5 females
   ANIMALS PER CAGE: 2 or 3 (males) or 5 (females)
   TIME HELD BEFORE STUDIES: 12 days
   AVERAGE AGE WHEN STUDY BEGAN: 5-6 weeks
   DURATION OF EXPOSURE: 105-106 weeks
   AVERAGE AGE AT NECROPSY: 111 to 112 weeks
   DIET:
      NTP-2000 Open Formula meal, available ad libitum; rats received nonirradiated feed from the beginning of the studies for 8 months and irradiated feed to the end of the studies.
   WATER: tap water, available ad libitum
   ANIMAL ROOM ENVIRONMENT:
      temperature: 72°F; relative humidity: 50 %; room fluorescent light: 12 hours/day; room air changes: 10 hour
   TYPE AND FREQUENCY OF OBSERVATION:
      observed twice daily, rats were weight initially, during week 4, and every 4 weeks thereafter; clinical findings were recorded at 4-week intervals, feed consumption was measured over a 1-week period every 4 weeks
   URINALYSIS:
      Urine was collected during a 24-hour period from 5 male and 5 female rats from each group at 2 weeks and 3, 12, and 18 months. Parameters evaluated included urine volume, creatinine, p-acetamidobenzoic acid and p-nitrobenzoic acid concentrations. The urinary metabolites were quantitated by HPLC (High-Performance Liquid Chromatography)

Remark :
   Exposure Time: 2 weeks; 3, 12, 18 months

The study was performed as part of a 2-year carcinogenicity study (for further details see also chapter on carcinogenicity): p-Acetamido benzoic acid and p-Nitrobenzoic acid - biomarkers of exposure.

Result :
   urine volume was measured:
m/f (2w-18months, control, low, mid, high dose):
4.0-5.5/3.6-8.7 ml/24hrs, 4.4-7.1/3.6-11.9 ml/24hrs, 4.4-6.8/5.4-10.6
ml/24hrs, 4.0-7.9/4.6-5.8 ml/24hrs

p-Acetamidobenzoic acid/creatinine ratio was determined
(m/f: control, low, mid, high dose):
week 2:
0.000645/0, 0247/0.241, 0.601/1.15, 2.12/2.10
month 3
0/0, 0.0848/0.164, 0.176/0.402, 1.16/1.34
month 12
0.00327/0.0442, 0.0487/0.107, 0.112/0.482, 0.491/0.895
month 18
0/0, 0.0786/0.116, 0.131/0.495, 0.614/1.55

p-Nitrobenzoic acid/ creatinine ratio was determined
(m/f: control, low, mid, high dose):
week 2
0/0, 2.14/1.86, 4.90/5.81, 8.29/7.15
month 3
0/0, 1.01/1.29, 1.80/2.32, 2.55/4.03
month 12
0/0, 0.805/0.927, 1.59/1.84, 3.11/2.93
month 18
0/0, 0.866/1.23, 1.83/2.02, 2.47/3.12

Conclusion: Ratios of p-nitrobenzoic acid and p-acetamidobenzoic acid to creatinine
were linearly related to exposure concentrations at each time point and for
each sex. The metabolite-to-creatinine ratio was generally larger at 2
weeks than at the later times. This can be explained with the highest
exposure on a weight basis to the young animals.
There appear to be differences in metabolism between male and female
rats; females excrete more p-acetamidobenzoic acid.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
03.11.2004

In Vitro/in vivo: In vivo
Type: Metabolism
Species: Mouse
Number of animals:
Males: 5
Females: 5
Doses:
Males: 0.1250, 2500, 5000 ppm
(approx. 0, 170, 345, 690 mg/kg bw)
Females: 0.1250, 2500, 5000 ppm
(approx. 0, 155, 315, 660 mg/kg bw)
Vehicle: other: none
Route of administration: oral feed
Exposure time:
Product type guidance:
Decision on results on acute tox. tests:
Adverse effects on prolonged exposure:
Half-lives: 1st:
2nd:
3rd:
Toxic behaviour:
Deg. product:
Method: other: see freetext Method
Year: 2001
GLP: Yes
Test substance: other TS; purity: 99 %

Method:
SIZE OF STUDY GROUPS: 5 males and 5 females
ANIMALS PER CAGE: 1 (males) or 5 (females)
TIME HELD BEFORE STUDIES: 12 days
AVERAGE AGE WHEN STUDY BEGAN: 5-6 weeks
DURATION OF EXPOSURE: 105-106 weeks
AVERAGE AGE AT NECROPSY: 111 to 112 weeks
DIET:
NTP-2000 Open Formula meal, available ad libitum; mice received nonirradiated feed from the beginning of the studies for 8 months and irradiated feed to the end of the studies.
WATER: tap water, available ad libitum
ANIMAL ROOM ENVIRONMENT:
temperature: 72°F; relative humidity: 50 %; room fluorescent light: 12 hours/day; room air changes: 10 hour
TYPE AND FREQUENCY OF OBSERVATION:
observed twice daily, rats were weight initially, during week 4, and every 4 weeks thereafter; clinical findings were recorded at 4-week intervals, feed consumption was measured over a 1-week period every 4 weeks
URINALYSIS:
Urine was collected during a 24-hour period from 5 male and 5 female mice from each group at 2 weeks and 3, 12, and 18 months. Parameters evaluated included urine volume, creatinine, p-acetamidobenzoic acid and p-nitrobenzoic acid concentrations. The urinary metabolites were quantitated by HPLC (High-Performance Liquid Chromatography)

Remark:
Exposure Time: 2 weeks; 3, 12, 18 months

The study was performed as part of a 2-year carcinogenicity study (for further details see also chapter on carcinogenicity): p-Acetamido benzoic acid and p-Nitrobenzoic acid - biomarkers of exposure.

Result:
urine volume was measured:
m/f (2w-18months, control, low, mid, high dose):
0.3-0.7/0.5-0.7 ml/24 hrs, 0.4-1.1/0.3-1.0 ml/24 hrs, 0.5-1.2/0.2-1.1 ml/24 hrs, 0.4-1.2/0.2-1.0 ml/24 hrs

p-Acetamidobenzoic acid/creatinine ratio was determined (m/f: control, low, mid, high dose):
week 2:
0.0395/0.0077, 0.0479/0.105, 0.138/0.162, 0.408/1.32
month 3
0/0, 0.0445/0.0270, 0.0674/0.108, 0.117/0.326
month 12
0/0, 0.00609/0.0179, 0.0112/0.0220, 0.0986/0.267
month 18
0/0, 0.0186/0.00829, 0.0522/0.0564, 0.101/0.236

p-Nitrobenzoic acid/ creatinine ratio was determined (m/f: control, low, mid, high dose):
week 2
0/0, 0.0447/0.513, 0.640/1.34, 1.58/325
month 3
0/0, 0/0.253, 0.194/1.20, 0.667/2.58
month 12
0/0, 0/0.0472, 0/0.137, 0.210/0.931,
month 18
0/0, 0/0, 0/0.217, 0.218/0.907

Conclusion:
The urinary concentrations of p-acetamidobenzoic acid and p-nitrobenzoic acid in mouse urine were often below the level of detection, and no detailed comparisons with exposure levels and between genders were attempted.

Reliability:
(1) valid without restriction
Flag : Critical study for SIDS endpoint
03.11.2004 

In Vitro/in vivo : In vivo 
Type : Toxicokinetics 
Species : Rat 
Number of animals 
Males : 4 
Females : 4 
Doses 
Males : 2, 200 mg/kg bw in corn oil 
Females : 2, 200 mg/kg bw in corn oil 
Vehicle : other: corn oil 
Route of administration : gavage 
Exposure time : 
Product type guidance : 
Decision on results on acute tox. tests : 
Adverse effects on prolonged exposure : 
Half-lives 
1st: 1 hour 
2nd: 
3rd: 

Toxic behaviour : 
Deg. product : 
Method : other: see freetext Method 
Year : 2001 
GLP : Yes 
Test substance : other TS: purity: 99 % 

Method : Groups of 3 or 4 male and 3 or 4 female rats received single oral dose of 2 or 200 mg/kg bw radiolabelled p-nitrotoluene in corn oil by gavage: 
--- Urine was collected 4, 8, 24, 48 and 72 hours post dosing and radioactivity measured. 
--- Feces were collected 24, 48 and 72 hours post dosing. 
--- Urinary metabolites were quantitated and identified by HPLC. 
--- Blood and plasma were analyzed for radiolabel concentration: 
- serial blood samples were obtained from rats via indwelling jugular cannula at 2, 4, 6, 8 and 24 hours and by cardiac puncture at terminal sacrifice at 72 hours 
- 200 mg-dosed rats only: plasma concentration was measured using gas-chromatography at 5,15, 30, 60, 120, 240 and 480 minutes after dosing. 
--- 200 mg-dose rats only: Bile was collected via an indwelling cannula 30, 60, 90, 120, 180, 240, 300, 360 minutes after dosing and total radiolabel was measured by liquid scintillation spectrometry. Metabolites were identified by comparison with metabolite standards using HPLC 

Result : Excretion: 
More than 70 % of the 2 and 200 mg/kg bw doses to male or female rats were recovered in urine 24 hours post dosing and by 72 hours more than 80 %, from feces 2-5 % was recovered. 
Metabolites: 
Urinary metabolite profile was similar for rats receiving single 2 or 200 mg/kg bw. The major metabolites excreted in the urine of male/ female rats included: 
p-Nitrobenzoic acid (2 mg-dose: 30% /47%; 200 mg-dose: 36% /45%); p-acetamidobenzoic acid (2 mg-dose: 16% /9%; 200 mg-dose: 16% /19%), p-nitrohippuric acid (2 mg-dose: 14% /8%; 200 mg-dose: 10% /9%), and p-nitrobenzylmercaptoic acid (2 mg-dose: 7% /1%; 200 mg-dose: 7% /1%); 

blood and plasma concentration (blood/plasma): 
2 mg-dose: 
highest value of radiolabel was reached after 2 hours: 
m: 0.406/0.705 equivalents; f: 0.715/1.28 equivalents
200 mg-dose:
highest value of radiolabel was reached after 6 hours:
m: 99.9/145 equivalents; f: 114/160 equivalents
then radioactivity declined

200 mg-dose: plasma concentration:
male: highest concentration 8637 ng/g plasma 15 minutes after dosing,
then declining to 313 ng/g plasma 480 min after dosing
females: highest concentration 8657 ng/g plasma 5 minutes after dosing,
then declining to below the limit of quantitation (305 mg/g plasma) 480
minutes after dosing.
The half-live in plasma was approximately 1 hour for females and slightly
less for males.

200 mg-dose, male:
Cumulative excretion of radioactivity in bile:
Ranging from 0.4 % of the dose 30 minutes post dosing up to 7 % of the
dose 360 minutes post dosing
metabolite profile:
S-(p-nitrobenzyl)-glutathione > p-Nitrobenzoic acid > p-nitrobenzyl-
glucuronide

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03.11.2004 (129)

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<td>Half-lives</td>
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Method:
5 male rats received single daily gavage doses of 200 mg/kg bw p-
nitrotoluene in corn oil for 12 days, with radiolabel added to the dose on d
1, 5, and 9. Cumulative excretion of radioactivity in urine was measured 4,
8, 24, 48, 72, and 96 hours after each radiolabelled dose and in feces 24,
48, 72 and 96 hours after each radiolabelled dose.
urinary metabolite profile was measured 24 hours after the day 5 dose and
4, 8, 24 and 48 hours after the day 9 dose.

Result:
No change in the rates and routes of excretion of radioactivity were
observed during the 12 day study when compared with single
administration:
urine/feces: d1: 90.1 % of the dose after 96 hrs / 4.5 % of the dose after 72
OECD SIDS
4-NITROTOLUENE
5. TOXICITY
ID: 99-99-0
DATE: 09.09.2004

hrs; d 5: 91.6 % of the dose after 96 hrs / 3.6 % of the dose after 96 hrs; d 9: 88.1 % of the dose after 96 hrs /6.2 % of the dose after 96 hrs

urinary metabolites (summary range of all collection time):
p-nitrobenzoic acid (40-52 %), p-acetamidobenzoic acid (8-27 %), p-nitrohippuric acid (11-18 %), and p-nitrobenzylmercapturic acid (3-13 %)

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.08.2004 (129)

In Vitro/in vivo : In vivo
Type : Toxicokinetics
Species : Mouse
Number of animals
Males : 3
Females : 3
Doses
Males : 2, 200 mg/kg bw in corn oil
Females : 2, 200 mg/kg bw in corn oil
Vehicle : other: corn oil
Route of administration : gavage
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :

Half-lives :
1st:
2nd:
3rd:

Toxic behaviour :
Deg. product :
Method : other: see freetext Method
Year : 2001
GLP : Yes
Test substance : other TS: purity: 99 %

Method : Groups of 3 male and 3 female mice received single oral dose of 2 or 200 mg/kg bw radiolabelled p-nitrotoluene in corn oil by gavage:
--- Urine was collected 4, 8, 24, 48 and 72 hours post dosing and radioactivity measured.
--- Feces were collected 24, 48 and 72 hours post dosing.
--- Urinary metabolites were quantitated and identified by HPLC.
--- Blood and plasma were analyzed for radiolabel concentration, 200 mg-dosed mice only:
- serial blood samples were obtained from mice via indwelling jugular cannula at 2, 4, 6, 8 and 24 hours and by cardiac puncture at terminal sacrifice at 72 hours
- plasma concentration was measured using gas-chromatography at 5, 15, 30, 60, 120, 240 and 480 minutes after dosing.

Result :
Excretion:
More than 70 % of the 2 and 200 mg/kg bw doses to male or female rats were recovered in urine 24 hours post dosing and by 72 hours more than 80 %, from feces 7-14 % was recovered.
Urinary Metabolites:
The major metabolites excreted in the urine of male/ female mice included:
p-Nitrobenzoic acid (2 mg-dose: 0 % /0%; 200 mg-dose: 5.5% /10.3%); p-acetamidobenzoic acid (2 mg-dose: 0% /0%; 200 mg-dose: 4.2% /7%); p-nitrohippuric acid (2 mg-dose: 9.9% /14.2%; 200 mg-dose: 20.5% 14.7%), 2-methyl-5-nitrophenyl-sulfate (2 mg-dose: 11.2% /11.5%; 200 mg-dose: 19% /12%); 2-methyl-5-nitrophenyl-glucuronide (2mg-dose: 42% /34.5 %; 200 mg-dose: 12.7% / 18.7%)
blood and plasma concentration (blood/plasma):
200 mg-dose:
highest value of radiolabel was reached after 40 minutes, m: 125/183 equivalents; after 20 minutes, f: 131/202 equivalents
then radioactivity declined

200 mg-dose: plasma concentration:
males: highest concentration 5451 ng/g plasma 10 minutes after dosing, then declining to 72.5 ng/g plasma 240 min after dosing.
females: highest concentration 12779 ng/g plasma 10 minutes after dosing, then declining below the limit of quantitation (145 mg/g plasma) 240 minutes after dosing.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
03.11.2004 (129)

In Vivo/in vivo : In vivo
Type : Toxicokinetics
Species : Rat
Number of animals
Males : 3
Females :
Doses
Males : 200 mg/kg bw
Females :
Vehicle : other: corn oil
Route of administration : gavage
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives :
1st:
2nd:
3rd:

Toxic behaviour :
Deg. product :
Method : other: see freetext Method
Year : 1984
GLP : no data
Test substance : other TS: no data on purity

Method : 3 male Fisher rats were given a single oral dose of radiolabelled p-nitrotoluene by gavage, dissolved in corn oil and immediately afterwards placed in metabolism cages. Urine and feces were collected up to 72 hours post dosing; charcoal traps were changed up to 72 hours after dose. Metabolites were identified by HPLC and mass spectrometry.

Result : Excretion of radioactivity 72 hours after dosing (mean of 3 rats):
total : 82.8 % of the dose
Urine: 76.7 % of the dose (peak excretion rate: 10 hours after dose), feces: 6.1 % of the dose (peak excretion rate: within the first 24 hours after dose), expired air: 0 % 
(peak excretion rate 12 hours after dose, data not shown)
identification of the urinary metabolites (mean of 3 rats 72 hours post treatment:
4-aminobenzoic acid (0.8 % of the dose), 4-acetamidobenzoic acid (27 % of the dose), 4-nitrobenzoic acid (28 % of the dose), 4-nitrohippuric acid (13 % of the dose), S-(4-nitrobenzyl)-N-acetylcysteine (3.7 % of the dose), 4-nitrobenzyl glucuronide (1.4 % of the dose), 5-methyl-2-nitrophenylsulfate (0.2 % of the dose)

Reliability : (2) valid with restrictions
no data on purity of test substance, and no information on GLP, small
number of animals of one sex only, and only 1 dose level applied

Flag: Critical study for SIDS endpoint
03.11.2004

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: Rat

Number of animals:
- Males: 3
- Females: 3

Doses:
- Males: 200 mg/kg bw
- Females: 200 mg/kg bw

Vehicle: other: corn oil

Route of administration: gavage

Exposure time:

Product type guidance:

Decision on results on acute tox. tests:

Adverse effects on prolonged exposure:

Half-lives:
- 1st:
- 2nd:
- 3rd:

Toxic behaviour:

Deg. product:

Method:
other: see freetext Method

Year: 1985

GLP: no data

Test substance:
other TS: purity >99 %

Method:
Single oral doses of 200 mg/kg bw radiolabelled substance were given to controls, sham operated and bile duct cannulated Fisher rats, each with 3 males and 3 females and placed in metabolism cages. Urine, feces and bile were collected over a period of 12 hours. Metabolites in the urine and in the bile were identified by HPLC. Hepatic macromolecular covalent binding was determined by the exhaustive extraction method.

Result:
Excretion 12 hrs after dose (male/female, % of the dose):
TOTAL: control: 41.1/38.7, sham-operated: 49.4/42.4, bile duct-cannulated: 37.9/24.8
URINE: control: 40/38.6, sham operated: 49.2/42.2, bile duct-cannulated:
27.7/23.4
BILE: control: Not Determined (ND), sham operated: ND, bile-duct cannulated: 9.8/1.3
FECES: control: 1.1/0.1, sham operated: 0.2/0.3, bile duct-cannulated: 0.4/0.1

Determination of the metabolites (male/female, % of the dose):
URINE: aminobenzoic acid: ND/<0.1, acetamidobenzoic acid: 0.7/0.1, nitrohippuric acid: 0.4/<0.1, nitrobenzoic acid: 2.8/0.8, S-(nitrobenzyl)-gluthadione: 2.8/0.1, S-(nitrobenzyl-N-acetylcysteine: 0.7/<0.1, nitrobenzyl glucuronide: 0.9/0.1, nitrobenzylsulfate: <0.1/<0.1, 2-methyl-5-nitrophenyl glucuronide: 0.2/0.1
BILE: aminobenzoic acid: 0.7/0.4, acetamidobenzoic acid: 8.5/6.6, nitrohippuric acid: 3.9/4.5, nitrobenzoic acid: 9.2/9.4, S-(nitrobenzyl)-gluthadione: 0.1/0.4, S-(nitrobenzyl-N-acetylcysteine: 1.7/0.7, nitrobenzyl glucuronide: 1.5/0.5, 2-methyl-5-nitrophenyl glucuronide: 0.2/0.3, 2-methyl-5-nitrophenyl sulfate: 0.2/0.1

Hepatic macromolecular covalent binding:
TOTAL (male/female, nmol NT equivalent/g liver): control: 647/430, sham-operated: 241/421, bile duct-cannulated: 468/261
Effect was not altered by pretreatment with sulfotransferase inhibitors (PCP or DCNP)

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**In Vitro/in vivo**

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<tr>
<td>Doses Males</td>
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<td>Doses Females</td>
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<td>Vehicle Males</td>
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<tr>
<td>Vehicle Females</td>
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<tr>
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<td>Decision on results on acute tox. tests</td>
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<td>Adverse effects on prolonged exposure</td>
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<tr>
<td>Half-lives</td>
<td>1st, 2nd, 3rd</td>
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**Remark**

1.07, 10.7, 107 mg/kg bw radiolabelled p-nitrotoluene was orally administered to 3 male Fischer 344 rats. The major route of excretion was urinary with 80, 64 and 80 % of dose appearing in 24 hr for low, medium, and high doses, respectively. Fecal excretion accounted for approximately 2 to 4 % of dose in 24 hr, and 4 to 5 % by eight days. Analysis of variance of the excreta revealed no significant difference between dosed. It was therefore concluded that excretion was not dose dependent following single oral exposure for the range 1.07 to 107 mg/kg bw.

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**In Vitro/in vivo**

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<tbody>
<tr>
<td>Species</td>
<td>Rat</td>
</tr>
<tr>
<td>Number of animals</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>3</td>
</tr>
<tr>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>Doses Males</td>
<td>8 mg/kg bw in ethanol/polyoxyethylene sorbitanoleate/water mixture</td>
</tr>
<tr>
<td>Doses Females</td>
<td></td>
</tr>
<tr>
<td>Vehicle Males</td>
<td>other: ethanol/polyoxyethylene sorbitanoleate/water mixture (2:1:7)</td>
</tr>
<tr>
<td>Vehicle Females</td>
<td></td>
</tr>
<tr>
<td>Route of administration</td>
<td>i.v.</td>
</tr>
<tr>
<td>Exposure time</td>
<td></td>
</tr>
<tr>
<td>Product type guidance</td>
<td></td>
</tr>
<tr>
<td>Decision on results on acute tox. tests</td>
<td></td>
</tr>
<tr>
<td>Adverse effects on prolonged exposure</td>
<td></td>
</tr>
<tr>
<td>Half-lives</td>
<td>1st:</td>
</tr>
</tbody>
</table>
Toxic behaviour:
Deg. product:
Method:
Year:
GLP:
Test substance:

Remark:

Reliability:
Flag: Critical study for SIDS endpoint

5.1.1 ACUTE ORAL TOXICITY

Type: LD50
Value: > 2250 mg/kg bw
Species: Rat
Strain: other: Wistar-II-R
Sex: male/female
Number of animals: 30
Vehicle: other: Polyethylene glycol 400
Doses: 100, 250, 500, 1000, 2250 mg/kg bw in Polyethylenglycol 400
Method: other: 15 rats/sex and dose group, rat weight: 160-245 g, food and water ad libitum, 5 different dose levels (see remarks), application by gavage, dosing volume not reported, post dose observation period: 1-2 weeks

Year: 1976
GLP: No
Test substance: as prescribed by 1.1 - 1.4

Remark: M A L E S: no male rat died: No. of rats with --------clin.signs/total // onset/end of clin. signs
100 mg/kg bw (as 2% solution): 0/15 // -/-
200 mg/kg bw (as 5% solution): 15/15 // 29/ 2d
500 mg/kg bw (as 10% solution): 15/15 // 21/ 4d
1000 mg/kg bw (as 20% solution): 15/15 // 18/ 4d
2250 mg/kg bw (as 30% solution): 15/15 // 4/ 6d
F E M A L E S: no female died: No. of rats with --------clín. signs/total //
onset/end of clín. signs
100 mg/kg bw (as 2% solution): 0/15 // - / -
200 mg/kg bw (as 5% solution): 15/15 // 40'/ 3d
500 mg/kg bw (as 10% solution): 15/15 // 35'/ 4d
1000 mg/kg bw (as 20% solution): 15/15 // 20'/ 5d
2250 mg/kg bw (as 30% solution): 15/15 // 12'/ 5d

clinical signs: difficulties in breathing for up to 3 days after dosing, poor
condition for up to 6 days post application

Reliability : (2) valid with restrictions
no necropsy and no histopathological examinations were performed
Flag 30.08.2004 : Critical study for SIDS endpoint

Type : LD50
Value : = 4700 mg/kg bw
Species : Rat
Strain : Wistar
Sex : Male
Number of animals : 10
Vehicle : other: Methylcellulose 1%
Doses : 1000, 2000, 3000, 5000, 7000, 9000 mg/kg bw suspension in
methylcellulose
Method : other: rat mean weight: 200 g, food and water ad libitum, appl. by gavage,
dosing volume: 1 ml, 6 different doses, observation period: 14 d, statistical
evaluation according to Bartlett, determination of LD50 with probit method
Year : 1978
GLP : No
Test substance : other TS: purity: 99%
Remark : No of death rats, 0-5/5-18/18-24 hrs // 2/3/4-7/8-14 days ------------------
after appl. // total No of dead rats:
1000 mg/kg bw (5% solution): 0/0/ 0 // 0/0/0/0 // 0/10
2000 mg/kg bw (10% solution): 0/0/ 1 // 0/0/0/0 // 1/10
3000 mg/kg bw (15% solution): 0/0/ 2 // 0/0/0/0 // 2/10
5000 mg/kg bw (25% solution): 0/0/ 5 // 0/0/0/0 // 5/10
7000 mg/kg bw (35% solution): 0/0/ 8 // 0/0/0/0 // 8/10
9000 mg/kg bw (45% solution): 0/0/10 // 0/0/0/0 // 10/10

LD50 = 4700 +/-330 mg/kg

clin. signs: onset: 5-10 min after administration of the test substance
excitation, tachypnea, convulsions, then somnolence, atony,
and wheezing for up to 24 hours; full recovery within 1 week

Reliability : (2) valid with restrictions
no necropsy and no histopathological examinations were performed
Flag 07.03.2003 : Critical study for SIDS endpoint

Type : LD50
Value : = 2140 mg/kg bw
Species : Rat
Strain : no data
Sex : Male
Number of animals : no data
Vehicle : no data
Doses : no data

<table>
<thead>
<tr>
<th>Date</th>
<th>Value</th>
<th>Species</th>
<th>Strain</th>
<th>Sex</th>
<th>Number of animals</th>
<th>Vehicle</th>
<th>Doses</th>
<th>Method</th>
<th>Year</th>
<th>GLP</th>
<th>Test substance</th>
<th>Reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.12.2002</td>
<td>3200 mg/kg bw</td>
<td>Rat</td>
<td>Wistar</td>
<td>Female</td>
<td>10</td>
<td>Methylcellulose 1 %</td>
<td>1000, 2000, 3000, 5000, 7000 mg/kg bw suspended in methylcellulose</td>
<td>1977</td>
<td>No</td>
<td>no data</td>
<td>(4) not assignable</td>
<td>documentation insufficient for assessment</td>
</tr>
<tr>
<td>07.03.2003</td>
<td>1960 mg/kg bw</td>
<td>Rat</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
<td>other: no data</td>
<td>1976</td>
<td>No</td>
<td>no data</td>
<td>(2) valid with restrictions</td>
</tr>
</tbody>
</table>

Remark: No of death rats, 0-5/5-18/18-24 hrs // 2/3/4-7/8-14 days --------------------
| After appl. // total No of dead rats: |
| 1000 mg/kg bw (5% solution): 0/0/0 // 0/0/0/0 // 0/10 |
| 2000 mg/kg bw (10% solution): 0/0/1 // 0/0/0/0 // 1/10 |
| 3000 mg/kg bw (15% solution): 0/0/4 // 0/0/0/0 // 4/10 |
| 5000 mg/kg bw (25% solution): 0/0/9 // 0/0/0/0 // 9/10 |
| 7000 mg/kg bw (35% solution): 0/0/10 // 0/0/0/0 // 10/10 |

LD50 = 3200 +/-200 mg/kg

Clin. signs: Onset: 5-10 min after administration of the test substance: excitation, tachypnea, convulsions, then somnolence, atony, wheezing for up to 24 hours; full recovery within 1 week.

Flag: Critical study for SIDS endpoint
<table>
<thead>
<tr>
<th>Type</th>
<th>Value</th>
<th>Species</th>
<th>Strain</th>
<th>Sex</th>
<th>Number of animals</th>
<th>Vehicle</th>
<th>Doses</th>
<th>Method</th>
<th>Year</th>
<th>GLP</th>
<th>Test substance</th>
<th>Reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD50</td>
<td>= 7100 mg/kg bw</td>
<td>Rat</td>
<td>no data</td>
<td>Male</td>
<td></td>
<td>other: water suspension of gum arabicum</td>
<td>2100-16000 mg/kg bw</td>
<td>other: according to Deichmann, LeBlanc, J. Industr. Hyg. Toxicol. 25, 415 (1943)</td>
<td>1959</td>
<td>No</td>
<td>other TS: no data on purity</td>
<td>(4) not assignable documentation insufficient for assessment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(142) (143)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD50</td>
<td>= 2144 mg/kg bw</td>
<td>Rat</td>
<td>no data</td>
<td>no data</td>
<td></td>
<td>no data</td>
<td>no data</td>
<td>other: no data</td>
<td>1972</td>
<td>No</td>
<td>no data</td>
<td>(4) not assignable documentation insufficient for assessment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(144)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD50</td>
<td>= 2144 mg/kg bw</td>
<td>Rat</td>
<td>ChR-CD</td>
<td>Male</td>
<td></td>
<td>other: corn oil</td>
<td>LD50 study: 1000, 2000, 4000 mg/kg bw</td>
<td>other: see freetext Method</td>
<td>1972</td>
<td>No</td>
<td>other TS: no data on purity</td>
<td>The test material, as suspension or solution in corn oil, was administered by intragastric intubation (dosing volume not stated) to young adult rats in single doses. 5 rats per dose level were used in the LD50 study. No data on number of rats in range-finding study. Survivors were sacrificed 14 days post-dosing. The LD50 value was calculated from mortality data, using the method of C.S. Weil, Biometrics 8, 249 (1952)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Range-finding study: 130 (1% solution), 670 (10 % solution), as 30 % suspension: 2250, 3400, 5000, 7500 (administered in divided doses), 11000 (administered in divided doses) mg/kg bw</td>
<td></td>
<td></td>
<td>(144)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Result:
Rats fed with 130, 670 and 2250 mg/kg bw survived and were sacrificed 14 days after dosing.
Rats fed 3400, 5000, 7500 and 11000 mg/kg bw were found dead 1 day after dosing.

Clinical signs:
Nonlethal doses:
irregular respiration on day of dosing, belly to cage posture and prostration on day of dosing at 2250 mg/kg bw, initial weight loss one day after dosing.
Lethal doses:
irregular respiration, belly-to cage posture and cyanosis on day of dosing, prostration on day of dosing at 5000 mg/kg bw and above; moribundity on day of dosing; salivation and lacrimation on day of dosing at 7500 mg/kg bw and above.
Cyanogenic effect at 3400 mg/kg bw and above.

LD50 Study:
4000 mg/kg bw: mortality 5/5
2000 mg/kg bw: mortality 2/5
1000 mg/kg bw: mortality 0/5
LD 50: 2144 mg/kg bw (95% confidence limits 1449 to 3171 mg/kg bw)

Reliability: (2) valid with restrictions
number of rats not mentioned, Purity of Test substance not given, no necropsy, no histopathological examination and no hematology was performed.

Flag: Critical study for SIDS endpoint

Type: LD50
Value: = 1230 mg/kg bw
Species: Mouse
Strain: no data
Sex: Male
Number of animals: no data
Vehicle: no data
Doses: no data
Method: other: according to Smyth et al., Am. Ind. Hyg. Ass. J. 23, 95-107 (1962), LD50 was calculated by the use of probit method
Year: 1977
GLP: No
Test substance: other TS: purity: no data
Reliability: (4) not assignable
documentation insufficient for assessment

Type: LD50
Value: = 1231 mg/kg bw
Species: Mouse
Strain: no data
Sex: no data
Number of animals: no data
Vehicle: no data
Doses: no data
Method: other: no data
Year: 1972
GLP: no data
Test substance: other TS: no data on purity
Reliability: (4) not assignable
documentation insufficient for assessment
5. TOXICITY

Value : = 1280  mg/kg bw
Species : Mouse
Strain : 
Sex : no data
Number of animals : 
Vehicle : no data
Doses : 
Method : other: no data
Year : 1976
GLP : No
Test substance : other TS: no data on purity

Reliability : (4) not assignable
secondary literature
09.12.2002

Type : LD50
Value : = 1750  mg/kg bw
Species : Rabbit
Strain : 
Sex : no data
Number of animals : 
Vehicle : Other
Doses : 
Method : other: no data
Year : 1976
GLP : No
Test substance : other TS: no data on purity

Reliability : (4) not assignable
secondary literature
09.12.2002

5.1.2 ACUTE INHALATION TOXICITY

Type : LC0
Value : ca. 1  mg/l
Species : Rat
Strain : other: CD
Sex : Male
Number of animals : 10
Vehicle : other: air
Doses : 0.78 - 1.24  mg/l air
Exposure time : 1 hour(s)
Method : other: Test substance was metered into a heated stainless steel tube; air carried the vapors into a 20-liter battery jar; Neither gross nor histopathological examination was performed
Year : 1972
GLP : No
Test substance : other TS: no data on purity

Result : mortality 0/10; clinical signs of intoxication during exposure: face-pawing; grooming, labored respiration, red tinged discharge from the eyes; signs of intoxication post exposure: none
Reliability : (4) not assignable
documentation insufficient for assessment
31.08.2004

Type : LC50
Value : > 4167  mg/m³
Species : Rat
Strain : Wistar
Sex : Male
Number of animals : 5
Vehicle : other: air
Doses : 
Exposure time : 1 hour(s)
Method : other: exposure to dust, post exposure observation time: 7 days
Year : 1976
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : mortality: 0/5
Result : mortality: 0/5, no signs of intoxication during and post exposure
Reliability : (2) valid with restrictions
exposure time only 1 hour, no gross or histopathological examination
Flag : Critical study for SIDS endpoint
04.02.2003 (136)

Type : Other
Value : 
Species : Rat
Strain : no data
Sex : no data
Number of animals : 
Vehicle : other: no data
Doses : 1000 mg/kg
Exposure time : 1 hour(s)
Method : other: no data
Year : 1990
GLP : no data
Test substance : other TS: no data on purity

Result : 4-Nitrotoluene has no effect on either lung or liver microsomes. (no further information available)
Reliability : (4) not assignable
Documentation insufficient for assessment
02.09.2004 (147)

Type : other: exposure to an atmosphere essentially saturated with test substance
Value : 
Species : Rat
Strain : Sprague-Dawley
Sex : Male
Number of animals : 10
Vehicle : other: air
Doses : 152 ppm (67 % of saturation) = 851 mg/m³
Exposure time : 4 hour(s)
Method : other: an excess of the test substance was sealed into a 120 l chamber for 24 hrs, the saturation conc. at 22 °C were calculated from Antoine equation, whole body exposure, post exposure observation 14 d, gross pathological examination
Year : 1977
GLP : no data
Test substance : other TS: no data on purity

Result : No death occurred during exposure or during the subsequent 14-d observation period. Gross pathologic examination of rats sacrificed after 14 days revealed no lesions which could be attributed to exposure (no further information given)
### Toxicity

#### ID: 99-99-0

**Reliability:**
- (2) valid with restrictions
  - no information on purity of test substance, no individual animal data reported

**Flag:**
- Critical study for SIDS endpoint
  - 02.09.2004

<table>
<thead>
<tr>
<th>Type</th>
<th>LC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>&gt; 4167 mg/m³</td>
</tr>
<tr>
<td>Species</td>
<td>Mouse</td>
</tr>
<tr>
<td>Strain</td>
<td>NMRI</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Number of animals</td>
<td>10</td>
</tr>
<tr>
<td>Vehicle</td>
<td>other: air</td>
</tr>
<tr>
<td>Doses</td>
<td></td>
</tr>
<tr>
<td>Exposure time</td>
<td>1 hour(s)</td>
</tr>
<tr>
<td>Method</td>
<td>other: exposure to dust, post exposure observation time: 7 days</td>
</tr>
<tr>
<td>Year</td>
<td>1976</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>as prescribed by 1.1 - 1.4</td>
</tr>
</tbody>
</table>

**Result:**
- mortality: 0/10, no signs of intoxication during and post exposure

**Reliability:**
- (2) valid with restrictions
  - exposure time only 1 hour, no gross or histopathological examination

**Flag:**
- Critical study for SIDS endpoint
  - 04.02.2003

<table>
<thead>
<tr>
<th>Type</th>
<th>other: exposure to an atmosphere essentially saturated with test substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Mouse</td>
</tr>
<tr>
<td>Strain</td>
<td>other: CF-1</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Number of animals</td>
<td>10</td>
</tr>
<tr>
<td>Vehicle</td>
<td>other: air</td>
</tr>
<tr>
<td>Doses</td>
<td>228 ppm (100 % saturation) = 1277mg/m³</td>
</tr>
<tr>
<td>Exposure time</td>
<td>4 hour(s)</td>
</tr>
<tr>
<td>Method</td>
<td>other: an excess of the test substance was sealed into a 120 l chamber for 24 hrs, the saturation conc. at 22 °C were calculated from Antoine equation, whole body exposure, post exposure observation 14 d, gross pathological examination</td>
</tr>
<tr>
<td>Year</td>
<td>1977</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: no data on purity</td>
</tr>
</tbody>
</table>

**Result:**
- No death occurred during exposure or during the subsequent 14-d observation period. Gross pathologic examination of rats sacrificed after 14 days revealed no lesions which could be attributed to exposure (no further information given)

**Reliability:**
- (2) valid with restrictions
  - no information on purity of test substance, no individual animal data reported

**Flag:**
- Critical study for SIDS endpoint
  - 02.09.2004

<table>
<thead>
<tr>
<th>Type</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Strain</td>
<td>no data</td>
</tr>
<tr>
<td>Sex</td>
<td>no data</td>
</tr>
<tr>
<td>Number of animals</td>
<td>1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>other: no data</td>
</tr>
</tbody>
</table>
### Doses: 250-625 mg/l air (see Method)

### Exposure time: 10.5 hour(s)

### Method: other: see freetext Method

### Year: 1903

### GLP: No

### Test substance: other TS: isomer not specified, no data on purity

#### Method

Animals lived in glass boxes with air ventilation. The content of the test substance in the air was measured every 2-3 hours. Animals were observed for respiration, observation of pupils, coordination of movement, convulsions, if the animal died: necropsy and pathologic evaluation.

#### Result

**Test substance content in the glass box:**
- In the first 3 hrs: 625 mg/l air
- In the next 4.5 hrs: 290 mg/l air
- In the next 3 hrs: 250 mg/l air

Rabbit survived, symptoms during and after exposure: no symptoms

#### Reliability

(3) invalid

Isomer not specified

---

### Type: Other

### Value:

### Species: Rabbit

### Strain: no data

### Sex: no data

### Number of animals: 1

### Vehicle: other: no data

#### Method: animals lived in glass boxes with air ventilation. The content of the test substance in the air was measured every 2-3 hours. Animals were observed for respiration, observation of pupils, coordination of movement, convulsions, if the animal died: necropsy and pathologic evaluation.

#### Result

**Test substance content in the glass box:**
- In the first 4 hrs: 200 mg/l air
- In the next 4 hrs: 250 mg/l air

Rabbit survived, during and after exposure rabbit showed no reaction.

#### Reliability

(3) invalid

Isomer not specified

---

### Type: Other

### Value:

### Species: Cat

### Strain: no data

### Sex: no data

### Number of animals: 1

### Vehicle: other: no data

#### Method: animals lived in glass boxes with air ventilation. The content of the test substance in the air was measured every 2-3 hours. Animals were observed for respiration, observation of pupils, coordination of movement, convulsions, if the animal died: necropsy and pathologic evaluation.

#### Result

**Test substance content in the glass box:**
- In the first 4.5 hrs: 200 mg/l air
- In the next 4.5 hrs: 250 mg/l air

Rabbit survived, during and after exposure rabbit showed no reaction.

#### Reliability

(3) invalid

Isomer not specified
### Method

animals lived in glass boxes with air ventilation, content of the Test substance in the air was measured every 2-3 hours. Animals were observed for respiration, observation of pupils, coordination of the movement, convulsions, if the animal died: necropsy and pathologic evaluation

### Result

Test substance content in the glass box:
- In the first 3 hrs: 625 mg/l air, in the next 4.5 hrs: 290 mg/l air, in the next 3 hrs: 250 mg/l air
- cat survived, symptoms during exposure: drowsiness, respiration rate: 20-25/min symptoms after exposure: decreased feed intake at the first day after exposure, then normal again

### Reliability

(3) invalid

<table>
<thead>
<tr>
<th>Date</th>
<th>(150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.08.2004</td>
<td></td>
</tr>
</tbody>
</table>

### 5.1.3 ACUTE DERMAL TOXICITY

<table>
<thead>
<tr>
<th>Type</th>
<th>LD50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>&gt; 16000 mg/kg bw</td>
</tr>
<tr>
<td>Species</td>
<td>Rat</td>
</tr>
<tr>
<td>Strain</td>
<td>no data</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Number of animals</td>
<td>no data</td>
</tr>
<tr>
<td>Vehicle</td>
<td>no data</td>
</tr>
<tr>
<td>Doses</td>
<td>2100-16000 mg/kg bw</td>
</tr>
<tr>
<td>Method</td>
<td>other: exposure time: 6 hours, then cleaning of the treated skin area</td>
</tr>
<tr>
<td>Year</td>
<td>1959</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: no data on purity</td>
</tr>
</tbody>
</table>
**Remark**

- mortality: all animals survived (no further details reported)
- within 72 hours methemoglobinemia up to 25 %, reversible
  (no further details reported)

**Reliability**

- (2) valid with restrictions
  documentation limited but hematology reported

**Flag**

- Critical study for SIDS endpoint
  02.09.2004

---

**Type**

- LD50

**Value**

- > 750 mg/kg bw

**Species**

- Rat

**Strain**

- Wistar

**Sex**

- male/female

**Number of animals**

- 10

**Vehicle**

- other: polyethylene glycol 400

**Doses**

- 750 mg/kg bw in polyethylene glycol 400

**Method**

- other: 5 rats/sex/dose group, 1 dose only as 30 % emulsion covered by
  aluminium foil fixed by broad stripes of adhesive plaster to back and belly
  for a 24 hour-exposure period: cleaning with soap and water, observation
  period: 1 week

**Year**

- 1976

**GLP**

- no data

**Test substance**

- as prescribed by 1.1 - 1.4

---

**Remark**

- mortality: 0/10, 18 hours post application up to 4 days:
  poor general condition

**Reliability**

- (2) valid with restrictions
  one dose only, no gross and histopathologic examination

**Flag**

- Critical study for SIDS endpoint
  06.03.2003

---

**Type**

- Other

**Species**

- Mouse

**Strain**

- Sex

**Number of animals**

- Vehicle

**Doses**

- Dermal application of 4-nitrotoluene to the tail of mice has no effect on
  respiration frequency or motility

**Reliability**

- (4) not assignable
  no validated test method

**Result**

- 31.08.2004

---

**Type**

- LD50

**Value**

- > 20000 mg/kg bw

**Species**

- Rabbit

**Strain**

- New Zealand white

**Sex**

- Female

**Number of animals**

- 3

**Vehicle**

- other: undiluted

**Doses**

- Various dose levels up to and including 20000 mg/kg bw (data not shown)

**Method**

- other: 4-NT was applied undiluted to the clipped back; kept in place by 8-
  ply gauze patches, latex rubber dental dam, elastoplast tape for 24 hrs;
  after removal tape, latex, and gauze rabbits observation for 14 d

**Year**

- 1977

**GLP**

- no data

**Test substance**

- other TS: purity not mentioned
**Remark:**
No observable toxic effect at this dose level; during subsequent 14-day observation period all rabbits were symptoms free and gained normal weight.

**Reliability:**
(2) valid with restrictions
individual animal data not shown

**Flag:**
Critical study for SIDS endpoint

**30.08.2004**

**Type:** LD0
**Value:** ca. 200 mg/kg bw
**Species:** Rabbit
**Strain:** no data
**Sex:** Male
**Number of animals:** 6
**Vehicle:** no data
**Doses:** 200 mg/kg bw
**Method:** other: TS was applied to the clipped dorsal skin for 24 hours and wrapped with a layer, stretch gauze bandage and elastic adhesive tape. Afterwards wrapping was removend, skin washed with water and dried; further observation: 48 hours
**Year:** 1972
**GLP:** No
**Test substance:** other TS: no data on purity

**Result:** mortality at 72 hrs: 0/6; no clinical signs of intoxication

**Reliability:**
(4) not assignable
no data on purity, only 1 dose, no data on vehicle, no gross or histopathological evaluation

**31.08.2004**

**Type:** Other
**Value:**
**Species:** Rabbit
**Strain:** no data
**Sex:** Female
**Number of animals:** 1
**Vehicle:** other: no vehicle used
**Doses:** 1000 mg/kg bw
**Method:** other: powdered TS was applied on the unshaved skin (200 cm2). TS was covered from cloth, then fixed with an abdominal binder, followed by staniol foile, and finally bandaged, not removed until the end of the observation time: 50 hrs
**Year:** 1908
**GLP:** No
**Test substance:** other TS: no data on purity

**Result:** 3 hours after application of Test substance rabbit dosen't move and dosen't feed; recoveryv occurred within 12 hours

**Reliability:**
(4) not assignable
unusual experiment which does not comply with the methods of today

**31.08.2004**

**Type:** Other
**Value:**
**Species:** Rabbit
**Strain:** no data
**Sex:** no data
**Number of animals:** 1
**Vehicle:** other: none
**Doses:** 50 cm3
**Method:** other: see freetext Method
OECD SIDS  4-NITROTOLUENE
5. TOXICITY  ID: 99-99-0
DATE: 09.09.2004

| Year       | 1903 |
| GLP        | No   |
| Test substance | other TS: isomer not specified, no data on purity |

**Method**
Test substance was applied on the skin (no information about the application area, or wether the animal was shaved or not), then wrapped with cloths and put into a cage with the head looking outside the cage enabling it to breathe fresh air.
Test duration: 2.5 hours; necropsy and pathological examinations were performed.

**Result**
At the beginning tachypnea, restlessness; after 1 hour apathy, slow reactions, bradypnea, dilated pupils; after 2 hours head and ears cold, nose, tonguemucous membraane livid, laboured breathing, wheezing, convulsions, coma and death after 2.5 hours.
Necropsy: early rigor mortis, lung: bleeding in the right lower lobe, trachea: increased mucous membranes, anemic, heart contracted, atonic right venticle, vein filled with blood, blood: dark red, urine: nitrotoluene odor

**Reliability**
(3) invalid

30.08.2004

| Type     | Other |
| Value    | |
| Species  | Cat   |
| Strain   | no data |
| Sex      | no data |
| Number of animals | 1 |
| Vehicle  | other: none |
| Doses    | 50 cm3 |
| Method   | other: see freetext Method |
| Year     | 1903 |
| GLP      | No |
| Test substance | other TS: isomer not specified, no data on purity |

**Method**
Test substance was applied on the skin (no information about the application area, or wether the animal was shaved or not), then wrapped with cloths and put into a cage with the head looking outside the cage enabling it to breathe fresh air.
Test duration: 2.5 hours; necropsy and pathological examinations were performed.

**Result**
At the start of treatment and 1 hour later: restlessness, crying, groaning, sneezing, lacrimation with increasing intensity within 2 hours after beginning of the treatment.
After 3 hours: deep and long inspiration, apathy, staring; after 4 hours: no reactions, livid tongue, hypersalivation, wheezing, cornea reflex absent, cornea drying, bradypnea, head cold, coma and death after 6 hours.
Necropsy: early rigor mortis, skin and lung no findings, heart contracted, vein filled with blood, blood: dark red, clotting, no methemoglobinemia, urine: cloudy, dark, nitrotoluene odor

**Reliability**
(3) invalid

02.09.2004

<p>| Type     | LD50 |
| Value    | = 940 mg/kg bw |
| Species  | Rat              |
| Strain   |     |</p>
<table>
<thead>
<tr>
<th><strong>Sex</strong></th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of animals</strong></td>
<td>other: water suspension of gum arabicum</td>
</tr>
<tr>
<td><strong>Vehicle</strong></td>
<td>i.p.</td>
</tr>
<tr>
<td><strong>Doses</strong></td>
<td>2.04, 3.06, 4.53, 6.85, 10.21 15.30 mmol/kg bw (= 280, 420, 621, 940, 1400, 2098 mg/kg bw)</td>
</tr>
<tr>
<td><strong>Route of admin.</strong></td>
<td>i.p.</td>
</tr>
<tr>
<td><strong>Exposure time</strong></td>
<td>other: according to Deichmann, LeBlanc, J.Industr.Hyg. Toxicol. 25, 415 (1943)</td>
</tr>
<tr>
<td><strong>Year</strong></td>
<td>1959</td>
</tr>
<tr>
<td><strong>GLP</strong></td>
<td>No</td>
</tr>
<tr>
<td><strong>Test substance</strong></td>
<td>other TS: no data on purity</td>
</tr>
<tr>
<td><strong>Remark</strong></td>
<td>within 3 hours methemoglobinemia up to 27 %, reversible within 24 hours.</td>
</tr>
</tbody>
</table>

**Type**: other: determination of methemoglobinemia  
**Value**:  
**Species**: Rat  
**Strain**: no data  
**Sex**: no data  
**Number of animals**:  
**Vehicle**:  
**Doses**: 30 mg/kg bw (n=1), 80 mg/kg bw (n=2), 200 mg/kg bw (n=1), 300 mg/kg bw (n=1), 500 mg/kg bw (n=6), 5000 mg/kg bw (n=1)  
**Route of admin.** | i.p.       |
| **Exposure time** |  

**Remark**: 30 mg resulted in 5 % Methemoglobin (methb) (max. 1 hr after application) and no Heinz bodies (Hb), 80 mg in 0 % methb and 30% Hb day before and 32 % Hb a day after exposure, 200 mg in 8 % methb 2 hrs after exposure and 35 % Hb day before and 34 % Hb a day after exposure, 300 mg in no methb and no Hb, 500 mg in 3, 26, 6, 11, 20, 7 % methb, respectively (max: -, 26, 9, 2, 8, 24 hrs after application) and HB (day before - day after exposure: 0-98, 35-96, 0-0, 50-80, 0-92, 79-81 %, respectively), cats died 2-3 days after the injection, at necropsy no unresorbed substance in the belly, 5000 mg: cat died 6 hours post application; necropsy revealed unresorbed
substance in the belly
histopathological examination showed fatty degeneration of the livers.

**Conclusion**
According to the study authors, the measured MetHb levels were difficult to evaluate because the blood became extremely turbid within a very short time. Only in one single animal, MetHb (26%) could be identified unambiguously following the injection of 500 mg/kg bw.

**Reliability**
(4) not assignable
methodological deficiencies

**5. TOXICITY**

**ID:** 99-99-0

**DATE:** 09.09.2004

<table>
<thead>
<tr>
<th>Type</th>
<th>LD50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>= 6800 mg/kg bw</td>
</tr>
<tr>
<td>Species</td>
<td>Mouse</td>
</tr>
<tr>
<td>Strain</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>Doses</td>
<td></td>
</tr>
<tr>
<td>Route of admin.</td>
<td>other: no data</td>
</tr>
<tr>
<td>Exposure time</td>
<td></td>
</tr>
</tbody>
</table>

**31.10.1999**

<table>
<thead>
<tr>
<th>Type</th>
<th>LD50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>= 1042 mg/kg bw</td>
</tr>
<tr>
<td>Species</td>
<td>Mouse</td>
</tr>
<tr>
<td>Strain</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
</tr>
<tr>
<td>Number of animals</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>Doses</td>
<td></td>
</tr>
<tr>
<td>Route of admin.</td>
<td>other: no data</td>
</tr>
<tr>
<td>Exposure time</td>
<td></td>
</tr>
</tbody>
</table>

**31.10.1999**

**5.2.1 SKIN IRRITATION**

<table>
<thead>
<tr>
<th>Species</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Exposure</td>
<td>Semiocclusive</td>
</tr>
<tr>
<td>Exposure time</td>
<td>24 hour(s)</td>
</tr>
<tr>
<td>Number of animals</td>
<td>2</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>PDII</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>not irritating</td>
</tr>
<tr>
<td>Classification</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>other: 2 rabbits, 500 mg/rabbit, to the hairless side of the ear, kept in place by adhesive plaster, time of reading when the wrapping was removed, 24 hrs and daily up to 7 days</td>
</tr>
<tr>
<td>Year</td>
<td>1976</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>as prescribed by 1.1 - 1.4</td>
</tr>
</tbody>
</table>

**Reliability**
(2) valid with restrictions
limited documentation: skin reaction grading scheme not documented

**31.08.2004**

<table>
<thead>
<tr>
<th>Species</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>20 other: mg</td>
</tr>
<tr>
<td>Exposure</td>
<td>no data</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Exposure time</td>
<td>24 hour(s)</td>
</tr>
<tr>
<td>Number of animals</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>PDII</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>not irritating</td>
</tr>
<tr>
<td>Classification</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>other: according to Draize et al., J.Pharmacol.Exper.Therap.82, 377 (1944)</td>
</tr>
<tr>
<td>Year</td>
<td>1959</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: no data on purity; melt.p.:128-129 grade Celsius</td>
</tr>
<tr>
<td>Reliability</td>
<td>(4) not assignable Documentation insufficient for assessment</td>
</tr>
<tr>
<td>16.12.2002</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Exposure</td>
<td>Semiocclusive</td>
</tr>
<tr>
<td>Exposure time</td>
<td>4 hour(s)</td>
</tr>
<tr>
<td>Number of animals</td>
<td>3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>other: Polyethylenglycol 400 (see Method)</td>
</tr>
<tr>
<td>PDII</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>not irritating</td>
</tr>
<tr>
<td>Classification</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>OECD Guide-line 404 &quot;Acute Dermal Irritation/Corrosion&quot;</td>
</tr>
<tr>
<td>Year</td>
<td>1986</td>
</tr>
<tr>
<td>GLP</td>
<td>Yes</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: purity: 99 %</td>
</tr>
<tr>
<td>Method</td>
<td>3 New Zealand rabbits (weight: 2.2-2.9 kg, age: 3-5 months) were put individually in cages. Room temperature 20+/-3 °C, relative humidity: 50+/-20 %, light: 12 hours/day, water and feed ad libitum 24 hours before start of the experiment rabbits were shaved at the dorsal region of the trunk (area 25 cm², intact skin). 500 mg Test substance was moistened with 0.2 ml polyethylene glycol 400 and applied on a special tape with additional gauze (area: 2.5 cm²). This tape was fixed on the shaved area and covered by a semiocclusive dressing for 4 hours. After this time tape and Test substance were carefully removed from the skin with warm water. Reading: 30-60 min, 24, 48 and 72 hours after removing of the tape</td>
</tr>
<tr>
<td>Result</td>
<td>rabbit: 1/2/3: 30-60 minutes after removal of the tape: erythema: 0/0/0, edema: 0/0/0 24 hrs after removal of the tape: erythema: 0/0/0, edema: 0/0/0 48 hrs after removal of the tape: erythema: 0/0/0, edema: 0/0/0 72 hrs after removal of the tape: erythema: 0/0/0, edema: 0/0/0</td>
</tr>
<tr>
<td>Source</td>
<td>Hoechst AG Frankfurt/Main</td>
</tr>
<tr>
<td>Reliability</td>
<td>(1) valid without restriction</td>
</tr>
<tr>
<td>Flag</td>
<td>Critical study for SIDS endpoint</td>
</tr>
<tr>
<td>31.08.2004</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>.5 other: mg</td>
</tr>
<tr>
<td>Exposure</td>
<td>Semiocclusive</td>
</tr>
<tr>
<td>Exposure time</td>
<td>4 hour(s)</td>
</tr>
<tr>
<td>Number of animals</td>
<td>6</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>PDII</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>not irritating</td>
</tr>
<tr>
<td>Classification</td>
<td></td>
</tr>
</tbody>
</table>
### 5. TOXICITY

**Method**: Test substance was applied to the clipped free of hair on the back under cotton gauze pads, the trunk was then loosely wrapped with rubber sheetings for 4 hours. Then wrapping and gauze pads were removed, skin reactions evaluated and the test sites washed. Reading again 24 and 48 hours after initial application.

**Reliability**: (4) not assignable
- technical purity is not defined, results of the readings are not reported in detail

---

#### 5.2.2 EYE IRRITATION

<table>
<thead>
<tr>
<th>Species</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Dose</td>
<td>50 mg</td>
</tr>
<tr>
<td>Exposure time</td>
<td>Unspecified</td>
</tr>
<tr>
<td>Comment</td>
<td>no data</td>
</tr>
<tr>
<td>Number of animals</td>
<td>2</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>not irritating</td>
</tr>
<tr>
<td>Classification</td>
<td>other: 2 rabbits, 50 mg/rabbit eye, other rabbit eye served as control, reading after 2h, 24h, and daily up to 7 days (end of the observation time)</td>
</tr>
</tbody>
</table>

**Method**: other: according to Draize Pharmacol. Exper. Therap. 82, 377 (1944)

**Year**: 1959
**GLP**: No
**Test substance**: other TS: no data on purity; melting point: 128-129 centigrade Celsius

**Result**: Cornea and iris without findings, no conjunctival edema but conjunctival injections in both test eyes (score 1) up to day 6, recovery at day 7

**Reliability**: (2) valid with restrictions
- limited documentation: grading scheme not documented

---

<table>
<thead>
<tr>
<th>Species</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>no data</td>
</tr>
<tr>
<td>Dose</td>
<td>20 mg</td>
</tr>
<tr>
<td>Exposure time</td>
<td>Unspecified</td>
</tr>
<tr>
<td>Comment</td>
<td>no data</td>
</tr>
<tr>
<td>Number of animals</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>slightly irritating</td>
</tr>
<tr>
<td>Classification</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>other: according to Draize Pharmacol. Exper. Therap. 82, 377 (1944)</td>
</tr>
<tr>
<td>Year</td>
<td>1959</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: no data on purity; melting point: 128-129 centigrade Celsius</td>
</tr>
</tbody>
</table>

**Remark**: method: according to Draize
**Reliability**: (4) not assignable
documentation insufficient because lack of details

---

<table>
<thead>
<tr>
<th>Species</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td></td>
</tr>
<tr>
<td>Dose</td>
<td>100 mg</td>
</tr>
</tbody>
</table>
Exposure time: 24 hour(s)
Comment: rinsed after (see exposure time)
Number of animals: 3
Vehicle: 
Result: not irritating
Classification: 
Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1986
GLP: Yes
Test substance: other TS: purity: 99 %

Method: 3 New Zealand rabbits (weight: 2.7-3.7 kg, age: 3-5 months) were put individually in cages. Room temperature 20+/-3 °C, relative humidity: 50+/-20 %, light: 12 hours/day, water and feed ad libitum
24 hours before the start of the test fluorescein instillation should discover damage of the cornea of the rabbit’s eyes, because only rabbits with eyes without impairment should be included in the test.

100 mg Test substance was applied into the conjunctival sac of the left eye of each of 3 rabbits. The right eye served as control. 24 hours post application only the eyes with white discharge were rinsed with water. Reading was performed 1, 24, 48 and 72 hours post application according to Draize. Additionally, cornea was examined under UV light using fluorescein solution.

Result: rabbit 1/2/3:
Chemosis: 1h: 1/1/1; 24h: 0/0/0; 48h: 0/0/0; 72h: 0/0/0
Erythema: 1h: 1/1/1; 24h: 1/2/1; 48h: 0/0/0; 72h: 0/0/0
Iris: 1h: 0/0/0; 24h: 0/0/0; 48h: 0/0/0; 72h: 0/0/0
Opacity: 1h: 0/0/0; 24h: 0/0/0; 48h: 0/0/0; 72h: 0/0/0
fluorescein-test: 24h: 0/0/0 72h: 0/0/0

white discharge was observed in the first hour post application of test substance

Calculated mean values following 24, 48 and 72 hours of observation:
all rabbits/rabbit1/2/3
Opacity: 0/0/0/0 Iris: 0/0/0/0
Erythema: 0.4/0.3/0.7/0.3 Chemosis: 0/0/0/0

Source: Hoechst AG Frankfurt/Main
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
02.09.2004 (162)

Species: Rabbit
Concentration: 
Dose: 10 other: mg
Exposure time: 
Comment: 
Number of animals: 2
Vehicle: 
Result: not irritating
Classification: 
Method: other: solid test substance was placed in the concunctival sac of one eye, 20 sec. later one eye was washed, observation: 1 and 4 hrs and at 1, 2, 3 days after treatment
Year: 1981
GLP: no data
Test substance: other TS: purity: 99.5 %
Reliability: (4) not assignable
5.3 SENSITIZATION

Type: Buehler Test
Species: guinea pig
Concentration: 1st: Induction 50 % other: chamber
              2nd: Challenge 10 % other: chamber
              3rd:
Number of animals: 20
Vehicle: other: acetone
Result: not sensitizing
Classification: Method: OECD Guide-line 406 "Skin Sensitization"
Year: 1996
GLP: no data
Test substance: other TS: no data on purity

Method: TEST ANIMALS:
Young adult, male and female Hartley guinea pigs were used. The animals
weighed 326 to 521 grams at the start of the study.
20 test animals 10 naive control animals and 8 pilot animals were used;
equal numbers of males and females were included in each animal group.
HOUSING AND ANIMAL CARE:
Prior to use, all animals were acclimated for at least 5 days; animals were
individually housed in wire mesh suspension cages; diet and tap water ad
libitum during acclimatization and test period; 12-hour light/12-hour dark
cycle; Temperature: 64-79°F; relative humidity: 30-70%
TEST MATERIAL ADMINISTRATION:
-------1) Irritation screening (Pilot)
The irritation potential of the test material at levels of 50%, 25%, 10%, 5%,
2.5%, 1%, 0.5% and 0.25% was evaluated in 2 groups of 4 animals each. 4
levels of the test material were evaluated per animal such that each animal
in a given pilot group was exposed to the same levels. Dilutions of the test
material were formulated w/v in acetone.
On the day prior to test material exposure the hair was removed from each
of the animals' backs. A 0.3 ml quantity of each test preparation was
applied into a 25 mm chamber which was applied to the clipped surface of
the animals in restrainers and occluded with rubber dental dam pulled out
and fastened to the bottom of the restrainer with clips. The day following
the irritation exposure all animals were depilated and scored.
-------2) Induction Phase
The left shoulder (site 1) of each test animal was clipped the day before
exposure. The animals were restrained and a 0.3 ml quantity of the test
preparation was applied as previously described. The procedure was
repeated at the same site once a week for the next 2 weeks for a total of 3
approximate 6-hour exposures. After the last induction exposure, the
animals were left untreated for approximately 2 weeks (13 days) before
primary challenge.
-------3) Primary Challenge Phase
The test animals were again exposed in the challenge phase. In addition
10 naive animals which had never been exposed to the test material were
concurrently treated with the same test material concentration. The same
exposure procedure was used as for the "Induction Phase" but the
chambers were applied to a skin side that had not been exposed
previously. Each animal received one patch of the test material using site
2.
-------Observations
The day following primary challenge exposure all animals were depilated
within no more time than 15 minutes. Following a minimum of 2 hours after
depilation, the test sides were graded.

Based on these results an assessment by comparison of responses in the
test group to that of the corresponding control group was carried out.

Historical positive control data, Buehler Test, 1996:
1-Chloro-2,4-Dinitrobenzene,
alpha-Hexylcinnamaldehyde

Result:

Based on the Irritation Screening (Pilot):
a 50% w/v concentration of 4-nitrotoluene in acetone was chosen for use at
induction.
a 10% w/v concentration of 4-nitrotoluene in acetone was chosen for use at
primary challenge.

Test animals, 20 guinea pigs,
at 24 hours: 10/10 females with slight, patchy erythema, 10/10 males with
slight, patchy erythema: numerical mean score: 0.5;
at 48 hours: 5/10 females with slight, patchy erythema, 5/10 females with
no reaction, 6/10 males with slight, patchy erythema, 4/10 males with no
reaction: numerical mean score: 0.3

Control:
Naive animals, 10 guinea pigs,
24 hours: 5/5 females, 5/5 males: with slight, patchy erythema, numerical
mean score: 0.5,
48 hours: 3/5 females with slight patchy erythema, 2/5 females with no
reactions, 2/5 males with slight patchy erythema, 3/5 males with no
reaction: numerical mean score: 0.3

Historical positive control data
1-Chloro-2,4-dinitrobenzene, incidences-mean score:
0.1% in acetone: 10/10-11.7 (24 hrs), 10/10-1.4 (48 hrs)
alpha-Hexylcinnamaldehyde, techn. = 85 %, incidences-mean score:
5 % in acetone: 10/10-1.1 (24 hrs), 7/10-0.9 (48 hrs)
2.5 % in acetone: 7/10-0.9 (24 hrs), 4/10-0.8 (48 hrs)

Reliability:
(1) valid without restriction

Flag:
Critical study for SIDS endpoint

03.11.2004 (164)

Type:
other: Single Injection Adjuvant Test (SIAT)

Species:
guinea pig

Concentration:
1st: Induction 7.6 other: mM intracutaneous
2nd: Challenge 19.2 other: mM other: chamber

3rd:

Number of animals:
10

Vehicle:
no data

Result:
not sensitizing

Classification:

Method:
other: according to Goodwin et al., Contact Dermatitis 7, 248 (1981)

Year:
1983

GLP:
no data

Test substance:
other TS: purity > 99 %

Method:
TEST ANIMALS:
10 guinea pigs were used and were housed in single sex pairs for the
duration of the experiment in a room maintained at a constant temperature
of 20°C. They were fed pelleted diet, additional hay and cabbage daily,
water ad libitum

PREPARATION OF TEST SOLUTION:
the substance was dissolved in a minimum amount of a suitable vehicle
before mixing with FCA.

TEST PROCEDURES:
---------1) Preliminary irritation test
tests were carried out on groups of 4 guinea-pigs to select the appropriate
concentrations for induction and challenge

2) SIAT procedure

A moderately irritant concentration was selected for the intradermal induction concentration (0.001 ug/ml); Challenge was made using the maximum non-irritant concentration (0.0026 ug/ml, 6-hour patch).

Sensitization was induced by a single intradermal injection of the test substance in complete FCA in the nuchal region of the test guinea pigs. The guinea pigs were challenged 12 to 14 days later (without further treatment) by a 6-hour occluded chamber application of the test substance on the prepared razored flank of the guinea pig. Groups of 4 untreated control guinea pigs of similar age and weight to the test guinea pigs were included in this challenge.

Reactions were assessed 18 and 42 hours after removal of the chamber, according to the degree of erythema and edema.

The challenge was repeated at weekly intervals (3-4 challenges in total) on opposite flanks using further groups of untreated control guinea pigs at each challenge.

Result: 
p-Nitrotoluene did not induce sensitization in guinea pigs by the SIAT procedure (no further details given).

Reliability: 
(2) Valid with restrictions

Flag:
Critical study for SIDS endpoint

5.4 REPEATED DOSE TOXICITY

Type: Sub-acute
Species: Rat
Sex: male/female
Strain: other: F344/N
Route of admin.: oral feed
Exposure period: 14 d
Frequency of treatm.: Daily
Post exposure period: No
Doses: 0, 1250, 2500, 5000, 10000, 20000 ppm (=ca.94, 188, 375, 750, 1500 mg/kg bw/day)
Control group: yes, concurrent no treatment
Method: other: 5 rats/sex/dose group, observed for mortality, clinical signs of toxicity; weighed initially, after 1 week and at necropsy, feed consumption measured weekly
Year: 1992
GLP: Yes
Test substance: other TS: purity: >96 %
Remark: dose finding study for the 13 week toxicity study
Result: no effects on survival, 20000 ppm (male, female): weight loss, no other signs of toxicity
Reliability: (2) Valid with restrictions
dose finding study

30.08.2004

(166) (167)
Control group : yes, concurrent no treatment
LOAEL : ca. 625 ppm
Method : other: see freetext Method
Year : 1992
GLP : Yes
Test substance : other TS: purity:>96 %

Method : --Size on study groups:
10 males and 10 females
--dose levels:
males: 0, 42, 82, 165, 342, 723 mg/kg bw/day
females: 0, 44, 82, 164, 335, 680 mg/kg bw/day
--Type and frequency of observation:
observed 2x/day for mortality/moribundity, body weight and clinical
observations recorded weekly and at necropsy; feed consumption was
measured weekly.
--Necropsy and histologic examinations
complete necropsy performed on all animals. Protocol-required tissues
examined in all control animals, all early death animals and all animals in
the highest dose group with 60 % survivors.
- The following tissues were examined:
gross lesions tissue masses or suspect tumors and regional lymph nodes,
skin, mandibular and mesenteric lymph nodes, mammary glands with
adjacent skin, salivary gland, thigh muscle, ileum, colon, caecum, rectum,
liver, femur, (to include diaphysis with marrow cavity and epiphysis),
thymus, trachea, lungs, and bronchi, heart, thyroid, parathyroid,
esophagus, stomach, duodenum, jejunum, pancreas, spleen and kidneys,
adrenal glands, urinary bladder, seminal vesicles, prostate, testis,
epididymides,
owaries, uterus, nasal cavity, nasal cavity and nasal turbinates, brain with
stem, pituitary, preputial or clitoral glands. The following organs were
weighed at termination of the study: heart,
liver, lungs, right kidneys, thymus, and right testicle
--clinical chemistry/hematology:
blood, and samples analyzed at 1 week, 3 weeks and at the end of 13
week study
--Reproductive System evaluation
male and female rats from 0, 2500, 5000 and 10000 ppm (see also
chapter: 5.8.1
--time held before study: 10-15 days
--Age when placed on study: 6 weeks
--Age when killed: 19 weeks
diet: NIH-07 ad libitum
--animal room environment:
5/cage, 66-79°F; 32-90 % humidity, 12 hours fluorescent light/day, 16-29
air changes per hour
--Statistical methods:
Parametric multiple comparisons procedures of Williams and Dunnett
Nonparametric multiple comparisons methods of Shirley and Dunn
Jonckheere's test
The outlier test of Dixon and Massey

Remark : See also Chapter 5.8.1 for reproductive organ evaluation
Result : SURVIVAL and CLINICAL SIGNS:
--no effects on survival;
--no clinical signs of toxicity which could be attributed to p-nitrotoluene
BODY WEIGHT :
--10000 ppm, males and females: slightly reduced body weight gain (not
significant) when compared to the control;
-- males: necropsy body weight significantly reduced from 5000 ppm
onwards (315 g, 253 g versus 350 g of controls)
-- females: necropsy body weight significantly reduced from 5000 ppm
-SIGNIFICANT ABSOLUTE and RELATIVE ORGAN WEIGHT CHANGES:

-------MALES:

HEART: absolute reduced from 1250 ppm onwards 0.966 g, 0.858 g versus 1.121 g of controls;
RIGHT KIDNEY: absolute reduced at 10000 ppm (0.926 g versus 1.133 g of controls and relative increased from 5000 ppm onwards (3.49, 3.69 versus 3.21 of controls);
LIVER: absolute reduced at 10000 ppm (9.60 g versus 11.35 g of controls), and relative increased from 5000 ppm onwards (35.3, 38.3 versus 32.2 of controls);
LUNGS: absolute reduced at 10000 ppm (1.220 g versus 1.509 g of controls) and relative increased from 5000 ppm onwards (4.83, 4.87 versus 4.28 of controls);
RIGHT TESTIS absolute reduced from 5000 ppm onwards (1.348 g, 1.030 g versus 1.447 g of controls);
THYMUS: absolute reduced at 10000 ppm (0.222 g versus 0.338 g of controls)

-------FEMALES

HEART: absolute reduced at 10000 ppm (0.591 g versus 0.710 g of controls),
RIGHT KIDNEY: absolute reduced from 5000 ppm onwards (0.632 g, 0.637 g versus 0.696 g of controls) and relative increased at 10000 ppm (3.66 versus 3.44 of controls),
LIVER: relative increased at 10000 ppm (36.1 versus 29.3 of controls),
LUNGS: absolute reduced at 10000 ppm (0.970g versus 1.081g of controls),
THYMUS: absolute reduced from 5000 ppm onwards (0.244g, 0.240 versus 0.287g of controls)

HISTOPATHOLOGICAL EVALUATION:
males, females:
incidences with increasing concentration (cont., low to high dose increasing in severity
(average severity is based on the number of animals with lesions:
1=minimal, 2=mild, 3=moderate, 4=marked)

-------- KIDNEY:
-- hyaline droplet nephropathy
  (males: 0/10, 10/10(1), 10/10(1), 10/10(1), 10/10(2), 10/10(2)),
-- karyomegaly
  (males: 0/10, 0/10, 3/10(1), 5/10(1),10/10(2), 10/10(2);
  females:0/10, 10/10(1) 10/10(1), 10/10(2), 10/10(2), 10/10(2));
-- pigment
  (males: 0/10, 0/10, 0/10, 0/10, 0/10, 0/10(1);
  females: 0/10, 10/10(1), 10/10(1), 10/10(1), 10/10(2), 10/10(2)
-- alpha-2u globulin concentration in male rats (in percent of supernatant protein)
  7.2, 16.6, 14.1, 13.7, 15.1, 20.3
-------- SPLEEN
-- hematopoiesis
  (males: 0/10, 6/10(1), 9/10(1), 10/10(1.2), 10/10(2.2),
   females: 0/10, 4/10(1), 4/10(1.7), 5/10(1.2), 9/10(1.2), 10/10(1.8));
-- hemosiderin pigment
  (males: 0/10, 10/10(1), 8/10(1), 10/10(1.1), 9/10(1.3), 10/10(2.4);
  females: 0/10, 5/10(1), 6/10(1), 10/10(1.6), 10/10(1.9), 10/10(2.0);
-- congestion
(males: 0/10, 8/10(1), 10/10(1), 9/10(1), 10/10(1), 10/10(1); females: 0/10, 4/10(1), 6/10(1), 10/10(1), 10/10(1), 10/10(2)

--------- REPRODUCTIVE ORGANS:
-- TESTIS degeneration ( 0/10, 0/10, 1/10(2), 0/10, 1/10(2), 4/10(1.8)

HEMATOLOGY and CLINICAL CHEMISTRY DATA:
male and female:
--HEMATOCRIT (%):
male: significantly increased at 10000 ppm week1 (48.1 versus 44.8 in control),
female: significantly increased from 5000 ppm onwards at week 1 (46.5, 46.8 versus 44.3 of controls), decreased at 10000 ppm at week13 (43.7 versus 45.7 at controls)
--HEMOGLOBIN (g/dl):
male: significantly increased at 10000 ppm at week 1 (17.0 versus 15.7 of controls), significantly decreased from 5000 ppm onwards at week 13 (15.2 and 15.0 versus 15.9 of controls),
female: f: significantly increased at 10000 ppm at week1 (16.7 versus 16.0 of controls) and significantly decreased at 10000 ppm at week 3 and at week 13 (16.6 versus 17.4 of controls and 14.7 versus 15.9 of controls, respectively)
--ERYTHROCYTES(10 exp.6/ul):
male: significantly increased at 10000 ppm at week 1 (8.33 versus 7.73 of controls), significantly decreased at 10000 ppm at week3 (7.88 versus 8.31 of controls) and significantly decreased from 5000 ppm onwards at week 13 (8.56, 8.33 versus 8.97 of controls),
female: significantly increased from 5000 ppm onwards at week 1 (8.17, 8.15 versus 7.75 of controls) and significantly decreased at 10000 ppm at week 13 (8.0 versus 8.5 controls)
--MEAN CELL VOLUME (fL):
male significantly increased from 2500 ppm onwards at week 3 (58, 58.1, 60.3 versus 56.8 of controls) and at week 13 at 10000 ppm (54.5 versus 51.1 of controls),
female: significantly increased at 10000 ppm at week 13 (54.7 versus 53.7 of controls)
--MEAN CELL HEMOGLOBIN (pg):
male: from 625 ppm week3 (19.9, 20.0, 20.3, 20.1, 20.8 versus 19.4 of controls)
--MEAN CELL HEMOGLOBIN CONCENTRATION (g/dl):
male: significantly increased from 2500 ppm onwards at week 3 (34.9, 34.7, 34.6 versus 34.2 of controls) and significantly decreased at 10000 ppm at week 13 (33.1 versus 34.8 of controls),
female: significantly decreased at 10000 ppm week 3 (35.3 versus 36.2) and from 5000 ppm onwards week 13 (33.8, 33.7 versus 34.8)
--PLATELETS (10³/ul):
female: significantly decreased from 2500 ppm onwards at week 1 (882.2, 931.4, 965.8 versus 1013.8 of controls) and at week 13 (565.4, 677.6, 785 versus 791.7 of controls)
--RETICULOCYTES (10 exp 6/ul):
male: significantly decreased at 10000 ppm at week 1 (0.08 versus 0.21 of controls),
female: significantly decreased at 10000 ppm at week 1 (0.08 versus 0.13) and significantly increased from 1250 ppm onwards at week13 (0.1, 0.11, 0.15, 0.21 versus 0.06 of controls)
--NUCLEATED ERYTHROCYTES/100 LEUCOCYTES.
male: significantly increased at 10000 ppm at week 3 and at week 13 (2.00 versus 0.3 of controls and 2.40 versus 0.4 of controls, respectively),
female: significantly increased at 10000 ppm at week 3 (1.1 versus 0 of controls) and significantly increased from 5000 ppm onwards at week 13 (1.0, 5.22 versus 0.22 of controls)
--UREA NITROGEN (mg/dl):
   male: significantly decreased from 5000 ppm onwards at week 3 (13.8, 14.9 versus 18.5 of controls),
--CREATININE (mg/dl):
   male: significantly increased from 2500 ppm onwards at week 13 (0.81, 0.83, 0.94 versus 0.72 of controls),
   female: significantly increased from 2500 ppm onwards at week 13 (0.83, 0.81, 0.83 versus 0.73 of controls)
--TOTAL PROTEIN (g/dl):
   male: significantly decreased from 625 ppm onwards at week 1 (6.2, 6.2, 6.0, 6.0, 6.0 versus 6.4 of controls) and from 1250 ppm onwards at week 13 (6.6, 6.6, 6.6, 6.4 versus 7.0 of controls),
   ALBUMIN (g/dl):
   male: significantly increased at 10000 ppm at week 3 (4.2 versus 3.9 of controls)
   --METHEMOGLOBIN (%):
   male: significantly increased at 10000 ppm week 3 (7.1 versus 5.65) and week 13 (8.08 versus 6.54),
   female: significantly increased at 10000 ppm week 13 (9.02 versus 6.36)
   --ALKALINE PHOSPHATASE (IU/l):
   male: significantly decreased from 5000 ppm week 3 (265, 246 versus 299),
   female: significantly increased from 5000 ppm week 3 (265, 246 versus 299),
   --ALANINE AMINOTRANSFERASE (IU/l):
   male: significantly increased at 10000 ppm at week 3 (43 versus 33 of controls) and significantly decreased from 5000 ppm onwards at week 13 (36, 35 versus 46 of controls),
   female: significantly decreased at 10000 ppm at week 3 (38 versus 44 of controls) and from 5000 ppm onwards at week 13 (41.42 versus 45 of controls)
   --SORBITOL DEHYDROGENASE (IU/l):
   male: significantly decreased from 1250 ppm onwards at week 1 (6, 6, 6, 6, 5 versus 7 of controls) and from 5000 ppm onwards at week 13 (7, 7 versus 10 of controls),
   female: significantly decreased from 625 ppm onwards at week 3 (10, 11, 9, 9, 7 versus 13 of controls)
   --BILE ACID (umol/l):
   male: at 10000 ppm at week 13 (16.2 versus 6.1 of controls),
   female: significantly increased at 10000 ppm at week 3 (41.6 versus 16.3 of controls)
### Test substance
- Other TS: p-Nitrotoluene, purity > 99%

### Method
- **SIZE OF STUDY GROUPS:** 50 males and 50 females
- **ANIMALS PER CAGE:** 2 or 3 (males) or 5 (females)
- **TIME HELD BEFORE STUDIES:** 12 days
- **AVERAGE AGE WHEN STUDY BEGAN:** 5-6 weeks
- **DURATION OF EXPOSURE:** 105-106 weeks
- **AVERAGE AGE AT NECROPSY:** 111 to 112 weeks

**DIET:**
- NTP-2000 Open Formula meal, available ad libitum; rats received nonirradiated feed from the beginning of the studies for 8 months and irradiated feed to the end of the studies.
- **WATER:** tap water, available ad libitum

**ANIMAL ROOM ENVIRONMENT:**
- Temperature: 72°F; relative humidity: 50±5%; room fluorescent light: 12 hours/day; room air changes: 10 hour

**TYPE AND FREQUENCY OF OBSERVATION:**
- Observed twice daily, rats were weight initially, during week 4, and every 4 weeks thereafter; clinical findings were recorded at 4-week intervals, feed consumption was measured over a 1-week period every 4 weeks

**METHOD OF SACRIFICE:** Carbon dioxide asphyxiation

**NECROPSY:** Necropsy was performed on all animals

**URINALYSIS:** See chapter 5.0
- Urine was collected during a 24-hour period from 5 male and 5 female rats from each group at 2 weeks and 3, 12, and 18 months. Parameters evaluated included urine volume, creatinine, p-acetamidobenzoic acid and p-nitrobenzoic acid.

**HISTOPATHOLOGY:**
- Complete histopathology was performed on all animals.
- In addition to gross lesions and tissue masses, the following tissues were examined:
  - adrenal gland, bone, brain, clitoral gland, esophagus, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, skin, spleen, stomach (foregut and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus

Haematology or clinical chemistry was not performed.

No interim kill was performed.

**STATISTICAL METHODS:**
- Poly-k test, continuity corrected Poly-3 test, Fisher's least significant difference test, Mann-Whitney U test

**Remark**
- See chapter 5.0 for Urinalysis
- See chapter 5.7 for Neoplastic effects

**Result**
- **SURVIVAL RATE:**
  - M, (control, low, mid, high dose): 31/50, 38/50, 38/50, 40/50; f, (control, low, mid, high dose): 39/50, 37/50, 39/50, 41/50
  - **MEAN BODY WEIGHT AT THE END OF THE STUDY:**
    - M, (control, low, mid, high dose): 402 g, 409 g, 402 g, 366 g (91 % of controls);
    - F, (control, low, mid, high dose): 294 g, 272 g, 262 g, 210 g
  - **CLINICAL SIGNS OF TOXICITY:**
    - All exposed male and female rats: nasal- and eye-discharge

HEMATOLOGY DATA or CLINICAL CHEMISTRY DATA were not reported.
NON-NEOPLASTIC EFFECTS (male, female, control, low, mid, high dose):

---------- KIDNEY:
---renal tubule hyaline droplet, m: 2/50, 23/50, 27/50, 18/50 f: 8/50, 41/50, 49/50, 46/50;
---renal tubule pigmentation, m: 10/50, 28/50, 47/50, 46/50 f: 9/50, 43/50, 49/50, 50/50;
---mineralization, f: 15/50, 21/50, 32/50, 40/50;
---oncocytic renal tubule hyperplasia, f: 0/50, 2/50, 4/50, 6/50

The oncocytic hyperplasia was characterized by individual tubules that were slightly enlarged and filled by large polygonal epithelial cells containing abundant eosinophilic granular cytoplasm and centrally located nuclei (oncocyes). Oncocytic proliferation is thought to arise from the distal renal tubule and is not a part of the spectrum of lesions in the development of proximal tubular neoplasms. No oncocytic neoplasms were observed in the current study.

---------- SPLEEN:
---hemapoietic cell proliferation, m: 9/50, 13/50, 19/50, 25/50 f: 26/50, 26/50, 45/50, 43/50;
---pigmentation, m: 10/50, 12/50, 24/50, 38/50 f: 24/50, 32/50, 45/50, 48/50;

---------- LIVER
---basophilic focus, m: 31/50, 39/50, 42/50, 45/50; clear cell focus, m: 20/50, 27/50, 30/50, 32/50;
---eosinophilic focus, m: 5/50, 5/50, 5/50, 9/50 f: 1/50, 2/50, 7/50, 9/50;

---------- TESTIS:

(average severity of lesions: 1=minimal, 2=mild, 3=moderate, 4=marked)
---germinial epithel atrophy, m: 7/50(2.1), 11/50(2.7), 8/50(3.1), 30/50(3.5)

Neoplastic effects: see chapter 5.7

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
02.09.2004

Type : Species : Rat
Sex : no data
Strain : no data
Route of admin. : Gavage
Exposure period : 30 d
Frequency of treatm. : Daily
Post exposure period : no data
Doses : 1/5 LD50, no other information
Control group : other: no data
Method : other: no data
Year : 1973
GLP : No
Test substance : no data

Result : sulphhemoglobinemia, forming of methemoglobin and Heinz bodies, anemia, erythrocytosis, reticulocytosis,
Reliability : (4) not assignable
documentation insufficient for assessment
17.12.2002

Type : Species : Rat
Sex : no data
Strain : no data
Route of admin. : Gavage
<table>
<thead>
<tr>
<th>Exposure period</th>
<th>12 w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of treatm.</td>
<td>3/w</td>
</tr>
<tr>
<td>Post exposure period</td>
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</tr>
<tr>
<td>Doses</td>
<td>1/5 LD50, no other information</td>
</tr>
<tr>
<td>Control group</td>
<td>other: no data</td>
</tr>
<tr>
<td>Method</td>
<td>other: no data</td>
</tr>
<tr>
<td>Year</td>
<td>1973</td>
</tr>
<tr>
<td>GLP</td>
<td>No</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
</tbody>
</table>

**Result**: sulphhemoglobinemia, prolongation of clotting time

**Reliability**: (4) not assignable
Documentation insufficient for assessment

17.12.2002

| Type | Rat |
| Species | male/female |
| Strain | Wistar |
| Route of admin. | Gavage |
| Exposure period | 24 w |
| Frequency of treatm. | once/d, 5 d/w |
| Post exposure period | no data |
| Doses | 400 mg/kg bw/day as suspension in 1 % methylcellulose |
| Control group | yes, concurrent vehicle |
| Method | other: see freetext Method |
| Year | 1980 |
| GLP | No |
| Test substance | other TS: purity: 99 % |

**Method**: 10 rats/dose/sex, the rats were paired with exposed animals of the other sex after 3 months of exposure and the treatment was continued for another 3 months. Hematology and biochemistry parameters were measured and histological examinations performed; Reproductive function was analysed

**Remark**: see also chapter 5.8.1

**Result**: BOTH SEXES:
no signs of intoxication, number of erythrocytes and leucocytes not altered, decrease in hemoglobin content (about 10 %);
MALES:
reduced body weight gain, atrophy of testes, necroses of seminiferous tubules
The severity of the effects was mild in controls to moderate-severe in the dosed animals.

FEMALES:
no apparent effects, except for a loss of hair
OFFSPRING:
no apparent effects.

In a study by Ciss (1980) the effects of 4-nitrotoluene on Wistar rats were investigated by exposing groups of males and females to 400 mg/kg bw/day by oral gavage daily for 3 months. The rats were paired with exposed animals of the other sex and the treatment was continued for another 3 months. The males showed testicular atrophy, necrosis of the seminiferous tubules and an increase in spleen weight. No significant effect on the reproduction or on the offspring were observed.

**Reliability**: (2) valid with restrictions
only one dose level used

**Flag**: Critical study for SIDS endpoint

31.08.2004

(168)
5. TOXICITY

Type : Sub-chronic
Species : Rat
Sex : male/female
Strain : Fischer 344
Route of admin. : Gavage
Exposure period : 13 w
Frequency of treatm. : no data
Post exposure period : no data
Doses : 0, 90, 180, 360 mg/kg bw/day in corn oil
Control group : Yes
NOAEL : = 180 mg/kg bw
Method : other: Sperm Morphology and Vagina Cytology Examination (SMVCE), see also freetext Test condition
Year : 1988
GLP : no data
Test substance : other TS: no data
Remark : see also chapter 5.8.1
Result : MALES 360 mg/kg bw/day decrease in terminal body weight, decrease in absolute cauda epididymis, epididymides and testis weights and relative epididymides weight, but no alteration of sperm parameters
FEMALES 360 mg/kg bw/day: no effect (including estrous cycle length) reported
Test condition : Groups of 10 Fischer 344/N per sex were used
The sperm morphology and vaginal cytology examinations were carried out at the end of 13 week exposure studies and included evaluations of
- motility, concentration and head morphology of sperm from the caudal epididymis
- male reproduction organ (cauda epididymis, epididymis and testis) weights
- average estrous cycle length and relative frequency of different estous stages in females
Reliability : (2) valid with restrictions
documentation insufficient for assessment
Flag : Critical study for SIDS endpoint
31.08.2004

Type : Sub-acute
Species : Rat
Sex : male/female
Strain : Wistar
Route of admin. : Gavage
Exposure period : 4 w
Frequency of treatm. : once/d, 5 d/w
Post exposure period : no data
Doses : 0, 500, 1000 mg/kg bw/day as suspension in 1 % methylcellulose
Control group : yes, concurrent vehicle
Method : other:10 rats/sex and dose group,
Year : 1980
GLP : No
Test substance : other TS: no data
Result : for both sexes: no death reported
Reliability : (2) valid with restrictions
documentation insufficient for assessment
30.08.2004

Type : Sub-acute
Species : Mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 14 d
Frequency of treatm. : Daily
Post exposure period : No
Doses : 675, 1250, 2500, 5000, 10000 ppm (=ca. 101, 187, 375, 750, 1500 mg/kg bw/day)
Control group : yes, concurrent no treatment
Method : other: 5 mice/sex/dose group, observed for mortality, clinical signs of toxicity; weighed initially, after 1 week and at necropsy, feed consumption measured weekly
Year : 1992
GLP : Yes
Test substance : other TS: purity: > 96 %
Remark : dose finding study for the 13 week toxicity study
Result : no effects on survival, reduced body weight gain in the highest dose groups, no clinical signs of toxicity
Reliability : (2) valid with restrictions
dose-finding study

31.08.2004

Type : Sub-chronic
Species : Mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 13 w
Frequency of treatm. : Daily
Post exposure period : No
Doses : 0, 625, 1250, 2500, 5000, 10000 ppm (see also freetext Method)
Control group : yes, concurrent no treatment
NOAEL : 2500 ppm
LOAEL : 5000 ppm
Method : other: 10 mice/sex/dose group, observed for mortality, body weight, clinical signs, feed consumption; complete necropsy performed on all rats
Year : 1992
GLP : Yes
Test substance : other TS: >96 %

Method : --Size of study groups:
10 males and 10 females
--Dose levels:
males: 0, 131, 212, 439, 813, 1491 mg/kg bw/day
females: 0, 164, 320, 625, 1075, 1634 mg/kg bw/day
--Type and frequency of observation:
observed 2x/day for mortality/moribundity, body weight and clinical observations recorded weekly and at necropsy; feed consumption was measured weekly.
--Necropsy and histologic examinations
complete necropsy performed on all animals. Protocol-required tissues examined in all control animals, all early death animals and all animals in the highest dose group with 60 % survivors.
- The following tissues were examined:
gross lesions tissue masses or suspect tumors and regional lymph nodes, skin, mandibular and mesenteric lymph nodes, mammary glands with adjacent skin, salivary gland, thigh muscle, ileum, colon, caecum, rectum, liver, femur, (to include diaphysis with marrow cavity and epiphysis), thymus, trachea, lungs, and bronchi, heart, thyroid, parathyroid,
esophagus, stomach, duodenum, jejunum, pancreas, spleen and kidneys, adrenal glands, urinary bladder, seminal vesicles, prostate, testes, epididymides, ovaries, uterus, nasal cavity, nasal cavity and nasal turbinates, brain with stem, pituitary, preputial or clitoral glands. The following organs were weighed at termination of the study: heart, liver with gallbladder, lungs, right kidney, thymus, and right testicle.

--clinical chemistry/hematology:
not performed

--Reproductive System evaluation
male and female mice from 0, 2500, 5000 and 10000 ppm (see also chapter: 5.8.1)
--time held before study: 12-14 days
--Age when placed on study: 6 weeks
--Age when killed: 19 weeks
--diet: NIH-07 ad libitum
--animal room environment:
mice were housed individually, 66-79°F; 32-90 % humidity, 12 hours fluorescent light/day, 16-29 air changes per hour

--Statistical methods:
Parametric multiple comparisons procedures of Williams and Dunnett
Jonckheere's test

Remark: See also chapter 5.8.1. for reproductive organ evaluation

Result:
SURVIVAL.
no effects on survival;

CLINICAL SIGNS
There were no clinical signs which could be attributed to the administration of p-nitrotoluene.

Necropsy Body Weight (g):
(control, low to high dose):
male: 33.5, 33.1, 33.8, 32.3, 31.4 (sign.), 29.9 (sign.)
female: 29.6, 33.0, 33.3, 27.9, 27.1, 24.9 (sign.)

Liver Weight and Pathology
-- dose-related increase in relative liver weights in all groups(control, low to high dose):
male: 43.2, 45.7, 49.6, 46.3, 52.1, 58.3,
female: 45.1, 48.0, 49.4, 49.7, 52.7, 56.6
-- As no treatment-related gross lesions and no histopathological liver lesions were observed, this weight increase was judged to be not treatment related.

Hematology and Clinical Chemistry.
not performed;

Reproductive System Evaluation:
no adverse effects on reproductive parameters

Reliability: (2) valid with restrictions
hematology and clinical chemistry were not performed

Flag: Critical study for SIDS endpoint

Type: Chronic
Species: Mouse
Sex: male/female
Strain: B6C3F1
Route of admin.: oral feed
Exposure period: 105-106 weeks
Frequency of treatm.: Daily
Post exposure period: No
Doses: 0, 1250, 2500, 5000 ppm  
(males: approx. 0, 170, 345, 690 mg/kg bw/day)  
(females: approx. 0, 155, 315, 660 mg/kg bw/day)

Control group: yes, concurrent no treatment

LOAEL: 1250 ppm

Method: other: in accordance with OECD TG 453, see freetext Method

Year: 2001

GLP: Yes

Test substance:

Method:

SIZE OF STUDY GROUPS: 50 males and 50 females  
ANIMALS PER CAGE: 1 (males) or 5 (females)  
TIME HELD BEFORE STUDIES: 12 days  
AVERAGE AGE WHEN STUDY BEGAN: 5-6 weeks  
DURATION OF EXPOSURE: 105-106 weeks  
AVERAGE AGE AT NECROPSY: 111 to 112 weeks  
DIET:  
NTP-2000 Open Formula meal, available ad libitum; mice received nonirradiated feed from the beginning of the studies for 8 months and irradiated feed to the end of the studies.  
WATER: tap water, available ad libitum  
ANIMAL ROOM ENVIRONMENT:  
temperature: 72°F; relative humidity: 50%; room fluorescent light: 12 hours/day; room air changes: 10 hour  
TYPE AND FREQUENCY OF OBSERVATION:  
observed twice daily, rats were weight initially, during week 4, and every 4 weeks thereafter; clinical findings were recorded at 4-week intervals, feed consumption was measured over a 1-week period every 4 weeks  
METHOD OF SACRIFICE: Carbon dioxide asphyxiation  
NECROPSY: necropsy was performed on all animals  
URINALYSIS: see chapter 5.0  
Urine was collected during a 24-hour period from 5 male and 5 female mice from each group at 2 weeks and 3, 12, and 18 months. Parameters evaluated included urine volume, creatinine, p-acetamidobenzoic acid and p-nitrobenzoic acid.  
HISTOPATHOLOGY:  
Complete histopathology was performed on all animals.  
In addition to gross lesions and tissue masses, the following tissues were examined:  
adrenal gland, bone, brain, clitoral gland, esophagus, gall bladder, heart and aorta, large intestine (ecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland (except male mice), nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus  
STATISTICAL METHODS:  
Poly-k test, continuity corrected Poly-3 test, Fisher's least significant difference test, Mann-Whitney U test  

Remark: See also Chapter 5.7 for neoplastic lesions

Result:

SURVIVAL RATE (control, low, mid, high dose):  
male: 46/50, 46/50, 45/50, 42/50;  
female: 46/50, 47/50, 43/50, 49/50  
BODY WEIGHT AT THE END OF THE STUDY (control, low, mid, high dose):  
male: 41.3 g, 39.7 g, 37.3 g, 36.2 g;  
female: 38.5 g, 39.1 g, 38.9 g, 32.9 g  
CLINICAL SIGNS of toxicity.  
no clinical findings were attributed to p-nitrotoluene exposure
HEMATOLOGY and CLINICAL CHEMISTRY:
were not reported.

NON-NEOPLASTIC EFFECTS (male, female, control, low, mid, high dose):
---------- LUNG-- alveolar epithelial bronchiolization,
        male: 0/50, 20/50 (sign.), 30/50 (sign.), 48/50 (sign.)
        female: 0/50, 33/50 (sign.), 41/50 (sign.), 49/50 (sign.);
        --alveolar epithel hyperplasia (not significant),
        male: 1/50, 1/50, 4/50, 6/59;
        female: 2/50, 1/50, 2/50, 1/50
        --no evidence of viral infection
---------- LIVER
        -- syncytia focal alterations only in males:
        2/50, 13/50, 17/50, 33/50

NEOPLASTIC EFFECTS: see chapter 5.7

Reliability : (2) valid with restrictions
hematology and clin.chemistry are not performed
Flag : Critical study for SIDS endpoint
31.08.2004 (129)

Type:
Species: Mouse
Sex: Female
Strain: B6C3F1
Route of admin.: Gavage
Exposure period: 14 d
Frequency of treatm.: Daily
Post exposure period: no data
Doses: 200, 400, 600 mg/kg bw/day in corn oil
Control group: yes, concurrent vehicle
Method: other
Year: 1991
GLP: no data
Test substance: other TS: no data

Result: Suppression of IgM antibody forming cell response to the
T-dependent antigen from sheep erythrocytes (no further
information) in a dose dependent manner; max. suppression:
61 %
Reliability: (4) not assignable
documentation insufficient for assessment
05.03.2003 (171) (172)

Type:
Species: Mouse
Sex: male/female
Strain: B6C3F1
Route of admin.: Gavage
Exposure period: 13 w
Frequency of treatm.: no data
Post exposure period: no data
Doses: 0, 40, 80, 160 mg/kg bw/day in corn oil
Control group: yes, concurrent vehicle
NOAEL: = 160 mg/kg bw
Method: other: Sperm Morphology and Vagina Cytology Examination (SMVCE), see
also freetext Test condition
Year: 1988
GLP: no data
Test substance: other TS: no data on purity
Remark : see also chapter 5.8.1

Result : no alteration of body weight, reproduction organ weights (testis, epididymis, cauda epididymis) and no effect on sperm parameters and estrous cycle length

Test condition : Groups of 10 B6C3F1 mice per sex were used

The sperm morphology and vaginal cytology examinations were carried out at the end of 13 week exposure studies and included evaluations of - motility, concentration and head morphology of sperm from the caudal epididymis - male reproduction organ (cauda epididymis, epididymis and testis) weights - average estrous cycle length and relative frequency of different estrous staged in females

Reliability : (2) valid with restrictions

limited documentation: (no quantitative data reported)

Flag : Critical study for SIDS endpoint 31.08.2004 (170)

Type :
Species : Mouse
Sex : Female
Strain : B6C3F1
Route of admin. : Gavage
Exposure period : 14 d
Frequency of treatm. : Daily
Post exposure period : No
Doses : 0, 200, 400, 600 mg/kg bw/day in corn oil
Control group : yes, concurrent vehicle
Method : other: see freetext Method
Year : 1994
GLP : no data
Test substance : other TS: no data on purity

Method :
Experimental protocol:
Mice (total number not mentioned) were 6-8 weeks of age at the start of each study (no detailed information of the different performed studies with respect to the number of rats per study)
Housing in plastic cages: 4/cage
Free access to tap water
Applied dose volume: 0.1 mg/10 gr bodyweight
Toluene was used for comparison
Body weight termination on day 1, day 8, day 15 when necropsied gross and histopathologic examination of: brain, liver, thymus, spleen, lungs, kidney, lymph nodes

day 15: Test procedures:
---hematology and serum chemistry:
erythrocyte and leukocyte lymphocytes, polymorphonuclear leukocytes, monocytes, eosinophils) number, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) Alanine aminotransferase (ALT), urea nitrogen(UN), glucose, albumin and total protein
---bone marrow
nucleated bone marrow cells were enumerated and evaluated for DNA-synthesis and colony-forming ability
---T and B Cell enumeration
---Spleen IgM and IgG Antibody response to the T-dependent Antigen, sRBC
---Spleen cell proliferative response to the mitogens PHA, CinA and LPS
5. Toxicity

---Mixed Leukocyte response (MLR) to DBA/2 spleen cells
---Delayed hypersensitivity Response to Keyhole Limpet Hemocyanin
---Serum Complement proteins
---Macrophage Phagocytosis of Fluorescent Covyspheres and chicken erythrocytes (cRBC)
---Clearance of sheep erythrocytes by the Reticulo-Endothelial System (RES)
---Macrophage enzyme profiles
---Natural Killer (NK) cells and serum Interferon activity
---Host resistance to Microbial and tumor Challenge

Statistical analysis:
Bartlett's test for homogenicity
One-way analysis of variance
Wilcoxon Rank Test
Jonckheere's Test
Proportional Hazards General Linear Model
Fisher's Exact Test

Result:

BODY WEIGHT
--Body weight gain
comparable in all groups (test and control groups),
--Body weight change
significantly increased at 600 mg-gr intervall day 15 to day1: 1.40 g versus control: 0.93 g

ORGAN WEIGHTS
Absolute and relative organ weights without findings except
--LIVER:
dose-dependant increase in absolute liver weight at 400 and 600 mg with
significantly increased relative liver weights at 600 mg of 5.1 % versus 4.7 % of controls: mild to moderate swelling of hepatocytes adjacent to the central vein (appeared to be reversible, no evidence of necrosis).

HEMATOLOGY:
values comparable in all groups (test and control group)
--Leukocyte differential blood count: no pathological findings except
------Monocytes (vehicle, low, mid, high dose: 2.1%, 1.1%, 1.3%, 1.1%,
trend analysis: p<0.01) and
------Eosinophiles (vehicle, low, mid, high dose: 0.3%, 0.1%, 0.8%, 0.4%,
trend analysis p<0.01)

Dose-dependant increase in phagocytosis by peritoneal cells from mice
(vehicle, low, mid, high dose: 1109, 1171, 2480, 3787, trend analysis:
p<0.01)
Serum chemistry, bone marrow cellularity, number of CFU-M and CFU-GM not affected;
suppression of IgM-response to sRBC and the DHR response to KLH;
24 % decrease in the percentage of CD4+ T lymphocytes in the spleen;
no increase in unstimulated natural killer cell activity, response to B cell mitogen LPS, C3 activity or interferon levels;
Decreased resistance to Listeria monocytogenes but not to Streptococcus pneumoniae, Plasmodium yoelli or the B16F10 melanoma;
Increased resistance to the PYB6 tumor

Conclusion:
The functional impairment im PFCA (Plaque Forming Assay) and against HR (Listerien) face the functional improvement of phagocytosis and defence against infections or tumor cells. Therefore the hypothesis (impairment of T-cells by 4-nitrotoluene treatment as main target) is not conclusive because T-cells are necessary for all above mentioned functions. In addition, Natural Killer (NK) cells are not affected.
Due to the lack of information on toxicology, especially histopathology, it is not clear whether the reported findings are secondary to toxic effects and thus of any biological relevance.

Reliability:
(2) valid with restrictions
The document provides limited documentation for the toxicity of 4-nitrotoluene. The data was collected on 31.08.2004 and 17.12.2002.

### Toxicity

**Type:**
- Species: Dog
- Sex: no data
- Strain: no data
- Route of admin.: oral unspecified
- Exposure period: several d
- Frequency of treatm.: Daily
- Post exposure period: no data
- Doses: 5 g/dog/day
- Control group: other: no data
- Method: other: no further information
- Year: 1874
- GLP: No
- Test substance: no data

**Result:**
- irritation of gastric mucous, vomiting, loss of weight, icterus, symptoms reversible
- Reliability: (4) not assignable
documentation insufficient for assessment

### Genetic Toxicity 'In Vitro'

**Type:**
- System of testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538
- Test concentration: 0, 0.1, 0.5, 1, 5, 10 mg/plate dissolved in DMSO
- Cycotoxic concentr.: 10 mg/plate
- Metabolic activation: Without
- Result: Positive
- Method: other: a pour-plate method according to Ames et al., Proc. Natl. Acad. Sci.(USA) vol.70, 782 (1973), pos. and neg. and solvent control, tested up to toxic effect, all tests performed in duplicate, repeated at least 3 times
- Year: 1986
- GLP: no data
- Test substance: other TS: purity 99 %

**Remark:**
- positive only in Salmonella typhimurium TA 100: dose-related increase up to 5 mg/plate; 10 mg/plate: cytotoxic, no data on revertants
- Reliability: (2) valid with restrictions
evaluation only performed in the absence of a metabolic activation system; one trial with solvent control and with the positive controls for all 37 tested substances

**Flag:**
- Critical study for SIDS endpoint

**Type:**
- System of testing: Chinese Hamster ovary (CHO) cells
- Test concentration: -S9-mix: (1)0, 300, 400, 500 ug/ml (harvest time 20 hrs), (2) 300, 400, 500 ug/ml (harvest time 21 hours); +S9-mix: (1) 500, 550, 600 ug/ml (harvest time: 20.3 hours), (2) 400, 500, 550 ug/ml (harvest time: 21 hours)
- Cycotoxic concentr.: 500 ug/ml dimethylsulfoxid
- Metabolic activation: with and without
- Result: Positive
- Year: 1992
- GLP: Yes
- Test substance: other TS: purity: > 96 %

---

OECD SIDS 4-NITROTOLUENE

5. TOXICITY

DATE: 09.09.2004

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Method: CHO cells were incubated with 4-nitrotoluene or solvent (Dimethylsulfoxides (DMSO, solvent control)
-------1) in the absence of S9-mix for 18 hours at 37°C cells, were then washed and fresh medium containing Colcemid was added for additional 2 to 3 hours (=arrestin the first metaphase); cells were harvested by mitotic shake-off, fixed and stained in 6% Giemsa.
-------2) in the presence of S9-mix for 2 hours at 37°C, cells were then washed and fresh medium was added and incubation was continued for 18 to 19 hours. Colcemid was added for the last 2 to 3 hours of incubation before harvest by mitotic shake-off, fixed and stained in 6 % Giemsa

Because of significant chemical-induced cell cycle delay, incubation time prior to addition of colcemid was lengthened from the usual 8- to 10-hour period to provide sufficient metaphases at harvest

-------Preparation of S9-mix
Liver S9-fraction was routinely prepared from male Sprague-Dawley rats that were injected, i.p., with Aroclor 1254. 5 days after injection, the animals were sacrificed and the livers were removed aseptically. Liver homogenates were prepared aseptically at 0-4°C: first rinsed, then minced, homogenized, centrifuged and finally distributed into freezing ampules and stored at -70°C.
-------Positive Controls
without S9-mix: Mitomycin-C;
with S9-mix: Cyclophosphamide
-------Data Evaluation
Armitrage test: Significance of percent cells with aberrations tested by linear regression trend test versus log of the dose

Result:
without S9-mix: negative
with S9-mix:
Trial (1): 500-600 precipitate was formed at these concentrations; 600 mg: positive (>=20% increase over solvent control)
trial (2): 400, 500 mg: negative; 600 mg: positive (>=20% increase over solvent control)

Reliability:
(1) valid without restriction

Flag:
Critical study for SIDS endpoint

Year: 1978
GLP: No
Test substance: other TS: no data on purity

Method:
a sterile paper disc containing 10 ul of DMSO solution of 4-nitrotoluene was placed on the center of the top-agar layer containing S. typhimurium TA 98 or TA100. The plate was inverted and incubated at 37°C in the dark for 48 hours. The diameter of the clear zone produced by antibacterial activity was measured. A disc containing 10 ug of DMSO only was applied the control plate.

Result: 4-nitrotoluene was considered as not having growth inhibition
Reliability: (4) not assignable
### 30.08.2004 (178)

**Type:** Ames test  
**System of testing:** Salmonella typhimurium TA98, TA100  
**Test concentration:** not given  
**Cytotoxic concentr.:** not given  
**Metabolic activation:** with and without  
**Result:** Negative  
**Method:** Other  
**Year:** 1987  
**GLP:** no data  
**Test substance:** other TS  
**Remark:** TA100 +/- S9 equivocal  
**Reliability:** (4) not assignable  

2 strains only, documentation insufficient

### 18.12.2002 (179)

**Type:** Cytogenetic assay  
**System of testing:** Chinese Hamster lung (CHL) cells  
**Test concentration:**  
**Cytotoxic concentr.:**  
**Metabolic activation:** Without  
**Result:** Negative  
**Method:**  
**Year:**  
**GLP:**  
**Test substance:**  
**Remark:** Significant increase of polyploid cells  
**Reliability:** (4) not assignable  

Secondary literature

### 18.12.2002 (180)

**Type:** Ames test  
**System of testing:** Salmonella typhimurium TA98, TA100  
**Test concentration:** no data  
**Cytotoxic concentr.:**  
**Metabolic activation:** with and without  
**Result:** Positive  
**Method:** other: according to Ames et al., Mutat. Res. 31, 347 (1975)  
**Year:** 1981  
**GLP:** No  
**Test substance:** other TS: no data on purity  
**Reliability:** (4) not assignable  

Documentation insufficient for assessment, only two strains used, no data on test concentration

### 30.08.2004 (181)

**Type:** Ames test  
**System of testing:** Salmonella typhimurium TA98, TA100  
**Test concentration:** no data  
**Cytotoxic concentr.:** no data  
**Metabolic activation:** with and without  
**Result:** Positive  
**Method:** other: according to Ames et al., Mutat. Res. 31, 347 (1975)  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS: no data on purity
Remark : positiv only in TA100 with S9
Reliability : (4) not assignable
documentation insufficient for assessment: only performed with two strains, no data on test concentration, cytotoxicity, GLP or purity of the Test substance, no positive and no negative control reported

31.08.2004 (182)

Type : other: Micronucleus Test
System of testing : Chinese Hamster lung (CHL) cells
Test concentration : no data
Cytotoxic concentr. :
Metabolic activation : Without
Result : Negative
Method : Other
Year : 1991
GLP : No
Test substance : other TS: no data

Remark : test done in presence of Cyto-B
Reliability : (4) not assignable
Information given is insufficient for assessment

29.01.2003 (183) (184)

Type : Bacillus subtilis recombination assay
System of testing : Bacillus subtilis H17, M45
Test concentration : various concentrations (no other information)
Cytotoxic concentr. :
Metabolic activation : Without
Result : Positive
Year : 1986
GLP : no data
Test substance : other TS: purity: 99 %

Reliability : (4) not assignable
documentation insufficient for assessment

18.12.2002 (175)

Type : Ames test
System of testing : Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538
Test concentration : 5 dose levels: 10-5000 ug/plate, concurrent solvent control
Cytotoxic concentr. : not given
Metabolic activation : with and without
Result : Positive
Method : other: plate incorporation assay according to de Serres and Shelby, Environ. Mutagen. 1, 87 (1979)
Year : 1982
GLP : no data
Test substance : other TS: no data

Reliability : (4) not assignable
the report describes the results of the screening of 2,4,6-trimitrotoluene wastewater condensate products;
lack of relevant information : cytotoxic concentration, detailed doses

30.08.2004 (185) (186)

Type : Ames test
System of testing : Salmonella typhimurium TA98, TA100
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : Negative
Method : other: according to Yahagi, Tanpakushitsu Kakusan Koso 29, 1178 (1975)
Year : 1983
GLP : no data
Test substance : other TS: chromatographically pure

Remark : TA98 strains additional with norharman
Reliability : (4) not assignable
   no data on test concentration and cytotoxicity
01.09.2004

Type : Ames test
System of testing : Salmonella typhimurium TA98, TA100
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : Negative
Method : other: according to Ames, Mutat. Res. 31, 347 (1975)
Year : 1981
GLP : no data
Test substance : other TS
Reliability : (4) not assignable
   Documentation insufficient for assessment, only two strains used, no data
   on test concentration
25.02.2003

Type : Unscheduled DNA synthesis
System of testing : rat spermatogenic cells
Test concentration : 0, 10, 100, 1000 uM in DMSO
Cycotoxic concentr. : 1000 uM
Metabolic activation : Without
Result : Negative
Method : other: Preparing a cell suspension composed of post-s-phaser primary
   spermatocytes and spermatids, incubation with test substance for 18 hrs
Year : 1984
GLP : no data
Test substance : other TS: purity: 99 %
Reliability : (4) not assignable
   not the established cell line for this test method, only tested without an
   activation system
30.08.2004

Type : Ames test
System of testing : Salmonella typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537
Test concentration : 0, 30, 100, 300, 1000, 3000 ug/plate in DMSO
Cycotoxic concentr. : 
Metabolic activation : with and without
Result : Positive
Method : other: preincubation method, highest concentration: toxic, positive,
   negative and solvent control
Year : 1981
GLP : no data
Test substance : other TS
Reliability : (4) not assignable
   documentation insufficient for assessment
12.04.2003

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<table>
<thead>
<tr>
<th>Type</th>
<th>Unscheduled DNA synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>System of testing</td>
<td>rat hepatocytes</td>
</tr>
<tr>
<td>Test concentration</td>
<td>10, 100, 1000 uM dissolved in DMSO</td>
</tr>
<tr>
<td>Cytotoxic concentr.</td>
<td>1000 uM</td>
</tr>
<tr>
<td>Metabolic activation</td>
<td>Without</td>
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<tr>
<td>Result</td>
<td>Negative</td>
</tr>
<tr>
<td>Method</td>
<td>other: according to Williams, Cancer Res. 37,1845 (1977); Cancer Lett. 4, 69 (1978); see also freetext Method</td>
</tr>
<tr>
<td>Year</td>
<td>1983</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: purity: 99 %</td>
</tr>
</tbody>
</table>

**Method**

Hepatocytes were isolated from untreated male rats by an EGTA-collagenase procedure. 4-nitrotoluene was dissolved in DMSO and combined with Williams Medium E containing radio-labelled thymidine. The mixture of hepatocytes, 4-nitrotoluene and labelled thymidine was incubated for 18 hours. Then autoradiography and scoring was performed.

**Result**

10 uM, 100 uM: there were more grains in the cytoplasm than in the nucleus

**Reliability**

(2) valid with restrictions

only one trial with neg (solvent) control and one trial with the positive control for all three isomers, which were tested in this experiment

**Flag**

30.08.2004 Critical study for SIDS endpoint (191) (192)

<table>
<thead>
<tr>
<th>Type</th>
<th>Ames test</th>
</tr>
</thead>
<tbody>
<tr>
<td>System of testing</td>
<td>Salmonella typhimurium TA98, TA100, TA1535, TA1537</td>
</tr>
<tr>
<td>Test concentration</td>
<td>+/- S9-mix: 0.0, 3.3, 10.0, 33.0, 100.0, 333.0, 500.0, 667.0, 1000 ug/plate in DMSO</td>
</tr>
<tr>
<td>Cytotoxic concentr.</td>
<td>from 500 ug/plate</td>
</tr>
<tr>
<td>Metabolic activation</td>
<td>with and without</td>
</tr>
<tr>
<td>Result</td>
<td>Negative</td>
</tr>
<tr>
<td>Method</td>
<td>other: preincubation protocol according to Ames, Mutat. Res. 31, 347 (1975) positive control, solvent control, S9 from male Sprague-Dawley rat and male Syrian hamster livers, 2 trials each (see also freetext Method)</td>
</tr>
<tr>
<td>Year</td>
<td>1983</td>
</tr>
<tr>
<td>GLP</td>
<td>Yes</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: purity: &gt; 96 %</td>
</tr>
</tbody>
</table>

**Method**

-------Preparation of S-9 fraction:

Liver S9-fraction was routinely prepared from male Sprague-Dawley rats and male Syrian hamster that were injected, i.p., with Aroclor 1254. 5 days after injection, the animals were sacrificed and the livers were removed aseptically. Liver homogenates were prepared aseptically at 0-4°C: first rinsed, then minced, homogenized, centrifuged and finally distributed into freezing ampules and stored at -70°C.

-------Dose Setting Experiment

to select the dose range. The test chemical were checked for toxicity to TA100 up to a concentration of 10 mg/plate or the limit of solubility, both in the presence and absence of S-9 mix.

-------Positive Controls

Positive control chemicals were tested concurrently.

---in the presence of rat and hamster S-9

-2-aminoanthraceene (all strains)

---without S-9mix

-4-Nitro-o-phenylenediamine (Strain TA98)

-Sodium azide (Strain TA100 and TA1535)
-9-Aminoacridine (Strain TA1537)

Data Evaluation

a positive response was indicated by a reproducible, dose-related increase whether it be twofold over the background or not.

Remark

The positive controls were functional. The background revertant numbers in controls were within expectation

Result

No effects were seen at the top-dose in test cultures

Reliability

(2) valid with restrictions

four strains only

Flag

Critical study for SIDS endpoint

31.08.2004

(193) (167) (129) (194)

Type

Mouse lymphoma assay

System of testing

mouse lymphoma L5178Y/tk+/- cells

Test concentration

-S9-mix: ethanol: (1) 0, 75, 100, 150, 180, 200, 240 (2) 0, 25, 50, 75, 100, 150, 250 +S9-mix: l. acetone: (1)(2)(3) 0, 50, 75, 100, 150, 200, 300, 500, 500, II. Ethanol: 50, 100, 150, 200, 250, 300 ug/ml

Cytotoxic concentr.:

500 ug/ml

Metabolic activation

with and without

Result

Positive

Method

other: protocol presented by Myhr et al., Prog. Mutat. Res. 5, 555-568 (1985), highest dose was determined by limit of solubility and toxicity, colony size not reported (see also freetext Method)

Year

1992

GLP

Yes

Test substance

other TS: purity: > 96 %

Method

in brief:

Cells (6X10[exp.5]) were treated for 4 hours at 37°C in medium with 4-nitrotoluene/solvent or with solvent alone or with 4-nitrotoluene/S9-mix/solvent or with S9-mix/solvent.

Then cells were washed, resuspended in medium and incubated for 48 hours at 37°C. After expression cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (Tk) locus, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.

Preparation of S-9 fraction:

Liver S9-fraction was routinely prepared from male Fisher 344 rats that were injected, i.p., with Aroclor 1254. 5 days after injection, the animals were sacrificed and the livers were removed aseptically. Liver homogenates were prepared aseptically at 0-4°C: first rinsed, then minced, homogenized, centrifuged and finally distributed into freezing ampules and stored at -70°C.

Positive Controls:

in the presence of S9-mix: methylcholantrene

in the absence of S9-mix: methyl methansulfonate

Data Evaluation

based on a statistical model developed by NIEHS for the mouse lymphoma assay (no further information)

Result

in the presence of metabolic activation system:

Solvent acetone:

Trial (1): 300 ug/ml: precipitate of 4-nitrotoluene; 500 ug/ml: lethal; 50-200 ug/ml: significant positive response

Trial (2): 300 ug/ml: precipitate of 4-nitrotoluene; 500 ug/ml: lethal; 300 ug/ml: significant positive response

Trial (3): 300 ug/ml: precipitate of 4-nitrotoluene; 500 ug/ml: lethal; 50-300 ug/ml: significant positive response
---Solvent ethanol:
Trial (1): 250, 300 ug/ml: precipitate of 4-nitrotoluene; 50, 100 ug/ml and 200, 250, 300 ug/ml: significant positive response, 150 ug/ml: negative
-----in the absence of a metabolic activation system:
---Solvent ethanol
Trial (1): no cytotoxicity or precipitation observed; 240 ug/ml: significant positive response
Trial (2): no cytotoxicity or precipitation observed; 150, 250 ug/ml: significant positive response

Reliability : (2) valid with restrictions
no differentiation between small and large colonies

Flag : Critical study for SIDS endpoint
30.08.2004 (167) (129)

Type : Sister chromatid exchange assay
System of testing : Chinese Hamster ovary (CHO) cells
Test concentration : -S9-mix: (1) 0, 50, 167, 500 (2) 200, 300, 400, 500 ug/ml; +S9-mix: (1) 0, 50, 167, 500 (2) 0, 600, 700 (3) 0, 550, 600, 650 ug/ml (solvent DMSO)
Cytotoxic concentr. : -S9-mix: 500 ug/ml; +S9-mix: no data
Metabolic activation : with and without
Result : Positive
Method : other: described in Galloway 1987, highest concentration was determined by solubility
Year : 1992
GLP : Yes
Test substance : other TS: purity: > 96 %

Method : CHO cells were incubated with 4-nitrotoluene or solvent (Dimethylsulfoxide=DMSO)
-----in the absence of S9-mix
for 2 hours at 37 °C. then BrdU was added and incubation was continued for 23.5 hours. Cells were washed, fresh medium containing BrdU and colcemid was added and incubation was continued for 2 to 3 hours. Cells were then collected by mitotic shake-off, fixed, air-dried and stained.
-----in the presence of S9-mix
for 2 hours at 37°C. the cells were then washed and medium containing BrdU was added. Cells were incubated for further 25,5 hours with colcemid present for the final 2 to 3 hours. Cells were then collected by mitotic shake-off, fixed, air-dried and stained.

-----Preparation of S-9 fraction:
Liver S9-fraction was routinely prepared from male Sprague-Dawley rats that were injected, i.p., with Aroclor 1254. 5 days after injection, the animals were sacrificed and the livers were removed aseptically. Liver homogenates were prepared aseptically at 0-4°C: first rinsed, then minced, homogenized, centrifuged and finally distributed into freezing ampules and stored at -70°C.
-----Positive Controls
in the presence of S9-mix: Cyclophosphamide
in the absence of S9-mix: Mitomycin C
-----Data Evaluation
Significance of relative SCEs/chromosome tested by linar regression versus log of the dose

Result : without S9-mix:
Trial (1): 500 ug/ml: precipitate of 4-nitrotoluene; 500 ug/ml: positive response (20 % increase over the solvent control); Summary: weak positive
Trial (2): 200-500 ug/ml: precipitate of 4-nitrotoluene; 200-500 ug/ml: positive response (20 % increase over the solvent control); Summary: positive
with S9-mix:
Trial (1): 500 ug/ml: precipitate of 4-nitrotoluene; 50-500 ug/ml: negative; Summary: negative
Trial (2): 600 and 700 ug/ml: precipitate of 4-nitrotoluene; 700 ug/ml: positive response (20 % increase over the solvent control); Summary: weak positive
Trial (3): 550-650 ug/ml: precipitate of 4-nitrotoluene; 550-650 ug/ml: positive response (20 % increase over the solvent control); Summary: positive

Reliability: (2) valid with restrictions
in the presence of metabolic activation system not tested up to cytotoxicity

Flag: Critical study for SIDS endpoint

Type: other: Serum Free Unscheduled DNA Synthesis Assay (SFUDS)
System of testing: rat hepatocytes
Test concentration: 0, 0.1, 0.5, 1, 5, 10, 50, 100 ug/ml 1 % DMSO
Cytotoxic concentr.: 100 ug/ml
Metabolic activation: no data
Result: Positive
Method: other: test substance dissolved in DMSO, DMSO control, hepatocytes cultured in serum free defined medium (WEM)
Year: 1995
GLP: no data
Test substance: other TS: purity: 98 %

Result: Trial (1): 0.1-5 ug/ml: dose-related significant increase in net nuclear silver grains when compared with the concurrent solvent control; 10 and 50 ug/ml: reduced number in net nuclear silver grains when compared with 0.1-5 ug/ml-slides but significant when compared to the concurrent solvent control; 100 ug/ml toxic
Trial (2): 0.1-50 ug/ml: significant, but not dose-related, increase in net nuclear silver grains when compared with the concurrent solvent control; 100 ug/ml toxic

Reliability: (4) not assignable
special study

Type: Cytogenetic assay
System of testing: human peripheral lymphocytes
Test concentration: 0, 0.005, 0.05, 0.4, 1,0 mmol/l in DMSO
Cytotoxic concentr.: no data
Metabolic activation: Without
Result: Positive
Method: other: see freetext Method
Year: 1995
GLP: no data
Test substance: no data

Method: Lymphocytes from a healthy male donor, 4-nitrotoluene dissolved in DMSO was added to cultures at 48 h after cultur initiation and incubated for additional 24 hours, colchicine was added 2 hours before the end of the incubation. Chromosome preparation were made and stained with Giemsa: The number of cells with chromosome aberrations (gaps were excluded) among 100 cells was recorded, no statistical evaluation. The percentage of aberrant cells was calculated.

Result: dose related increase in percentage of aberrant cells
Reliability: (4) not assignable
not tested up to cytotoxicity, no data on purity of TS, not tested in the presence of an activation system, only one negative (solvent) control for 22 tested substances, no positive control
02.09.2004

Type: Ames test
System of testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102, TA104
Test concentration: +/−S9-mix: (1) 0, 0.0763, 0.305, 1.22, 4.88, 19.5, 78.1, 313, 1250, 5000 ug/plate; (2) 0, 9.7719.5, 39.1, 78.1, 156, 313, 625, 1250 ug/plate in DMSO
Cytotoxic concentr.: from 313 ug/ml
Metabolic activation: with and without
Result: Negative
Method: other: preincubation method according to Ames, Mutat. Res. 31, 347 (1975); Maron, Mutat. Res.113, 173 (1983); highest doses used: cytotoxic, positive controls, solvent control (see also freetext Method)
Year: 1996
GLP: no data
Test substance: other TS: purity 99 %

Method:

------positive controls:
---without S9-mix:
2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (Salmonella typhimurium TA100, TA98, Escherichia coli WP2uvrA, WP2uvrA/pKM101)
Sodium azide (Salmonella typhimurium TA1535)
4-Nitroquinoline-N-oxide (Salmonella typhimurium TA1538)
9-Aminoacridine (Salmonella typhimurium TA1538)
Bleomycin (Salmonella typhimurium TA102)
Pyruvic aldehyde (Salmonella typhimurium TA104)
---with S9-mix
2-Aminoanthracene (for all strains)

------Preparation of S9 Fraction:
Male Sprague-Dawley rats were used for the preparation of liver fractions. Sodium phenobarbital and 5,6-benzoflavone were used as an inducer of the rat metabolic activation system. Sodium phenobarbital was injected intraperitoneally into the rats 4 days before killing and 1,2 and 3 days before killing 5,6 benzoflavone was injected intraperitoneally. From these rats liver S9 fraction was prepared according to Ames et al. (1975), Methods for detecting carcinogens and mutagens in the Salmonella /mammalian microsome mutagenicity test, Mutat. Res. 31, 347-364. S9 was dispensed into freezing ampules and stored at -80°C. Once the stock S9 had been thawed, remained S9 was not reused.

Evaluation criteria:
Twohold rule was used for data evaluation. The chemicals are considered to be mutagenic when dose-related increase in revertant colonycount is observed and the number of revertant colonies per plate with the test substance is more than twice that of the negative control (solvent control) and when a reproducibility of test result is observed.

Remark: The positive controls were functional. The background revertant numbers of the controls were with expectation.

Result: No effects were seen at the top dose of the test cultures.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31.08.2004

5.6 GENETIC TOXICITY ‘IN VIVO’

Type: Cytogenetic assay
Species: other: mouse bone marrow cells
Sex: Male
Strain: other: BDF1
Route of admin.: i.p.
<table>
<thead>
<tr>
<th>Exposure period</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Doses</td>
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</tr>
<tr>
<td>Result</td>
<td>Negative</td>
</tr>
<tr>
<td>Method</td>
<td>other</td>
</tr>
<tr>
<td>Year</td>
<td>1989</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: no data</td>
</tr>
<tr>
<td>Result</td>
<td>no signs of intoxication reported; Chromosomal aberrations show no difference from controls; no further information given</td>
</tr>
<tr>
<td>Reliability</td>
<td>(4) not assignable documentation insufficient for assessment</td>
</tr>
</tbody>
</table>

26.02.2003 (184) (200)

**Type**: Micronucleus assay
**Species**: other: mice bone marrow
**Sex**: Male
**Strain**: B6C3F1
**Route of admin.**: i.p.
**Exposure period**: 3 times at 24 hour intervall
**Doses**: (1) (2) 0, 150, 300, 600 mg/kg bw in corn oil
**Result**: Negative
**Method**: other: as described by Shelby (1993), Environm. Mol. Mutagen 21, 160-179: 5 mice/group, positive (Cyclophosphamide) and solvent control, micronucleated PCE's/1000 PCE's, one-tailed trend test followed by pairwise comparison dosed vs.co
**Year**: 2001
**GLP**: Yes
**Test substance**: other TS: purity: > 99 %

**Method**: Primary range finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by 4-nitrotoluene exposure. (data not shown)

Standard three-exposure protocol:
Male B6C3F1 mice were injected intraperitoneally (three times at 24 hour intervals) with 4-nitrotoluene dissolved in corn oil. Solvent controls mice were injected with corn oil only. The positive control mice received injections of cyclophosphamide. The mice were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2000 polychromatic erythrocytes (PCE's) were scored up to five mice per dose group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group.

Statistical evaluation:
statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-armitage trend test followed by pairwise comparison between each dosed group and the control group.

**Result**: Signs of intoxication were not reported.

Trial (1):

corn oil control: micronucleated PCE's/1000 PCEs: 0.90 (P<=0.0000)
cyclophosphamide control: micronucleated PCE's/1000 PCEs: 6.20 (P<=0.0000)

150 mg/kg: micronucleated PCE's/1000 PCEs: 2.20 (P<=0.0097),
300 mg/kg: micronucleated PCE's/1000 PCEs: 2.50 (P<=0.0030),
600 mg/kg: micronucleated PCE's/1000 PCEs: 1.70 (P<=0.0582).
Summary: positive (trend not significant: P=0.166)
Trial (2):

corn oil control: micronucleated PCE's/1000 PCEs: 1.50

cyclophosphamide control: micronucleated PCE's/1000 PCEs: 4.67
\(P<=0.0001)\)

150 mg/kg: micronucleated PCE's/1000 PCEs: 1.90 \((P<=0.2462)\),
300 mg/kg: micronucleated PCE's/1000 PCEs: 1.60 \((P<=0.4287)\),
600 mg/kg: micronucleated PCE's/1000 PCEs: 2.20 \((P<=0.1247)\).

Summary: negative

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
31.08.2004 (129)

Type : Micronucleus assay
Species : other: rat bone marrow
Sex : Male
Strain : other: F344/N
Route of admin. : i.p.
Exposure period : 3 times at 24 hour intervall
Doses : 0, 150, 300, 600 mg/kg bw in corn oil
Result : Negative
Method : other: as described by Shelby (1993), Environm. Mol. Mutagen 21, 160-179: 5 rats/group, positive (cyclophosphamide) and solvent control, micronucleated PCE's/1000 PCE's, one-tailed trend test followed by pairwise comparison dose vs. co

Year : 2001
GLP : Yes
Test substance : other TS: purity: > 99 %

Method : Primary range finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by 4-nitrotoluene exposure. (data not shown)
Standard three-exposure protocol:
Male Fisher344/N rats were injected intraperitoneally (three times at 24 hour intervals) with 4-nitrotoluene dissolved in corn oil. Solvent controls rats were injected with corn oil only. The positive control rats received injections of cyclophosphamide. The rats were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2000 polychromatic erythrocytes (PCE's) were scored up to five rats per dose group.
The results were tabulated as the mean of the pooled results from all animals within a treatment group.
Statistical evaluation:
statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-armitage trend test followed by pairwise comparison between each dosed group and the control group.

Result : no signs of intoxication were reported;
no increases in micronucleated PCE's in the bone marrow of male rats
\((P<=0.466):\)

corn oil control: micronucleated PCE's/1000 PCEs: 0.80

cyclophosphamide control: micronucleated PCE's/1000 PCEs: 10.30
\((P<=0.0000)\)

150 mg/kg: micronucleated PCE's/1000 PCEs: 1.00 \((P<=0.3186)\),
300 mg/kg: micronucleated PCE's/1000 PCEs: 0.80 \((P<=0.5)\),
600 mg/kg: micronucleated PCE's/1000 PCEs: 0.90 \((P<=0.4041)\).

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
02.09.2004 (129)

Type : Micronucleus assay
Species : Mouse
Sex : Male
Strain : 
Route of admin. : i.p.
Exposure period : Once
Doses : no data
Result : Negative
Method : other: no data
Year : 1989
GLP : 
Test substance : other TS

Result : no increase of micronuclei
Reliability : (4) not assignable
documentation insufficient for assessment

17.12.2002 (201)

Type : Micronucleus assay
Species : other: mouse bone marrow
Sex : Male
Strain : other: BDF1
Route of admin. : i.p.
Exposure period : no data
Doses : no data
Result : Negative
Method : other: no details reported
Year : 1989
GLP : no data
Test substance : other TS: no data

Result : no signs of intoxication reported; no increase in the frequencies of MNPCEs.
Reliability : (4) not assignable
documentation insufficient for assessment

30.08.2004 (200)

Type : Unscheduled DNA synthesis
Species : Rat
Sex : Male
Strain : Fischer 344
Route of admin. : Gavage
Exposure period : Once
Doses : 100, 500 mg/kg bw in corn oil
Result : Negative
Method : other: according to Mirsalis, Carcinogenesis 1, 621 (1980), 12 hrs after application of TS, hepatocytes were isolated, cultured in the presence of 3H-TdR; incorporation of label measured by quant. autoradiography, pos. and neg. control
Year : 1982
GLP : no data
Test substance : other TS: no data on purity

Result : no induction of UDS in hepatocytes
Reliability : (2) valid with restrictions
limited description of the method

26.02.2003 (202)

Type : Unscheduled DNA synthesis
Species: Rat  
Sex: Male  
Strain: Fischer 344  
Route of admin.: Gavage  
Exposure period: Once  
Doses: 0, 100, 200, 500 mg/kg bw in corn oil  
Result: Negative  
Method: other: according to Mirsalis, Carcinogenesis 6, 1521-1524 (1985): 12 week old male rats, UDS measured in primary cultures of hepatocytes derived from rats 12 hrs after treatment  
Year: 1991  
GLP: no data  
Test substance: other TS: purity: >96 %  

Method: 3 male rats/group (12 week old), no information on dose selection available, 12 hrs after application of 4-nitrotoluene hepatocytes were isolated. Cells were cultured in Williams Medium E. After a labelling period of 4 hrs with [3H]thymidine incubation with unlabelled thymidine was continued for 14-19 hrs. The incubation was terminated by washing the cells, fixed on slides and stained. For each dose, 3 slides were scored for each of the 3 rats (6000 cells). Significance of response was determined using Student's t-test modified for unpaired observations with unequal variance.  
Result: no signs of intoxication were reported; no induction of UDS in hepatocytes  
Reliability: (1) valid without restriction  
Flag: Critical study for SIDS endpoint  
31.08.2004  

Type: Unscheduled DNA synthesis  
Species: Rat  
Sex: Male  
Strain: Fischer 344  
Route of admin.: Gavage  
Exposure period: Once  
Doses: 0, 200, 500 mg/kg bw in corn oil  
Result: Negative  
Method: other: see freetext Method  
Year: 1983  
GLP: no data  
Test substance: other TS: purity: 99 %  

Method: 3 rats/dose, 1 rat as neg. control, 1 rat as positive control, application by gavage, 12 hrs after treatment hepatocytes were isolated, incubation with [3H]thymidine for 4 hrs and then for 14 hrs with unlabeled thymidine, pos. (Dimethylnitrosamine) and solvent control. Then the cells were fixed: 3 slides per animal were prepared and 50 cells were scored per slide by autoradiography  
Remark: no induction of UDS in rat hepatocytes  
Reliability: (2) valid with restrictions  
only one trial with negative (solvent) control and one trial with the positive control for all three isomers, which were tested in this experiment  
02.09.2004  

Type: Unscheduled DNA synthesis  
Species: Rat  
Sex: Male  
Strain: Fischer 344  
Route of admin.: Gavage  
Exposure period: Once  
Doses: 0, 50, 200, 1000 mg/kg bw in corn oil
Result : Negative
Method : other: see freetext Method
Year : 1989
GLP : no data
Test substance : other TS: purity: > 96 

Method : 3 doses, dose selection in general: 80, 40, 10% of LD50, highest dose of 1000 mg/kg bw was chosen if the LD50 exceeded this value; 2 and 12 hrs after application, hepatocytes were isolated, cultures were incubated with [3H]thymidine for 4 hrs at 37 °C, then for 14-18 hrs with unlabelled thymidine and then fixed on slides and stained. Quantitative autoradiographic grain counting was performed from 50 morphologically unaltered cells per slide, 3 slides per rat.

Result : no signs of intoxication were reported;
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint

Type : Unscheduled DNA synthesis
Species : Rat
Sex : Male
Strain : 
Route of admin. : oral unspecified
Exposure period : Once
Doses : no data
Result : Negative
Method : 
Year : 
GLP : 
Test substance : 

Result : no induction of UDS in hepatocytes
Reliability : (4) not assignable
documentation insufficient for assessment

Type : Unscheduled DNA synthesis
Species : Rat
Sex : no data
Strain : 
Route of admin. : oral unspecified
Exposure period : no data
Doses : no data
Result : Negative
Method : 
Year : 
GLP : 
Test substance : 

Result : no induction of UDS in hepatocytes
Reliability : (4) not assignable
documentation insufficient for assessment

Type : other: induction of scheduled DNA synthesis (S-Phase)
Species : Rat
Sex : Male
Strain : Fischer 344
Route of admin. : Gavage
Exposure period : Once
5.1 TOXICITY

<table>
<thead>
<tr>
<th>Doses</th>
<th>100 - 750 mg/kg bw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>Negative</td>
</tr>
<tr>
<td>Method</td>
<td>other: 12 week old rats, S-phase was measured in primary hepatocytes 24 and 48 hrs after treatment</td>
</tr>
<tr>
<td>Year</td>
<td>1991</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>no data</td>
</tr>
<tr>
<td>Remark</td>
<td>p-NT failed to induce S-Phase activity</td>
</tr>
<tr>
<td>Reliability</td>
<td>(4) not assignable</td>
</tr>
<tr>
<td></td>
<td>special study</td>
</tr>
</tbody>
</table>

5.2 CARCINOGENICITY

<table>
<thead>
<tr>
<th>Species</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
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</tr>
<tr>
<td>Strain</td>
<td>Sencar</td>
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<tr>
<td>Route of admin.</td>
<td>Dermal</td>
</tr>
<tr>
<td>Exposure period</td>
<td>30 w</td>
</tr>
<tr>
<td>Frequency of treatm.</td>
<td>initiator: once, promotor: 1/w</td>
</tr>
<tr>
<td>Post exposure period</td>
<td>no data</td>
</tr>
<tr>
<td>Doses</td>
<td>initiator: 50, 250, 400 mg/kg; promoter: 4 ug/kg TPA</td>
</tr>
<tr>
<td>Result</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>other: no data</td>
</tr>
<tr>
<td>Method</td>
<td>other: tumor initiation promotion test</td>
</tr>
<tr>
<td>Year</td>
<td>1985</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: purity: 98 %</td>
</tr>
<tr>
<td>Remark</td>
<td>Initiation-promotion-test, initiator: 4-nitrotoluene, promoter: 12-O-tetradecanoylphorbol-13-acetate (TPA), both given in acetone</td>
</tr>
<tr>
<td>Reliability</td>
<td>(2) valid with restrictions</td>
</tr>
<tr>
<td></td>
<td>limited documentation of the test procedure, no data on GLP, no data on sex of the mice used</td>
</tr>
<tr>
<td>Flag</td>
<td>Critical study for SIDS endpoint</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
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</tr>
<tr>
<td>Strain</td>
<td>A/Jax</td>
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<tr>
<td>Route of admin.</td>
<td>i.p.</td>
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<td>Exposure period</td>
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<td>Frequency of treatm.</td>
<td>3/w</td>
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<tr>
<td>Post exposure period</td>
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<td>Doses</td>
<td>1800, 4500, 9000 mg/kg given in corn oil</td>
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<td>Control group</td>
<td>Yes</td>
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<td>Method</td>
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</tr>
<tr>
<td>Year</td>
<td>1985</td>
</tr>
<tr>
<td>GLP</td>
<td>no data</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: purity: 98 %</td>
</tr>
<tr>
<td>Result</td>
<td>for all groups: dose related increase in lung tumor incidence, but no statistical significance: 1800 mg group: mortality: 4/30, 12 % of the survivors with tumors 4500 mg group: mortality: 1/30, 14 % of the survivors with tumors 9000 mg group: mortality: 1/30, 24 % of the survivors</td>
</tr>
</tbody>
</table>
survivors with tumors

Reliability: (3) invalid
the test is of sufficient good quality, but application procedure is not relevant for the human situation and there are longterm study with mice which give more evidence for hazard assessment.

12.04.2003 (207)

Species: Rat
Sex: male/female
Strain: other: F344/N
Route of admin.: oral feed
Exposure period: 105 -106 weeks
Frequency of treatm.: Daily
Post exposure period: No
Doses: 0, 1250, 2500, 5000 ppm
(males: approx. 0, 55, 110, 240 mg/kg bw)
(females: approx. 0, 60, 125, 265 mg/kg bw)

Result: yes, concurrent no treatment
Control group: yes, concurrent no treatment
Method: other: in accordance with OECD TG 453, see freetext Method
Year: 2001
GLP: Yes
Test substance: other TS: purity > 99%

Method: SIZE OF STUDY GROUPS: 50 males and 50 females
ANIMALS PER CAGE: 2 or 3 (males) or 5 (females)
TIME HELD BEFORE STUDIES: 12 days
AVERAGE AGE WHEN STUDY BEGAN: 5-6 weeks
DURATION OF EXPOSURE: 105-106 weeks
AVERAGE AGE AT NECROPSY: 111 to 112 weeks
DIET:
NTP-2000 Open Formula meal, available ad libitum; rats received nonirridiated feed from the beginning of the studies for 8 months and irradiated feed to the end of the studies.
WATER: tap water, available ad libitum
ANIMAL ROOM ENVIRONMENT:
temperature: 72°F; relative humidity: 50 5; room fluorescent light: 12 hours/day; room air changes: 10 hour
TYPE AND FREQUENCY OF OBSERVATION:
Observed twice daily, rats were weight initially, during week 4, and every 4 weeks thereafter; clinical findings were recorded at 4-week intervals, feed consumption was measured over a 1-week period every 4 weeks
METHOD OF SACRIFICE: Carbon dioxide asphyxisation
NECROPSY: Necropsy was performed on all animals
URINALYSIS:
Urine was collected during a 24-hour period from 5 male and 5 female rats from each group at 2 weeks and 3, 12, and 18 months. Parameters evaluated included urine volume, creatinine, p-acetamidobenzoic acid and p-nitrobenzoic acid.
HISTOPATHOLOGY:
Complete histopathology was performed on all animals.
In addition to gross lesions and tissue masses, the following tissues were examined:
adrenal gland, bone, brain, clitoral gland, esophagus, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejenum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, skin, spleen, stomach (foresomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus
Haematology or clinical chemistry was not performed. No interim kill was performed.

STATISTICAL METHODS:
Poly-k test, continuity corrected Poly-3 test, Fisher’s least significant difference test, Mann-Whitney U test

Result:
Survival rate (control, low, mid, high dose):
m: 31/50, 38/50, 38/50, 40/50; f: 39/50, 37/50, 39/50, 41/50
mean body weights at the end of the study (control, low, mid, high dose):
m: 402 g, 409 g, 402 g; f: 294 g, 272 g, 262 g, 210 g.
Clinical signs of toxicity:
all exposed male and female rats: nasal- and eye discharge

Haematology data or clinical chemistry data were not reported

Non-Neoplastic effects (control, low, mid, high dose):
kidney:
renal tubule hyaline droplet, m: 2/50, 23/50, 27/50, 18/50; f: 8/50, 41/50, 49/50, 46/50; renal tubule pigmentation, m: 10/50, 28/50, 47/50, 46/50; f: 9/50, 43/50, 49/50, 50/50; mineralization, f: 15/50, 21/50, 32/50, 40/50;
'oncocytic renal tubule hyperplasia, f: 0/50, 2/50, 4/50, 6/50
spleen:
hemapoietic cell proliferation, m: 9/50, 13/50, 19/50, 25/50; f: 26/50, 26/50, 45/50, 43/50; pigmentation, m: 10/50, 12/50, 24/50, 38/50; f: 24/50, 32/50, 45/50, 48/50;

liver:
basophilic focus, m: 31/50, 39/50, 42/50, 45/50; clear cell focus, m: 20/50, 27/50, 30/50, 32/50; eosinophilic focus, m: 5/50, 5/50, 5/50, 9/50; f: 1/50, 2/50, 7/50, 9/50;
testis:
germinl epithel atrophy, m: 7/50, 11/50, 8/50, 30/50

Neoplastic effects (control, low, mid, high dose):
clitoral gland:
adenoma or carcinoma, f: 8/50, 12/50, 20/50 (sign.), 8/50
(historical control: 84/636 = 13.2 %, range: 2-24 %)
skin (subcutaneous):
fibroma, m: 1/50, 2/50, 7/50, 1/50 (historical control: 33/609 = 5.4 %, range: 0-12%)

fibroma or fibrosarcoma, m: 1/50, 2/50, 9/50, 1/50 (historical control: 41/609 =m 6.7 %, range: 2-14%)
mononuclear cell leukemia:
m: 24/50, 12/50, 5/50, 4/50; f: 13/50, 12/50, 3/50, 1/50
testis
interstitial cell adenoma, m: 49/50, 46/50 45/50 34/50

Reliability:
(2) valid with restrictions

Flag:
30.08.2004: Critical study for SIDS endpoint (129)

Species: Mouse
Sex: male/female
Strain: B6C3F1
Route of admin.: oral feed
Exposure period: 105 - 106 weeks
Frequency of treatm.: Daily
Post exposure period: No
### Doses

<table>
<thead>
<tr>
<th>Doses</th>
<th>0, 1250, 2500, 5000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(males: approx. 0, 170, 345, 690 mg/kg bw)</td>
</tr>
<tr>
<td></td>
<td>(females: approx. 0, 155, 315, 660 mg/kg bw)</td>
</tr>
</tbody>
</table>

### Result

<table>
<thead>
<tr>
<th>Control group</th>
<th>yes, concurrent vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>in accordance with OECD TG 453, see freetext Method</td>
</tr>
<tr>
<td>Year</td>
<td>2001</td>
</tr>
<tr>
<td>GLP</td>
<td>Yes</td>
</tr>
<tr>
<td>Test substance</td>
<td>other TS: purity &gt; 99 %</td>
</tr>
</tbody>
</table>

### Method

| SIZE OF STUDY GROUPS: 50 males and 50 females |
| ANIMALS PER CAGE: 1 (males) or 5 (females) |
| TIME HELD BEFORE STUDIES: 12 days |
| AVERAGE AGE WHEN STUDY BEGAN: 5-6 weeks |
| DURATION OF EXPOSURE: 105-106 weeks |
| AVERAGE AGE AT NECROSPEY: 111 to 112 weeks |

#### DIET:
- NTP-2000 Open Formula meal, available ad libitum; mice received nonirradiated feed from the beginning of the studies for 8 months and irradiated feed to the end of the studies.
- WATER: tap water, available ad libitum

#### ANIMAL ROOM ENVIRONMENT:
- temperature: 72°F; relative humidity: 50 5; room fluorescent light: 12 hours/day; room air changes: 10 hour

#### TYPE AND FREQUENCY OF OBSERVATION:
- Observed twice daily, rats were weight initially, during week 4, and every 4 weeks thereafter; clinical findings were recorded at 4-week intervals, feed consumption was measured over a 1-week period every 4 weeks

#### METHOD OF SACRIFICE:
- Carbon dioxide asphyxiation

#### NECROPSY:
- Necropsy was performed on all animals

#### URINALYSIS:
- Urine was collected during a 24-hour period from 5 male and 5 female mice from each group at 2 weeks and 3, 12, and 18 months. Parameters evaluated included urine volume, creatinine, p-acetamidobenzoic acid and p-nitrobenzoic acid.

#### HISTOPATHOLOGY:
- Complete histopathology was performed on all animals.
- In addition to gross lesions and tissue masses, the following tissues were examined:
  - adrenal gland, bone, brain, clitoral gland, esophagus, gall bladder, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland (except male mice), nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, skin, spleen, stomach (foregut and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus

Haematology or clinical chemistry was not performed.
No interim kill was performed.

### Statistical Methods:
Poly-k test, continuity corrected Poly-3 test, Fisher's least significant difference test, Mann-Whitney U test

### Result

<table>
<thead>
<tr>
<th>Survival rate (control, low, mid, high dose):</th>
</tr>
</thead>
<tbody>
<tr>
<td>m: 46/50, 46/50, 45/50, 42/50; f: 46/50, 47/50, 43/50, 49/50</td>
</tr>
<tr>
<td>body weight at the end of the study (control, low, mid, high dose):</td>
</tr>
<tr>
<td>m: 41.3 g, 39.7 g, 37.3 g, 36.2 g; f: 38.5 g, 39.1 g, 38.9 g, 32.9 g</td>
</tr>
<tr>
<td>no clinical findings were attributed to p-nitrotoluene exposure</td>
</tr>
<tr>
<td>Haematology data or clinical chemistry data were not reported.</td>
</tr>
</tbody>
</table>
5. TOXICITY

ID: 99-99-0

**Non-Neoplastic effects (control, low, mid, high dose):**
- lung
  - alveolar epithelial bronchiolization, m: 0/50, 20/50, 30/50, 48/50 f: 0/50, 33/50, 41/50, 49/50; alveolar epithel hyperplasia, m: 1/50, 1/50, 4/50, 6/50

**Neoplastic effects (control, low, mid, high dose):**
- lung
  - alveolar/bronchiolar adenoma or carcinoma, m: 8/50, 14/50, 12/50, 19/50
  (historical control: 176/659 = 26.7 %, range: 12-44 %)

**Reliability:**
- (2) valid with restrictions
  no interim kill was performed and no hematology data or clinical chemistry data were noted

**Flag:**
- Critical study for SIDS endpoint

30.08.2004 (129)

5.8.1 TOXICITY TO FERTILITY

**Type:**
- Fertility

**Species:**
- Rat

**Sex:**
- male/female

**Strain:**
- Wistar

**Route of admin.:**
- Gavage

**Exposure period:**
- 24 w

**Frequency of treatm.:**
- once/d, 5 d/w

**Premating exposure period**
- Male: 12 w
- Female: 12 w

**Duration of test:**
- 24 w

**No. of generation studies:**
- Doses:
  - 400 mg/kg as suspension in 1 % methylcellulose

**Control group:**
- yes, concurrent vehicle

**Method:**
- other: 10 rats/dose/sex, mating in the 13th. week of treatment, hematol.,biochem.,histol. examination; analysis of the function of reproduction

**Year:**
- 1980

**GLP:**
- No

**Test substance:**
- other TS: purity: 99 %

**Remark:**
- see also chapter 5.4

**Result:**
- Both sexes: no signs of intoxication, number of erythrocytes and leucocytes not altered, decrease in hemoglobin content (about 10 %);
  - Males: reduced body weight gain, atrophy of testes, necroses of seminiferous tubules. The severity of the effects was mild in controls to moderate-severe in the dosed animals.
  - Females: no apparent effects, except for a loss of hair
  - Offspring: no apparent effects.
  In a study by Ciss (1980) the effects of 4-nitrotoluene on Wistar rats were investigated by exposing groups of males and females to 400 mg/kg bw/day by oral gavage daily for 3 months. The rats were paired with exposed animals of the other sex and the treatment was continued for another 3 months. The males showed testicular atrophy, necrosis of the seminiferous tubules and an increase in spleen weight. No significant effect on the reproduction or on the offspring were observed.

**Reliability:**
- (2) valid with restrictions
  only one dose used, therefore no dose response and no NOAEL or LOAEL can be derived

**Flag:**
- Critical study for SIDS endpoint
Type: Fertility  
Species: Rat  
Sex: male/female  
Strain: Wistar  
Route of admin.: Gavage  
Exposure period: males: 35 days, females: up to 46 days  
Frequency of treatm.: Daily  

<table>
<thead>
<tr>
<th>Premating exposure period</th>
<th>Male</th>
<th>2 weeks</th>
<th>Female</th>
<th>2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of test</td>
<td>47 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of generation</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Doses: 0, 25, 100, 400 mg/kg bw in polyethylene glycol 400  
Control group: yes, concurrent vehicle  
other: NOAEL (reproductive toxicity) = 25 mg/kg bw  
other: NOAEL (general toxicity, males) = 25 mg/kg bw  
other: LOAEL (general toxicity, females) = 25 mg/kg bw  

Result: see freetext Result  
Method: other: in compliance with OECD 421, additional evaluation: liver, spleen, kidney, pituitary gland, uterus, uterine cervix and vagina, mammary gland, seminal vesicle and prostate  
Year: 2002  
GLP: Yes  
Test substance: as prescribed by 1.1 - 1.4  

Method: Experimental animals  
Adaption Period: 7 days  
Age at study start: m/f: 12 weeks  
Body weight at study start, m: 320-344 g; f: 196-220 g  
Size of study group: 12 male and 12 female rats per group  
-females used were nulliparous and not pregnant  
Housing condition:  
- during adaption period in groups  
- at study start: individually  
- during pairing: 1 male and 1 female/cage  
- during lactation: individual females with their pups  
- room temperature: 22°C  
- relative humidity: approx. 50%  
- air change: at least 10 times per hour  
Nutrition:  
- standart rat diet and tap water ad libitum  
Dosing:  
- The dose levels used were selected according to a preceding maternal tolerability study in rats.  
- males: 
  received p-nitrotoluene 2 weeks prior to mating, during the following mating and remating period up to the day before necropsy. Necropsy was performed on d 36  
- females: 
  received p-nitrotoluene 2 weeks prior to mating, during the following mating and remating period, during gestation and up to the day before necropsy (treatment time 41-46 d). Necropsy was performed on d 4 or 5 post partum.  
Pairing for mating:  
During the two-week mating period each female was paired daily. Females
in which insemination had not been detected by the end of this period were mated for another week.

Investigation in parent animals:
appearance, behaviour, excretory products, mortality twice daily body weight twice a week or weekly of inseminated females up to (and on day of delivery), on day 4 pp and on day of necropsy feed consumption weekly, water consumption daily gross pathological examination of all male and female rats at necropsy weight of liver, spleen and kidneys of all rats at necropsy histopathologic evaluated organs:
epididymides, testes, prostate, seminal vesicles, coagulation glands ovaries with oviduct, uterus, uterine cervix and vagina, mammary gland with mamilla, liver, kidneys, pituitary, spleen and all other organs with macroscopic findings.
Time to insemination during the mating and remating period insemination index and fertility index following male specific data were recorded and evaluated: testicular and epididymal weight (left and right testis/epididymis individually following female specific data were recorded and evaluated: duration of gestation, gestation index course of birth, lactation behavior, number of corpora lutea in the right and the left ovary, number of implantation sites

Investigations in the F1 pups at birth and up to day 5 pp:
twice daily: Appearance, general behavior, mortality Clinical findings, sex ratio of pups at birth, individual pup weights at birth and on d 4 after birth, viability index Pups were sacrificed on day 4 to 5 pp.

Statistical methods:
Analysis of variance (ANOVA), in case of significant results Dunnett’s test 2 By N Chi²test, in case of significant differences Fisher’s exact test with Bonferroni correction

Result:
F0-GENERATION
all rats including controls showed severe salivation (most probably due to vehicle),

MORTALITY, 400 mg-dose group:
3 males and 5 females in the premating period and
1 female sacrificed moribund on day 22 p.c.: ventral posture, hypoactivity, piloerection, intrauterine death of its litter)

FOOD INTAKE
----MALE, 400 mg-dose group, significantly different from control:
week1: 23.9g/d(control males) versus 14.32g/d (p<0.01)
week2: 23 g/d(control males) versus 26.7g/d (p<0.01)

---FEMALE, control, low, mid, high dose [g/day]:
week1: 14.6, 14.8, 14.9, 14.7 (p<0.01)
week2: 15.7, 15.7, 16.2, 18.7 (p<0.05)
day 0-7p.c.: 19.7, 19.9, 21.1, 19.6
day 14-20p.c.: 25.2, 24.2, 24.8, 20.9 (p<0.05)
day 0-4p.p.: 38.9, 32.4, 34.6, 27.2 (p<0.01)

DEVELOPMENT OF BODY WEIGHT GAIN(d1-d15); significant changes:
----male/female, 400 mg-group:
d1-4: -20.8g/-17.6g (control:8.1g/1.3g);
d4-8: 9.5g/-10.4g (control: 8.4g/2.8g);
d8-15: 19.4g/17.0g (control 9.6g/5.5g);
females body weight gain reduced when compared to controls
during gestation: control, low, mid, high dose: 111.3g, 109.3g, 105.5g, 72.2g (p<0.01)
during lactation: control, low, mid, high dose: 27.2g, 16.1g, 16.6g, 4.0g (p<0.01)

CLINICAL SIGNS OF INTOXICATION,
400 mg-group:
m/f: piloerection, respiratory sound, sunken flanks, increased water intake and urination, reduced amount of feces
females: hypoactivity, alteration of gait and increased incidence of soft and light colored feces
-- 100 mg-group: no findings
-- 25 mg-group:
1 female: ventral posture, hypoactivity, high stepping gait, piloerection

EFFECTS ON REPRODUCTION (control, low to high dose):
--- INSEMINATION INDEX
(no of females inseminated/no of females paired X 100):
100%, 100%, 100%, 100%.

--- FERTILITY INDEX
(no of females with implantation sites/no of females inseminated X 100):
75%, 75%, 100%, 100%

--- GESTATION INDEX
(no of females with viable pups/no of females with implantation sites X 100): 100%, 100%, 100%, 85.7%
----- No of corpora lutea: 21.56, 18.44, 16.42 (stat. sign.), 17.67
----- No of implantation sites/litter: 13, 12.89, 12.75, 12.33
----- Mean number of pups delivered (living and dead): 12.1, 11.6, 12.1, 9.2

--- PRENATAL LOSS
(Difference between no of implantation sites and the total no of pups littered (living and dead)):
0.89, 1.33, 0.67, 3.17 (stat. sign.)
----- Duration of gestation (days): 22.78, 22.44, 22.33, 22.67

--- COURSE OF BIRTH:
----- 400 mg-group: 1 female was sacrificed on d22 p.c.: all fetuses of its litter were dead
----- 25 mg-group: delivering of only 1 pup with a huge hematoma, loss of all other pups on day of birth

F1-PUPS
--- LACTATION BEHAVIOUR:
1 pup of the 100 mg-group, 2 pups of the 400 mg-group: no milk ingestion

POSTNATAL DEVELOPMENT OF F1 PUPS (control, low to high dose):
--- NO OF PUPS DELIVERED (mean-no):
12.11, 11.56, 12.08, 9.17
--- NO OF LIVE PUPS (mean-no, d0/d4):
12.11/12, 11.56/11.25, 11.92/11.33, 9.17/8.33
--- LIVE BIRTH INDEX (%):
100, 100, 98.61, 100
--- VIABILITY INDEX (d4 pp, %):
98.99, 88.89, 95.41, 92.06
--- SEX RATIO (d0, % males/litter):
49.94, 48.66, 44.00, 46.43

--- PUP CLINICAL OBSERVATIONS (frequency/pups/litters, day 0-day 5):
----- Found dead: 1/1/1; 14/14/1; 5/5/1; 1/1/1
----- Missing: 0/0/0; 0/0/0; 2/2/1; 4/4/2
Hypoactivity: 0/0/0; 1/1/1; 0/0/0; 1/1/1
Hematoma: 0/0/0; 0/0/0; 0/0/0; 5/2/2
Pale skin: 0/0/0; 5/1/1; 1/1/1; 1/1/1
Tip of tail dark discolored: 0/0/0; 4/1/1; 0/0/0; 0/0/0
Milk spot not detectable: 0/0/0; 0/0/0; 1/1/1; 2/2/2
Tip of tail missing: 0/0/0; 1/1/1; 0/0/0; 0/0/0

MEAN PUP WEIGHT:
d0: m/f/total and d4: m/f/total:
control group:
6.30/5.96/6.12 and 9.64/9.22/9.43;
25 mg-group:
6.27/5.94/6.07 and 9.42/9.09/9.22;
100 mg-group:
5.43(p<0.01)/5.28(p<0.5)/5.36(p<0.05) and 8.31(p<0.05)/8.20/8.26;
400 mg-group:
4.89(p<0.01)/4.69(p<0.01)/4.80(p<0.01) and 6.79(p<0.01)/6.55(p<0.01)/6.68(p<0.01)

F0-GENERATION
GROSS PATHOLOGY:
MEAN ORGAN WEIGHTS (control, low to high dose, abs(g)/rel[%]):
MALE
Liver:
15.23/3.88, 15.52/3.96, 17.51(p<0.05)/4.26(p<0.05), 19.53(p<0.01)/5.04(p<0.01);
Spleen:
0.67/0.17, 0.69/0.18, 0.72/0.18, 1.06(p<0.01)/0.28(p<0.01)
Kidney, testes weights, epididymal weights were comparable to controls
FEMALE:
Liver and kidney weights were comparable to controls
Spleen:
0.56/0.21, 0.598/0.23, 0.59/0.22, 1.07(p<0.01)/0.45(p<0.01)

HISTOPATHOLOGY (MALES, FEMALES):
400 mg group:
Liver:
periportal pigment deposits (2/12 males, 4/12 females),
variable glycogen content (4/12 males, 3/12 females);
Kidney:
tubular pigment (3/12 females),
mononuclear infiltration (2/12 females),
tubular vacuolation (5/12 females),
single cell necrosis (2/12 males);
Spleen:
Congestion (12/12 males, 10/12 females),
increased pigment (2/12 males, 1/12 females)
Testes:
atrophy, 2/12;
Epididymides:
Cellular debris 4/12
100 mg-group:
Spleen:
Congestion (12/12 males, 2/12 females)
25 mg-groups:
no changes attributable to treatment
Reliability:
(1) valid without restriction
### Critical Study for SIDS Endpoint

**02.09.2004**

**Type**: Other  
**Species**: Rat  
**Sex**: male/female  
**Strain**: Fischer 344  
**Route of admin.**: Gavage  
**Exposure period**: 13 w  
**Frequency of treatm.**: no data  
**Premating exposure period**  
- Male:  
- Female:  
**Duration of test**: 13 w  
**No. of generation studies**:  
**Doses**: 0, 90, 180, 360 mg/kg bw/day given in corn oil  
**Control group**: yes, concurrent vehicle  
**Method**: other: Sperm Morphology and Vagina Cytology Examination (SMVCE), see also freetext Test substance  
**Year**: 1988  
**GLP**: no data  
**Test substance**: other TS: no data  
**Remark**: see also chapter 5.4  
**Result**: 360 mg/kg bw/day, males: decrease in terminal body weight, decrease in absolute cauda epididymis, epididymides and testis weights and relative epididymis weight, but no alteration of sperm parameters  
360 mg/kg bw/day, females: no effect (including estrous cycle length) reported  
**Test condition**: Groups of 10 Fischer 344/N rats per sex were used  

The sperm morphology and vaginal cytology examinations were carried out at the end of 13 week exposure studies and included evaluations of  
- motility, concentration and head morphology of sperm from the caudal epididymis  
- male reproduction organ (cauda epididymis, epididymis and testis) weights  
- average estrous cycle length and relative frequency of different estrous staged in females  
**Reliability**: (2) valid with restrictions  
limited documentation  
**Flag**: Critical study for SIDS endpoint

**31.08.2004**

**Type**: Other  
**Species**: Mouse  
**Sex**: male/female  
**Strain**: other: B6C3F1  
**Route of admin.**: Gavage  
**Exposure period**: 13 w  
**Frequency of treatm.**: no data  
**Premating exposure period**  
- Male:  
- Female:  
**Duration of test**: 13 w  
**No. of generation studies**:  
**Doses**: 0, 40, 80, 160 mg/kg given in corn oil  
**Control group**: yes, concurrent no treatment
**Method**: other: Sperm Morphology and Vagina Cytology Examination (SMVCE), see also freetext Test substance

**Year**: 1988

**GLP**: no data

**Test substance**: other TS: no data

**Remark**: see also chapter 5.4

**Result**: no alteration in body or reproductive organ weights (testis, epididymis, cauda epididymis), no effect on sperm motility, no effect on estrous cycle length

**Test condition**: Groups of 10 B6C3F1 mice per sex were used

The sperm morphology and vaginal cytology examinations were carried out at the end of 13 week exposure studies and included evaluations of:
- motility, concentration and head morphology of sperm from the caudal epididymis
- male reproduction organ (cauda epididymis, epididymis and testis) weights
- average estrous cycle length and relative frequency of different estrous staged in females

**Reliability**: (2) valid with restrictions

**31.08.2004** (170)

**Type**: other: reproductive system evaluation

**Species**: Rat

**Sex**: male/female

**Strain**: other: Fischer 344/N

**Route of admin.**: oral feed

**Exposure period**: 13 w

**Frequency of treatm.**: Daily

**Premating exposure period**

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Duration of test**: 13 w

**No. of generation studies**: 

**Doses**: Only controls and the 3 highest dosages from the repeated dose tox study 0, 2500, 5000, 10000 ppm (m: ca. 165, 342, 723 and f: ca. 164, 335, 680 mg/kg bw/day)

**Control group**: yes, concurrent no treatment

**Method**: other: 10 rats/sex/dose group, reproductive system evaluation

**Year**: 1992

**GLP**: Yes

**Test substance**: other TS: > purity: 96 %

**Remark**: see also chapter 5.4

**Result**: male, 10000 ppm:
- degeneration of testis (weight reduction): 1.09g (p<0.01) versus 1.51g of controls,
- reduced number of sperm: [mean/10exp.4 ml suspension]: 50.15 (p<0.01) versus 76.43 of controls reduced motility of sperm [%]: 59 (n.s.) versus 79 of controls;
- female, :
  - increased proportion of rats in diestrus [as % of cycle]: from 5000 ppm onwards: 55, 78.3 versus 45.8 of controls
  - estrous cycle: lengthened estrous cycle : 5.15d, 6.05 d, 5.00 d versus 5.15 d

for repeated dose toxicity parameters and detailed study description see
chapter 5.4

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
31.08.2004

Type : other: reproductive system evaluation
Species : Mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 13 w
Frequency of treatm. : Daily

Premating exposure period:
Male : 
Female : 

Duration of test : 13 w
No. of generation studies:

Doses : Only controls and the 3 highest dosages from the repeated dose tox study
0, 2500, 5000, 10000 ppm (m: ca. 439, 813, 1491 and f: 625, 1075, 1634 mg/kg bw/day
Control group : yes, concurrent no treatment
Method : other: 10 rats/sex/dose group, reproductive system evaluation
Year : 1992
GLP : Yes
Test substance : other TS: Purity: > 96 %

Remark : see also chapter 5.4
Result : no adverse effects on reproductive parameteres observed.
for repeated dose toxicity parameters and detailed study description see chapter 5.4

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
31.08.2004

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : male/female
Strain : Wistar
Route of admin. : Gavage
Exposure period : males: 35 days, females: up to 46 days
Frequency of treatm. : Daily
Duration of test : 46 days
Doses : 0, 25, 100 or 400 mg/kg bw/day in polyethylene 400
Control group : yes, concurrent vehicle
NOAEL teratogen. : = 25 mg/kg bw
LOAEL Maternal : 25 mg/kg bw
Toxicity Method : other: in compliance with OECD 421, additional evaluation: liver, spleen, kidney, pituitary gland, uterus, uterine cervix and vagina, mammary gland, seminal vesicle and prostate
Year : 2002
GLP : Yes
Test substance : as prescribed by 1.1 - 1.4

Method : Experimental animals
Adaption Period: 7 days
Age at study start: m/f: 12 weeks
Body weight at study start, m: 320-344 g; f: 196-220 g
size of study group: 12 male and 12 female rats per group
-females used were nulliparous and not pregnant

Housing condition:
-- during adaption period in groups
-- at study start: individually
-- during pairing: 1 male and 1 female/cage
-- during lactation: individual females with their pups - - room temperature : 22°C
- relative humidity: approx. 50 %
- air change: at least 10 times per hour

Nutrition:
- standart rat diet and tap water ad libitum

Dosing
- The dose levels used were selected according to a preceding maternal tolerability study in rats.
- males: received p-nitrotoluene 2 weeks prior to mating, during the following mating and remating period up to the day before necropsy. Necropsy was performed on d 36
- females: received p-nitrotoluene 2 weeks prior to mating, during the following mating and remating period, during gestation and up to the day before necropsy (treatment time 41-46 d). Necropsy was performed on d 4 or 5 post partum.

Pairing for mating
During the two-week mating period each female was paired daily. Females in which insemination had not been detected by the end of this period were mated for another week.

Investigation in parent animals:
appearance, behaviour, excretory products, mortality twice daily
body weight twice a week or weekly of inseminated females
up to (and on day of delivery), on day 4 pp and on day of necropsy
feed consumption weekly, water consumption daily
gross pathological examination of all male and female rats at necropsy.
weight of liver, spleen and kidneys of all rats at necropsy

histopathologic evaluated organs:
epididymides, testes, prostate, seminal vesicles, coagulation glands
ovaries with oviduct, uterus, uterine cervix and vagina, mammary gland
with mamilla, liver, kidneys, pituitary, spleen and all other organs with macroscopic findings.

Time to insemination during the mating and remating period
insemination index and fertility index
following male specific data were recorded and evaluated: testicular and epididymal weight (left and right testis/epididymis individually
following female specific data were recorded and evaluated: duration of gestation, gestation index/course of birth, lactation behavior, number of corpora lutea in the right and the left ovary, number of implantation sites

Investigations in the F1 pups at bith and up to day 5 pp:
twice daily: Appearance, general behavior, mortality
Clinical findings, sex ratio of pups at birth, individual pup weights at birth
and on d 4 after birth, viability index
Pups were sacrificed on day 4 to 5 pp.

Statistical methods:
Analysis of variance (ANOVA), in case of significant results Dunnett's test
2 By N Chi²test, in case of significant differences Fisher's exact test with Bonferroni correction

Remark : see also chapter 5.8.1
Result : F0-GENERATION
all dosed rats including controls showed severe salivation (probably due to vehicle),

MORTALITY; 400 mg-dose group:
3 males and 5 females in the premating period and 1 female sacrificed moribund on day 22 p.c.: ventral posture, hypoactivity, piloerection, intrauterine death of its litter

FOOD INTAKE, 400mg-group
m/f: severely decreased,

DEVELOPMENT OF BODY WEIGHT GAIN
25 and 100 mg-groups: body weight gain comparable to controls
400 mg-group, m/f:
  d1-4: -20.8g/-17.6g (control:8.1g/1.3g);
  d4-8: 9.5g/-10.4g (control: 8.4g/2.8g);
  d8-15: 19.4g/17.0g (control 9.6g/5.5);
400 mg, females body weight gain reduced when compared to controls during
[...]gestation (72.2 g versus 111.3g) and
[...]lactation (4.0 g versus 27.2 g)

CLINICAL SIGNS OF INTOXICATION
-----400 mg-group:
m/f: piloerection, respiratory sound, sunken flanks, increased water intake and urination, reduced amount of feces
females: hypoactivity, alteration of gait and increased incidence of soft and light colored feces

-- 25 mg-group:
1 female: ventral posture, hypoactivity, high stepping gait, piloerection

EFFECTS ON REPRODUCTION (control, low to high dose):
--INSEMINATION INDEX
(no of females inseminated/no of females paired X 100):
100%, 100%, 100%, 100%.
Determination of the time to insemination did not reveal toxicologically relevant effects in comparison to control values

--FERTILITY INDEX
(no of females with implantation sites/ no of females inseminated X 100):
75%, 75%, 100%, 100%

--GESTATION INDEX
(no of females with viable pups/no of females with implantation sites X 100): 100%, 100%, 100%, 85.7%

-----No of corpora lutea: 21.56, 18.44, 16.42(stat. sign.), 17.67
-----No of implantation sites/litter: 13, 12.89, 12.75, 12.33
-----Duration of gestation(days): 22.78, 22.44, 22.33, 22.67

PRENATAL LOSS
(Difference between no of implantation sites and the total no of pups littered (living and dead)):
0.89, 1.33, 0.67, 3.17 (stat. sign.)

- COURSE OF BIRTH:
-----400 mg-group: 1 female was sacrificed of d22 p.c.: all fetuses of its litter were dead
-----25 mg-group: delivering of only 1 pup with a huge hematoma, loss of all other pups on day of birth

F1-PUPS
--LACTATION BEHAVIOUR:
1 pup of the 100 mg- group, 2 pups of the 400 mg-group: no milk ingestion

POSTNATAL DEVELOPMENTof F1 PUPS (control, low to high dose):
-----No pups delivered(mean-no):
12.11, 11.56, 12.08, 9.17
-----No live pups (mean-no,d0/d4):
Live birth index (%): 100, 100, 98.61, 100
Viability index (d4 pp, %): 98.99, 88.89, 95.41, 92.06
Sex ratio (d0, % males/liter): 49.94, 48.66, 44.00, 46.43
Pup clinical observations (frequency/pups/litters, day 0-day 5):
---Found dead: 1/1/1; 14/14/1; 5/5/1; 1/1/1
---Missing: 0/0/0; 0/0/0; 2/2/1; 4/4/2
---Hypoactivity: 0/0/0; 1/1/1; 0/0/0; 1/1/1
---Hematoma: 0/0/0; 0/0/0; 0/0/0; 5/2/2
---Pale skin: 0/0/0; 5/1/1; 1/1/1; 1/1/1
---Tip of tail dark discolored: 0/0/0; 4/1/1; 0/0/0; 0/0/0
---Milk spot not detectable: 0/0/0; 0/0/0; 1/1/1; 2/2/2
---Tip of tail missing: 0/0/0; 1/1/1; 0/0/0; 0/0/0

MEAN PUP WEIGHT:
d0: m/f/total and d4: m/f/total:
---control-group
6.30/5.96/6.12 and 9.64/9.22/9.43;
---25 mg-group:
6.27/5.94/6.07 and 9.42/9.09/9.22;
---100 mg-group:
5.43(p<0.01)/5.28(p<0.5)/5.36(p<0.05) and 8.31(p<0.05)/8.20/8.26;
---400 mg-group:
4.89(p<0.01)/4.69(p<0.01)/4.80(p<0.01) and 6.79(p<0.01)/6.55(p<0.01)/6.68(p<0.01)

F0 GENERATION
GROSS PATHOLOGY:
-------mean organ weights control, low to high dose, abs(g)/rel[%]):
MALE
liver:
15.23/3.88, 15.52/3.96, 17.51(p<0.05)/4.26(p<0.05),
19.53(p<0.01)/5.04(p<0.01);
spleen:
0.67/0.17, 0.69/0.18, 0.72/0.18, 1.06(p<0.01)/0.28(p<0.01)
kidney weights, testes weight, epididymal weights were comparable to controls
---FEMALE:
liver and kidney weights were comparable to controls
spleen:
0.56/0.21, 0.598/0.23, 0.59/0.22, 1.07(p<0.01)/0.45(p<0.01)

HISTOPATHOLOGY (MALE; FEMALE):
-------400 mg group:
LIVER:
periportal pigment deposits (2/12 males, 4/12 females),
variable glycogen content (4/12 males, 3/12 females);
KIDNEY:
tubular pigment (3/12 females),
mononuclear infiltration (2/12 females),
tubular vacuolation (5/12 females),
single cell necrosis (2/12 males);
Spleen:
congestion (12/12 males, 10/12 females),
increased pigment (2/12 males, 1/12 females)
TESTES:
atrophy, 2/12;
EPIDIDYMIDES:
cellular debris 4/12

----------100 mg- and 25 mg-groups:
no changes attributable to treatment

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
31.08.2004 (208)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Type of experience : Human
Remark : An 1898 survey of poisoning in an aniline factory mentions 10 mixed cases involving o- and p-nitrotoluene mixture ("red oil"). p-Nitrotoluene was characterized as relatively nonpoisonous, while o-nitrotoluene was described as comparable to that of nitrobenzene.
Reliability : (4) not assignable mixed exposure
Flag : Critical study for SIDS endpoint
12.04.2003 (209)

Type of experience : Human
Remark : A 1930 communication describes a case of mixed poisoning by a nitrotoluene and nitrochlorobenzene mixture (ortho- and para-isomers) including cyanosis of the lips, gingiva, nose, paleness, difficulties in breathing, increased heart rate. Observed effects cannot definitely be attributed to nitrotoluene.
Reliability : (4) not assignable mixed exposure
Flag : Critical study for SIDS endpoint
12.04.2003 (210)

Type of experience : Human
Remark : Cases of poisoning from nitrotoluene are uncommon. There is some evidence that the different isomers vary in toxicity. It is stated that nitrotoluene is a methemoglobin former.
Reliability : (4) not assignable information is insufficient for assessment
Flag : Critical study for SIDS endpoint
12.02.2003 (211)

Type of experience : Human
Remark : The levels of 4-toluidine-adducts in blood and of 4-toluidine in urine are measured at least once a year in each worker of the 4-nitrotoluene manufacturing plant of the Bayer AG as part of the Bayer health
surveillance program. The measured values for hemoglobin-adducts were not higher than in the unexposed population. Average levels of 4-toluidine-adducts in blood (ng/l) [and of 4-toluidine in urine (µg/l)] were:
Worker Nitration 2002: 11 (max. 40) [< 2]
Worker Distillation 2002: < 20 [<2]
General Population Non-Smoker: 26
General Population Smoker: 70

**Reliability**
- (2) valid with restrictions
- Basic data given

**Flag**
- Critical study for SIDS endpoint
- 22.10.2004

**Type of experience**
- Human

**Remark**
4-Nitrotoluene is used in the production of 4,4'-diaminostilbene-2,2'-disulphonic acid (DAS), a stilbene intermediate in the manufacture of fluorescent whitening agents. Occupational exposure to DAS has been associated with alterations in male reproductive hormone levels and effects on male sexual function (Whelan 1996). These effects, however, cannot be attributed to 4-nitrotoluene, which is used in the process, but are more likely the effect of the stilbene compound.

**Result**
The internal 4-toluidine-level is associated with smoking habits.

**Reliability**
- (2) valid with restrictions
- Basic data given

**Flag**
- Critical study for SIDS endpoint
- 22.10.2004

**Type**
- Biochemical or cellular interactions

**Remark**
In an effort to develop a potency ranking for methemoglobin forming agents, a linear regression analysis of methemoglobin formation in sheep erythrocytes by direct acting and bioactivated agents was conducted. Methemoglobin formation was determined following the incubation of Dorset-sheep erythrocytes with varying concentrations of various direct acting agents (sodium-nitrite, copper, sodium-chlorate, chloride, p-dinitrobenzene, and o-dinitrobenzene) or bioactivated agents (alpha-naphthol, o-nitrotoluene, m-nitrotoluene, p-nitrotoluene (2.5; 5.0; 7.5; 10.0 mM), aniline, o-nitroaniline, m-nitroaniline, and p-nitroaniline. A dose dependent enhancement of methemoglobin formation was seen following treatment with each of the direct acting and bioactivated agents with or without the presence of a bioactivation system. A significant effect of the bioactivating system was seen for aniline, o-nitroaniline, p-nitroaniline, m-nitroaniline, and m-nitrotoluene but not for the remaining bioactivated compounds. Based upon three different methods of analysis, the ranking of the direct acting agents from most to least potent inducer of methemoglobin formation was determined to be p-dinitrobenzene, o-dinitrobenzene, copper and nitrite, chloride, and chlorate while that for the bioactivated agents was alpha-naphthol, p-nitroaniline, m-nitroaniline, o-nitroaniline, p-nitrotoluene and aniline, and m-nitrotoluene and o-nitrotoluene.

**Reliability**
- (4) not assignable
- special study
### TOXICITY

<table>
<thead>
<tr>
<th>Date</th>
<th>Type</th>
<th>Remark</th>
<th>Reliability</th>
<th>Flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>06.03.2003</td>
<td>Cytotoxicity</td>
<td>1 mmol 4-nitrotoluene was not cytotoxic to aerobic or hypoxic mammalian cells (V79 resp. EMT6 fibroblasts) and did not inhibit growth in air</td>
<td>(4) not assignable</td>
<td>Critical study for SIDS endpoint</td>
</tr>
<tr>
<td>28.01.2003</td>
<td>Other</td>
<td>Addition of 4-nitrotoluene to isolated rat seminiferous tubules or Sertoli cell cultures at concentrations of 1 mmol, 10 umol or 100 nmol had no effect on basal or stimulated inhibin secretion; corresponding in vivo studies (rats, single oral dose, no further information) did not alter interstitial fluid inhibin levels significantly</td>
<td>(4) not assignable</td>
<td></td>
</tr>
<tr>
<td>12.04.2003</td>
<td>Other</td>
<td>4-Nitrotoluene (no. 47) is one of the chemicals in the list of suspected endocrine disrupters (EDs) published by the Japan Environment Agency. 4-Nitrotoluene was therefore tested in a screening assay using Yeast Two-Hybrid system based on the ligand-dependent interaction of nuclear hormone receptors with coactivators. 4-Nitrotoluene was judged to be negative [positive Control: 17ß-Estradiol(E2)]</td>
<td>(4) not assignable</td>
<td></td>
</tr>
<tr>
<td>30.08.2004</td>
<td>other: in vitro</td>
<td>Human breast cancer ZR-75 cells were incubated with 10^{exp-5}M p-nitrotoluene with or without 17-beta-estradiol (10 nM) for 10 days and counted on days 0, 3, 6, 8 and 10. p-Nitrotoluene did not enhance breast cancer cell growth at this concentration to any significant degree.</td>
<td>(4) not assignable</td>
<td></td>
</tr>
<tr>
<td>03.11.2004</td>
<td>other: in vitro</td>
<td>Incubation of freshly isolated rat hepatocytes with labeled 4-nitrotoluene dissolved in ethanol (no further information) resulted in formation of 5 major metabolites: 4-nitrobenzoic acid, 4-nitrobenzyl alcohol, 4-nitrobenzyl alcohol glucuronide, 4-nitrobenzyl alcohol sulfate and S-(4-nitrobenzyl)gluthatione</td>
<td>(4) not assignable</td>
<td></td>
</tr>
</tbody>
</table>
Remark: Incubation of labeled 4-nitrotoluene (100 umol in ethanol) with rat liver post-mitochondrial supernatant produced 4-nitrobenzyl alcohol, S-(4-nitrobenzyl)glutathione and 4-nitrobenzyl sulfate. Omission of glutathione in the incubation mix decreased glutathione conjugation and increased the amount of 4-nitrobenzyl sulfate.

Reliability: (4) not assignable special investigation

Type: other: in vitro investigation

Remark: Incubation of 4-nitrotoluene with liver homogenate of male rabbits resulted in forming of 4-nitrobenzoic acid by TPNH and DPN depending enzyme systems.

Reliability: (4) not assignable special investigation

Type: other: in vitro investigation

Remark: After incubation of 4-nitrotoluene with male rat liver homogenate the nitroreductase activity was reduced to 5%.

Reliability: (4) not assignable special investigation

Type: other: in vitro investigation

Remark: 4-Nitrotoluene was hydroxylated to 4-nitrobenzyl alcohol by isolated hepatocytes of rats, mice, guinea pigs, hamsters and rabbits; animals pretreated with or without phenobarbital (75 mg/kg, i.p., 4 days) or 3-methylchlanthrene 20 mg/kg, single dose).

Reliability: (4) not assignable special investigation

Type: other: in vitro investigation

Remark: Oxidation of 4-nitrotoluene to 4-nitrobenzyl alcohol and 4-nitrobenzoic acid occurred in rat or mouse liver homogenate as well as in grass grubs.

Reliability: (4) not assignable special investigation

Type: other: in vitro investigation

Remark: Incubation of isolated hepatocytes from male F-344 rats with labeled 4-nitrotoluene resulted in forming 4-nitrobenzyl alcohol (5%) and unidentified metabolites (42%).

Reliability: (4) not assignable special investigation

Type: other: in vitro investigation

Remark: Groups (n=Number) of immature female CD Sprague-Dawley rats (age and mean body weight not given) received single i.p. injections (dosing volume: 10 ml/kg bw):

- 0.01 (n=5), 0.1 (n=5), 1 (n=6), 10 (n=10), 30 (n=10), 100 (n=18), 300
Concurrent solvent control groups were included for each dose group separately containing n = 5, 5, 5, 10, 18, 10, 5 rats, respectively. DES served as positive control: 0.001 (n=10), 0.01 (n=23), 0.1 (n=5) mg/kg bw including concurrent solvent controls (n =10, 24, 5 rats, respectively).

Result:
All rats that received 1000 mg/kg bw p-nitrotoluene became heavily sedated and remained in this condition until sacrifice at 24 hrs after treatment.

Relative uterine weight (uterine weight X 10(exp.-3)/body weight):
- p-Nitrotoluene/concurrent solvent control:
  - 0.01 mg: 0.67/0.67 (n.s.=not significant); 0.1 mg: 0.69/0.67 (n.s.); 1 mg: 0.70/0.67 (n.s.); 10 mg: 0.81/0.73 (n.s.); 30 mg: 1.78/1.40 (p=0.011); 100 mg: 1.04/0.76 (p=0.10); 300 mg: 1.53/1.40 (n.s.); 1000 mg: 0.98/0.8 (n.s.)
- DES/concurrent solvent control:
  - 0.0001 mg: 1.65/1.40 (n.s.); 0.01 mg: 1.60/1.16 (p<0.001); 0.1 mg: 1.65/0.80 (p<0.001)

Reliability: (3) invalid exposures not carried out in a single study but in 3 separate experiments, marked variability in the level of control uterine weights which raises issues as to whether the rats used in the different experiments were of the same age, no dose-response relationship

Remark:
Incubation of liver homogenate from male rats with 4-nitrotoluene resulted in forming of 4-nitrobenzyl alcohol and 4-nitrobenzoic acid

Reliability: (4) not assignable special investigation

Remark:
Incubation of male rat liver homogenate with 1 mMol 4-nitrotoluene inhibited the delta-aminolevulinic acid synthetase and stimulated the ferrochelatase activities

Reliability: (4) not assignable special investigation

Remark:
Incubation of vertebrates liver homogenates (rabbit, coypu, hamster, guinea pig, cat) resulted in formation of 4-nitrobenzoic acid, no detection of alcoholic metabolites.

Reliability: (4) not assignable special investigation

Remark:
Incubation of Ehrlich ascites cells or Chinese hamster V79 lung cells with 1 mMol 4-nitrotoluene inhibited the oxygen utilization, results independent of additionally glucose

Reliability: (4) not assignable special investigation

Remark:
Single oral dose of 500 mg/kg 4-nitrotoluene to mice pretreated with pyrazole (100 mg/kg i.p.) or 4-bromopyrazole (50 mg/kg i.p.) resulted in reduced renal excretion of
<table>
<thead>
<tr>
<th>Reliability</th>
<th>4-nitrobenzoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(4) not assignable</td>
</tr>
<tr>
<td></td>
<td>no validated test method</td>
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<tr>
<td>28.01.2003</td>
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</tbody>
</table>

**Remark**

Incubation of rat liver homogenates resulted in formation of 4-nitrobenzoic acid, no detection of alcoholic metabolites; pretreatment of rats with sodium phenobarbitone (35 mg/kg i.p. twice daily for 4 days) or 3,4-benzopyrene (25 mg/kg single dose) doubled the activity of liver enzymes.

**Reliability**

(4) not assignable

**Remark**

Incubation of rat liver homogenates resulted in formation of 4-nitrobenzoic acid, no detection of alcoholic metabolites; pretreatment of rats with sodium phenobarbitone (35 mg/kg i.p. twice daily for 4 days) or 3,4-benzopyrene (25 mg/kg single dose) doubled the activity of liver enzymes.

**Reliability**

special study

**Remark**

In vitro, nitrotoluol (isomer not specified) inhibited the oxygen utilization of V79 cells and reduced the survival of the cells after radiation (2100 rad) drastically.

**Reliability**

(3) invalid

**Remark**

In vitro, nitrotoluol (isomer not specified) inhibited the oxygen utilization of V79 cells and reduced the survival of the cells after radiation (2100 rad) drastically.

**Reliability**

special investigation

**Remark**

It is reported that 11 industrial chemicals were analysed for their estrogen receptor (ER) binding capacity using ER-α and for estrogen (ES)-like activities by measuring uterus weights in mice. For 4-nitrotoluene, ER binding capacity is reported at more than 0.1 μg/mL in a dose-dependent manner but 4-nitrotoluene did not show significant effect on uterus weight in mice.

**Reliability**

(4) not assignable

**Remark**

Diethylstilbestrol (DES), 17β-estradiol, and 11 industrial chemicals were analysed for their estrogen receptor (ER) binding capacity using ER-α and for estrogen (ES)-like activities by measuring uterus weights in mice. DES and 17β-estradiol showed ER binding at 0.000000819 μg/ml, whereas 4-nitrotoluene did not show significant effect on uterus weight in mice.

**Reliability**

(4) not assignable


(3) Römpp (1998) Römpp Lexion Chemie (10. Auflage), G. Thieme Verlag, Stuttgart


(7) IARC (1996) Monograph 65

(8) BUA (1989) GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA) Report 41, Nitrotoluenes. VCH, Weinheim


6. REFERENCES

(22) Bayer AG (2002) Calculation of

- log Octanol-Water-Partition Coefficient with SRC-KOWWIN v. 1.66, 2000
- Henry's Law Constant with SRC-HENRYWIN v. 3.10, 2000
- Indirect Photodegradation with SRC-AOPWIN v. 1.89, 2000
- Soil Adsorption Coefficient with SRC-PCKOCWIN v. 1.66, 2000
- Mackay-Distribution Level I according to Mackay, D., 1991


(35) Bayer AG (1978) Unpublished data: Experimental Data of Flash Point


(64) Bayer AG (1973) Internal study: Test on Ready Biodegradability According to Closed Bottle Test (Internal report written 2000-07-04)


(73) Lang P-Z (1996) QSAR for the acute toxicity of nitroaromatics to the carp (Cyprinius carpio). Chemosphere 32 (8): 1547 - 1552


(89) Bayer AG (1985) Internal study: Acute fish toxicity test with Brachydanio rerio (1985-03-05)

(90) Bayer AG (1985) Internal study: Acute fish toxicity test with Leuciscus idus (1985-03-12)


(105) Hoechst AG (1982) Unpublished study W82-082


<table>
<thead>
<tr>
<th>Number</th>
<th>Reference</th>
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<tbody>
<tr>
<td>135</td>
<td>Siepes IG, Carter DE (no date) Pharmacokinetics of xenobiotics: p-nitrotoluene. NIEHS-Contract-No. NO1-ES-8-2130</td>
</tr>
</tbody>
</table>
(139) Vernot EH, MacEwen JD, Haun CC, Kinkead ER (1977) Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicol Appl Pharmacol 42: 417-423


(146) El DuPont De Nemours & CO (1972) Inhalation class poison study of o,m,p-nitrotoluenes in male CHR-CD rats. March 15, 1972: NTIS/OTS 0557155

(147) Zewdie T, Furman G, Roberts A, Schatz R (1990) Structure activity realtionship of toluene, p-xylene, p-nitrotoluene (PNT) and p-chlorotoluene (PCT) on rat lung and liver microsomal function and composition. The Toxicologist 10: 120


(149) Kinkead ER, MacEwen JD, Haun CC, Vernot EH (1977) Toxic hazards evaluation of five atmospheric pollutants from army ammunition plants. US AMBRD: TR 7703, NTIS ADA043957


(152) Alexandrow IS (1936) Über die relative toxische Wirkung der aromatischen Amino- und Nitroverbindungen auf weiße Mäuse durch die Haut. Journal Physiol USSR 20: 1100-1109


(160) Hoechst AG (1986) p-Nitrotoluol Prüfung auf Hautreizung am Kaninchen. Unveröffentlichter Bericht Nr. 86.0361, 26.03.1986


(162) Hoechst AG (1986) p-Nitrotoluol Prüfung auf Augenreizung beim Kaninchen. Unveröffentlichter Bericht Nr. 86.0362, 26.03.1986


(164) Chemfirst Inc (1997) Delayed contact hypersensitivity study in guinea pigs (Buehler Technique), paranitrotoluene (at the request of First Mississippi Corporation), January 14, 1997 NTIS/OTS 0559506


(167) US Department of Health and Human Services (1992) National Toxicology Program, NTP Technical report on toxicity studies of o-, m-, and p-Nitrotoluenes, administered in dosed feed to F344/N rats and B6C3F1 mice. NTP technical report series 23 (author: Dunnick JK), NIH Publ.-No. 96-3346


(174) Jaffe M (1874) Über das Verhalten des Nitrotoluols im thierischen Organismus. Ber dt chem Ges 7: 1673-1679


6. REFERENCES


6. REFERENCES


(216) Allenby G, Foster PMD, Sharpe RM (1991) Inhibit secretion as a marker of testicular toxicity in isolated seminiferous tubules (ST), sertoli cell (SC) cultures and in vivo. The Toxicologist 11: 938


(219) DeBethizy JD, Rickert DE (1983) Sequential enzyme-catalyzed metabolism of 4-nitrotoluene to s-(4-nitrobenzyl)glutathione. Biochem Biophys Res Commun 114: 500-504


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<tr>
<td>224</td>
<td>Hook GER, Smith JN (1967) Oxidation of methyl groups by grass grubs and vertebrate liver enzymes. Biochem J 102: 504-510</td>
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