FOREWORD INTRODUCITON

4-Nitrotoluene

CAS N°: 99-99-0

SIDS Initial Assessment Report

For

SIAM 17

Arona, Italy, 11 – 14 November 2003

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:

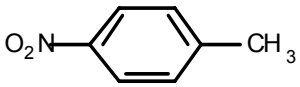
OECD/ICCA - The BUA* Peer Review Process

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)

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	99-99-0
Chemical Name	4-Nitrotoluene
Structural Formula	

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 predominent 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, α2u-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 temperatur) 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 $Pa \cdot m^3 \cdot mol^{-1}$ indicates a moderate potential for volatilization of 4-nitrotoluene from aqueous solution. In the atmosphere 4-nitrotoluene is degraded due to indirect photolysis ($t_{1/2air}$: 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-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 were found. 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-ErC_{50} 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 m^3/s), and the dilution factor (700), for the receiving water a Predicted Environmental Concentration (PEC) of < 2.8 ng/l is calculated.
In Gemany 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.

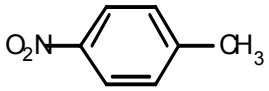
Environment:
The chemical possesses properties indicating a hazard for the environment. Based on data presented by the Sponsor country, exposure to the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate 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 IDENTITY

1.1 Identification of the Substance

CAS Number: 99-99-0
IUPAC Name: 4-Nitrotoluene
Molecular Formula: $C_7H_7NO_2$
Structural Formula:



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

IUCLID	Parameter	Value	Source
	Molar mass	137.13 Dalton	
2.1	Melting point	51.3 °C	Verschueren, 1996
2.2	Boiling point at 1013 hPa	238 °C	Verschueren, 1996
2.3	Density at 20 °C	1.29 g/cm³	Verschueren, 1996
2.4	Vapour pressure at 20 °C	13 Pa	Auergesellschaft, 1988
2.5	Octanol/water partition coefficient (log K_{ow}) at 25 °C	2.37 (measured)	Fujita et al., 1964
2.6.1	Water solubility at 20 °C	345 mg/l (measured)	Bayer AG, 1987
	Solubility in organic solvents	Soluble in most organic solvents	Booth, 2002
2.7	Flash point	103 °C (DIN 51758)	Bayer AG, 2001
2.8	Auto flammability (ignition temperature)	450 °C	Hommel, 1997
2.14	Conversion factors for the vapour phase	1 mg/m^3 = 0.18 ppm 1 ppm = 5.70 mg/m^3	Verschueren, 1996
2.14	Vapour density in relation to air (= 1)	4.72	Verschueren, 1996
2.14	Decomposition	Decomposes on heating producing toxic fumes (nitrogen oxides)	CEC/IPCS, 2002

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 nitration 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 m^3/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 decommisioned 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/cm^3 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 m^3 mol^{-1} and a log K_{ow} of 2.37 the following results were obtained (Bayer AG, 2003a):

% to air	0.8
% to water	56.2
% to sludge	4.3
% degraded	38.6
% removal (sum of losses to air, removal with sludge and degradation)	43.8

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 K_{ow} 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: Nitration 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:

	Worker Nitration 2002	**Worker Distillation 2002**	**General Population Non-Smoker**	**General Population Smoker**
4-Toluidine-adducts in blood (ng/l)	11 (max. 40)	< 20	26	70
4-Toluidine in urine (µg/l)	< 2	< 2		

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. Excrection 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 nitro-benzylglucuronides) 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 adjuvans 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 α-hexylcinnamaldehyde (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

Type	Species	Dose levels	Effects	Reference
13-week	Rat F344/N - dietary	0; 625; 1,250; 2,500; 5,000; 10,000 ppm (m: 0; 42; 82; 165; 342, 723 mg/kg bw/day) (f: 0; 44; 82; 164; 335, 680 mg/kg bw/day)	10,000 ppm: bile acids ↑ m: testis degeneration, liver weight (abs) ↓, 8.1% Met-Hb f: lengthened estrous cycle, liver weight (rel) ↑, 9.0% Met-Hb	U.S. Department of Health and Human Services, 1992. Dunnick et al.,1994
			≥ 5,000 ppm: m: body weight ↓, liver weight (rel) ↑	
			≥ 1,250 ppm: f: body weight ↓	
			≥ 625 ppm (**LOAEL**): splenic hematopoiesis↑ hemosiderin deposition, congestion ↑ m: α-2u nephropathy	
13-week	Mouse B6C3F1 - dietary	0; 625; 1,250; 2,500; 5,000; 10,000 ppm (m: 0; 131; 212; 439; 813, 1491 mg/kg bw/day) (f: 0; 164; 320; 625; 1075, 1634 mg/kg bw/day)	10,000 ppm: f: body weight ↓	U.S. Department of Health and Human Services, 1992 Dunnick et al.,1994
			≥ 5,000 ppm: m: body weight ↓	
			2,500 ppm: **NOAEL**	
13-week	Rat F33/N - gavage	0; 90, 180, 360 mg/kg bw/day (in corn oil)	360 mg/kg bw/day: m: terminal body weight ↓ absolute cauda epididymis, absolute epididymis, absolute testis weights ↓ relative testis weight ↓ sperm parameters: not affected f: estrous length not affected	Morrissey et al., 1983
			180 mg/kg bw/day: **NOAEL**	
13-week	Mouse / gavage	0; 40, 80, 160 mg/kg bw/day (in corn oil)	160 mg/kg bw/day: **NOAEL** (no adverse effects on male reproductive organ weights, sperm parameters, estrous cycle)	Morrissey et al., 1983
26-week	Rat Wistar - gavage	0; 400 mg/kg bw/day (susp. in 1% methylcellulose) mated in week 13	400 mg/kg bw/day: **LOAEL** m: reduced body weight gain, testicular atrophy, necroses of seminiferous tubules f, offspring: no apparent effects	Ciss et al., 1980a

Type	Species	Dose levels	Effects	Reference
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 ↓ m: eosinophilic foci↑ m: testis degeneration, interstitial cell adenoma ↓ f: oncocytic renal tubule hyperplasia	U.S. Department of Health and Human Services, 2002
			≥ 2,500 ppm m: liver basophilic and clear cell foci f: eosinophilic liver foci at 2,500 ppm only: f: clitoris gland adenomas/carcinomas ↑ m: subcutaneous fibromas/fibrosarcomas ↑	
			≥ 1,250 ppm :**LOAEL** m. f: splenic hematopoiesis↑, hemosiderin deposition, congestion; nasal and eye discharge m: α-2u nephropathy f: body weight ↓	
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: m: hepatocyte syncytial alterations f: body weight ↓	U.S. Department of Health and Human Services, 2002
			≥ 1,250 ppm: **LOAEL** m: body weight ↓, m,f: alveolar bronchiolar epithelialization ↑ (no evidence of viral infection)	
14-day, immunotoxi-city study	Mouse B6C3F1 - gavage	0; 200; 400; 600 mg/kg bw/day (females only)	≥ 400 mg/kg bw/day: swelling of hepatocytes adjacent to the central veins, no necroses eosinophils ↑, macrophage activity ↑	Burns et al., 1994
			≥ 200 mg/kg bw/day: Antibody response to sRBC↓, CD4+ splenic T cells ↓, delayed antigen response	

m = male, f = female; rel = relative, abs = absolute; ↑ = increase, ↓ = 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 (≥ 5,000 ppm, corresponding to 342 mg/kg bw/day). In male rats, α2u-globulin nephropathy was seen in all dosed groups, i.e. at ≥ 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. α2u-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 interprete 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. pneumonniae* 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, α2u-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 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 similiar. 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 α-2u-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 decresed 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 hematocrit 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 ($NOAEL_{reproductive\ toxicity}$: 25 mg/kg bw/day; $NOAEL(male)_{general\ toxicity}$: 25 mg/kg bw/day (male), $LOAEL(female)_{general\ toxicity}$: 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 ($NOAEL_{developmental\ toxicity}$: 25 mg/kg bw/day; $LOAEL_{developmental\ toxicity}$: 100 mg/kg bw/day; $LOAEL_{maternal\ toxicity}$: 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 nitrochloro-benzene 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, α2u-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 qualitiy 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 ($NOAEL_{reproductive\ toxicity}$: 25 mg/kg bw/day; $NOAEL_{developmental\ toxicity}$: 25 mg/kg bw/day, $LOAEL_{developmental\ toxicity}$: 100 mg/kg bw/day; $NOAEL(male)_{general\ toxicity}$: 25 mg/kg bw/day (male), $LOAEL(female)_{general\ toxicity}$: 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.

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_{50} 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_{50} 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_{50} 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_{50} 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_{50} 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_{50}) 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-EC_{50} 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-EC_{50} of 11.8 mg/l (Liu et al., 1983). With *Daphnia pulex* Yen and coworkers (2002) obtained an 4h-EC_{50} of 41 mg/l. Endpoint in all these studies was immobilisation.

For the algae *Chlorella pyrenoidosa* a 96 h-EC_{50} 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-EC_{50} 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:

Fish	*Carassius auratus*	48 h-LC_{50}	10.5 mg/l
Daphnia	*Daphnia magna*	48 h-EC_{50}	4.2 mg/l
Algae	*Chlorella pyrenoidosa*	96 h-EC_{50}	22.2 mg/l

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:

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

*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:

Inoculum	**Endpoint**	**Result (mg/l)**	**Reference**
Activated sludge	3 h-EC_{50}	100	Yoshioka et al., 1986
Pseudomonas putida	16 h-EC_3	26	Bringmann and Kühn, 1976; Bringmann and Kühn, 1977; Bringmann and Kühn, 1980a
Entosiphon sulcatum	72 h-NOEC	8.6	Bringmann and Kühn, 1980a ; Bringmann and Kühn, 1981
Uronema parduzci:	20 h-NOEC	0.89	Bringmann and Kühn, 1980b

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-EC_{50} 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 EC_{50} 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 EC_{50} 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_rC_{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 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. 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.

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I U C L I D

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
Flags (profile)	:	Flags: without flag, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type : lead organisation
Name : Bayer AG
Contact person :
Date :
Street :
Town : 51368 Leverkusen
Country : Germany
Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

02.11.2004

Type : cooperating company
Name : Ciba Geigy AG
Contact person :
Date :
Street :
Town : 4002 Basel
Country : Switzerland
Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

23.01.2003

Type : cooperating company
Name : Hickson & Welch Ltd.
Contact person :
Date :
Street :
Town : WF10 2JT Castleford
Country : United Kingdom
Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

Type : cooperating company
Name : Hoechst AG
Contact person :
Date :
Street :
Town : 65903 Frankfurt/Main
Country : Germany
Phone :
Telefax :
Telex :

Cedex :
Email :
Homepage :

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 :

Remark : Booth (1991) describes 4-nitrotoluene as colourless to light yellow rhombic-bipyramidal crystals, Roempp (1998) as colourless crystals, Budavari (1989) as yellowish crystals, and Verschueren (1996) as needle-shaped colourless crystals.
Flag : Critical study for SIDS endpoint
23.01.2003 (1) (2) (3) (4)

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 (5)

Type of spectra : UV

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

26.01.2003 (6)

1.2 SYNONYMS AND TRADENAMES

1-Methyl-4-nitrobenzene

Remark : IUPAC name
Flag : Critical study for SIDS endpoint
29.11.2002

4-Methylnitrobenzene

Flag : Critical study for SIDS endpoint
29.11.2002

4-Nitro-1-methylbenzene

Flag : Critical study for SIDS endpoint
29.11.2002

4-Nitrotoluene

Remark : EINECS name
Flag : Critical study for SIDS endpoint
29.11.2002

4-Nitrotoluol

Flag : Critical study for SIDS endpoint
19.12.2002

Benzene, 1-methyl-4-nitro-

Remark : CA Index name
Flag : Critical study for SIDS endpoint
29.11.2002

p-Methylnitrobenzene

Flag : Critical study for SIDS endpoint
29.11.2002

p-Nitrotoluene

Remark : Common name
Flag : Critical study for SIDS endpoint
23.01.2003

p-Nitrotoluol

Flag : Critical study for SIDS endpoint
29.11.2002

PNT

Flag : Critical study for SIDS endpoint

Toluene, p-Nitro

Flag : Critical study for SIDS endpoint
29.11.2002

1.3 IMPURITIES

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 :
CAS-No : 88-72-2
EC-No : 201-853-3
EINECS-Name : 2-nitrotoluene
Molecular formula : C7H7NO2
Value : <= .5 % w/w

04.02.2003 (7)

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

23.01.2003 (7)

Purity :
CAS-No :
EC-No :
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

Labelling	:	as in Directive 67/548/EEC
Specific limits	:	
Symbols	:	T, N, ,
Nota	:	, ,
R-Phrases	:	(23/24/25) Toxic by inhalation, in contact with skin and if swallowed (33) Danger of cumulative effects (51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S-Phrases	:	(28) After contact with skin, wash immediately with plenty of water and soap, if possible with Polyethylnglycol 400, too (37) Wear suitable gloves (45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible) (61) Avoid release to the environment. Refer to special instructions/Safety data sets
Remark	:	EC-Index-No. 609-006-00-3
Flag	:	Critical study for SIDS endpoint

26.08.1999

1.6.2 CLASSIFICATION

Classified	:	as in Directive 67/548/EEC
Class of danger	:	toxic
R-Phrases	:	(23/24/25) Toxic by inhalation, in contact with skin and if swallowed
Specific limits	:	
Flag	:	Critical study for SIDS endpoint

23.01.2003

Classified	:	as in Directive 67/548/EEC
Class of danger	:	other: danger of cumulative effects
R-Phrases	:	(33) Danger of cumulative effects
Specific limits	:	
Flag	:	Critical study for SIDS endpoint

23.01.2003

Classified	:	as in Directive 67/548/EEC
Class of danger	:	dangerous for the environment
R-Phrases	:	(51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Specific limits	:	
Flag	:	Critical study for SIDS endpoint

23.01.2003

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

Flag : Critical study for SIDS endpoint
27.01.2003

Type of search : Internal and External
Chapters covered : 3, 4
Date of search : 20.09.2002

Flag : Critical study for SIDS endpoint
27.01.2003

Type of search : Internal and External
Chapters covered : 5
Date of search : 01.09.2002

Flag : Critical study for SIDS endpoint
27.01.2003

1.13 REVIEWS

Memo : BUA (1989) Report 41, Nitrotoluenes

Flag : Critical study for SIDS endpoint
29.11.2002 (8)

2.1 MELTING POINT

Value : 51.3 °C
Sublimation :
Method :
Year : 1996
GLP :
Test substance :

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
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
06.08.2003 (4)

Value : 51.3 °C
Sublimation :
Method :
Year : 1992
GLP :
Test substance :

Reliability : (2) valid with restrictions
Reliable source
06.08.2003 (9)

Value : 51 °C
Sublimation :
Method :
Year : 1997
GLP :
Test substance :

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (10)

Value : 51.6 °C
Sublimation :
Method :
Year : 2000
GLP :
Test substance :

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (11)

Value : 51.9 °C
Sublimation :
Method :
Year : 1968
GLP :
Test substance :

Remark : Booth (1991) reports the melting point to be 51.9 °C for the stable and 44.5 °C for an unstable form of p-nitrotoluene without giving further explanations [Booth G (1991) Nitro Compounds, Aromatic. In: Ullmann´s Encyclopedia of Industrial Chemistry (5th ed), Verlag Chemie, Weinheim, vol 17, p 420]
Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (1) (12)

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 (13)

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 (3)

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 (2) (10) (4)

Value : 238 °C at 1013 hPa

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (3)

Value : 238.3 °C at 1013 hPa

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (14) (1) (11) (9) (12)

Value : 237.7 - 239 °C at 1013 hPa

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (15)

Value : 105 °C at 12 hPa

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (3)

Value : 53.7 °C at 1.3 hPa

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

2.3 DENSITY

Type : density
Value : 1.29 g/cm³ at 20 °C

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
06.08.2003 (4)

Type : density
Value : 1.286 g/cm³ at 20 °C

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

Type : density
Value : 1.14 g/cm³ at 55 °C

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (15)

Type : density
Value : 1.1038 g/cm³ at 75 °C

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (11)

Type : density
Value : 1.1 g/cm³ at 80 °C

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (15)

Type : density
Value : 1.1 g/cm³ at 100 °C

Reliability : (4) not assignable
Not assignable/manufacturer data without proof
06.08.2003 (16)

Type : density
Value : 1.104 g/cm³ at °C

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (3)

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

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (12)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .13 hPa at 20 °C

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
06.08.2003 (14)

Value : .055 hPa at 25 °C

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (15)

Value : .27 hPa at 30 °C

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (14)

Value : 1 hPa at 50 °C

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (14)

Value : = 1.3 hPa at 53.7 °C

Remark : Reference not available
Reliability : (4) not assignable
Not assignable/manufacturer data without proof
06.08.2003 (17)

Value : = 13.3 hPa at 100 °C

Remark : Reference not available
Reliability : (4) not assignable
Not assignable/manufacturer data without proof
06.08.2003 (17)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : 2.37 at 25 °C
pH value :
Method : other (measured)
Year : 1964
GLP :
Test substance :

Remark : Hansch C, Leo A, Hoekman D (1995) cite the work of Fujita T, Iwasa J, Hansch C (1964)
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
06.08.2003 (18) (19)

Partition coefficient : octanol-water
Log pow : 2.42 at °C
pH value :
Method : other (measured)
Year : 1989
GLP :
Test substance :

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (20)

Partition coefficient : octanol-water
Log pow : 2.4 at °C
pH value :
Method : other (calculated)
Year : 2002
GLP :
Test substance :

Method : Calculated according to Leo et al. 1971
Reliability : (2) valid with restrictions
Accepted calculation method
06.08.2003 (21)

Partition coefficient : octanol-water
Log pow : 2.36 at 20 °C
pH value :
Method : other (calculated): with SRC-KOWWIN v.1.66, 2000
Year : 2002
GLP :
Test substance :

Reliability : (2) valid with restrictions
Accepted calculation method
06.08.2003 (22)

Partition coefficient : octanol-water
Log pow : 2.42 at °C
pH value :

Method :
Year : 1971
GLP :
Test substance :

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (23)

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 :

Remark : The authors cite a work of Verhaar HJM, van Leeuwen CJ, Hermens JLM (1992) Classifying Environmental Pollutants. 1: Stucture-activity relationships for prediction of aquatic toxicity. Chemosphere 25: 471 - 491
Reliability : (4) not assignable
Secondary literature
06.08.2003 (26)

Partition coefficient : octanol-water
Log pow : 2.38 at °C
pH value :

Method : other (calculated)
Year : 1998
GLP :
Test substance :

Result : log Pow was calculated with CLOGP ver 3.55 software
Reliability : (2) valid with restrictions
Accepted calculation method
06.08.2003 (27)

Partition coefficient : octanol-water
Log pow : 2.52 at °C
pH value :
Method :
Year : 1997
GLP :
Test substance :

Method : not specified, whether measured or calculated
Reliability : (4) not assignable
Not assignable
06.08.2003 (28)

Partition coefficient : octanol-water
Log pow : 2.52 at °C
pH value :
Method :
Year : 2002
GLP :
Test substance :

Remark : Only short abstract in English available
Reliability : (4) not assignable
Original reference not translated
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
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

experimentally determined or calculated.
Reliability : (4) not assignable
Secondary literature
06.08.2003 (31)

Partition coefficient : octanol-water
Log pow : 2.34 at °C
pH value :
Method :
Year : 1998
GLP :
Test substance :

Remark : Value taken from "Wood CA (1994), 2nd Ann.SPMD Workshop, Missouri"
Reliability : (4) not assignable
Secondary literature
06.08.2003 (32)

Partition coefficient : octanol-water
Log pow : 2.53 at °C
pH value :
Method :
Year : 1993
GLP :
Test substance :

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)
Reliability : (4) not assignable
Not assignable
06.08.2003 (33)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : .345 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: measured via HPLC
Year : 1987
GLP :
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
Study meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
16.10.2003 (34)

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 (1)

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 :

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (4)

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 (8)

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 alcohol, benzene, ether, chloroform, and acetone
Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (2)

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 : 2000
GLP :
Test substance :

Result : Soluble in ethanol, and acetone
Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (11)

Solubility in : Water
Value : .419 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1997
GLP :
Test substance :

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (10)

Solubility in : Water
Value : .44 g/l at 30 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :

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

2.6.2 SURFACE TENSION

Method :
Year : 2002
GLP :
Test substance : other TS: presumably molten substance

Result : Beilstein's reported values for surface tension are:
37.41 - 20.91 g/s2 at 56 - 220 °C
Reliability : (2) valid with restrictions
Data from handbook or collection of data
16.10.2003 (15)

2.7 FLASH POINT

Value : 103 °C
Type : closed cup
Method : other: DIN 51758
Year : 1978
GLP :
Test substance :

Reliability : (2) valid with restrictions
Test procedure in accordance with national standard methods with acceptable restrictions
Flag : Critical study for SIDS endpoint
06.08.2003 (35) (16)

Value : 103 °C
Type :
Method :
Year : 1997
GLP :
Test substance :

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (10)

Value : 106 °C
Type :
Method :
Year : 1989
GLP :
Test substance :

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

Value : 106 °C
Type : closed cup
Method :

Year : 1991
GLP :
Test substance :

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

2.8 AUTO FLAMMABILITY

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

Reliability : (2) valid with restrictions
Data from handbook or collection of data
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
Data from handbook or collection of data
06.08.2003 (15)

2.14 ADDITIONAL REMARKS

Memo : Beilstein Reference No. 4-05-00-00848

Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.08.2003 (15)

Memo : Merck No. 6572

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

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 (4)

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 (4)

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 (13)

Memo : Vapour density

Remark : vapour densitiy 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

Type	:	other: (calculated) with SRC-AOPWIN v. 1.90 (2000)
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	500000 molecule/cm³
Rate constant	:	.0000000000007722 cm³/(molecule*sec)
Degradation	:	50 % after 20.8 day(s)
Deg. product	:	
Method	:	
Year	:	2002
GLP	:	
Test substance	:	
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 Accepted calculation method
Flag	:	Critical study for SIDS endpoint

06.02.2003 (22)

Type	:	water
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Deg. product	:	
Method	:	other (measured): see Method
Year	:	1986
GLP	:	no data
Test substance	:	no data
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: t1/2 = 5.9 hours
Reliability	:	(2) valid with restrictions Study well documented and meets acceptable scientific priniciples
Flag	:	Critical study for SIDS endpoint

26.01.2003 (36)

Type	:	water
Light source	:	

Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	.1 mmol/l at 30 °C
Deg. product	:	no
Method	:	other (measured): see TC
Year	:	1997
GLP	:	no data
Test substance	:	other TS: purity not stated
Result	:	The following initial rates of degradation were obtained: - 0.49 µmol/lxmin - 1.01 µmol/lxmin in the presence of 20 mmol/l H2O2 (photooxidation with hydrogenperoxide) - 1.45 µmol/lxmin in the presence of 100 µmol/l Fe2(SO4)3 - 14.5 µmol/lxmin 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 priniciples

24.01.2003 (37)

Type	:	water
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	.1 mmol/l at 30 °C
Deg. product	:	not measured
Method	:	other (measured): see TC
Year	:	1999
GLP	:	no data
Test substance	:	other TS: purity not stated
Result	:	The following initial rates of degradation were obtained: - 6.9 µmol/lxmin in the presence of TiO2 (photocatalysis) - 14.5 µmol/lxmin in the presence of 100 µmol/l Fe2(SO4)3, without oxalate (photo-Fenton reaction) - 60 µmol/lxmin 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 priniciples

24.01.2003 (38)

Type	:	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 k1 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 mol/l x 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)

3.1.2 STABILITY IN WATER

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)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

Type of measurement : background concentration
Media : surface water
Concentration : <= .05 µg/l
Method :

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)

Type of measurement : background concentration
Media : surface water
Concentration :
Method :

Remark : Electronic update of the publication of Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit (ed, 2001) Umweltpolitik Wasserwirtschaft in Deutschland Teil II Gewässergüte oberirdischer Binnengewässer, Umweltbundesamt, Berlin, pp 19, 36
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)

Type of measurement : concentration at contaminated site
Media : other: sewage treatment plant
Concentration : < .01 µg/l
Method : GC/MS

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 accoring 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

treatment plants in Japan from July 1998 to March 1999. The occurence of 4-nitrotoluene was only monitored in autumn 1998.
4-Nitrotoluene level was below limit of detection (0.01 µg/l) in sewage.

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles. Basic data given.

Flag : Critical study for SIDS endpoint
03.07.2003 (42)

Type of measurement : concentration at contaminated site
Media : ground water
Concentration : 14 mg/l
Method : GC/MS

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
Study meets generally accepted scientific principles. Basic data given.

Flag : Critical study for SIDS endpoint
03.07.2003 (43)

Type of measurement : other: tobacco smoke
Media : other: smoke from tobacco product
Concentration :
Method : GC/MS

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
Study meets generally accepted scientific principles and is well documented.

Flag : Critical study for SIDS endpoint
03.07.2003 (44)

Type of measurement : concentration at contaminated site
Media : ground water
Concentration :
Method :

Result : 4-Nitrotoluene is present in groundwater from a decommissioned munitions production facility near Melbourne, Australia. No numeric data given.
Reliability : (4) not assignable
Documentation insufficient for assessment
Flag : Critical study for SIDS endpoint
04.12.2003 (45)

Type of measurement : concentration at contaminated site
Media : ground water
Concentration :
Method : reverse phase HPLC/UV detection

Result : 4-Nitrotoluene is present in ground water from decommissioned munitions site
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
04.12.2003 (46)

Type of measurement : concentration at contaminated site
Media : ground water
Concentration : ca. .3 mg/l
Method : GC/FID- and ECD-Detectors

Result : 4-Nitrotoluene is present in leachates from landfill in Grindstedt, Denmark
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
04.12.2003 (47)

Type of measurement : concentration at contaminated site
Media : other: wastewater
Concentration : ca. .295 mg/l
Method : HPLC and GC

Remark : Condensate wastewater from US munitions production site
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
04.12.2003 (21)

Type of measurement : other: untreated wastewater from munition production facility
Media : other: wastewater
Concentration :
Method : GC/MS

Result : Untreated wastewater from ADI Mulwala Munition Production Facility (Australia) contains about 20 mg/l 4-nitrotoluene and other nitroaromatic compounds
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
04.12.2003 (48)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

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: see Test condition
Year	:	1996
Result	:	Adsorption of 4-nitrotoluene (and other nitroaromatic compounds) to 3 homoionic kalium ion clay minerals was determined: 1. Kaolinite - Distribution coefficient Kd (l/kg dry matter) 4.9 2. Illite - Distribution coefficient Kd (l/kg dry matter) 24 3. Montmorillonite - Distribution coefficient Kd (l/kg dry matter) 45 Further results were: - 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+ - Highest adsorption coefficients are found for polynitroaromatic compounds - Ionic strength (in the range of 0.0001 - 0.1 M) had no measurable effect on the adsorption It is rationalized that electron donor-acceptor complex formation occurs with oxygene 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
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 (49)

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: measured (thermodynamic method)
Year : 1999

Method : Thermodynamic column method of Brunner et al. 1990 applied [Brunner S, Hornung E, Santl H, Wolff E, Pringer OG, Altschuh J, Brueggemann R (1990) Henry´s law constants for polychlorinated biphenyls: Experimental determination and structure-property relationship. Environ Sci Technol 24, 1751 - 1754]:
- 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 x 8,314 Pa m3/mol K x 298 K = 0.57 Pa m3 mol-1 at 25°C
Test condition : Temperature 25 °C
Gas pahse: Nitrogen
Liquid phase: Demineralized, distilled water
Analysis: GC/ECD
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
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

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

Result : Koc = 309
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
07.02.2003 (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: (calculation) Kenaga & Goring 1978
Year : 1990

Method : - Calculation of soil adsorption coefficient from Kow (octanol/water partition coefficient):
log Koc = 0.544 log Kow + 1.377
- Calculation of soil adsorption coefficient from S (water solubility):
log Koc = - 0.55 log S + 3.64 (S 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 : Koc = 494 (based on log Kow)
Koc = 175 (based on log S)
Reliability : (2) valid with restrictions
Accepted calculation method
07.02.2003 (51)

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: 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 Kd (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

- Distribution coefficient Kd (l/kg dry matter) 3.1
3. clay mineral containing fine sand with coal particles
- Effective grain size (mm) 0.029
- Organic content (g/kg dry matter) 3.1
- Clay content (g/kg dry matter) 13
- Distribution coefficient Kd (l/kg dry matter) 3.9

Test condition : Method according to Hildenbrand M, Haderlein S (1997) Sorptionskinetik von Nitroaromaten in Grundwasserleitern. Prodeedings des DGFZ e.V. ISSN 1430-0176

Reliability : (2) valid with restrictions
Basic data given

19.12.2002 (52)

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 :
Year : 1999

Result : 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 contaminats 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)

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: see TC
Year : 1998

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 l/µmol
- Maximum sorbed-phase solute concentration 980 µmol/kg
Mobility was influenced by the presence of other test substances since they compeate for binding sites of the mineral (competitive sorption)

Test condition : - Experimental procedure as described in Fresch C, Simon W, Haderlein SB, Reichert P, Schwarzenbach RP (1998) Nonlinear sorption and nonequilibrium solute transport in aggregated porous media: experiments, process identification and modeling. J Contam Hydrol 31: 373 - 404
- 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

03.01.2003 (53)

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*10^9 m³
water: 7*10^6 m³
soil: 4.5*10^4 m³ 1500 kg/m³ 2 % org. C
sediment: 2.1*10^4 m³ 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

30.07.2003 (22)

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

Contact time	:	
Degradation	:	.8 (±) % after 14 day(s)
Result	:	under test conditions no biodegradation observed
Deg. product	:	
Method	:	other: Japanese Guideline by MITI of 1974; corresponds to OECD 301C Modified MITI Test I
Year	:	1992
GLP	:	no data
Test substance	:	no data
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). Sludge conc. : 30 mg/l
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint
24.01.2003		(9)

Type	:	aerobic
Inoculum	:	activated sludge, industrial, adapted
Contact time	:	21 day(s)
Degradation	:	100 (±) % after 21 day(s)
Result	:	other: ultimate biodegradation
Deg. product	:	not measured
Method	:	OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
Year	:	1990
GLP	:	no
Test substance	:	no data
Test condition	:	- 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
Reliability	:	(2) valid with restrictions Guideline study without detailed documentation
Flag	:	Critical study for SIDS endpoint
30.01.2003		(54)

Type	:	aerobic
Inoculum	:	activated sludge, adapted
Concentration	:	200 mg/l related to COD (Chemical Oxygen Demand) related to
Contact time	:	
Degradation	:	98 (±) % after 120 hour(s)
Result	:	
Deg. product	:	
Method	:	other: similar to OECD 302B "Zahn-Wellens-Test"
Year	:	1976
GLP	:	no
Test substance	:	no data
Result	:	Rate of biodegradation: 32.5 mg COD/g x h
Test condition	:	- Duration of the test: 120 h - Concentration tested of the test substance: 200 mg COD/l - Inocolum was adapted during 20 days. Inocolum concentration applied: 100 mg/l dry matter

- 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 : Critical study for SIDS endpoint
26.01.2003 (55)

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

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)

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

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)

Type :
Inoculum : domestic sewage, non-adapted
Concentration : 10 mg/l related to Test substance
related to
Contact time : 14 day(s)
Degradation : 75 - 100 (±) % after 14 day(s)
Result :
Deg. product : Yes
Method : other: see below Test conditions
Year : 1983
GLP : No
Test substance : other TS: substance of the highest purity available
Deg. products : 106-49-0 203-403-1 p-toluidine

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-absorpion 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
Basic data supplied

26.01.2003 (58)

Type : Anaerobic
Inoculum :
Contact time :
Degradation : > 90 (±) % after 150 day(s)
Result :
Deg. product : Yes
Method : other: see remarks
Year : 1998
GLP : no data
Test substance : no data

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

laboratory batch reactors.
Test substance: a mixture of 14 different organic chemicals were added to the reactors, approx. 500 µg/l was the intial concentration of the nitro-substituted benzenes.

Result : In all test systems more than 90 % degradation was observed within 150 days
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles. Basic data given.
30.01.2003 (59)

Type : Aerobic
Inoculum : other: mixed culture of bacteria acclimated to mono- and dinitrotoluenes
Contact time :
Degradation : 100 (±) % after 90 minute(s)
Result :
Deg. product : not measured
Method : other: see Test condition
Year : 1999
GLP : No
Test substance : no data

Result : 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).
Test condition : 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.
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles. Basic data given.
30.01.2003 (60)

Type : Aerobic
Inoculum : other: Mycobacterium strain HL 4-NT-1
Deg. product : Yes
Method : other: e.g. enzyme tests
Year : 2000
GLP : No
Test substance : no data

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
Study on transformation, low relevance in the context of risk assessment.
Study meets generally accepted scientific principles. Basic data given.
26.01.2003 (61)

Type : Aerobic
Inoculum : other: aquatic microcosms
Concentration : 20 mg/l related to Test substance
related to
Contact time : 36 day(s)
Degradation : (±) % after
Result : other: Calculated half-life in aquatic microcosm: 12 days (non-sterile)
Deg. product : not measured
Method : other: see test conditions
Year : 1999

GLP : No
Test substance : other TS: proceeding from a wastewater sample

Result : Percentage decrease of the TS
- Sterile: 25 %
- Nonsterile (microbial consortium): 38 %
- Nonsterile half-strength: 32 %
Calculated half-life in the nonsterile microcosm: 12 days

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.

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.

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 (62)

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 treament 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 (63)

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 : other: not readily biodegradable
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:

Conc. (mg/l)	Degradation (%) 5 days	10 days	20 days

		1,8	0	0	0
		5,3	47	55	61
		17,8	0	9	Oxygen exhausted
		53,3	0	0	Oxygen exhausted

Test condition : - Tested concentrations: 1.8, 5.3, 17.8, 53.3 mg/l
- Inocolum 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)
2) Repetitive Die Away Test: Blok (1979) A repetitive Die Away test combining several biodegradability test procedures. Int Biodeterior Bull 15: 57 - 63
3) Pitter test: Pitter P (1976) Determination of biological degradability of organic substances. Water Res 10: 231 – 235

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 aviod 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 choosen 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

06.08.2003 (65)

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 :
Method : other: see test conditions
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

06.08.2003 (66)

Type :
Inoculum : other: specific isolated bacteria from different soils
Contact time :
Degradation : (±) % after
Result : other: biodegradation influenced by several soil-dependent factors
Deg. product :
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 (68)

3.6 BOD5, COD OR BOD5/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 (9)

Species : Poecilia reticulata (Fish, fresh water)

Exposure period : at °C
Concentration :
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)
BCF: 39 (theoretical value, calculated from log Pow-value=2.4)
Result : BCF-Value is related to the weight
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 (30)

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 fat content.
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 (25)

BCF : 39.26
Elimination :
Method : other: calculated
Year : 1983
GLP :
Test substance : no data

Reliability : (2) valid with restrictions
Acceptable calculation method
17.12.2002 (21)

Species : Carassius auratus (Fish, fresh water)
Exposure period : at °C
Concentration :
BCF : < 1
Elimination :

Method	:	other: Bioaccumualtion to Crassius auratus in continuous-flow
Year	:	2002
GLP	:	no data
Test substance	:	no data
Reliability	:	(4) not assignable Yi et al. (1998): Literature not available Wang (2002): Only short abstract in English
19.12.2002		(29) (69)
Species	:	Carassius auratus (Fish, fresh water)
Exposure period	:	20 day(s) at 15 °C
Concentration	:	
BCF	:	234
Elimination	:	
Method	:	other: see Test condition
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 Secondary literature not available: DeVita WM, Crunkilton RL (1994): 2nd Ann.SPDM Workshop, Missouri
03.02.2003		(32)

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.
30.01.2003		(70)

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 Kumamot 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

- Probit analysis of data

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

Flag : Critical study for SIDS endpoint

01.08.2003 (28)

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
Method : other: Method by US-EPA 1975 (EPA-660/3-75-009)
Year : 1983
GLP : no data
Test substance : other TS: 95 to 99% purity

Method : "Methods for Acute Toxicity Testing with Fish, Macroinvertebrates, and Amphibians," Ecological Research Series, EPA-660/3-75-009, National Environmental Research Center, Office of Research and Development, U.S.

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 concetration 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 (71) (21) (72)

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 (73)

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 (74)

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 (75) (76)

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 stabilty of the compound was analysed before testing - The method is described in Canton JH, Slooff W (1982) Substitutes for phosphate containing washing products: their toxicity and biodegradability in the aquatic environment. Chemosphere 11 (9): 891 - 907
Result	:	Nominal concentrations.
Test condition	:	- Semistatic conditions - 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 (30)

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
Method	:	other: Method by US-EPA 1975 (EPA-660/3-75-009)
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 (77)

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

02.12.2003 (25) (78) (26) (79) (80) (81)

Type	:	flow through
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 day(s)
Unit	:	mg/l
LC50	:	23.8
Limit test	:	No
Analytical monitoring	:	Yes
Method	:	other: Method by US-EPA 1975 (EPA-660/3-75-009)
Year	:	1984
GLP	:	No
Test substance	:	other TS: >95% purity
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 Secondary literature, presumably error in first citation

06.02.2003 (82) (83) (84) (85) (86) (87) (33) (88) (27)

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

Year	:	1985
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	LC50 value is the geometric mean
Test condition	:	- 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

31.01.2003 (89)

Type	:	other: not specified
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
Test condition	:	10 fish per test concentration in 10 l water were applied. Solvent: aceton 1:1 Concentrations tested: 1, 10 und 100 mg/L
Reliability	:	(4) not assignable Only raw data available

31.01.2003 (90)

Type	:	other: not specified in abstract
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	69
Limit test	:	No
Analytical monitoring	:	no data
Method	:	
Year	:	1986
GLP	:	no data
Test substance	:	no data
Remark	:	- The results of Yoshioka Y, Nagase H, Ose Y, Sato T (1986) [Evaluation of the Test Method "Activated Sludge, Respiration Inhibition Test" Proposed by the OECD. Ecotoxicol Environ Safety 12: 206 - 212] are cited in Yoshioka Y, Ose Y, Sato T (1986) [Testing and evaluation of chemical toxicity on Tubifex. Eisei-Kagaku 32, 308 - 311] and in Walker JD (1989) [Effects of chemicals on microorganisms: Fate and effects of pollutants. J Water Pollut Control Fed 61 (6): 1077 - 1095] - Yoshioka Y, Ose Y, Sato T (1986) [Testing and evaluation of chemical toxicity on Tubifex. Eisei-Kagaku 32: 308 - 311]: Only abstract and tables in English, the rest in Japanese
Reliability	:	(4) not assignable Not assignable/Original reference not translated

06.08.2003 (91) (92)

Type	:	other: not specified
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l

LC50	:	104
Limit test	:	No
Analytical monitoring	:	no data
Method	:	other: not specified
Year	:	1999
GLP	:	no data
Test substance	:	
Reliability	:	(4) not assignable Secundary literature

31.01.2003 (93)

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 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

06.08.2003 (94) (95)

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
Result	:	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.
Reliability	:	(4) not assignable Secondary literature not specified

31.01.2003 (96)

Type	:	
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	
Unit	:	
Method	:	other: see below
Year	:	1995
GLP	:	
Test substance	:	
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

Reliability	:	(4) not assignable Unsuitable test system for the hazard assessment of chemicals. Test with liver of rainbow trout
03.02.2003		(97)
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		(98)

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
Method	:	other: Method by US-EPA 1975 (EPA-660/3-75-009)
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.

Test substance : Source: Matheson Chemical Co.
Reliability : (2) valid with restrictions
Test procedure according to national standards. Basic data given
Flag : Critical study for SIDS endpoint
30.07.2003 (21)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : 7.5
Limit Test : No
Analytical monitoring : Yes
Method : other: Analogy with the OECD 202 proposal to short-term toxicity tests performed on crustaceans (Daphnia magna) (1979)
Year : 1985
GLP : no data
Test substance : other TS: > 99.5 % Purity

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
- Circadic lighting
- Endpoint: Mortality/immobility

Reliability : (2) valid with restrictions
Comparable to guideline study, without detailed documentation
Flag : Critical study for SIDS endpoint
06.08.2003 (30)

Type : other: not specifed
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : ca. 4.2
Analytical monitoring : no data
Method : other: ISO6341
Year : 1997
GLP : no data
Test substance : other TS: >=98%

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 : Critical study for SIDS endpoint
01.08.2003 (28)

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 cultering 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 : Critical study for SIDS endpoint
16.10.2003 (87) (88) (27)

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
Apparently Daphnia(?) data omitted in the publication process (Table 3 of the publication of Yen J-H, Lin K-H, Wang Y-S (2002) Acute lethal toxicity of environmental pollutants to aquatic organisms. Ecotoxicol Environ Safety 52: 113 - 116)

Flag : Critical study for SIDS endpoint

04.12.2003 (75) (76)

Type	:	Static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	12.1
Limit Test	:	No
Method	:	other: Method by US-EPA 1975 (EPA-660/3-75-009)
Year	:	1979
GLP	:	No
Test substance	:	no data

Method : "Methods for Acute Toxicity Testing with Fish, Macroinvertebrates, and Amphibians," Ecological Research Series, EPA-660/3-75-009, National Environmental Research Center, Office of Research and Development, U.S.

Remark : Ecological Research Series, EPA-66013-75-009, National Environmental Research Center, Office of Research and Development, U.S., Environmental Protection Agency, Corvallis, OR, 1975
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 (77)

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

Test condition	:	- The fastest profilerating 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

16.10.2003 (99)

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

04.12.2003 (100) (101) (102)

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
Method	:	Method of the Dutch Standardization Organization, Rijswijk, The Netherlands (1980), with slight modifications according to Van Leeuwen, C.J. et al, Aquatic toxicological aspects of dithiocarbamates and related compounds. Short-term tests. Aquat. Toxicol. 7, 145-164 (1985)
Remark	:	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

(IC50 µmol/l). Maas-Diepeveen and van Leeuwen (1986) calculatedLC-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 Lichtfield 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.

04.12.2003 (103) (79)

Type : Static
Species : other: Daphnia carinata
Exposure period : 48 hour(s)
Unit : mg/l
IC50 : ca. 14.4
Limit Test : No
Analytical monitoring : no data
Method : other: comparable to OECD 202 part I (Daphnia, Acute Toxicity, 1984)
Year : 1997
GLP : no data
Test substance : other TS: purity not given

Result : Results are given as log IC50: log IC50 = 3.98 (IC50 mol/l)
Reliability : (2) valid with restrictions
According to guideline study with acceptable restrictions

16.10.2003 (74)

Type : Semistatic
Species : other aquatic mollusc: Lymnaea stagnalis
Exposure period : 7 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 : - Snails from laboratory culture, fed with lettuce, 5 month old animals
- 5 Egg capsules per group
- 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

16.10.2003 (104)

Type : Static
Species : other aquatic arthropod: aquatic insect larvae of Aedes aegyptii
Exposure period : 48 hour(s)
Unit : mg/l
LC50 : 65
Limit Test : No

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	:	
Species	:	other aquatic worm: Tubifex
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)

Type	:	
Species	:	Daphnia magna (Crustacea)
Exposure period	:	
Unit	:	
Method	:	
Year	:	1990
GLP	:	

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
Method	:	Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
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	:	
Analytical monitoring	:	no data
Method	:	other: OECD Guideline 201 (1984)
Year	:	1986
GLP	:	
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.

Flag : Critical study for SIDS endpoint
05.12.2003 (106) (103) (79)

Species : Scenedesmus pannonicus (Algae)
Endpoint : Biomass
Exposure period : 4 day(s)
Unit : mg/l
NOEC : 10
Limit test :
Analytical monitoring : No
Method :
Year : 1983
GLP : no data
Test substance : no data

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

Flag : Critical study for SIDS endpoint
04.12.2003 (104)

Species : other aquatic plant: Lemna minor
Endpoint : growth rate
Exposure period : 7 day(s)
Unit : mg/l
NOEC : 10
Limit test :
Analytical monitoring : No
Method :
Year : 1983
GLP : no data
Test substance :

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

04.12.2003 (104)

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

Method	:	other: OECD-Guideline 201 (algae, Growth inhibition test, 1981)
Year	:	1995
GLP	:	no data
Test substance	:	other TS: no purity given
Remark	:	The result of Liu and Lang (1995) was also reported by Lu et al. 2001 to be obtained with the same method except that the light intensity was reported to be 4000 lux.
Test condition	:	The test was performed under the followimg conditions: 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 (107) (108)

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
Year	:	1997
GLP	:	No
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 (74)

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	:	other: OECD Guide-line 201 (1981) and OJEC (1983)
Year	:	2000
GLP	:	no data
Test substance	:	no data
Method	:	Continuous light was applied. During the test, temperature was of 20 +/- 1°C. Aqueaous medium was prepared according to Lang (1994). 5 concencentrations 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
04.12.2003		(109)

Species	:	Scenedesmus quadricauda (Algae)
Endpoint	:	Biomass
Exposure period	:	8 day(s)
Unit	:	mg/l
TT	:	15
Limit test	:	
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 artifical 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		(110) (111)

Species	:	other algae: Scenedesmus vacuolatus
Endpoint	:	growth rate
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC50	:	17.2
Limit test	:	
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		(112)

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 controling unit - Two algae were used for 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

16.10.2003 (113)

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

06.08.2003 (30)

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

04.12.2003 (114)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	Aquatic
Species	:	activated sludge of a predominantly domestic sewage
Exposure period	:	3 hour(s)
Unit	:	mg/l
EC50	:	100
Analytical monitoring	:	No
Method	:	OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year	:	1986
GLP	:	no data
Test substance	:	no data
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	:	Critical study for SIDS endpoint

16.10.2003 (91)

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

Flag : Critical study for SIDS endpoint
16.10.2003 (115) (110) (111)

Type : Aquatic
Species : Entosiphon sulcatum (Protozoa)
Exposure period : 72 hour(s)
Unit : mg/l
TT : 8.6
Analytical monitoring : No
Method : other: cell multiplcation 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
Flag : Critical study for SIDS endpoint
16.10.2003 (111) (116)

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 condition : - 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
Flag : Critical study for SIDS endpoint
04.12.2003 (117)

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

Test substance	:	
Method	:	Cell counting by microscope and Coulter counter
Test condition	:	Incubation at 30°C without agitation
Reliability	:	(2) valid with restrictions Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
16.10.2003		(91) (118)
Type	:	Aquatic
Species	:	Uronema parduzci (Protozoa)
Exposure period	:	20 hour(s)
Unit	:	mg/l
TT	:	46
Analytical monitoring	:	No
Method	:	other: cell multiplcation inhibition test
Year	:	1980
GLP	:	
Test substance	:	
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
19.12.2002		(116)
Type	:	Aquatic
Species	:	Pseudomonas fluorescens (Bacteria)
Exposure period	:	7 hour(s)
Unit	:	mg/l
NOEC	:	10
Analytical monitoring	:	No
Method	:	other: see test conditions
Year	:	1983
GLP	:	No
Test substance	:	no data
Test condition	:	- Pseudomonads were in the log-phase - About 10 000 000 000 organisms in each assay - Test volume 100 ml - Temperature 22 +/- 2 °C - Incubation in the dark, static - Endpoint: specific growth rate - Incubation period is reported to be 0.3 days! - not clearly indicated how growth was determined
Reliability	:	(2) valid with restrictions Test procedure in accordance with generally accepted scientific standards
04.12.2003		(104)
Type	:	Aquatic
Species	:	Microcystis aeruginosa (Bacteria)
Exposure period	:	
Unit	:	mg/l
TGK (EC1)	:	3.3
Analytical monitoring	:	no data
Method	:	other: see TC
Year	:	1976
GLP	:	no data
Test substance	:	no data

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 artifical light at 27 °C - Every 10 days new cultures are cultivated by transfering 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 transfered 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 (119) (115)

Type	:	Aquatic
Species	:	Chilomonas paramaecium (Protozoa)
Exposure period	:	48 hour(s)
Unit	:	mg/l
TT	:	16
Analytical monitoring	:	No
Method	:	other: cell multiplcation inhibition test
Year	:	1980
GLP	:	no data
Test substance	:	
Result	:	TT (Toxicity Threshold) comparable to EC5; value refers to
Test condition	:	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 (116)

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
Remark	:	Although author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmoles / liter.
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

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

16.10.2003 (120)

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

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)

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

Remark : Although author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmoles / liter.
Result : 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

Study with acceptable restrictions: up to date method by the time the study was undertaken

16.10.2003 (120)

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 bioluminiscence 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 (103)

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 (79)

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 glas or polystyrene plastic

(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 comparision to control cultures

Reliability : (3) invalid
Unsuitable test system

16.10.2003 (121)

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 fischeri 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
- O.5 ml of bacterial culture were added to 2 ml of test solution
- Toxicities were determined from the reduction of bioluminescence (Microtox test)

Reliability : (3) invalid
Unsuitable test system

16.10.2003 (122)

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.
Reliability : (3) invalid
Insufficient documentation for assessment

03.02.2003 (27)

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 these 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		(87) (33) (88)

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) Tetratox: 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		(123)

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) Tetratox: 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 (124)

Type : Aquatic
Species : other bacteria: not specified
Exposure period :
Unit :
EC0 : = 7
Analytical monitoring : No
Method : other: Gährröhrchentest
Year : 1984
GLP : No
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
Insufficent documentation for risk assessment. No unit for the effect concentration is given
03.02.2003 (125)

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

Remark : Vibrio fischeri 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 fischeri. In: Devillers (ed) Comparative QSAR. Taylor and Francis, Philadelphia, pp 51 - 109. This observed result was about 11 mg/l
Reliability : (4) not assignable
Calculation which yields result far below the observed toxicity.
03.02.2003 (126)

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

Reliability : (4) not assignable
Original data not available
03.02.2003 (8)

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

04.12.2003 (104)

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	:	Test similar to the method described by Canton and Slooff (1982) [Canton JH, Slooff W (1982) Substitutes for phosphate containing washing products: their toxicity and biodegradability in the aquatic environment. Chemosphere 11 (9): 891 - 907] but - 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

04.12.2003 (30)

Species	:	Poecilia reticulata (Fish, fresh water)
Endpoint	:	other: mortality, swimming behaviour, and growth

Exposure period	:	28 day(s)
Unit	:	mg/l
NOEC	:	10
Analytical monitoring	:	No
Method	:	other: (semi)chronic toxicity test
Year	:	1983
GLP	:	no data
Test substance	:	no data
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 Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

04.12.2003 (104)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	Daphnia magna (Crustacea)
Endpoint	:	reproduction rate
Exposure period	:	21 day(s)
Unit	:	mg/l
NOEC	:	1
Analytical monitoring	:	No
Method	:	other: see below test conditions
Year	:	1983
GLP	:	no data
Test substance	:	no data
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 Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

06.08.2003 (104)

Species	:	other aquatic mollusc: Lymnaea stagnalis
Endpoint	:	reproduction rate
Exposure period	:	40 day(s)
Unit	:	mg/l
NOEC	:	.32
Analytical monitoring	:	No

Method	:	other: see TC
Year	:	1983
GLP	:	no data
Test substance	:	no data
Result	:	For the endpoint mortality, the NOEC was 10 mg/l
Test condition	:	- Semistatic conditions - 5 Month old snails from laboratory culture - 20 Snails per group - 20 l Test volume per group - Food: Lettuce - Temperature 20 +/- 1 °C - Circadic illumination - 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 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.
Flag	:	Critical study for SIDS endpoint

04.12.2003 (104)

Species	:	Daphnia magna (Crustacea)
Endpoint	:	reproduction rate
Exposure period	:	21 day(s)
Unit	:	mg/l
NOEC	:	.7
EC50	:	1.8
Analytical monitoring	:	Yes
Method	:	other: in analogy with the OECD 202 proposal 1979
Year	:	1985
GLP	:	No
Test substance	:	other TS: > 99.5 % Purity (Origin: Fluka)
Remark	:	21d-LC50 : 3.2 mg/l 21d-EC50 (mortality and behaviour): 3.2 mg/l
Test condition	:	- Daphnias were 1 day old at start of incubation - Culturing and test medium: NaHCO3 100 mg/l, CaCl2*2H2O 200 mg/l, KHCO3 20 mg/l, MgSO4*7H2O 180 mg/l - Food during incubation: Chlorella - 25 organisms per 1 litre of test medium, 2 replicates - Incubation temperature 19 +/- 1 °C - Circadic lighting - Endpoint: Mortality/reproduction
Reliability	:	(2) valid with restrictions Comparable to guideline study, without detailed documentation
Flag	:	Critical study for SIDS endpoint

30.07.2003 (30)

Species	:	other aquatic arthropod: aquatic larvae of Culex pipiens
Endpoint	:	other: development, mortality
Exposure period	:	25 day(s)
Unit	:	mg/l
NOEC	:	3.2
Analytical monitoring	:	No
Method	:	other: see TC
Year	:	1983
GLP	:	no data
Test substance	:	no data
Remark	:	For both endpoints, the same NOEC were obtained

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

04.12.2003 (104)

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

16.10.2003 (104)

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	:	

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

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

04.12.2003 (103) (79)

Species : other aquatic mollusc: Lymnaea stagnalis
Endpoint :
Exposure period :
Unit : mg/l
NOEC : .2
Analytical monitoring : Yes
Method : other
Year : 1985
GLP : No
Test substance : other TS: > 99.5 % Purity

Reliability : (3) invalid
Insufficiently documented. Test period, endpoint, and other important data not reported.

03.02.2003 (30)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

Species : Tubifex
Endpoint : Mortality
Exposure period : 48 other: days
Unit : other: mg/l
LC50 : 36 - 64
Method : other: not specified
Year : 1989
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Secondary literature

31.01.2003 (127)

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species : Phaseolus aureus (Dicotyledon)
Endpoint : Growth
Exposure period : 6 day(s)
Unit : mg/l
EC50 : ca. 200
Method : other: germination and growth of seedlings in sand

Year	:	1962
GLP	:	No
Test substance	:	other TS: recrystallized
Remark	:	Although author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmoles / liter.
Result	:	1.5 mmol/l equals about 0.2 g/l
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 containig 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 approcing 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

16.10.2003 (120)

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
Year	:	1962
GLP	:	No
Test substance	:	other TS: recrystallized
Remark	:	Although author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmoles / liter.
Result	:	0.3 g/l equals about 2 mmol/l
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 containig 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 approcing 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

16.10.2003 (120)

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"
Year	:	1993

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 = -0.33 x + 2.83
Using log kow = 2.37, log EC50 = 2.048 and EC50 = 15 mg/l
Reliability : (2) valid with restrictions
Guideline study with acceptable restrictions
Flag : Critical study for SIDS endpoint
31.01.2003 (128)

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: neattle 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
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
19.12.2002 (104)

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
- Tetrahymena pyriformis EC50 82 mg/l

17.12.2002 (127)

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 incubatred in 1 l of test solution
- animals were not fed during incubation
- temperature was 21 +/- 2 °C
- circadic illumination
- dosing was semistatic, once per day
- endpoint mortality

10.12.2002 (30)

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 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 %; 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
0.00327/0.00442, 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 (129)

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 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 %; 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 comparisions with exposure levels and between genders were attempted.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint
03.11.2004 (129)

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-nitrobenzylmercapturic 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 minuts 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

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
03.11.2004 (129)

In Vitro/in vivo : In vivo
Type : Toxicokinetics
Species : Rat
Number of animals
Males : 5
Females :
Doses
Males : 200 mg/kg bw in corn oil
Females :
Vehicle : other: corn oil
Route of administration : gavage
Exposure time : 12 day(s)
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 : 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

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:
male: 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 Vitro/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 (130) (131)

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)

Reliability : (2) valid with restrictions
small number of animals

Flag : Critical study for SIDS endpoint

03.11.2004 (132) (131) (133) (134)

In Vitro/in vivo : In vivo
Type :
Species : Rat
Number of animals
Males : 3
Females :
Doses
Males : 1.07, 10.7, 107 mg/kg bw in ethanol/DMSO/water
Females :
Vehicle : other: ethanol/DMSO/water-mixture
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 Remark
Year :
GLP : no data
Test substance : other TS: radiolabelled p-nitrotoluene

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.

Reliability : (2) valid with restrictions
limited documentation

Flag : Critical study for SIDS endpoint

03.11.2004 (135)

In Vitro/in vivo : In vivo
Type : Toxicokinetics
Species : Rat
Number of animals
Males : 3
Females :
Doses
Males : 8 mg/kg bw in ethanol/polyoxyethylene sorbitanoleate/water mixture
Females :
Vehicle : other: ethanol/polyoxyethylene sorbitanoleate/water mixture (2:1:7)
Route of administration : i.v.
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 Remark
Year :
GLP : No
Test substance : other TS: radiolabelled p-nitrotoluene

Remark : 8 mg/kg bw radiolabelled p-nitrotoluene was administered i.v. to 3 male Fischer 344 rats.
4-Nitrotoluene is rapidly distributed to the tissues and rapidly cleared with peak tissue levels reached between 15 and 30 minutes (highest tissue levels 5 min. post dosing were in muscles (6.9 % of the dose) and liver (7.1%), at 15 min post dosing in liver (10 %), skin (9%), small intestine (6.6%), whole blood (6%), fat (5%) and kidneys (3.5%); at 30 minutes, highest tissue levels were found in liver (6%), small intestine (6%), and skin (9%). Less than 1% remained in the analysed tissues by 10 hours. By 4 hr no parent 4-nitrotoluene was detected in kidney or liver. Whole blood contained less then 0.02 % of dose as parent.
24 hr following the injection approximately 80 % of the dose appeared in the urine.
As main metabolites were identified: p-nitrobenzoic acid, 2-hydroxy-4-nitrotoluene, and the glucuronide of p-nitrobenzylalcohol.
In bile cannulation experiments, 3 anesthetised rats were given an intravenous dose of 8 mg/kg bw. These experiments showed that approximately 30 % of the dose was excreted into bile by 4 hours. Since only 6 % of the dose appeared in the feces by 7 days in the animals from the intravenous distribution study, the authors concluded that 4-nitrotoluene was rapidly excreted into the bile and underwent significant enterohepatic circulation. No parent compound was found in bile.

Reliability : (2) valid with restrictions
limited documentation
Flag : Critical study for SIDS endpoint
30.08.2004 (135)

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 ----------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 // 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 : Critical study for SIDS endpoint
30.08.2004 (136)

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, 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 : Critical study for SIDS endpoint
07.03.2003 (137) (138)

Type : LD50
Value : = 2140 mg/kg bw
Species : Rat
Strain : no data
Sex : Male
Number of animals :
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

09.12.2002 (139)

Type : LD50
Value : = 3200 mg/kg bw
Species : Rat
Strain : Wistar
Sex : Female
Number of animals : 10
Vehicle : other: Methylcellulose 1 %
Doses : 1000, 2000, 3000, 5000, 7000 mg/kg bw suspended in methylcellulose
Method : other: rat mean weight: 200 g, food and water ad libitum, appl. by gavage, dosing volume: 1 ml, 5 different doses, post dose observation period: 14 d, statistical evaluation according to Bartlett, determination of LD50 with probit meth
Year : 1978
GLP : no data
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/ 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
Reliability : (2) valid with restrictions
no necropsy and no histopathological examinations were performed
Flag : Critical study for SIDS endpoint
07.03.2003 (137) (138)

Type : LD50
Value : = 1960 mg/kg bw
Species : Rat
Strain : no data
Sex : no data
Number of animals :
Vehicle :
Doses :
Method : other: no data
Year : 1976
GLP : No
Test substance : other TS: no data on purity

Reliability : (4) not assignable
secondary literature

30.08.2004 (140) (141)

Type	:	LD50
Value	:	= 7100 mg/kg bw
Species	:	Rat
Strain	:	no data
Sex	:	Male
Number of animals	:	
Vehicle	:	other: water suspension of gum arabicum
Doses	:	2100-16000 mg/kg bw
Method	:	other: according to Deichmann, LeBlanc, J. Industr. Hyg. Toxicol. 25, 415 (1943)
Year	:	1959
GLP	:	No
Test substance	:	other TS: no data on purity

Reliability : (4) not assignable
documentation insufficient for assessment

31.08.2004 (142) (143)

Type	:	LD50
Value	:	= 2144 mg/kg bw
Species	:	Rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Doses	:	
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

09.12.2002 (144)

Type	:	LD50
Value	:	= 2144 mg/kg bw
Species	:	Rat
Strain	:	other: ChR-CD
Sex	:	Male
Number of animals	:	
Vehicle	:	other: corn oil
Doses	:	LD50 study: 1000, 2000, 4000 mg/kg bw 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
Method	:	other: see freetext Method
Year	:	1972
GLP	:	No
Test substance	:	other TS: no data on purity

Method : 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 sacrficed 14 days post-dosing. The LD50 value was calculated from mortality data, using the method of C.S. Weil, Biometrics 8, 249 (1952)

Result : Range Finding Study:
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

31.08.2004 (145)

Type : LD50
Value : = 1230 mg/kg bw
Species : Mouse
Strain : no data
Sex : Male
Number of animals :
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

09.12.2002 (139)

Type : LD50
Value : = 1231 mg/kg bw
Species : Mouse
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
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

09.12.2002 (144) (140)

Type : LD50

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 (141)

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 (141)

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 (146)

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 leasions 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		(148) (149)
Type	:	LC50
Value	:	> 4167 mg/m³
Species	:	Mouse
Strain	:	NMRI
Sex	:	Male
Number of animals	:	10
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
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		(136)
Type	:	other: exposure to an atmosphere essentially saturated with test substance
Value	:	
Species	:	Mouse
Strain	:	other: CF-1
Sex	:	Male
Number of animals	:	10
Vehicle	:	other: air
Doses	:	228 ppm (100 % saturation) = 1277mg/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 leasions 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		(148) (149)
Type	:	Other
Value	:	
Species	:	Rabbit
Strain	:	no data
Sex	:	no data
Number of animals	:	1
Vehicle	:	other: no data

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, 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 rabbit survived, symptoms during and after exposure: no symptoms
Reliability	:	(3) invalid isomer not specified

31.08.2004 (150)

Type	:	Other
Value	:	
Species	:	Rabbit
Strain	:	no data
Sex	:	no data
Number of animals	:	1
Vehicle	:	other: no data
Doses	:	200-250 mg/l air (see Method)
Exposure time	:	8 hour(s)
Method	:	other: see freetext Method
Year	:	1903
GLP	:	No
Test substance	:	other TS: isomer not specified, no dat on purity
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 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

31.08.2004 (150)

Type	:	Other
Value	:	
Species	:	Cat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	other: no data
Doses	:	approx. 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, purity not given

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 isomer not specified

31.08.2004 (150)

Type	:	Other
Value	:	
Species	:	Cat
Strain	:	no data
Sex	:	no data
Number of animals	:	1
Vehicle	:	other: no data
Doses	:	200-250 mg/l air
Exposure time	:	8 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, 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 4 hrs: 200 mg/l air, in the next 4 hrs 250 mg/l air cat survived, during exposure the cat showed no reaction: drowsiness, after the exposure indigestion, but soon recovery
Reliability	:	(3) invalid isomer not specified

31.08.2004 (150)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 16000 mg/kg bw
Species	:	Rat
Strain	:	no data
Sex	:	Male
Number of animals	:	
Vehicle	:	no data
Doses	:	2100-16000 mg/kg bw
Method	:	other: exposure time: 6 hours, then cleaning of the treated skin area
Year	:	1959
GLP	:	No
Test substance	:	other TS: no data on purity

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 (140) (142) (151)

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 (136)

Type	:	Other
Value	:	
Species	:	Mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	

Result	:	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

31.08.2004 (152)

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 (148) (149)

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 (153)

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 (154) (155)

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

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
Isomer not specified

30.08.2004 (150)

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
Isomer not specified

02.09.2004 (150)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : LD50
Value : = 940 mg/kg bw
Species : Rat
Strain :

Sex	:	Male
Number of animals	:	
Vehicle	:	other: water suspension of gum arabicum
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: according to Deichmann, LeBlanc, J.Industr.Hyg.Toxicol. 25, 415 (1943)
Year	:	1959
GLP	:	No
Test substance	:	other TS: no data on purity
Remark	:	within 3 hours methemoglobinemia up to 27 %, reversible within 24 hours.

31.10.1999 (140) (142) (143)

Type	:	other: determination of methemoglobinemia
Value	:	
Species	:	Rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	
Doses	:	2.04, 3.06, 4.53, 6.85, 10.21 15.30 mmol/kg bw (= 280, 420, 621, 940, 1400, 2098 mg/kg bw
Route of admin.	:	i.p.
Exposure time	:	
Result	:	Single i.p. application of 2.04, 3.06, 4.53, 6.85, 10.21, 15.30 mmol/kg to rats resulted in formation of 6.6, 6.9, 21.7, 23.6, 16.0, 27.1 % methemoglobin; animals of the three highest doses died within 2-4 days after treatment
Reliability	:	(2) valid with restrictions test description suffers from deficiencies

12.04.2003 (156)

Type	:	other: determination of methemoglobinemia
Value	:	
Species	:	Cat
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

12.04.2003 (157)

Type : LD50
Value : = 6800 mg/kg bw
Species : Mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Route of admin. : other: no data
Exposure time :

31.10.1999 (158)

Type : LD50
Value : = 1042 mg/kg bw
Species : Mouse
Strain :
Sex : Female
Number of animals :
Vehicle :
Doses :
Route of admin. : other: no data
Exposure time :

31.10.1999 (159)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Semiocclusive
Exposure time : 24 hour(s)
Number of animals : 2
Vehicle :
PDII :
Result : not irritating
Classification :
Method : 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
Year : 1976
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
limited documentation: skin reaction grading scheme not documented

31.08.2004 (136)

Species : Rabbit
Concentration : 20 other: mg

Exposure	:	no data
Exposure time	:	24 hour(s)
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: according to Draize et al., J.Pharmacol.Exper.Therap.82, 377 (1944)
Year	:	1959
GLP	:	No
Test substance	:	other TS: no data on purity; melt.p.:128-129 grade Celsius

Reliability : (4) not assignable
Documentation insufficient for assessment

16.12.2002 (142) (143)

Species	:	Rabbit
Concentration	:	Undiluted
Exposure	:	Semiocclusive
Exposure time	:	4 hour(s)
Number of animals	:	3
Vehicle	:	other: Polyethylenglycol 400 (see Method)
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year	:	1986
GLP	:	Yes
Test substance	:	other TS: purity: 99 %

Method : 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

Result : 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

Source	:	Hoechst AG Frankfurt/Main
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

31.08.2004 (160)

Species	:	Rabbit
Concentration	:	.5 other: mg
Exposure	:	Semiocclusive
Exposure time	:	4 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	
Result	:	not irritating
Classification	:	

Method	:	other: see Method
Year	:	1973
GLP	:	No
Test substance	:	other TS: technical grade
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. Rading 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

30.08.2004 (161)

5.2.2 EYE IRRITATION

Species	:	Rabbit
Concentration	:	Undiluted
Dose	:	50 other: mg
Exposure time	:	Unspecified
Comment	:	no data
Number of animals	:	2
Vehicle	:	
Result	:	not irritating
Classification	:	
Method	:	other: 2 rabbits, 50 mg/rabbit eye, other rabbit eye sered as control, reading after 2h, 24h, and daily up to 7 days (end of the observation time)
Year	:	1976
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
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

30.08.2004 (136)

Species	:	Rabbit
Concentration	:	no data
Dose	:	20 other: mg
Exposure time	:	Unspecified
Comment	:	no data
Number of animals	:	
Vehicle	:	
Result	:	slightly irritating
Classification	:	
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
Remark	:	method: according to Draize
Reliability	:	(4) not assignable documentation insuffient because lack of details

30.08.2004 (142) (143)

Species	:	Rabbit
Concentration	:	
Dose	:	100 other: mg

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

documentation insufficient: individual animal data are not given

02.09.2004 (163)

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 quantitiy 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-Hexylcinnamaldehyd, 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: nM intracutaneous
2nd: Challenge 19.2 other: nM 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) Priliminary 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 similiar 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
individual animal data not given
Flag : Critical study for SIDS endpoint
03.11.2004 (165)

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)

Type : Sub-chronic
Species : Rat
Sex : male/female
Strain : other: F344/N
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
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,
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, 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

onwards (182 g, 173 g versus 199 g of controls)

-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 numbetr 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, 10/10(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(1.1), 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, 6/10((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 100000 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 decreasd 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 (10^3/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 onwars 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 13 (231, 218 versus 195)
--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)

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
02.09.2004 (166) (167)

Type : Chronic
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/day)
(females: approx. 0, 60, 125, 265 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 : 1991
GLP : Yes

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 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 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 (forestomach 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;
---renale 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 renale 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 (oncocytes). 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)
---germinal 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
hematology and clinical chemistry was not performed
Flag : Critical study for SIDS endpoint
02.09.2004 (129)

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 (168)

Type :
Species : Rat
Sex : no data
Strain : no data
Route of admin. : Gavage

Exposure period	:	12 w
Frequency of treatm.	:	3/w
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, prolongation of clotting time
Reliability	:	(4) not assignable Documentation insufficient for assessment

17.12.2002 (168)

Type	:	
Species	:	Rat
Sex	:	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 appearent 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 (137) (169)

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 limited documentation (no quantitative data reported)
Flag	:	Critical study for SIDS endpoint

31.08.2004 (170)

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 (137) (138)

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 (166) (167)

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, testis, 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
The outlier test of Dixon and Massey

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

02.09.2004 (166) (167)

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 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 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 (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 (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
-- syncytial 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 erytrocytes (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 porgan (cauda epididymis, epididymis and testis) weights - average estous cycle length andrelative frequency of different estous 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

---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 unstimmulated 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

limited documentation
31.08.2004 (173)

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

17.12.2002 (174) (155)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
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

12.04.2003 (175)

Type	:	Cytogenetic assay
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
Method	:	other: Galloway, Environ. Mutagen.7,1-51 (1985) see also freetext Method
Year	:	1992
GLP	:	Yes
Test substance	:	other TS: purity: > 96 %

Method	:	CHO cells were incubated with 4-nitrotoluene or solvent (Dimetylsulfoxides (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
31.08.2004		(176) (177) (167) (129)

Type	:	other: antibacterial activity by a spot test
System of testing	:	Salmonella typhimurium TA98, TA100
Test concentration	:	10, 1, 0.1 umole/plate in DMSO
Cycotoxic concentr.	:	
Metabolic activation	:	Without
Result	:	Negative
Method	:	other: according to Ames et al., Proc.Natl. Acad. Sci (USA), vol.70, p. 782 (1973) see also freetext Method
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 zome 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

special study
30.08.2004 (178)

Type : Ames test
System of testing : Salmonella typhimurium TA98, TA100
Test concentration : not given
Cycotoxic 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 :
Cycotoxic 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
Cycotoxic 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
Cycotoxic 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, cytotoxicty, 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
Cycotoxic 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)
Cycotoxic concentr. :
Metabolic activation : Without
Result : Positive
Method : other: according to Kada, Mutat.Res 16,165 (1972), Hirano, Mutat.Res. 97,339 (1982)
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
Cycotoxic 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 (187)

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 (188)

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 (189)

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 (190)

Type	:	Unscheduled DNA synthesis
System of testing	:	rat hepatocytes
Test concentration	:	10, 100, 1000 uM dissolved in DMSO
Cycotoxic concentr.	:	1000 uM
Metabolic activation	:	Without
Result	:	Negative
Method	:	other: according to Williams, Cancer Res. 37,1845 (1977); Cancer Lett. 4, 69 (1978); see also freetext Method
Year	:	1983
GLP	:	no data
Test substance	:	other TS: purity: 99 %
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. neg. control: concurrent solvent control pos. control: Acetylaminofluorene
Result	:	10 uM, 100 uM: there were more grains in the cytoplasma 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	:	Critical study for SIDS endpoint

30.08.2004 (191) (192)

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA98, TA100, TA1535, TA1537
Test concentration	:	+/- S9-mix: 0.0, 3.3, 10.0, 33.0, 100.0, 333.0, 500.0, 667.0, 1000 ug/plate in DMSO
Cycotoxic concentr.	:	from 500 ug/plate
Metabolic activation	:	with and without
Result	:	Negative
Method	:	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)
Year	:	1983
GLP	:	Yes
Test substance	:	other TS: purity: > 96 %
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: I. acetone: (1)(2)(3) 0, 50, 75, 100, 150, 200, 300, 500, II. Ethanol: 50, 100, 150, 200, 250, 300 ug/ml
Cycotoxic 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 nonseletive 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: pracipitate of 4-nitrotoluene; 500 ug/ml: lethal; 50-200 ug/ml: significant positive response
Trial (2): 300 ug/ml: pracipitate of 4-nitrotoluene; 500 ug/ml: lethal; 300 ug/ml: significant positive response
Trial (3): 300 ug/ml: pracipitate 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)
Cycotoxic 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 wee 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

30.08.2004 (177) (167) (129)

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
Cycotoxic 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

30.08.2004 (195)

Type : Cytogenetic assay
System of testing : human peripheral lymphocytes
Test concentration : 0, 0.005, 0.05, 0.4, 1,0 mmol/l in DMSO
Cycotoxic 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 (196) (197) (198)

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
Cycotoxic 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 intraperitonally. 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 (199)

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.

Exposure period	:	no data
Doses	:	no data
Result	:	Negative
Method	:	other
Year	:	1989
GLP	:	no data
Test substance	:	other TS: no data

Result : no signs of intoxication reported; Chromosomal aberrations show no difference from controls; no further information given

Reliability : (4) not assignable
documentation insufficient for assessment

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
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 intraperitneally (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 (203) (167)

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 (191) (192) (131)

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; no induction of UDS in hepatocytes
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

02.09.2004 (204)

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

26.02.2003 (205)

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

26.02.2003 (206)

Type	:	other: induction of scheduled DNA synthesis (S-Phase)
Species	:	Rat
Sex	:	Male
Strain	:	Fischer 344
Route of admin.	:	Gavage
Exposure period	:	Once

Doses	:	100 - 750 mg/kg bw
Result	:	Negative
Method	:	other: 12 week old rats, S-phase was measured in primary hepatocytes 24 and 48 hrs after treatment
Year	:	1991
GLP	:	no data
Test substance	:	no data
Remark	:	p-NT failed to induce S-Phase activity
Reliability	:	(4) not assignable special study

30.01.2003 (203)

5.7 CARCINOGENICITY

Species	:	Mouse
Sex	:	no data
Strain	:	Sencar
Route of admin.	:	Dermal
Exposure period	:	30 w
Frequency of treatm.	:	initiator: once, promotor: 1/w
Post exposure period	:	no data
Doses	:	initiator: 50, 250, 400 mg/kg; promoter: 4 ug/kg TPA
Result	:	
Control group	:	other: no data
Method	:	other: tumor initiation promotion test
Year	:	1985
GLP	:	no data
Test substance	:	other TS: purity: 98 %
Remark	:	Initiation-promotion-test, initiator: 4-nitrotoluene, promoter: 12-O-tetradecanoylphorbol-13-acetate (TPA), both given in acetone
Result	:	no initiation of skin tumors
Reliability	:	(2) valid with restrictions limited documentation of the test procedure, no data on GLP, no data on sex of the mice used
Flag	:	Critical study for SIDS endpoint

30.08.2004 (207)

Species	:	Mouse
Sex	:	Male
Strain	:	other: A/Jax
Route of admin.	:	i.p.
Exposure period	:	8 w
Frequency of treatm.	:	3/w
Post exposure period	:	16 w
Doses	:	1800, 4500, 9000 mg/kg given in corn oil
Result	:	ambiguous
Control group	:	Yes
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	other TS: purity: 98 %
Result	:	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 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	:	
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, 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 (forestomach 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, 366 g (91 % of controls); 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; renale 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 renale 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:
germinal 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
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)

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)
(females: approx. 0, 155, 315, 660 mg/kg bw)
Result :
Control group : yes, concurrent vehicle
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: 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 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 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.
HISTOPATHOLLOGY:
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 (forestomach 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: 46/50, 46/50, 45/50, 42/50; f: 46/50, 47/50, 43/50, 49/50
body weight at the end of the study (control, low, mid, high dose):
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
no clinical findings were attributed to p-nitrotoluene exposure
Haematology data or clinical chemistry data were not reported.

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

Neoplasic 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 appearent 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

02.09.2004 (137) (169)

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
Premating exposure period		
Male	:	2 weeks
Female	:	2 weeks
Duration of test	:	47 days
No. of generation studies	:	1
Doses	:	0, 25, 100, 400 mg/kg bw in polethylene glycol 400
Control group	:	yes, concurrent vehicle
other:	:	= 25 mg/kg bw
NOAEL(reproductive toxicity)		
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 indexcourse 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, viabilty index
Pups were sacrificed on day 4 to 5 pp.

Statistical methods:
Analysis of variance (ANOVA), in case of sinificant 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 probabely 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%.
Determination of the time to insemination did not reveal toxicologically relvant 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
------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 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 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/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-grpup:
4.89(p<0.01)/4.69(p<0.01)/4.80p<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(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

Flag : Critical study for SIDS endpoint
02.09.2004 (208)

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 epididymidis 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 estous cycle length and relative frequency of different estous staged in females

Reliability : (2) valid with restrictions
limited documentation
Flag : Critical study for SIDS endpoint
31.08.2004 (170)

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 estous cycle length and relative frequency of different estous staged in females

Reliability : (2) valid with restrictions
limitd documentatuion

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
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. 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 (166) (167)

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
hematology and clinical chemistry was not performed
Flag : Critical study for SIDS endpoint
31.08.2004 (166) (167)

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 Toxicity : 25 mg/kg bw
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 indexcourse 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, viabilty index
Pups were sacrificed on day 4 to 5 pp.

Statistical methods:
Analysis of variance (ANOVA), in case of sinificant 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 (probabely 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 relvant 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 femle 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):

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/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)/4.80p<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 (212)

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.

Flag : Critical study for SIDS endpoint
06.08.2003 (213)

Type of experience : Human

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 (214)

5.11 ADDITIONAL REMARKS

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, chlorite, 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, chlorite, 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

06.03.2003 (215)

Type : Cytotoxicity

Remark : 1 mmol 4-nitrotoluene was not cyctotoxic to aerobic or hypoxic mammalian cells (V79 resp. EMT6 fibroblasts) and did not inhibit growth in air

Reliability : (4) not assignable
special investigation

28.01.2003 (216)

Type : Other

Remark : 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

Reliability : (4) not assignable
special investigation

12.04.2003 (217)

Type : Other

Remark : 4-Nitrotoluene (no. 47) is one of the chemicals in the list of suspected endocrine disrupturs (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)]

Reliability : (4) not assignable
no validated test method

Flag : Critical study for SIDS endpoint

30.08.2004 (218)

Type : other: in vitro

Remark : 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.

Reliability : (4) not assignable
not validated test method

Flag : Critical study for SIDS endpoint

03.11.2004 (97)

Type : other: in vitro investigation

Remark : Incubation of freshly isolated rat hepatocytes with labeled 4-nitrotoluene dissolved in ethanol (no further information) resulted in formation of 5 major metabolites: 4-nitrobenzioc acid, 4-nitrobenzyl alcohol, 4-nitrobenzyl alcohol glucuronide, 4-nitrobenzyl alcohol sulfate and S-(4-nitrobenzyl)gluthatione

Reliability : (4) not assignable
special investigation

28.01.2003 (219) (131)

Type : other: in vitro investigation

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 gluthatione conjugation and increased the amount of 4-nitrobenzyl sulfate
Reliability : (4) not assignable
special investigation

29.01.2003 (220) (131)

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

28.01.2003 (221)

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

28.01.2003 (222)

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

30.08.2004 (223)

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

29.01.2003 (224)

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 unidenfified metabolites (42 %)
Reliability : (4) not assignable
special investigation

29.01.2003 (225)

Type : other: uterotropic assay

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

(n=10), 1000 (n=5) mg p-Nitrotoluene/kg bw, dissolved in corn oil . Concurrent solvent control groups were included for each dose group separately containing n = 5, 5, 5, 10, 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.)
positive control 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 wether the rats used in the different experiments were of the same age, no dose-response relationship

Flag : Critical study for SIDS endpoint
30.08.2004 (226)

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
28.01.2003 (227)

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
28.01.2003 (228)

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
28.01.2003 (229)

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
28.01.2003 (230)

Remark : Single oral dose of 500 mg/kg 4-nitrololuene to mice pretreated with pyrazole (100 mg/kg i.p.) or 4-bromopyrazole (50 mg/kg i.p.) resulted in reduced renal excretion of

4-nitrobenzoic acid

Reliability : (4) not assignable
no validated testmethod

28.01.2003 (231)

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
special study

29.01.2003 (229)

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
isomer not specified

29.01.2003 (232)

Remark : In vitro, mitchondrial nitroreductase developed only very little activity to reduce p-nitrotoluene.

Reliability : (4) not assignable
special investigation

28.01.2003 (233)

Remark : It is reported that 11 industrial chemicals were analysed for their estrogen receptor (ER) binding capacity using ER-.alpha. 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.mu.g/mL in a dose-dependent manner but 4-nitrotoluene did not show significant effect on uterus weight in mice.
RL 4 (not assignable): publication in Japanese, limited documentation

Diethylstilbestrol (DES), 17-beta-estradiol, and 11 industrial chemicals were analysed for their estrogen receptor (ER) binding capacity using ER-.alpha. and for estrogen (ES)-like activities by measuring uterus weights in mice. DES and 17-beta-estradiol showed ER binding at 0.000000819 ug/ml, whereas 4-nitrotoluene did not show significant effectson uterus weights in mice.
(4-Nitrophenol was tested in CD-1 mice at 0.1, 1, and 10 mg/kg bw/day for 3 days. DES and 17-beta-estradiol were given at doses of 0.0001, 0.001 and 0.01 ug/kg bw/day)

Reliability : (4) not assignable
limited documentation

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