FOREWORD **INTRODUCTION**

1-CHLORO-4-NITROBENZENE

CAS N°:100-00-5

SIDS Initial Assessment Report

For

SIAM 15

Boston, USA, 22-25 October 2002

1.	**Chemical Name:**	1-Chloro-4-nitrobenzene
2.	**CAS Number:**	100-00-5
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): 25 May 2002 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms 27 May 2002 (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:		20. August 2002
10. Date of last Update:		

11. Comments:

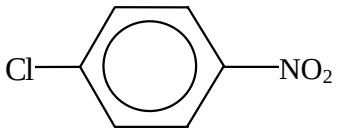
OECD/ICCA - THE BUA* PEER REVIEW PROCESS

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 o f 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.	100-00-5
Chemical Name	1-Chloro-4-nitrobenzene
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

1-Chloro-4-nitrobenzene is rapidly absorbed via skin, gastrointestinal tract or respiratory tract and distributed in the tissue predominantly in fat, blood cells, skeletal muscles, liver and kidney. Most of the substance was excreted with the urine followed by excretion with feces. 1-Chloro-4-nitrobenzene undergoes three major types of transformation *in vivo* in mammals: nitro-group reduction, displacement of the chloride in glutathione conjugation, and ring-hydroxylation. From accidental exposure of workers to 1-chloro-4-nitrophenol, large amounts of 2-chloro-5-nitrophenol, N-acetyl-S-(4-nitrophenyl)-L-cysteine, 4-chloroaniline and 4-chloroformanilide were identified.

The oral LD50 for 1-chloro-4-nitrobenzene in male rats is 294 or 694 mg/kg bw and in female rats 565 or 664 mg/kg bw. Cyanotic appearance was the predominant symptom. The 7-hour-inhalation of a highly saturated vapor-air mixture (concentration up to 77 mg/m^3) represented no acute hazard to male and female rats. In addition, the LC50 level could not be reached up to 16100 mg/m³ during a 4-hrs exposure against vapor and microcrystalline particles. The LD50 (dermal) for male rats is 750 mg/kg bw and for female rats 1722 mg/kg bw; the LD50 for male rabbits is 3550 mg/kg bw and for female rabbits 2510 mg/kg bw after acute dermal application. Cyanotic appearance was the predominant symptom. For the evaluation of acute toxicity it has to be taken into account that 1-chloro-4-nitrobenzene is a methaemoglobin forming chemical.

Experience with human exposure: all available reports relate to mixed exposure, frequently in combination with 1-chloro-2-nitrobenzene and/or nitrobenzene. A critical aspect in this context is that 1-chloro-4-nitrobenzene is rapidly absorbed via skin and the respiratory tract. The signs of acute intoxication include methaemoglobinaemia, vomiting, headache and in severe cases collapse.

The available study-reports on skin irritation have deficiencies with regard to the description of the results, nevertheless, 1-chloro-4-nitrobenzene is judged to be slightly irritating to the skin (intact or scarificed) of rabbits using a paste of the test substance and occlusive dressing and not irritating to the skin using undissolved, solid test substance and occlusive dressing.

In two available studies 1-chloro-4-nitrobenzene caused slight irritational effects to the eyes of rabbits which were reversible within 4 hours (first study: slight conjunctival injections, observed only in washed eye) resp. 8 days (second study: transient slight corneal cloudiness).

Due to the limited and poor quality information available regarding skin sensitization it cannot be concluded whether or not the chemical has a sensitizing activity.

The repeated dose toxicity via inhalation has been examined in rats for a period of 4 weeks and 13 weeks. In both studies, NOAECs were not achieved, the LOAECs were 5 mg/m³ (4 week-study) and 1.5 ppm (9.81 mg/m^3, 13 week-study), respectively, based on methemoglobinemia (3 % and 4 %, respectively) as the most sensitive effect. The maximum methemoglobin value was 42 % in females of the 24 ppm group in the 13-weeks study. The repeated dose toxicity via inhalation for a period of 13 weeks in mice revealed a NOAEC for histopathologic injury of 6 ppm (39.24 mg/m³). As target organs liver, kidney (rat only), spleen and blood were identified in both species.

Similarly, repeated dose toxicity by oral administration in rats [OECD TG 408 and 453] revealed changes predominantly consistent with methaemoglobinaemia. In the long term test a clear NOAEL could not be identified because histopathological examinations of most of the organs of the low- and mid-dose groups was performed only when macroscopic lesions were observed. The adverse effect level was 0.7 mg/kg bw/day. In the subchronic study the LOAEL was 3 mg/kg bw/day due to methaemoglobin formation and a NOAEL could not be derived. In both studies methaemoglobin formation and oxidative damage to red blood cells, leading to a regenerative anemia and a recognized spectrum of tissue damage and changes secondary to erythrocyte injury, were the main adverse effects.

1-Chloro-4-nitrobenzene induced reverse mutations in bacteria. It was not mutagenic in mammalian cells *in vitro* (HPRT test) and in insects *in vivo*. A mouse lymphoma assay was positive. *In vitro* it induced chromosomal aberrations and sister chromatid exchanges at high doses; no UDS in rat hepatocytes was reported.

The chemical induced micronuclei in mouse bone marrow *in vivo* at a toxic dose. In rat bone marrow it did not induce chromosomal aberrations *in vivo*. An *in vivo* SCE test was weakly positive in bone marrow cells of Chinese hamsters. DNA strand breaks were observed in liver, kidney and brain of mice. 1-Chloro-4-nitrobenzene is consequently capable of expressing mutgenic activity *in vivo* with low potency.

A combined chronic toxicity/carcinogenicity study (OECD Guideline 453) with 1-chloro-4-nitrobenzene in rats produced an increased incidence in interstitial cell tumours of the testes which were within the range of the historical control data and evaluated as not compound related. These tumours were described in literature as common tumours in male Sprague-Dawley rats. In another rat study which doesn't meet the criteria of today and is reported in brief, no tumours were found. In the available study with mice which doesn't meet the criteria of today and is only reported in brief, vascular tumors (localization not specified) were found. This tumor type is not uncommon in the substance class of substituted amino- or nitrobenzenes. Overall, taking into consideration the results of the genotoxicity tests and the limitations in the available long term studies, a carcinogenic potential cannot be ruled out.

Toxicity to reproduction of 1-chloro-4-nitrobenzene has been examined in rats and mice by oral administration. In a two generation study with rats [OECD guideline 416] no impairment of fertility was observed up to 5 mg/kg bw (high dose group), nevertheless, at this dose histopathological effects in testes were observed. But the evaluation of the effect on the male reproductive tract is limited because the testes in the low and mid dose group were not examined histopathologically. Therefore a NOAEL (male reproductive organ toxicity) was not established. The NOAEL for general toxicity of adults was not achieved. A LOAEL (adults) of 0.1 mg/kg bw/day based on histopathological effects in the spleen of F1 adults is indicated. The NOAEL for general toxicity of offspring is 0.1 mg/kg bw/day. In mice a study was performed using the NTP continuous breeding protocol. The NOAEL (fertility) is 125 mg/kg bw/day, the LOAEL (offspring general toxicity) is 62.5 mg/kg bw/day. The NOAEL (adult general toxicity) is 125 mg/kg bw/day, but full evaluation is not possible because evaluation of the animals of the two lower groups were very limited. Two subchronic inhalation studies with rats and mice with histopathologic evaluations on reproductive organs are available. There was evidence of decreased spermatogenesis (24 ppm) and decrease in average estrous cycle length in rats exposed to 1-chloro-4-nitrobenzene (6 ppm and above). In female mice an increase in estrous cycle length was noted at the highest exposure group (24 ppm).

Developmental toxicity of 1-chloro-4-nitrobenzene has been examined in rats and rabbits by oral administration [OECD TG 414]. In rats, a NOAEL for maternal toxicity was not achieved, the LOAEL(maternal toxicity) is 5 mg/kg bw/day; the NOAEL (developmental toxicity) is 15 mg/kg bw/day. The study with rabbits suffered from methodology deficiencies. Due to high mortality rate at the highest dose level, only two doses could be evaluated: the LOAEL (maternal toxicity) is 5 mg/kg bw/day and the LOAEL (developmental toxicity) is 5 mg/kg bw/day. Thus, in both species developmental toxicity occurred in the presence of maternal toxicity.

There are indications of immunotoxic potency following single and repeated applications of 1-chloro-4-nitrobenzene.

Environment

1-Chloro-4-nitrobenzene has a melting point of 83 °C, a solubility in water of 243 mg/l at 20 °C, and a vapour pressure of 8.5 Pa at 20°C. The measured log Kow is 2.39. The flash point is ca. 127 °C.

According to Mackay fugacity model level I the main target compartments for 1-chloro-4-nitrobenzene are air (65%) followed by water (33%). A measured Henry constant of 0.5 $Pa \cdot m^3 \cdot mol^{-1}$ indicates a moderate potential for volatilization of 1-chloro-4-nitrobenzene from aqueous solution. It is expected that in the atmosphere a degradation of 1-chloro-4-nitrobenzene occurs due to indirect photolysis ($t_{1/2air}$: ca. 62 days) and direct photolysis. 1-Chloro-4-

nitrobenzene is not readily biodegradable. Various tests showed adapted cultures to degrade 1-chloro-4-nitrobenzene. However, the degradation was inhibited at concentrations ≥ 8 mg/l. For *Pseudomonas putida* a O_2 consumption test resulted in a EC10 (30 min) of 59 mg/l. Bioconcentration factors determined for fish were in the range of 5.8– 20.9 and thus indicate no significant bioaccumulation potential of 1-chloro-4-nitrobenzene. A calculated Koc (Koc=309) suggests the substance to have a medium geoaccumulation potential.

Concerning the toxicity of 1-chloro-4-nitrobenzene towards aquatic species reliable experimental results of tests with fish, daphnia, and algae are available. The acute toxicity determined for fish (*Brachydanio rerio*) was of 14.36 mg/l (96 h LC50) and 2 mg/l (48 h) for *Leuciscus idus* and for daphnia (*Daphnia magna*) of 2.7 mg/l (48 h-EC50). In the growth rate tests with algae (*Scenedesmus subspicatus)* the values 4.9 mg/l (48 h-ErC10) and 16 mg/l (48 h-ErC50) were achieved while for *Chlorella pyrenoidosa* an effect value of 4.9 mg/l (96h-EC50) was found.

The prolonged toxicity to fish (*Brachydanio rerio)* for the endpoint sub-lethal effects (feeding, malposition) was evaluated through a 14 days test and a NOEC value of 1.53 mg/l was determined.

Two chronic tests with Daphnia (*Daphnia magna*) are available that were performed with analytical monitoring of the test substance concentration. In one test a 21 d-EC_{10} of 0.103 mg/l (effective concentration) was observed for the endpoint reproduction rate. The second test resulted in a 21d-NOEC of 0.19 mg/l (effective concentration) for the same endpoint. Calculating the geometric mean of these two values gives a NOEC of 0.14 mg/l. A PNECaqua = 2.8 µg/l is derived from this value, using an assessment factor of 50.

Exposure

About 220,900 tons 1-chloro-4-nitrobenzene were produced by about 30 producers worldwide in 1995 (excluding Eastern Europe). All 1-chloro-4-nitrobenzene is a basic chemical for the synthesis of intermediates which are further processed to pharmaceuticals, plant protection agents, auxiliaries in the rubber and plastics industry, dyestuffs/pigments, and others within the chemical industry. A direct use is not known.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

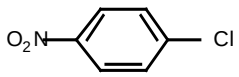
The chemical possesses properties indicating a hazard for human health and the environment. Based on data presented by the Sponsor country, exposure to humans and the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

SIDS Initial Assessment Report

1. IDENTITY

1.1 Identification of the Substance

CAS Number: 100-00-5
IUPAC Name: 1-Chloro-4-nitrobenzene
Molecular Formula: $C_6H_4ClNO_2$
Structural Formula:



1.2 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Parameter	Value	Source
Physical state	yellowish crystalline substance	Ullmann 2001
Melting point	83 °C	Ullmann 2001
Density at 22 °C	1.52 g/cm³	Ullmann 2001
Vapour pressure at 20 °C	8.5 Pa (calculated from measured values)	Bayer AG 1986a
Octanol/water partition coefficient (log K_{ow})	2.39 (measured)	Leo et al. 1971
Water solubility at 20 °C	243 mg/l (measured)	Bayer AG 1986b
Flash point	ca. 127 °C (measured)	Bayer AG 2001

2. GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

The world wide (excluding East Europe) production of 1-chloro-4-nitrobenzene amounted to 220,900 tons in 1995 (about 54,000 t in West Europe, 37,500 t in USA, 78,000 t in China, 29,000 t in India, 17,700 t in Japan, and 4,700 t in South Korea) by approximately 30 producers (Srour 1996). Bayer supposes a declined production volume and less producers in 2001.There is no information about production in East European countries (Bayer AG 2002b).

1-Chloro-4-nitrobenzene is a basic chemical, used industrially in intermediate chains, processed by nitration, sulfonation, reduction, and substitution. In the following an estimation of first stages in these chains and their percentage is given (Srour 1996):

4-nitrophenol (28 %),

4-aminodiphenylamine (26 %),

4-nitroaniline (11 %),

4-nitroanisole (8 %),

4-nitrophenetole (6 %),

2,4-dinitrochlorobenzene (6 %),

4-chloroaniline (3 %),

- others (13 %), including the manufacturing of chloronitrobenzenesulphonic acid, p-fluoroaniline, 2-amino-5-chlorobenzophenone and substituted diphenylethers.

These data relate to the above cited world wide production demand in 1995. The intermediates are used in the synthesis of pharmaceuticals (32 %), plant protection agents (27 %), auxiliaries in the rubber and plastics industry (15 %), dyestuffs/pigments and other (26 %). A direct use of 1-chloro-4-nitrobenzene is not known (Bayer AG 2002b). 1-Chloro-4-nitrobenzene is not listed in the Danish (Danish Environmental Protection Agency 2002) and in the Swedish (Swedish National Chemicals Inspectorate 2002) product registers. 1-Chloro-4-nitrobenzene is listed in the Swiss product register as a basic chemical but not as a consumer product (Bundesamt für Gesundheit 2002).

Production of 1-chloro-4-nitrobenzene takes place by mono-nitration of chlorobenzene in a continuously working closed system. Initially a mixture of chloronitrobenzenes is gained. This mixture is separated by distillation and crystallisation procedures yielding 1-chloro-4-nitrobenzene with a purity above 99 % (Bayer AG 2002b).

Releases into the environment may occur during production and processing.

Easily accessible information on exposure from production and processing of the chemical in the sponsor country is available at Bayer AG.

The processing plants are dedicated systems in which only chloronitrobenzenes are manufactured, separated and stored. Cleaning procedures take place only in the case of maintenance.

The exhausts from production and processing of 1-chloro-4-nitrobenzene are connected to air washing units and thermal exhaust purification plants. Thus during normal operation no 1-chloro-4-nitrobenzene is emitted. Following the Official German Emission Declaration of the year 2000, less than 25 kg/a 1-chloro-4-nitrobenzene were emitted into the atmosphere (Bayer AG 2002b).

The production process and the filling of the product are executed in a closed system (transport via pipings, sampling without dead volume, gas-shuttle pipe for filling processes).

Sewage flows leaving the production and processing facilities are generally pretreated at the production plant before reaching the industrial waste water treatment plant. 1-Chloro-4-nitrobenzene is monitored daily at the influent and the effluent of the waste water treatment plant. All values of the effluent measurement were below the detection limit of 20 µg/l in 2001. Additional the effluent was monitored on a fine analysis scale at 30 days. 27 values were below the detection limit of the fine analysis of 2 µg/l. For the receiving water a PEC of < $2.9x10^{-3}$ µg/l is calculated taking in account the 10 percentile of the river flow, the dilution factor, and the 90 percentile of the fine analysis measurements (Bayer AG 2002b).

To elucidate the possibility of environmental contamination by products containing 1-chloro-4-nitrobenzene residues from their production process, the content of 1-chloro-4-nitrobenzene in 4-amino-diphenylamine, a precursor of the rubber antioxidant 6PPD (N-1,3-dimethylbutyl-N´-phenyl-1,4-phenylenediamine), was measured by gas chromatograpy. No 1-chloro-4-nitrobenzene could be detected (detection limit of 100 ppm). Also for other products it is expected - due to the processing involved - that no significant 1-chloro-4-nitrobenzene residues occur in products manufactured from 1-chloro-4-nitrobenzene as an intermediate (Bayer AG 2002b).

Significant environmental releases from biological reformation of 1-chloro-4-nitrobenzene from end-products are not likely to occur. This is supported by monitoring data from German surface waters (see chapter 2.1). These data show that the environmental concentration of 1-chloro-4-nitrobenzene is in the range of < 0.01 µg/l to < 0.1 µg/l.

A significant exposure of the terrestrial compartment could not be identified.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

With regard to its chemical structure 1-chloro-4-nitrobenzene is not expected to hydrolyse under environmental conditions. The results of the stability experiments carried out by Canton et al. (1985) show no decay of the test compound (1-chloro-4-nitrobenzene, purity of 99.5 %) in the aquatic test medium after 8 days.

According to the Mackay Fugacity Model Level I, at 20 °C the main target compartments for 1-chloro-4-nitrobenzene are the air with 65 %, followed by the hydrosphere with 33% (UBA 2002), input parameters see IUCLID). The half-life for volatization from a standard model river is estimated to be 6 d and from a standard model lake 73 d (Bayer AG 2002a). The measured Henry constant is 0.5 Pa m^3 mol^{-1} at 25 °C (Altschuh et al. 1999), thus indicating a moderate volatility from aqueous solution.

Based on the available experimental data 1-chloro-4-nitrobenzene is not readily biodegradable.

In a closed bottle test in accordance with the later published method OECD 301 D 1-chloro-4-nitrobenzene as the only source of organic carbon was not degraded by non-adapted activated sludge (Bayer 1979a).

In contrast 1-chloro-4-nitrobenzene is biodegradable by adapted microorganisms and sewage from adapted wastewater treatment plants using the same test system:

After an adaptation period of 2 weeks, a mixed population of microorganisms from an industrial and domestic wastewater treatment plant, degraded (according to BOD measurements) 62 % of the

test substance during a 20 day incubation period at an initial 1-chloro-4-nitrobenzene concentration of 2.4 mg/l. At higher concentrations (>= 8 mg/l) of 1-chloro-4-nitrobenzene the degradation process was inhibited (Bayer 1979a).

There is only one standardized screening test on inherent biodegradation of 1-chloro-4-nitrobenzene available. In a MITI II test 0 % biodegradation after 2 weeks was found (MITI, 1992). However, it cannot be excluded that the inoculum was inhibited by the employed test substance concentration of 30 mg/l.

Cultures of *Rhodosporidium sp.* (fungus) metabolized 1-chloro-4-nitrobenzene to 4-chloroaniline and other aromatic compounds (> 90 % after 10 days), but the monocultures were not able to completely degrade the aromatic metabolites (Corbett and Corbett 1981).

Chloronitrobenzenes were degraded by isolated microbial cultures and adapted mixed sludge (> 80 % after 1.8 days) as long as there were additional sources of carbon and nitrogen in the nutrient media (Kuhlmann 1999).

Forest soil, rotten bark, wood chips, river sediments, sewage and other environmental materials were screened for microorganisms with the potential to degrade various industrial chemicals. In a soil pot or liquid culture reactor aerobic microorganisms were adapted to chloronitrobenzenes or other chemicals for up to 1 year with different methods. Adapted cultures were obtained which degraded 1-chloro-4-nitrobenzene within 7 days by more than 60 % (Voelskow 1984).

Under aerobic (Jakóbczyk et al. 1984) and anaerobic conditions (Gvozdyak et al. 1982) adapted microorganisms degraded 1-chloro-4-nitrobenzene in different types of model wastewater treatment plants during an average residence time of about one day (> 99 %). The activated sludge was first adapted for six month. It lasted another 8 month until the disturbance of the biological degradation processes by the test substance was finalized and the above described degradation was observed. The distribution / elimination of 1-chloro-4-nitrobenzene in a sewage treatment plant (microorganisms adapted to 1-chloro-4-nitrobenzene) 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 h^{-1}, a Henry constant of 0.5 Pa m3 mol^{-1} and a log Kow of 2.39 the following results were obtained:

% to air	1
% to water	96.1
% to sludge	2.9
% degraded	0

% removal (sum of losses to air, removal with sludge and degradation)	3.9

The comparison of influent and effluent concentrations of an industrial sewage treatment plant shows the substance to be removed by more than 98 % (Bayer AG 2002b). However, this removal cannot be transferred to other sewage treatment plants due to possible different waste water composition and adaptation processes. As environmental releases of 1-chloro-4-nitrobenzene occur from production and processing sites (local point sources), adapted microorganisms may be anticipated.

The indirect photochemical degradation in air by hydroxyl radicals (OH) is calculated with a half-life of 62 days (Bayer AG 2002a). The OH reactivity may be seen as an upper limit of stability, since direct photolysis due to the significant UV absorption in the sunlight range, is not taken into account (GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA) 1988).

Measured bioconcentration factors (BCF) determined for fish (*Cyprinus carpio*) according to OECD guideline 305 C, were in the range of 5.8 – 20.9. 1-Chloro-4-nitrobenzene concentrations of 0.15 and 0.015 mg/l were tested. Thus no significant potential for bioaccumulation of 1-chloro-4-nitrobenzene in aquatic organisms is indicated (MITI 1992).

There is no test available on geoaccumulation. Binding to soil organic matter has been calculated with Koc = 309 (Bayer AG 2002a). According to Blume (1990) 1-chloro-4-nitrobenzene can be regarded as a substance with medium geoaccumulation properties.

There are various data on concentrations of 1-chloro-4-nitrobenzene in German waters. In the river Elbe at Hamburg the concentration of 1-chloro-4-nitrobenzene decreased from about 0.4 µg/l in 1992 to less than 0.01 µg/l in 1998 (Umweltbehörde Hamburg 1999). In 1999 in the Elbe, the highest concentration of 1-chloro-4-nitrobenzene was 0.086 µg/l at Magdeburg, with an average of 0.022 µg/l for the 6 samples at this site. The German Federal Environmental Agency (UBA 1999) reported concentrations of 1-chloro-4-nitrobenzene for the year 1999 (UBA):

River	Measuring station	Type of value	Result
Danube	Ulm	90 % percentile	< 0.01 µg/l
Elbe	Schnackenburg	maximum	0.04 µg/l
Rhine	Kleve-Bimmen	90 % percentile	< 0.1 µg/l

In 2000, the concentration of 1-chloro-4-nitrobenzene was measured to be 0.01 µg/l in the river Main at Bischofsheim (personal communication BUA 2002).

Hendriks et al. reported levels of 1-chloro-4-nitrobenzene in suspended solids, in the zebra mussel (*Dreissena polymorpha*), and in the eel (*Anguilla anguilla*) for the Rhine (sampling site Lobith), Meuse (Eijsden), Ysselmeer and the Hollands Diep location in the Rhine-Meuse delta in 1993/94. In the Rhine the contents of 1-chloro-4-nitrobenzene were 31 µg/kg dry weight in suspended solids (1993/94), 0.12 µg/kg wet weight in zebra mussels (1994) and 1.5 µg/kg wet weight in eels (1994). These authors concluded that the bioaccumulation ratio is below 1 for the accumulation of 1-chloro-4-nitrobenzene from suspended solids in these species (Hendriks et al. 1998).

Although the measured environmental concentrations are low they indicate that environmental releases of 1-chloro-4-nitrobenzene occur.

2.3 Human Exposure

2.3.1 Occupational Exposure

To protect workers from exposure to 1-chloro-4-nitrobenzene at workplace, several different precautionary and protective measures are undertaken. In Germany the current workplace limit concentration according TRGS 901 for 1-chloro-4-nitrobenzene is 0.075 ppm (0.5 mg/m^3) (BMA 1995, BIA 2002).

Workplace monitoring is carried out periodically and appropriate personal protection equipment is used at the work place.

During the past five years (1997 - 2001) thirty three 8-hour shift samples were taken in different work place areas of Bayer production and processing plants. Thereof 23 measurements were below the detection limit. The detection limit depends on the air volume taken during sampling and was between 0.004 mg/m³ and 0.1 mg/m³. Nine measurements showed values between 0.016 and 0.061 mg/m³ and the maximum was 0.120 mg/m³ (Bayer AG 2002b).

2.3.2 Consumer Exposure

Due to some European product registers (Danish Environmental Protection Agency 2002; Swedish National Chemicals Inspectorate 2002; Switzerland: Bundesamt für Gesundheit 2002) there is no information about the occurrence of 1-chloro-4-nitrobenzene in consumer products.

3. HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

1-Chloro-4-nitrobenzene is rapidly absorbed by the body via skin (Nomeir et al. 1992, NTP 1993), gastrointestinal tract (Bray et al. 1956, Silveira et al. 1989, 1990, NTP 1993) and via respiratory tract (Yoshida et al. 1993d).

In rats, following oral dosing at least 78 % and following dermal application at least 62 % of the applied compound were absorbed. 72 hours after oral uptake of the compound up to 74 % of the dose was excreted with the urine and up to 12 % with the feces. After dermal application 45 % of the dose was excreted in the urine and 12 % in the feces within 72 hrs (up to 30 % of the dose was recovered in the protective device and ethanol trap (collects volatiles)). In both application routes, it was shown that at very high doses the initial urinary excretion rate is delayed and the initial fecal excretion is markedly depressed. These observations at high doses may reflect the reabsorption from greater billary excretion rates suggesting involvement of the enterohepatic cycle, but there are no signs of accumulation of 1-chloro-4-nitrobenzene or one of its metabolites (NTP 1993, Nomeir et al. 1992, Silveira et al. 1989).

24 hours post oral application the highest concentrations of the compound were found in the fat, followed by blood cells, skeletal muscles , liver and kidney. At 72 hours greatest concentration was found in the blood cells followed by fat, skeletal muscles and liver (NTP 1993).

1-Chloro-4-nitrobenzene undergoes three major types of transformation in vivo in mammals: nitro-group reduction, displacement of the chloride in glutathione conjugation, and ring-hydroxylation. As urinary metabolites in rats following i.p. injection 4-chloroaniline, 2,4-dichloroaniline, 4-nitrothiophenol, 2-chloro-5-nitrophenol, 2-amino-5-chlorophenol, 4-chloroformanilide, 4-chloro-2-hydroxyacetanilide and 4-chloroacetanilide were identified (Yoshida et al. 1991). Bray et al. (1956) reported that 63 % of 1-chloro-4-nitrobenzene administered to rabbits was excreted as metabolites in urine. Approximately 40 % of the administered dose was excreted as sulfate- or glucuronide-conjugated phenol metabolites and approximately 10 % as free 4-chloroaniline. From accidentally exposure of workers to 1-chloro-4-nitrophenol, large amounts of 2-chloro-5-nitrophenol, N-acetyl-S-(4-nitrophenyl)-L-cysteine, 4-chloroaniline and 4-chloroformanilide (produced by pyrolysis of 4-chloro-oxanilic acid, which originates from 1-chloro-4-nitrobenzene) and small amounts of 2,4-dichloroaniline, 2-amino-5-chlorophenol, 4-chloroacetanilide and 4-chloro-2-hydroxyacetanilide were identified (Yoshida et al. 1992, 1993c).

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

There is no study available which determined a LC50-value. However, the exposure (nose-only) of male and female rats to a highly saturated vapor-air mixture for 7 hours was tolerated without mortality. The analytical concentration in the chamber ranged from 53 mg/m³ measured 50 min after the start of the test up to 77 mg/m³ measured 290 min after the start of the test. The only signs of intoxication during exposure were narrowed palpebral fissures and tachypnoea. After termination of the exposure behaviour returned to normal. Pathologic examination after the 14 day-observation period revealed no remarkable findings (Hoechst AG, 1981). In another study 10 male rats were

exposed head-only to an atmosphere containing vapor and microcrystalline particles up to 16100 mg/m³ for 4 hours. Clinical signs of toxicity were observed during exposure, immediately and 14 days post exposure including cyanosis, pallor, lethargy, abnormal arched-back posture, tachypnoea, semi-prostration, alopecia, dermal irritation, corneal opacity, lacrimation, redish-brown nasal and frothy mouth discharges, and stained perineal area. Weight loss of 6-13 % was observed within the first 24 hrs but weight gain normalized thereafter. Three days post exposure death occurred in the highest dose group of 1/10 rats (Dupont de Nemours 1981).

Conclusion

The 7-hour-inhalation of a highly saturated vapor-air mixture (concentration up to 77 mg/m^3) represented no acute hazard to male and female rats. In addition, LC50 level could not be reached up to 16100 mg/m³ during a 4-hrs exposure against vapor and microcrystalline particles. For the evaluation of the acute inhalative toxicity it has to be taken into account that 1-chloro-4-nitrobenzene is a methaemoglobin forming chemical.

Dermal

There are no studies according to the current OECD guideline but there are study reports with rabbits and rats which give sufficient information to evaluate this endpoint:

Application of undiluted, but warmed to make suitable for dosing, 1-chloro-4-nitrobenzene (purity not given) on the skin of 2 male and 2 female rabbits for 24 hours yielded dermal LD50-value of 3550 mg/kg bw for males and 2510 mg/kg bw for females, respectively. During one day rabbits displayed lethargy resulting in increasing weakness, collapse and death which occurred within 4-6 days. At gross necropsy decedents showed haemorrhagic areas of the lungs, liver- and kidney-discoloration, darkened spleen and gastrointestinal inflammation. In survivors, the viscera appeared normal (Monsanto Co, 1983a). LD50 was 750 mg/kg bw (Bayer 1979b) when applied as polyethylene glycol solution to the skin of male rats for 24 hours and 1722 mg/kg bw following the application of 1-chloro-4-nitrobenzene dissolved in sesame oil to female rats (Hoechst 1975a). Male rats developed cyanotic appearance, sedation, unkempt fur, palmospasm and lowered body temperature. Clinical signs of intoxication in female rats were poor general condition, cyanotic appearance, brown colored urine, and the treated skin was grey-blue colored. Pathologic examination of the decedents was not possible because of ongoing autolysis. Survivors viscera appeared normal.

Conclusion

The LD50 (dermal) for male rats is 750 mg/kg bw and for female rats 1722 mg/kg bw; the LD50 for male rabbits is 3550 mg/kg bw and for female rabbits 2510 mg/kg bw after acute dermal application. Cyanotic appearance was the predominant symptom. For the evaluation of the acute dermal toxicity it has to be taken into account that 1-chloro-4-nitrobenzene is a methaemoglobin forming chemical.

Oral

There are no studies which were performed according to the current OECD guideline, but there are studies which give sufficient information to evaluate this endpoint.

One of these studies which meet the criteria of today yield a LD50-value of 294 mg/kg bw (male Wistar rat, Bayer AG 1979b). Signs of intoxication were reduced general condition, cyanotic appearance, diarrhea and increased excretion of urine. Death occurred within 3 days. In other studies LD50 were 565 mg/kg bw (female Wistar rat, Hoechst AG 1975b), or 664 mg/kg bw (female Wistar rat, Hoechst AG 1977d), and 694 mg/kg bw (male Wistar rat, Hoechst AG, 1977a), respectively. Imbalance, tremor, abdominal position and cyanotic appearance were the reported

signs of intoxication before death occurred. Pathologic examination of the decedents revealed brownish colored lungs and dark brown livers, whereas in survivors, 14 days post application, viscera appeared normal.

There are numerous additional publications available which determine LD50-values within the same range. None of them meets the criteria of today, and some of them suffer from deficiencies with regard to study reporting.

Conclusion

The oral LD50 for 1-chloro-4-nitrobenzene in male rats is 294 or 694 mg/kg bw and in female rats 565 or 664 mg/kg bw. Cyanotic appearance was the predominant symptom. For the evaluation of the acute oral toxicity it has to be taken into account that 1-chloro-4-nitrobenzene is a methaemoglobin forming chemical.

Studies in Humans

All available reports relate to mixed exposure, frequently in combination with 1-chloro-2-nitrobenzene and/or nitrobenzene. A critical aspect in this context is that 1-chloro-4-nitrobenzene is rapidly absorbed via skin and the respiratory tract. The signs of acute intoxication include methaemoglobinaemia, vomiting, headache and in severe cases collapse (Gerbis 1932, Renshaw and Ashcroft 1926, Linch 1974, Sekimpi and Jones 1986).

3.1.3 Irritation

Skin Irritation

Skin irritating potential was examined according Code of Federal Regulations Title 16 Section 1500.41 applying 500 mg 1-chloro-4-nitrobenzene mixed with 1-2 drops of water to the intact and to the scarificed skin of 6 rabbits for 24 hours covered by a occlusive dressing. When the dressings were removed (24 hrs-reading) no erythema but slight oedema (score 2.17/4 (maximum score)) in the intact skin and in the scarificed skin slight erythema (score 0.17/4) and slight oedema (score 1.67/4) were reported (48 hrs reading was not reported). 72 hrs post application the slight erythema of the scarificed skin had dissappeared and oedema showed a trend to disappear as it was evaluated with score of 1 for both, intact and scarificed skin. Although eight-day-reading was not documented, 1-chloro-4-nitrobenzene was judged to be slightly irritating to intact and scarificed skin of rabbits (Schreiber, 1980a).

In another study according Code of Federal Regulations Title 16 Section 1500.41 500 mg undissolved 1-chloro-4-nitrobenzene was applied to the skin of 6 rabbits for 24 hours using occlusive dressing. One rabbit showed slight cyanotic appearance after 24 hrs which was judged as sign of resorption through the skin. Details of the readings were not given; as result a slightly irritating reaction was reported and the irritation index of 0.1 was calculated (maximum possible value = 8), therefore 1-chloro-4-nitrobenzene was evaluated as no irritant (Hoechst AG, 1977b).

Conclusion

The available study-reports on skin irritation have deficiencies with regard to the description of the results, nevertheless, 1-chloro-4-nitrobenzene is judged to be slightly irritating to the skin (intact or scarificed) of rabbits using a paste of test substance and occlusive dressing or not irritating to the skin using undissolved, solid test substance and occlusive dressing.

Eye Irritation

100 µl of polymorphic substance was given into the right eye of each of 6 rabbits to determine the irritant potential of mucous membrane according Code of Federal Regulations, Title 16, Section 1500.42. 24 hours post application cornea and iris were not affected, only conjunctiva showed slight conjunctival injections in 6/6 eyes (score: 2/110) which showed a trend to disappear as it was evaluated with score 2 for 4/6 48 hrs, and 2/6 72 hrs post application and had disappeared after 8 days. As the primary irritant value is calculated to be 1.3/110, 1-chloro-4-nitrobenzene is regarded to be a non-irritant substance by the authors (Schreiber 1980b).

In another study 10 mg undissolved 1-chloro-4-nitrobenzene was placed into the right conjunctival sac of each of 2 rabbits: After 20 sec the treated eye of 1 rabbit was washed with tap water for 1 min. This eye showed small areas of transient slight corneal cloudiness which disappeared within 4 hours after treatment (scores not available), conjunctiva and iris were not affected. The unwashed eye of the other rabbit showed no effects on cornea, iris or conjunctiva. Based on the observations from the washed eye, 1-chloro-4-nitrobenzene is slighty irritating (Dupont de Nemours, 1982).

Conclusion

In two available studies 1-Chloro-4-nitrobenzene caused slight irritational effects to the eyes of rabbits which were reversible within 4 hours (first study: slight conjunctival injections, observed only in washed eye) resp. 8 days (second study: transient slight corneal cloudiness).

3.1.4 Sensitisation

Skin

There are no studies available which are in accordance with the current OECD guideline.

A very limited description of the testing is given by Sziza and Magos (1959) who used the Draize method and guinea pigs. A 3 % solution of 1-chloro-4-nitrobenzene in acetone was used for induction and a 0.3 % solution in acetone (skin painting) for challenge procedure. No sensitization was observed.

Rusakov et al. (1973), used test methods which are no longer in use and which are incompletely documented: In a modified Draize test induction was performed with an 1 % aceton solution of the compound on the shaved back of each of 10 guinea pigs for 5 consecutive days. At day 7 challenge was performed with the same solution. As there was no skin reaction observed, a modified Freunds complete adjuvant test was performed: the same guinea pigs were treated with a 10 % solution of 1-chloro-4-nitrobenzene. At day 22: 0.2 ml Freunds adjuvants together with 0.5 mg 1-chloro-4-nitrobenzene/kg bw was injected into the hind paw. 6 days later one drop of a 10 % solution of 1-chloro-4-nitrobenzene was applied on the shaved untreated skin as challenge. The author reported that all animals showed a positive reaction. Rats exposed via inhalation to 0.008 mg/m^3 for 5 months showed also positive reactions (Rusakov et al. 1973).

In another study (Schmidt and Chung 1992) the backs of 5 mice were shaved with electric clippers before induction was performed by applying 0.2 ml of a 0.05 M solution of the compound in acetone on day 1. Challenge application was performed with 20 µl of non-irritant 0.016 M solution to the surface of one ear of each mouse on day 5. Reactions were read after a further 24, 48 and 72 hours by comparison of the thickness of the ears between test mice and controls. 1-Chloro-4-nitrobenzene was reported to be a non-sensitizer; details of the reading were not given.

Conclusion

Due to the limited and poor quality information available regarding skin sensitization, it cannot be concluded whether or not the chemical has a sensitizing activity.

3.1.5 Repeated Dose Toxicity

Inhalation

Groups of 10 male and 10 female Sprague-Dawley rats were exposed to an aerosol of 1-chloro-4-nitrobenzene for 6 hrs a day, 5 days per week for a period of 4 weeks. For generation of the aerosol, 1-chloro-4-nitrobenzene was dissolved in ethylene glycol monoethyl ether (EGME) at concentrations of 0.33, 1.10, 3.3 % (weight/volume). The test solution was then atomized and conducted into the inhalation chambers. The chamber concentrations were 0, 5, 15 and 45 mg/m³. Control rats were exposed to the vehicle.

No mortality occurred. Mean weekly body weights were comparable to control groups. Concentration-dependent increasing degree of cyanosis, but no ocular abnormalities were observed at study termination.

According to the authors, clinical chemistry changes at week 4 were not related to treatment because they were within historical control limits, and exhibit no consistent dose-reponse relationship (no details available).

The significant haematological findings included increase in methaemoglobin and WBC in males and females (methb, males: week 2 at 45 mg/m³: 14.0 versus 3.2 % in controls(c) and week 4 at 5, 15, 45 mg/m³ (3.1, 3.1, 7.7 % versus 0.9 % in c; methb, females: week 2 at 15, 45 mg/m³: 11.5, 14.9 % versus 2.7 % in c and week 4 at 15, 45 mg/m³: 5.0, 12.3 % versus 1.8 % in c; WBC: males at week 2 and 4 in the 45 mg/m³ ; WBC, females, at week 2 in the 15 mg- and 45 mg-group and at week 4 in the 45 mg-group). Significant decreases were also noted in RBC at week 2 (males, 45 mg/m³ and females, 15, 45 mg/m³) and at week 4 in males and females (15, 45 mg/m³); decrease in haemoglobin at week 2 (males, females, 15, 45 mg/m³) and at week 4 (males, females, 45 mg/m³). Decreases in haematocrit: at week 2 (females, 15, 45 mg/m³) and week 4 (males, 45 mg/m³, females, 5, 15, 45 mg/m³) were also observed. At necropsy, liver (at 45 mg/m^3: male, absolute and relative, females relative) and spleen weights: (at 45 mg/m³: males, females, absolute and relative) were significant increased. Microscopically, increases in spleenic congestion, extramedullary haematopoiesis, haemosiderosis, and at 5 and 15 mg/m³ greater amounts of iron-positive pigments than in controls were recorded. A NOAEC could not be achieved, the LOAEC is 5 mg/m³ (Nair et al. 1986).

The repeated dose toxicity was also examined via whole-body inhalation in male and female Fischer 344/N rats and in male and female B6C3F1 mice for a period of 13 weeks (NTP 1993).

During exposure rats and mice were observed twice daily and were weighed at the start of the study, weekly thereafter and at necropsy. Clinical observations were recorded weekly. After cessation of exposure, complete necropsies were performed on all animals. Histopathologic evaluations, especially on target organs identified (rat: bone marrow, harderian gland, kidneys, liver, mediastinal lymph nodes, spleen and testes; mice: bone marrow, forestomach, liver and spleen) and on reproductive organs (see also chapter 3.2.10) were performed on all animals in the control and in the highest exposure groups and on all animals that died early. Target organs identified were also examined in all lower exposure groups.

Groups of 10 male and female rats were exposed to 0, 1.5, 3, 6, 12 or 24 ppm (approx. 0.0, 9.81, 19.62, 39.24, 78.48 or 156.96 mg/m³ air), 6 hours per day, 5 days per week over a period of 13

weeks. Additional 10 male and 10 female rats per group were exposed for clinical pathology studies on day 3 and day 23 consisting of haematology and clinical chemistry evaluations. Animals in the base study were evaluated at the end of the study.

In the 3 ppm-group 1 female rat had to be killed moribund due to malocclusion, no other rat died or had to be killed moribund. Mean body weight gain of the exposed rats was similar to those of the respective controls.

Haematology in males and females revealed concentration related increase in methaemoglobinemia significant in males and females from 1.5 ppm at day 3 and at all time points with maximum value of 4.13 g/dl (approx. 32%; males) and 5.90 g/dl (approx. 42%; females) at day 3 of the 24 ppm-group, respectively. Significant increase in reticulocyte count was noted in males at day 3 in the 1.5 ppm- and 24 ppm-group, at day 23 from 6 ppm onwards and at week 13 in all dose groups and in females at day 3 in the 23 ppm-group, at day 23 and week 13 from 3 ppm onwards. In addition, increased nucleated erythrocytes and increased leucocyte count (predominantly at the highest dose groups of males and females), concentration related decrease in haematocrit, haemoglobin and RBC at all time points.

Clinical chemistry in males and females showed changes in alanine aminotransferase (decrease), sorbitol dehydrogenase (increase) and alkaline phosphatase activities (decrease).

At necropsy an increase in abs. and rel. spleen weight (m >= 3 ppm, f >= 6 ppm), liver weight (m 24 ppm, f >= 6 ppm) and kidney weight (m and f 24 ppm) as well as a decrease in abs. and rel. testes weight (24 ppm) was noted. Increasing incidences of enlarged and darkened spleen with increasing concentrations were seen in males and females. Furthermore, in the 12 ppm- and 24 ppm-groups enlarged mediastinal lymph nodes (males and females) and darkened kidneys (only females) were recorded. Histopathologic examination showed haematopoietic cell proliferation in the bone marrow, chronic inflammation of the harderian gland, hyaline droplet nephropathy (only males) and tubule pigment in the kidneys, haemosiderin deposition in the liver, histiocytic hyperplasia in the mediastinal lymph nodes and in the spleen congestion, haemosiderin deposits haematopoietic cell proliferation and capsular fibrosis. All changes increased in severity and incidences with increasing concentration. Results of gonadal examinations see chapter 3.1.9 A NOAEC was not achieved, the LOAEC is 1.5 ppm (9.81 mg/m^3).

Groups of 10 male and 10 female mice were exposed to 0, 1.5, 3, 6, 12 or 24 ppm (approx. 0.0, 9.81, 19.62, 39.24, 78.48 or 156.96 mg/m^3 air), 6 hours per day, 5 days per week for a period of 13 weeks. No hematology evaluation was performed (for method description see page before).

No clinical signs of toxicity were observed which could be related to the exposure. 1 male rat of the 6 ppm-group died during week 8. Mean body weight gain of exposed mice was greater or equal than in the respective controls. The following organ weights were significantly increased: spleen (m, f: >= 12 ppm), liver (m, f: >= 12 ppm resp. 6), right kidney (m, f: >= 1.5 ppm resp. >=3 ppm). Histopathology revealed impairments increasing in severity with the dose from 12 ppm onwards: Haemosiderin deposition was recorded in bone marrow (males: 24 ppm: 10/10, females: 24 ppm: 8/10 and 9/10), in the liver (24 ppm: 10/10 males and 10/10 females) and spleen (12, 24 ppm: all males and females). Additionally, in the spleen excessive extramedullary haematopoiesis (males, 12 ppm 7/10, 24 ppm 10/10, females: all dose groups) and congestion (males, 12 ppm: 1/10, 24 ppm: 10/10, females, 24 ppm: 10/10) were seen. In the liver necrosis (males, 12 ppm: 1/10, 24 ppm: 5/10) and cytoplasmatic basophilia (males, 24 ppm: 4/10) and hyperplasia (males: 12 and 24 ppm: 3/10 and 9/10, females: 24 ppm: 10/10) and red blood cell fragments in the bone marrow were observed. One male and 7 females of the 24 ppm-group showed epithelial hyperplasia of the forestomach. Result of gonadal examinations see chapter 3.1.9. The NOAEC for histopathologic injury is 6 ppm (39.24 mg/m^3).

There are some studies with poor reliability available using other species and inhalatory exposure. As in the key studies with rats both cats and guinea pigs showed methaemoglobinaemia as main symptom.

Oral

The repeated dose toxicity was examined via oral application by gavage in a combined chronic toxicity/carcinogenicity study performed according OECD guideline 453 with some deviations (Monsanto 1985a) and in a subchronic study according OECD guideline 408 (Monsanto 1981a):

Groups of 60 male and 60 female Sprague-Dawley rats were dosed with 0, 0.1, 0.7 or 5 mg/kg bw dissolved in corn oil daily over a period of 24 months. The concurrent control groups received corn oil only (Monsanto 1985a).

For evaluation of the carcinogenic potency see also Chapter 3.1.8.

The two year survival rate ranged from 33-43 % for males and from 48 to 60 % for females. Some deaths of the rats were attributed to intubation accidents as was confirmed by gross and microscopic post mortem examinations. Physical abnormalities were seen in all dose groups including control groups. Slightly higher incidences in the high dosed rats than in controls were seen in the 2nd year of the study: In males between week 60 and week 102 yellow staining of the anogenital area and in females during the last months excessive lacrimation, chromodacryorrhea and alopecia were observed.

Haematology at 0.7/5.0 mg/kg bw/d revealed significant increases in methaemoglobin levels in males: 1.9/3.9 to 1.5/6.0 % (month 6 to 24) versus pretest value (pt) of 1.2 % and in females: 1.9/4.0 to 1.5/5.6 % (month 6 to 24) versus pt of 0.0 %. At 5.0 mg/kg bw/d: slight anaemia was noted as demonstrated by slightly decreased haemoglobin with min. in males/females month 18: 12.7/11.9 versus pt 14.4/14.1, haematocrit with min. in males month 24: 38 and in females month 18: 37 versus pt 44/43 in males/females, erythrocyte count with min. in males/females in month 18: 6.07/5.26 versus pt 6.43/6.15 and concomitant slight increases in numbers of reticulocytes with max. in males month 24: 10.9 and in females month 18: 5.6 versus control at month 6 of 1.0.

Clinical chemistry evaluations revealed no differences between values for control and treated groups. Urinalysis values for controls and treated rats were considered comparable.

Pathologic and histologic examination showed significantly increased absolute and relative spleen weights for high-dose male and female rats, consistent with the increase in incidence and/or severity (scores: 1=minimal, 2=mild, 3=moderate, 4=moderately severe, 5=severe) of accumulation of brown pigment (possibly haemosiderin) in the reticuloendothelial cells of the spleen: [control, low to high dose: male, 46/60 (1.6), 44/60 (1.2), 50/60 (1.7), 58/60 (3.2); female, 54/60 (2.3), 56/60 (2.5), 57/60 (2.8), 59/60 (3.7)] and extramedular haematopoiesis. At the highest dose, testes weights (absolute and relative) were slightly elevated. In all rats including control rats, bilateral degeneration of the germinal epithel, testicular periarteritis nodosa and uni- and bilateral oligospermie were noted, and pulmonary discolorations and pleural adhesions were seen. In some other organs there was a not dose-dependent increase in histopathological findings (e.g. uterus endometrial cysts 5/60, 5/11, 5/14, 7/60; uterus endometrial polyps 2/60, 2/11, 7/14, 3/60; see SIDS Dossier). The evaluation of these effects is limited by the fact that only macroscopically affected organs were examined microscopically in the low- and mid-dose groups. Endometrial changes are common in aging Sprague-Dawley rats (Attia 1996).

In summary, adverse effects were seen with 0.7 mg/kg bw/day. Whether or not 0.1 mg/kg bw/day is a NOAEL cannot be clarified due to the methodological deficiencies of the study.

In another study groups of male and female Sprague-Dawley rats received daily doses of 3, 10, or 30 mg/kg bw dissolved in corn oil by gavage for 13 weeks (90 days). Control groups received corn oil only (Monsanto 1981a).

One control female died due to physical trauma during dosing. General paleness immediately after dosing was observed in males and females of the 30 mg-group and in females of the 10 mg-group. Significantly increased food consumption was noted from males (10 mg-gr.: in 9 of 13 weeks; 30 mg-gr.: in 10 of 13 weeks) and females (10 mg-gr.: in 1 of 13 weeks; 30 mg-gr.: in 5 of 13 weeks). Body weight gain showed no statistical significant differences when compared to control rats.

Haematology revealed dose-dependent significant increases in methaemoglobin levels ranging in males/females from 4.5/4.9 % (3 mg-gr.) to 14.2/18,2 % (30 mg-gr.) versus 0.9/1.0 % in controls. Concomitant dose related increase in reticulocyte count up to 39.4 % versus 0.6 % in controls, dose related increase in WBC count, MCH- and MCV-values were reported. Dose-related significant decrease in erythrocyte count, Hgb, HCT and MCHC values were also noted. In the 10 mg- and 30 mg-groups total protein in males (d90) and females (d45) and SGPT only in males were significant reduced. Urinalysis showed increases in urinary urobilinogen in all dosed rats.

Gross and histopathological examination identified spleen, liver and kidney as target organs. Spleen in males and females showed dose-dependent increases in abnormal coloration, relative and/or absolute weight, enlargement due to vacuolisation of the congested red pulp, excessive haemosiderin and haematopoiesis. Livers of high dose males and females were enlarged and haemosiderosis and haematopoiesis were observed. In this dose groups, hyperplasia of bone marrow in males and females was noted. From kidneys of both sexes dose depending increases of discoloration, enlargement and haemosiderosis in the renal tubules were noted.

Thus, based on the observations in haematology and gross and histopathology, a NOAEL cannot be derived, the LOAEL is 3 mg/kg bw/day.

Conclusion

The repeated dose toxicity via inhalation has been examined in rats for a period of 4 weeks and 13 weeks. In both studies, NOAECs were not achieved, the LOAECs were 5 mg/m³ (4 week-study) and 1.5 ppm (9.81 mg/m^3, 13 week-study), respectively, based on methaemoglobinaemia as the most sensitive effect. The repeated dose toxicity via inhalation for a period of 13 weeks in mice revealed a NOAEC for histopathologic injury of 6 ppm (39.24 mg/m³). As target organs liver, kidney (rat only) and spleen and blood were identified in both species.

Similarly, repeated dose toxicity by oral administration in rats [OECD TG 408 and 453] revealed changes predominantly consistent with methaemoglobinaemia. In the longterm test a clear NOAEL could not be identified because histopathological examinations of most of the organs of the low- and mid-dose groups was performed only when macroscopic lesions were observed. The adverse effect level was 0.7 mg/kg bw/day. In the subchronic study the LOAEL was 3 mg/kg bw/day due to methaemoglobin formation and a NOAEL could not be derived. In both studies methaemoglobin formation and oxidative damage to red blood cells, leading to a regenerative anemia and a recognized spectrum of tissue damage and changes secondary to erythrocyte injury, were the main adverse effects.

3.1.6 Mutagenicity

In vitro Studies

(A) Gene mutation

There are numerous Ames tests which meet the criteria of today:

Salmonella typhimurium TA98, TA100, TA1535 and TA1537 were used with and without metabolic activation system (S9-mix) and doses ranging from 3.3 µg/plate up to 10000 µg/plate (NTP 1993: positive in TA100 and TA1535 with metabolic activation and in TA1535 without metabolic activation, Monsanto Co 1980b: positive in TA 1535 without S9-mix, Dupont de Nemours 1979b: positive in TA100 and TA1535 in the presence and in the absence of S9-mix, Haworth et al. 1983: positive in TA 100 with metabolic activation). The same strains and additional strain TA1538 were examined by Dupont de Nemours 1977, 1978 (100-10000 ug/plate with and without S9-mix) and by Shimizu 1983 (25.6 - 3276.8 µg/plate without S9-mix). Also in these studies 1-chloro-4-nitrobenzene exhibited mutagenic activity in TA100 and TA1535 without (Shimizu et al. 1983) and in TA100 and TA1535 in the presence of the activation system (Dupont de Nemours 1977, 1978). Cytotoxicity was determined in every study report.

In a study with deficiencies in the description of results, 1-Chloro-4nitrobenzene showed mutagenic activity in Salmonella typhimurium TA98 with metabolic activation and norharman (Suzuki et al. 1983).

Thus, it can be concluded that 1-chloro-4-nitrobenzene causes base-pair substitutions in Salmonella typhimurium.

In two HPRT assays which were performed with Chinese Hamster Ovary (CHO) cells according to OECD Guideline 476, 1-chloro-4-nitrobenzene did not induce gene mutations. The doses used by Dupont de Nemours, 1979a, ranged from 1.59 to 2.38 mM (approx. 250-375 ug/ml, solvent: aceton) in the presence (incubation time: 5 hrs) and in the absence (incubation time: 18 hrs) of S9-mix. Monsanto Co., 1983c, used 100 to 400 µg/ml dissolved in DMSO in the presence of S9-mix and in the absence of S9-mix 100 to 900 µg/ml dissolved in DMSO. In both experiments cytotoxicity was determined in preliminary tests.

A mouse lymphoma assay was performed according OECD Guideline 476 with the exception that no differentiation between small and large colonies was made. In the presence of metabolic activation system two trials were performed using doses of 42 - 350 µg/ml and 21 - 350 µg/ml , respectively. Without S9-mix doses up to 600 µg/ml were used. Under both conditions 1-chloro-4-nitrobenzene induced a positive response (Monsanto Co. 1983b).

Conclusion

1-Chloro-4-nitrobenzene exhibit mutagenic activity in Salmonella typhimurium (base-pair substitutions) and it gave positive results in the mouse lymphoma assay but not in the HPRT test in Chinese Hamster Ovary (CHO) cells.

(B) Cytogenicity

There is a study on cytogenicity using Chinese Hamster Lung (CHL) cells and doses ranging from 50 to 600 µg/ml without S9-mix and incubation time of 24 hrs and 48 hrs respectively. No cytogenetic activity was noted. In an additional trial doses of 200 - 600 µg/ml with S9-mix (incubation time: 6 hrs) and without S9-mix (treatment time not specified) were used. Without S9-mix no clastogenic activity was noted. In the presence of S9-mix 500 µg/ml yielded a questionable and 600 µg/ml a positive result (JETOC 1996). The studies were performed according the current OECD Guideline and cytotoxic concentration was determined.

In another cytogenicity assay with Chinese Hamster Ovary (CHO) cells several trials were reported (NTP 1993). Without S9-mix 3 trials were performed: Trial 1 with doses ranging from 50 - 500 µg/ml and harvest time of 10.5 hrs which was negative and trial 2 with 700 - 900 µg/ml (harvest time 10.6 hrs) which was weak positive and trial 3 with doses of 500 - 700 µg/ml and harvest time

of 19.5 hrs which was also weak positive. In the presence of S9-mix one trial with doses of 50 - 5000 µg/ml (harvest time: 10.5) showed no cytogenicity but in the second trial doses of 600 - 900 µg/ml and harvest time of 19.5 hrs gave positive results.

As described in the former section, 1-chloro-4-nitrobenzene induced a positive response in a mouse lymphoma assay (Monsanto Co. 1983b).

Conclusion

1-Chloro-4-nitrobenzene showed clastogenic activity in CHL cells at high doses only in the presence of metabolic activation. In Chinese hamster ovary cells clastogenic activity was observed with increasing dose and/or increasing incubation time in the presence and in the absence of metabolic activation. 1-Chloro-4-nitrobenzene induced a positive response in the mouse lymphoma assay.

(C) Indicator Tests

An increase in the Sister chromatid exchange rate was reported following treatment of Chinese Hamster Ovary (CHO) cells with 250 - 500 µg/ml in the presence of S9-mix, whereas 100 and 150 µg/ml without S9-mix was negative. Because of the observed cell cycle delay harverst time was extended up to 30.4 resp. 28.5 hrs in the test without resp. with metabolic activation . Dose selection was based on preliminary growth inhibition test (Galloway et al. 1987).

An UDS test was performed with commercial grade 1-chloro-4-nitrobenzene in rat hepatocytes according OECD Guideline 482 in the absence of a metabolic activation system. Doses of 0.1 up to 500 µg/ml were used with 100 and 500 µg/ml being cytotoxic doses. No unscheduled DNA-synthesis was observed (Monsanto Co 1985b). Another UDS test according OECD Guideline 482 in rat hepatocytes with doses up to 10000 µg/well in the absence of S9-mix yielded also negative results. Cytotoxicity was determined >= 1000 µg/well (Monsanto Co 1984b).

Conclusion

In the presence of metabolic activation 1-chloro-4-nitrobenzene induced Sister chromatid exchanges in Chinese Hamster Ovary cells. No UDS were reported after treatment of rat hepatocytes with 1-chloro-4-nitrobenzene in the absence of a metabolic activation system.

In vivo Studies

(A) Gene mutation

There are two Drosophila SLRL tests which are performed using different application routes, feeding and intraperitoneal injection with adults. Primarily post-meiotic cells were tested. Another Drosophila SLRL test used larval feeding. Both methods lead to negative results (NTP 1993, Zimmering et al. 1985, 1989).

Conclusion

1-Chloro-4-nitrobenzene showed no mutagenic activity in postmeiotic cells of Drosophila melanogaster and after larval feeding.

(B) Cytogenicity

A micronucleus assay in mouse bone marrow was performed according to OECD guideline 474 following a single intraperitoneal injection of 500 mg/kg bw dissolved in corn oil and yielded a positive result (Bayer 1990). A chromosome aberration assay (OECD Guideline 475) in rat bone

marrow following single application by gavage of 30 to 300 mg/kg bw dissolved in corn oil was negative (Monsanto Co. 1985c).

Conclusion

1-Chloro-4-nitrobenzene induced micronuclei in mouse bone marrow in vivo at a toxic dose. In rat bone marrow it did not induce chromosomal aberrations in vivo.

(C) Indicator test

Chinese Hamster bone marrow was examined for sister chromatid exchanges according the Guideline described in EPA OTS798.5915 following single intraperitoneal injections of 65 to 260 mg/kg bw dissolved in corn oil. A non dose-related increase in the frequency of Sister chromatid exchanges was noted (factor 1.5) (Bayer 1992).

Intraperitoneal injections of 60 or 1000 mg/kg bw (Cesarone et al. 1980) or 30 - 180 or 1000 mg/kg bw (Cesarone et al. 1983) 1-chloro-4-nitrobenzene into Swiss mice induced single DNA strand breaks in liver, kidney and brain which were identified by alkaline elution technique. Intraperitoneal injection, however, is not the recommended exposure route of the respective OECD guideline because it could expose the organs (liver and kidney) directly rather than via the circulatory system.

Conclusion

An in vivo SCE test was weakly positive in bone marrow cells of Chinese hamsters. Intraperitoneal injection of 1-chloro-4-nitrobenzene into mice resulted in DNA damage in liver, kidney and brain.

Conclusion

1-Chloro-4-nitrobenzene induced reverse mutations in bacteria. It was not mutagenic in mammalian cells in vitro (HPRT test) and in insects in vivo. A mouse lymphoma assay was positive. In vitro it induced chromosomal aberrations and sister chromatid exchanges at high doses; no UDS in rat hepatocytes was reported.

The chemical induced micronuclei in mouse bone marrow in vivo at a toxic dose. In rat bone marrow it did not induce chromosomal aberrations in vivo. An in vivo SCE test was weakly positive in bone marrow cells of Chinese hamsters. DNA strand breaks were observed in liver, kidney and brain of mice. 1-Chloro-4-nitrobenzene is consequently capable of expressing mutagenic activity in vivo with low potency.

3.1.7 Carcinogenicity

The carcinogenic potential was examined in a combined chronic toxicity/carcinogenicity study performed according OECD guideline 453 and administration of the compound via gavage (Monsanto Co 1985a).

Groups of 60 male and 60 female Sprague-Dawley rats were dosed with 0, 0.1, 0.7 or 5 mg/kg bw dissolved in corn oil daily over a period of 24 months. The concurrent control goups received corn oil only.

Haematology at 0.7/5.0 mg/kg bw/day revealed significant increases in methaemoglobin levels in males: 1.9/3.9 to 1.5/6.0 % (month 6 to 24) versus pretest value (pt) of 1.2 % and in females: 1.9/4.0 to 1.5/5.6 % (month 6 to 24) versus pt of 0.0 %. For further information on general toxicity see also Chapter 3..1.6.

Neoplasms were seen in both control and treated rats. The incidence and distribution of the observed pituitary gland adenomas, benign and malign neoplasms of the skin, mammary glands and

adrenal cortical or medullary neoplasms were similiar in control and treated groups of rats. The evaluation of the tumour incidences of these organs is limited by the fact that only macroscopically affected organs were examined microscopically in the low- and the mid-dose groups (exception: testes, epididymis, spleen: all animals were examined). Endocrine and mammary and skin neoplasms are common findings in Sprague-Dawley rats (Attia 1996, McMartin et. al. 1992).

Unilateral and bilateral interstitial cell tumors of the testes which occurred mainly in the old rats, were observed in controls (1.7 %) and in the low (6.8 %), the mid (8.3%) and high (10.0 %) dosed male rats (number of rats with interstitial cell tumours that died prior to term: (control): 0/39, (low to high dose): 2/38, 1/43, 2/39; number of rats sacrificed at term with interstitial cell tumors: (control): 1/21, (low to high dose): 2/21, 4/17, 4/21). According to an information given by the author, historical control data were compiled from 14 long-term studies of the same institute using the same strain of rats and showed incidence of 9.8 % interstitial cell tumors of testes (Monsanto Co 1985a), with a range of 3.45% to 23.48% (Huntingdon Life Sciences 2000). The incidences in the 1-chloro-4-nitrobenzene treatment groups were within the range of the historical control data, whereas the incidence in the control group was unusual low. Therefore the findings were not considered to be compound related. In summary, no clear compound related increase of tumours was shown in that study

The available studies to evaluate carcinogenicity in rats and mice by oral dosing via diet don't meet the criteria of today and are only reported in brief (Weisburger et al. 1978).

25 male CD rats/group were given 1-chloro-4-nitrobenzene in the diet for 18 months (50 % of MTD, MTD): 0, 2000, 4000 mg/kg diet (approx. 0, 150, 300 mg/kg bw/day). After 3 months of treatment, dosage was reduced to 250, 500 mg/kg diet (approx. 18.75, 37.5 mg/kg bw/day), because body weight gain was reduced by 10% when compared to the control group or deaths occurred from toxicity (no further information). After 2 month a dose of 500 and 1000 mg/kg diet (approx. 37.5 and 75 mg/kg bw/day) were given for the remaining 12 months. Following the 6-month-observation period, necropsy was performed. No tumours were found.

25 male and female CD1 HaM/ICR mice/group were given 1-chloro-4-nitrobenzene in the diet for 18 months (50 % of MTD, MTD): 0, 3000, 6000 mg/kg diet (approx. 0, 450, 900 mg/kg bw/day). Mice that died within the first 6 month were discarded without necropsy. Following the 3-month-observation period, necropsy was performed and mice with tumours were recorded: both male and female mice showed a significant increase in vascular tumors at the high dose level (localization of the vascular tumors was not specified): male mice: low dose level: 2/14, high dose level: 4/14, simultaneous control: 0/14, pooled control: 5/99; female mice: low dose level: 2/20, high dose level: 7/18, simultaneous control: 0/15, pooled control: 9/102. Male mice had also some liver tumors (hepatomas) at the low dose level: 4/14, high dose level: 0/14, simultaneous control: 1/14, pooled control: 7/99

Conclusion

A combined chronic toxicity/carcinogenicity study (OECD Guideline 453) with 1-chloro-4-nitrobenzene in rats produced an increased incidence in interstitial cell tumours of the testes which were within the range of the historical control data and evaluated as not compound related. These tumours were described in literature as common tumours in male Sprague-Dawley rats. In another rat study which doesn't meet the criteria of today and is reported in brief, no tumours were found. In the available study with mice which doesn't meet the criteria of today and is only reported in brief, vascular tumors (localization not specified) were found. This tumor type is not uncommon in the substance class of substituted amino- or nitrobenzenes. Overall, taking into consideration the results of the genotoxicity tests and the limitations in the available longterm studies, a carcinogenic potential cannot be ruled out.

3.1.8 Toxicity for Reproduction

Effects on Fertility

In a two-generation reproduction toxicity study, which was performed according OECD-Guideline 416, 15 male and 30 female CD rats per dose-group were given 0, 0.1, 0.7, and 5.0 mg/kg bw/day orally via gavage for 14 weeks prior to mating and throughout the mating, gestation and lactation periods. All F0 rats were sacrificed after weaning of the F1 generation. F1 weanlings were selected to produce F2 generation and received the test substance at the same dose levels noted above for approximately 18 weeks prior to mating and throughout the mating, gestation and lactation periods. All F1 rats were sacrificed after weaning of F2, F2 rats were sacrificed at weaning (d21 of lactation). Gross postmortem examinations (all animals) and histopathology examination (F0 adults: gross lesions, testes, epididymides, seminal vesicle, prostate, uterus, vagina; F1 adults (10 animals/sex/group), F1 and F2 weanlings (5 animals/sex/group): complete histopathology) were conducted (Monsanto 1984a). Hematology effects, especially level of methemoglobin were not measured.

None of control and treated F0 males but F0 females: 1, 0, 3, 2 (control to high dose) died. Some deaths were attributed to dosing-related injuries. Body weight gain and food consumption did not reveal an adverse effect of treatment.

Mating indices for F0 females were comparable between control (86.7 %) and low-dose-group (80.0 %) but not statistical significant lower in the mid- (71.4 %) and high- (71.4 %) -dose-group; for F0 males mating indices were 93.3 %, 86.7 %, 80.0 %, and 93.3 % (control to high dose), respectively. Fertility indices of males (control to high dose): 92.9 %, 100 %, 91.7 %, 71.4 %, and pregnancy rate (control to high dose): 80.8 % 83.3 %, 80.0 %, 70.0 % were (not statistical significant) lowered in the high dose-groups. Gestation length, parturition data, litter size data during lactation, mean pup weight data, pup sex distribution data were comparable between control and treated groups. Litter survival indices were slightly lower than control at the mid- and high-dose levels (93.8%, 85.7% resp. 100% (control)). 1 female of mid-dose group delivered 1 litter of 6 live pups, no live pups remained in the litter by d6 of lactation. Pup survival index was significantly reduced in the high dose group (d0-4: 85.6 % versus: 94.4 % in controls, d4-21: 91.6 % versus 98.7% in controls) because 2 high dose females experienced complete pup mortality within their litters.

No external malformations, or histopathological changes of tissues from selected organs were seen in the dead pups recovered at birth or during lactation in control, mid- and high-dose groups. 1 dead pup of the low dose group, recovered at birth, had no tail.

Mortality rates of F1 adult rats were (control to high dose): male: 6.7 %, 13.3 % 33.3 %, 6.7 %, respectively, and female: 3.3 %, 3.3 %, 3.3 %, 3.3 %, respectively. Some deaths were attributed to dosing-related injuries. Body weight gain and food consumption did not reveal an adverse effect of treatment.

Female mating indices were (not statistical significant, not dose-related) lower than the respective control data (control to high dose: 86.2 %, 76.7 %, 65.5 %, 72.4 %). Male mating and fertility indices and female pregnant rates were comparable between the control and the treated groups. No adverse effect was indicated in regard to gestation length, partiturition data, litter size data during lactation, litter survival indices (2 low- and 1 high-dose females failed to wean litters), mean pup weight data or pup sex distribution data. No external malformations, or histopathological changes of tissues from selected organs were seen in the dead pups recovered at birth or during lactation in control, low-, mid- and high-dose groups.

In F0 male rats of the high dose group histological changes in testes (bilateral degeneration/atrophy of epithelium in 2/15 animals and bilateral maturation arrest of the germinal epithelium in 1/15 animals) were seen. Epididymal observations (oligospermia) were also noted in these same F0 males which did not mate (males of the low- and mid-dose group were not examined).

In F1 adult rats, histopathological evaluation of tissues revealed extramedullary haematopoiesis and reticuloendothelial cells containing brown pigment in the spleens of all rats in all groups. These effects appeared more pronounced in males and females of high-dose group.

A NOAEL for general toxicity of adults was not established, the LOAEL (adults) of 0.1 mg/kg bw/day, based on histopathological effects in the spleen of F1 adults is indicated. The NOAEL for general toxicity of offspring is 0.1 mg/kg bw/day. Referring to fertility index of males in F0 and F1 in combination with the pregnancy rate in F0 and F1 no impairment of fertility was observed up to 5 mg/kg bw (high dose group), nevertheless, at this dose histopathological effects in reproductive organs of males were observed. But the evaluation of the effect on the male reproductive tract is limited because the testes in the low and mid dose group were not examined histopathologically.Therefore a NOAEL (male reproductive organ toxicity) was not established.

In another study male and female Swiss CD mice were exposed to 1-chloro-4-nitrobenzene dissolved in corn oil by gavage to assess reproduction and fertility using the NTP continuous breeding protocol (NTP 1991).

Data from a 2-week dose-range finding study were used to set exposure concentrations.

Groups of 20 breeding pairs received 0, 62.5, 125 or 250 mg 1-chloro-4-nitrobenzene /kg bw/day for 7 days prior to cohousing and for 98 days of continuous breeding. 40 breeding pairs received the corn oil vehicle only. The last litter born during the holding period following the continuous breeding phase from control and high dose group was reared by the dam until weaning, after which time treatment of the F1 animals was initiated by the same route and at the same concentration as the F0 animals. These F1 animals were used for the assessment of the second generation fertility.

In F0-generation 3, 3, 1, 4 (control to high dose) mice died, but were evaluated as not treatment related. In all groups of rats bodyweight gain throughout the study was noted, but water consumption was reduced in the highest dose group in males and females. None of the mice appeared cyanotic. Organ weights were not determined, histopathological and hematological evaluation were not done.

In F0, in the control and the 62.5 mg-groups 100 % of the pairs delivered at least four litters, in the 125 mg-groups 100% of the pairs delivered the 1st to 3rd litter and 95% the 4th. In the pairs of the 250 mg-groups 100 % of the pairs delivered the 1rst and 2nd litter and only 86 % delivered the 3rd and 79 % delivered the 4th litter, and the proportion of born pups alive of the final litter significant reduced. In all other groups average number of live pups per litter was comparable and sex ratio of pups born alive was not affected. Compared to the control group the dam weights in the mid- and high-dose group were increased at delivery of the final litter (113% resp. 110% compared to the control) and 21 days after the delivery of the final litter (112% resp. 118%). A trend test was positive.

In F1-pups of the 62.5 mg-groups (male and combined), 125, 250 mg-groups (male, female and combined), reduced live pup weights at birth were noted. In the 125- and 250 mg-groups the pup weight adjusted for litter size was significantly reduced with a dose-related response and in the final litter of the continuous breeding phase, the F1 weight gain during lactation was adversely affected. In the final litter of 250 mg-group the F1 pup survival was adversely affected as well. The proportion of pups born alive was significantly decreased. None of the pups were noted as being cyanotic.

In F1-adults (control and 250 mg-group) water consumption was comparable. At mating most of the 250 mg/kg bw/d group-F1-animals were cyanotic: eyes and skin had blue tint, and urine color was amber. The mating, pregnancy and fertility indices were comparable between control and the 250 mg-group mice, but number of live pups delivered by 250 mg-dosed pairs was lower than from control pairs. The proportion of F2 pups born alive and live F2 pups weights at birth were significantly reduced in the 250 mg-group.

Vaginal cytology and sperm parameters (F1, control and 250 mg-group) examination showed a significantly increased oestrous cycle length in the F1 females whereas epididymal sperm motility, sperm count and sperm morphology were not affected in the F1 males by 1-chloro-4-nitrobenzene treatment.

Gross pathology of 250 mg-group (F1) at terminal sacrifice revealed cyanotic appearance and absolute liver weight and liver-to-body weight ratios were increased and spleens were extremely enlarged and darkened while body weigh gain was not affected in both F1 sexes.

In males, absolute seminal vesicle weights were significantly decreased and seminal vesicle-to-body weight ratios were similarly affected at 250 mg/kg bw/d.

There was no evidence of an androgen deficiency lesion in the testes of the 5 high-dose males examined.

Thus, the NOAEL (fertility) is 125 mg/kg bw/day, the LOAEL (general toxicity, offspring) is 62.5 mg/kg bw/day. The NOAEL (adult general toxicity) is 125 mg/kg bw/day, but full evaluation is not possible because evaluation of the animals of the two lower groups were very limited and the most sensitive parameter for systemic toxicity (MetHb-formation) was not determined.

Groups of 10 male and female rats were exposed to 0, 1.5, 3, 6, 12 or 24 ppm (approx. 0.0, 9.81, 19.62, 39.24, 78.48 or 156.96 mg/m³ air), 6 hours per day, 5 days per week over a period of 13 weeks. Haematology in males and females revealed concentration related increase in methaemoglobinemia significant in males and females from 1.5 ppm with maximum value of 4.13 g/dl in males and 5.90 g/dl in females (approx. 32 resp. 42%). (For further description of the general toxicity see chapter 3.1.6). At the end of the exposure period also the gonadal organs were examined (groups 0, 6, 12 and 24 ppm). Among females of the 6, 12, and 24 ppm group significant decrease in average estrous cycle length was observed. In males of the 24 ppm-group left caudal epididymal and testicular weights , epididymal sperm (spermatozoa) count per gram of caudal tissue and total spermatid head count per testes were significantly decreased (NTP 1993).

Groups of 10 male and 10 female mice were exposed to 0, 1.5, 3, 6, 12 or 24 (approx. 0.0, 9.81, 19.62, 39.24, 78.48 or 156.96 mg/m³ air) 6 hours per day, 5 days per week for a period of 13 weeks. The most sensitive parameter for general toxicity, methaemoglobinaemia, was not determined in that study (For further description of the general toxicity see chapter 3.1.6). At the end of the exposure period also the gonadal organs were examined (groups 0, 6, 12 and 24 ppm). In females of the 24 ppm-group significant estrous cycle length were observed. In males no significant findings in sperm morphology were noted (NTP 1993).

Conclusion

Toxicity to reproduction of 1-chloro-4-nitrobenzene has been examined in rats and mice by oral administration. In the two generation study with rats [OECD guideline 416] no impairment of fertility was observed up to 5 mg/kg bw (high dose group), nevertheless, at this dose histopathological effects in reproductive organs of males were observed. But the evaluation of the effect on the male reproductive tract is limited because the testes in the low and mid dose group were not examined histopathologically, therefore a NOAEL (male reproductive organ toxicity) was

not established. The NOAEL for general toxicity of adults was not achieved. A LOAEL (adults) of 0.1 mg/kg bw/day based on histopathological effects in the spleen of F1 adults is indicated. The NOAEL for general toxicity of offspring is 0.1 mg/kg bw/day. In mice a study was performed using NTP continuous breeding protocol. The NOAEL(fertility) is 125 mg/kg bw/day, the LOAEL(offspring general toxicity) is 62.5 mg/kg bw/day. The NOAEL(adult general toxicity) is 125 mg/kg bw/day, but full evaluation is not possible because evaluation of the animals of the two lower groups were very limited. Two subchronic inhalation studies with rats and mice with histopathologic evaluations on reproductive organs are available. There was evidence of decreased spermatogenesis (24 ppm) and decrease in average estrous cycle length in rats exposed to 1-chloro-4-nitrobenzene (6 ppm and above). In female mice an increase in estrous cycle length was noted at the highest exposure group (24 ppm).

Developmental Toxicity

1-Chloro-4-nitrobenzene was examined for developmental toxicity in rats and rabbits according to OECD Guideline 414 with some deviations and application of the compound orally via gavage.

24 mated female CD rats received 0, 5, 15, or 45 mg 1-chloro-4-nitrobenzene/kg bw/day dissolved in corn oil via gavage during day 6 to 19 of gestation. All females were sacrificed on day 20 of gestation and fetuses were examined for malformations (Monsanto Co 1980a).

Pregnancy rates among dams were similar between the groups. No mortality occurred in the control or treated groups during day 6-20. Terminal body weight and body weight gain (day 6-20) were comparable between control, low- and mid-dose females, but significant reduced in high-dose females (71 g versus 118 g of controls). Several high-dose females were reported to have pale eye color during dosing interval. Comparable between control, low-, mid-, and high-dose groups were the mean numbers of implantations (13.3, 13.9, 14.1, 13.6), but mean number of resorptions were significant increased in the high-dose group: 5.6 versus 0.5 in controls, and mean number of live fetuses were significant decreased (8.0 versus 12.8 in controls) in this dose-group, 7/22 high-dose females (31.8%) had uterine implantation sites comprised entirely of resorption sites. Maternal gross postmortem observations revealed that mean spleen weigths were significant higher than control in each treated group, mean spleen-to-body weight ratios were higher in all treated groups, significant in the mid– and high-dose group (dose-related increase), from low-dose onwards significant dose-dependent lesions of the spleen: enlargement (up to 4 x normal size in high-dosed females), dark coloration and/or pitted surface. Thus, a NOAEL for maternal toxicity cannot be achieved, the LOAEL for maternal toxicity is 5 mg/kg bw/day.

The mean number of male and female fetuses and mean fetal weights were comparable between the control, low- and mid-dose groups. In the high dose group, the mean number of male and female fetuses (male: 4.2 versus 6.2 in control, female 3.8 versus 6.5 in control) and the respective mean weights (male: 3.33 g versus 3.95 g, female: 3.11 g versus 3.76 g in control) were markedly lowered. In this dose-group the incidence of ossification variations (i.e., asymmetrical/unossified sternebrae, incompletely ossified cervical vertebral transverse processes, rudimentary structures) was significant increased when compared to controls (95.7 % versus 85.5 % in control).

Fetal evaluations at low- and mid-dose levels did not reveal malformations. At high-dose level, a significant increase in the incidence of skeletal malformations (30.4 % versus 1.3 % in controls), predominantly angulated ribs alone or associated with misshapen and/or shortened bones of the forelimbs (i.e. humerus, radius, ulna) was noted. The NOAEL for developmental toxicity is 15 mg/kg bw/day.

Thus, developmental toxicity in rats occurred in the presence of significant maternal toxicity.

18 mated female New Zealand White rabbits received 0, 5, 15, or 40 mg/kg bw/day 1-chloro-4-nitrobenzene dissolved in corn oil via gavage during day 7-19 of gestation. All surviving females were sacrificed on day 30 of gestation and fetuses were examined for malformations (Monsanto Co 1982).

Mortality rate among dams was (control to 40 mg-group): 1/18, 1/18, 1/18, 8/18. Because of the high mortality rate in the 40 mg-group and the fact that two females had aborted their pregnancies, the decision was made to terminate that group on d20. No gross post mortem, reproduction or fetal evaluation data were taken for the high-dose females killed with the decision to terminate the group.

During treatment interval mean body weight loss in all groups, control and treated rabbits; mean body weight change during post-treatment interval comparable between control, low- and mid-dose groups.

In the 15-mg-group, anogenital staining was observed in a few dams at d19; the 5-, 15- and 40-mg-dosed rabbits suffered from soft stool at day 19 of gestation; and the 40 mg-rabbits females had grayish/pale appearing eyes at day 10, day 15 and day 19.

Pregnancy rates were 94.4, 88.9, 88.9 % (control, 5 and 15 mg/kg bw/day), respectively. Abortion occurred in 1 control female on day 25 and 2 high-dosed females on day 18 and day 20, respectively. Premature delivery occurred in the low dose group on day 30, day 27, day 29 and in the mid-dose group on day 26.

In all other surviving dams there were no significant changes in reproductive parameters (i.e. mean number of implantations, resorptions and fetuses).

At necropsy of the dams, there were no adverse effects on maternal spleen weights. Although mortality was notably increased in the high-dose level, no consistent treatment-related morphologic alterations were identified among the animals that died prior to the decision to terminate the group.

Fetal data:

Between the control, the low- and the mid-dose groups, mean fetal weights (male: 40.6gr, 39.4gr, 41.5gr, female: 39.0gr, 38.6gr, 39.0gr), mean number of fetuses (male: 3.5, 4.3, 3.6, female: 4.4, 4.1, 4.1) were comparable. Some variability in the sex distribution ratio (male/female) between control (53/66), the low-dose (51/49) and the mid-dose (51/57) was considered not to be treatment related. No treatment related effect was evident in fetal ossification data, in external and soft tissues evaluation.

During the skeletal evaluations, the incidence of fetuses with malformations (predominantly fused sternebrae) was (not significantly) increased in the low and in the mid dose levels. The number of affected litters were not dose-dependingly increased: control-gr.: 1/63 fetus (1.6 %) in 1/15 litters, 5 mg-gr.: 3/54 fetuses (5.9 %) in 2/12 litters, 15 mg-gr.: 4/58 (6.9 %) fetuses in 2/14 litters. There is no information about the severity of this finding. According to the authors, this finding is seen historically at low incidence with this strain of rabbit. As the increase of fused sternebrae was not statistically significant and there was no effect on the number of affected litters, the relevance of this finding remains questionable.

Thus, the LOAEL(maternal toxicity) is 5 mg/kg bw/day and the LOAEL(developmental toxicity) is 5 mg/kg bw/day.

Conclusion

Developmental toxicity of 1-chloro-4-nitrobenzene has been examined in rats and rabbits byoral administration [OECD TG 414]. In rats, a NOAEL for maternal toxicity was not achieved, the

LOAEL(maternal toxicity) is 5 mg/kg bw/day; the NOAEL(developmental toxicity) is 15 mg/kg bw/day. The study with rabbits suffered from methodology deficiencies. Due to high mortality rate at the highest dose level, only two doses could be evaluated: the LOAEL(maternal toxicity) is 5 mg/kg bw/day and the LOAEL(developmental toxicity) is 5 mg/kg bw/day. Thus, in both species developmental toxicity occurred in the presence of maternal toxicity.

3.2 Other relevant information

Immunotoxicity

Single i.p. application of 300 mg 1-chloro-4-nitrobenzene/kg bw to male BDF1 mice lead to significantly decrease of the percentages and numbers of B, T, subsets of T (CD4 and CD8) and NK cells compared to the respective control. Macrophages, nucleated erythrocytes and dead cells were markedly increased in the exposed mice (Li et al. 1999). In another acute experiment using the same doses and mouse strain mentioned above, the NK and cytotoxic T-lymphocytes (CTL) activity decreased (NK more inhibited than CTL acitivity). Stimulated B-lymphocyte proliferations (LPS) was inhibited by 1-chloro-4-nitrobenzene (Li et al. 1998). These findings indicate that 1-chloro-4-nitrobenzene has an immunotoxic effect in mice.

The subchronic i.p. application of 1-chloro-4-nitrobenzene to male BDF1 mice lead to immunotoxic effects (15 mice/group, 3 appl./week, 4 weeks). Natural killer cells and cytotoxic T-lymphocytes (CTL) activity and stimulated B-lymphocyte proliferations decreased. Hemoglobin concentration was not significantly affected but body weight was significantly lower. This subchronic experiment indicated as well as the acute i.p. experiment that 1-chloro-4-nitrobenzene has an immunotoxic effect on mice (Li et al. 1998, 1999).

3.3 Initial Assessment for Human Health

1-Chloro-4-nitrobenzene is rapidly absorbed via skin, gastrointestinal tract or respiratory tract and distributed in the tissue predominantly in fat, blood cells, skeletal muscles, liver and kidney. Most of the substance was excreted with the urine followed by excretion with feces. 1-Chloro-4-nitrobenzene undergoes three major types of transformation in vivo in mammals: nitro-group reduction, displacement of the chloride in glutathione conjugation, and ring-hydroxylation. From accidentally exposure of workers to 1-chloro-4-nitrophenol, large amounts of 2-chloro-5-nitrophenol, N-acetyl-S-(4-nitrophenyl)-L-cysteine, 4-chloroaniline and 4-chloroformanilide were identified.

The oral LD50 for 1-chloro-4-nitrobenzene in male rats is 294 or 694 mg/kg bw and in female rats 565 or 664 mg/kg bw. Cyanotic appearance was the predominant symptom. The 7-hour-inhalation of a highly saturated vapor-air mixture (concentration up to 77 mg/m3) represented no acute hazard to male and female rats. In addition, LC50 level could not be reached up to 16100 mg/m³ during a 4-hrs exposure against vapor and microcrystalline particles. The LD50 (dermal) for male rats is 750 mg/kg bw and for female rats 1722 mg/kg bw; the LD50 for male rabbits is 3550 mg/kg bw and for female rabbits 2510 mg/kg bw after acute dermal application. Cyanotic appearance was the predominant symptom. For the evaluation of acute toxicity it has to be taken into account that 1-chloro-4-nitrobenzene is a methaemoglobin forming chemical.

Experience with human exposure: All available reports relate to mixed exposure, frequently in combination with 1-chloro-2-nitrobenzene and/or nitrobenzene. A critical aspect in this context is that 1-chloro-4-nitrobenzene is rapidly absorbed via skin and the respiratory tract. The signs of acute intoxication include methaemoglobinaemia, vomiting, headache and in severe cases collapse.

The available study-reports on skin irritation have deficiencies with regard to the description of the results, nevertheless, 1-chloro-4-nitrobenzene is judged to be slightly irritating to the skin (intact or scarificed) of rabbits using a paste of test substance and occlusive dressing or not irritating to the skin using undissolved, solid test substance and occlusive dressing.

In two available studies 1-Chloro-4-nitrobenzene caused slight irritational effects to the eyes of rabbits which were reversible within 4 hours (first study: slight conjunctival injections, observed only in washed eye) resp. 8 days (second study: transient slight corneal cloudiness).

Due to the limited and poor quality information available regarding skin sensitization it cannot be concluded whether or not the chemical has a sensitizing activity.

The repeated dose toxicity via inhalation has been examined in rats for a period of 4 weeks and 13 weeks. In both studies, NOAECs were not achieved, the LOAECs were 5 mg/m³ (4 week-study) and 1.5 ppm (9.81 mg/m3, 13 week-study), respectively, based on methaemoglobinaemia as the most sensitive effect. The repeated dose toxicity via inhalation for a period of 13 weeks in mice revealed a NOAEC for histopathologic injury of 6 ppm (39.24 mg/m³). As target organs liver, kidney (rat only) and spleen and blood were identified in both species.

Similarly, repeated dose toxicity by oral administration in rats [OECD TG 408 and 453] revealed changes predominantly consistent with methaemoglobinaemia. In the longterm test a clear NOAEL could not be identified because histopathological examinations of most of the organs of the low- and mid-dose groups was performed only when macroscopic lesions were observed. The adverse effect level was 0.7 mg/kg bw/day. In the subchronic study the LOAEL was 3 mg/kg bw/day due to methaemoglobin formation and a NOAEL could not be derived,. In both studies methaemoglobin formation and oxidative damage to red blood cells, leading to a regenerative anemia and a recognized spectrum of tissue damage and changes secondary to erythrocyte injury, were the main adverse effects.

1-Chloro-4-nitrobenzene induced reverse mutations in bacteria. It was not mutagenic in mammalian cells in vitro (HPRT test) and in insects in vivo. A mouse lymphoma assay was positive. In vitro it induced chromosomal aberrations and sister chromatid exchanges at high doses; no UDS in rat hepatocytes was reported.

The chemical induced micronuclei in mouse bone marrow in vivo at a toxic dose. In rat bone marrow it did not induce chromosomal aberrations in vivo. An in vivo SCE test was weakly positive in bone marrow cells of Chinese hamsters. DNA strand breaks were observed in liver, kidney and brain of mice. 1-Chloro-4-nitrobenzene is consequently capable of expressing mutgenic activity in vivo with low potency.

A combined chronic toxicity/carcinogenicity study (OECD Guideline 453) with 1-chloro-4-nitrobenzene in rats produced an increased incidence in interstitial cell tumours of the testes which were within the range of the historical control data and evaluated as not compound related. These tumours were described in literature as common tumours in male Sprague-Dawley rats. In another rat study which doesn't meet the criteria of today and is reported in brief, no tumours were found. In the available study with mice which doesn't meet the criteria of today and is only reported in brief, vascular tumors (localization not specified) were found. This tumor type is not uncommon in the substance class of substituted amino- or nitrobenzenes. Overall, taking into consideration the results of the genotoxicity tests and the limitations in the available longterm studies, a carcinogenic potential cannot be ruled out.

Toxicity to reproduction of 1-chloro-4-nitrobenzene has been examined in rats and mice by oral administration. In the two generation study with rats [OECD guideline 416]no impairment of fertility was observed up to 5 mg/kg bw (high dose group), nevertheless, at this

dosehistopathological effects in reproductive organs of males were observed. But the evaluation of the effect on the male reproductive tract is limited because the testes in the low and mid dose group were not examined histopathologically. Therefore a NOAEL (male reproductive organ toxicity) was not established. The NOAEL for general toxicity of adults was not achieved. A LOAEL (adults) of 0.1 mg/kg bw/day based on histopathological effects in the spleen of F1 adults is indicated. The NOAEL for general toxicity of offspring is 0.1 mg/kg bw/day. In mice a study was performed using NTP continuous breeding protocol. The NOAEL(fertility) is 125 mg/kg bw/day, the LOAEL(offspring general toxicity) is 62.5 mg/kg bw/day. The NOAEL(adult general toxicity) is 125 mg/kg bw/day, but full evaluation is not possible because evaluation of the animals of the two lower groups were very limited. Two subchronic inhalation studies with rats and mice with histopathologic evaluations on reproductive organs are available. There was evidence of decreased spermatogenesis (24 ppm) and decrease in average estrous cycle length in rats exposed to 1-chloro-4-nitrobenzene (6 ppm and above). In female mice an increase in estrous cycle length was noted at the highest exposure group (24 ppm).

Developmental toxicity of 1-chloro-4-nitrobenzene has been examined in rats and rabbits by oral administration [OECD TG 414]. In rats, a NOAEL for maternal toxicity was not achieved, the LOAEL(maternal toxicity) is 5 mg/kg bw/day; the NOAEL(developmental toxicity) is 15 mg/kg bw/day. The study with rabbits suffered from methodology deficiencies. Due to high mortality rate at the highest dose level, only two doses could be evaluated: the LOAEL(maternal toxicity) is 5 mg/kg bw/day and the LOAEL(developmental toxicity) is 5 mg/kg bw/day. Thus, in both species developmental toxicity occurred in the presence of maternal toxicity.

There are indications of immunotoxic potency following single and repeated applications of 1-chloro-4-nitrobenzene.

4. HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute and ChronicToxicity Test Results

In this chapter only the lowest valid test concentrations of acute and chronic testing are presented.

Acute toxicity to fish (Brachydanio rerio) has been tested in a flow through system according to OECD Guideline 203 with analytical monitoring. A 96 h-LC50 of 14.36 mg/l was achieved (Roederer 1990). The result (LC50) of a test 48 h with the species *Leuciscus idus* in accordance to the German standard method for water, wastewater and sludges DIN 38412 Part 15 was 2 mg/l (Knie et al. 1983).

A prolonged fish test was performed with *Brachydanio rerio* during 14 days in a flow through system according to OECD Guideline 204 with analytical monitoring. As endpoint the following criteria were selected: feeding behaviour, respiration, weight and malposition. A NOEC of 1.53 mg/l was obtained for the endpoints feeding and malposition (Roederer 1990).

With *Daphnia* acute tests were performed according to three standard procedures. In a study according to the German standard method for water, wastewater and sludges DIN 38412 Part 11 the toxicity to *Daphnia magna* was tested during 24 h resulting in an EC50 of 15 mg/l (Kuehn et al. 1988, 1989). A test according to a Dutch standard test showed a 48 h-EC50 of 6.7 mg/l for *Daphnia magna* (Maas-Diepeveen and van Leeuwen 1986). Using a method analogue to OECD Guideline 202 Canton et al. (1985) reported an EC50 (48 h) of 2.7 mg/l for immobilization. All effect values for *Daphnia* are related to nominal concentrations and were obtained in open systems. Three long-term studies are available for *Daphnia magna*. The lowest effect value was a 21d-EC10 of 0.103 mg/l for reproduction of *Daphnia magna* (Bayer 1986c). This value was calculated from the nominal EC10 of 0.15 mg/l using the analytical recovery of 69.2 % from the test concentration 1.5 mg/l. In another reproduction test with *Daphnia magna* a 21d-NOEC of 0.19 mg/l was obtained. This value was derived from the nominal NOEC of 0.32 mg/l using the analytical recovery of 60 % found for the test concentrations 0.63 mg/l and 1.25 mg/l (Kuehn et al. 1988). In a third long-term study with *Daphnia magna* a 21d-LOEC of 1.8 mg/l was found. No information is available on the analytical monitoring of the test substance concentration (Maas-Diepeveen and van Leeuwen 1986). All these long-term studies were performed in semi-static open systems and analytical monitoring was not performed for all concentrations tested. Therefore, the resultant effect values may be somewhat uncertain. However, as the effect values found in these tests are in the same order of magnitude, the studies are regarded to be sufficient for the assessment of long-term effects of 1-chloro-4-nitrobenzene to *Daphnia magna*.

With the green alga *Scenedesmus subspicatus* the following effect values (related to nominal concentrations) were found (Kuehn and Pattard 1990):

48h-ErC50: 16 mg/l

48h-ErC10: 4.9 mg/l

For *Chlorella pyrenoidosa* a 96 h-EC50 on a decrease of 50% in the maximum density (yield) is reported with 4.9 mg/l (no information about analytical monitoring) (Maas-Diepeveen and van Leeuwen 1986).

Toxicity to Microorganisms

Regarding the toxicity to microorganisms, a O_2-consumption test in accordance to Robra with *Pseudomonas putida* during 30 minutes was performed and an EC10 of 59 mg/l was determined (Knie et al. 1983). In a biodegradation study using a mixed population of microorganisms from an industrial and domestic wastewater treatment plant it was found that the degradation process was inhibited at 1-chloro-4-nitrobenzene concentrations of >= 8 mg/l, while at concentrations of 2.4 mg/l 62 % of the test substance were degraded within 20 days (see section 2.1).The lowest available long-term test values without effects were the long-term Daphnia tests with EC10/NOEC results of 0.103 mg/l and 0.19 mg/l. Calculating the geometric mean from these two values results in a concentration of 0.14 mg/lwhich is used as basic value for the derivation of the PNECaqua. Since long-term tests with species from two trophic levels are available, an assessment factor of 50 was applied according to EU Technical Guidance Document. The PNECaqua is calculated to be 2.8 µg/l.

4.2 Terrestrial Effects

No test result with plants according to OECD-Guideline 208 (Terrestrial plant growth test) is known. In humid sand, the 6d-EC50 was 91 mg/l for *Phaseolus aureus* and 132 mg/l for *Cucumis sativus* (Eckert 1962). The 14 d EC50 of *Lactuca sativa* was measured for various chloro(nitro)benzenes including e.g. 1-chloro-2-nitrobenzene. An equation for the calculation of the EC for chloro(nitro)benzenes has been derived (Hulzebos et al. 1993), which was used to calculate the EC50 of 1-chloro-4-nitrobenzene to 3 mg/l.

4.3 Other Environmental Effects

No data available.

4.4 Initial Assessment for the Environment

The favourite target compartments of 1-chloro-4-nitrobenzene are air with 65 %, followed by water with 33 % according to a Mackay calculation level I. In air, the substance is indirectly photodegradable with t1/2 = 62 days. 1-Chloro-4-nitrobenzene is not readily biodegradable but is degradable by adapted microorganisms.

Measured bioconcentration factors in fish are in the range of 5.8 – 20.9 at a 1-chloro-4-nitrobenzene concentration of 0.15 to 0.015 mg/l. A calculated Koc of 309 suggests the substance to have a medium geoaccumulation potential.

The lowest valid acute test results of aquatic testing determined for fish, daphnia, algae and bacteria were as following:

Fish: *Brachydanio rerio* with a 96 h-LC50 of 14.36 mg/l, and *Leuciscus idus* with 48 h-LC50 of 2 mg/l

Daphnia: *Daphnia magna* with a 24 h-EC50 of 15 mg/l and 48 h-EC50 of 2.7 mg/l,

Algae: *Scendesmus subspicatus* with a 48h-ErC50 of 16 mg/l and a 48h-ErC10 of 4.9 mg/l. For *Chlorella pyrenoidosa* a 96 h-EC50 of 4.9 mg/l was achieved.

Microorganisms: *Pseudomonas putida* with a 10 min EC10 of 59 mg/l.

In a prolonged fish test with *Brachydanio rerio* a 14d-NOEC of 1.53 mg/l was obtained for the endpoints feeding and malposition.

Chronic toxicity has been tested for *Daphnia magna* with a 21d-NOEC of 0.103 mg/l on reproduction.

The lowest measured 6d-EC50 for was 91 mg/l for the plant *Phaseolus aureus*. For Lactuca sativa a 14d EC50 of 1-chloro-4-nitrobenzene was calculated to be 3 mg/l.

Chronic toxicity has been tested for Daphnia magna with a 21d-NOECs of 0.103 mg/l and 0.19 mg/l on reproduction. Calculating the geometric mean from these two values results in a concentration of 0.14 mg/l. Following the EU Technical Guidance Document, for the derivation of the PNECaqua an assessment factor of 50 is appropriate in the case of 2 chronic endpoints from different trophic levels. Using the mean Daphnia NOEC of 0.14 mg/l, a PNECaqua of 2.8 µg/l is calculated.

5. RECOMMENDATIONS

The chemical is currently of low priority for further work.

The chemical possesses properties indicating a hazard for human health and the environment. Based on data presented by the Sponsor country, exposure to human and 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.

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I U C L I D

Data Set

Existing Chemical	:	ID: 100-00-5
CAS No.	:	100-00-5
EINECS Name	:	1-chloro-4-nitrobenzene
EC No.	:	202-809-6
TSCA Name	:	Benzene, 1-chloro-4-nitro-
Molecular Formula	:	C6H4ClNO2

Producer related part

Company	:	Bayer AG
Creation date	:	18.06.1993

Substance related part

Company	:	Bayer AG
Creation date	:	18.06.1993
Status	:	
Memo	:	X Update 1998 AKTUELL EG / ICCA
Printing date	:	22.08.2003
Revision date	:	02.06.1994
Date of last update	:	22.08.2003
Number of pages	:	1
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

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

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type :
Substance type : organic
Physical status : solid
Purity : > 99.8 % w/w
Colour : yellow
Odour :

Flag : Critical study for SIDS endpoint
29.04.2002

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

1-CHLOR-4-NITROBENZOL

Flag : Critical study for SIDS endpoint

1-NITRO-4-CHLORBENZOL

Flag : Critical study for SIDS endpoint

4-CHLOR-1-NITROBENZOL

Flag : Critical study for SIDS endpoint

4-CHLORNITROBENZOL

Flag : Critical study for SIDS endpoint

4-NITRO-1-CHLOROBENZOL

Flag : Critical study for SIDS endpoint

4-NITROCHLORBENZOL

Flag **:** Critical study for SIDS endpoint

BENZENE, 1-CHLORO-4-NITRO-

Flag **:** Critical study for SIDS endpoint

CHLOR-P-NITROBENZOL

Flag **:** Critical study for SIDS endpoint

P-CHLORNITROBENZOL

Flag **:** Critical study for SIDS endpoint

P-NITROCHLORBENZOL

Flag **:** Critical study for SIDS endpoint

P-NITROPHENYLCHLORID

Flag **:** Critical study for SIDS endpoint

PNCB

Flag **:** Critical study for SIDS endpoint

1.3 IMPURITIES

Purity **:** typical for marketed substance
CAS-No **:** 88-73-3
EC-No **:** 201-854-9
EINECS-Name **:** 1-chloro-2-nitrobenzene
Molecular formula **:**
Value **:**

Remark **:** information for commercial 1-chloro-4-nitrobenzene pure
Flag **:** Critical study for SIDS endpoint
02.05.2002

Purity **:** typical for marketed substance
CAS-No **:** 121-73-3
EC-No **:** 204-496-1
EINECS-Name **:** 1-chloro-3-nitrobenzene
Molecular formula **:**
Value **:**

Remark **:** information for commercial 1-chloro-4-nitrobenzene pure
Flag **:** Critical study for SIDS endpoint
02.05.2002

1.4 ADDITIVES

1.5 TOTAL QUANTITY

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 Polyethylenglykole 400 too (36/37) Wear suitable protective clothing and 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	**:**	EEC Index No. 610-005-00-5
Flag	**:**	Critical study for SIDS endpoint

15.07.2002

1.6.2 CLASSIFICATION

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

28.03.2000

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 (33) Danger of cumulative effects
Specific limits	**:**	
Remark	**:**	EEC Index No. 610-005-00-5
Flag	**:**	Critical study for SIDS endpoint

15.07.2002

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 : other: use 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 :

Remark : canc. cat. 3 (possible carcinogen)
mutagen cat. 3 (possible mutagen)
Flag : Critical study for SIDS endpoint
02.05.2002 (1)

Type of limit : MAK (DE)
Limit value : .075 ml/m3
Short term exposure limit value
Limit value : .3 ml/m3
Time schedule : 15 minute(s)
Frequency : times

Remark : risk of cutaneous absoption
Flag : Critical study for SIDS endpoint
02.05.2002 (2)

Type of limit : MAK (DE)
Limit value : .5 mg/m3
Short term exposure limit value
Limit value : 2 mg/m3
Time schedule : 15 minute(s)
Frequency : times

Remark : risk of cutaneous absorption
Flag : Critical study for SIDS endpoint
02.05.2002 (2)

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)

1.8.4 MAJOR ACCIDENT HAZARDS

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

Remark : Anhang II, 4 c

1.8.5 AIR POLLUTION

Classified by : other: Bayer AG
Labelled by : other: Bayer AG
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

1.12 LAST LITERATURE SEARCH

Type of search : Internal
Chapters covered : 3, 4
Date of search : 27.02.2002
Flag : Critical study for SIDS endpoint
16.07.2002

Type of search : Internal
Chapters covered : 2
Date of search : 01.04.2002

Flag : Critical study for SIDS endpoint
16.07.2002

1.13 REVIEWS

Memo : BUA Report 11

Flag : Critical study for SIDS endpoint
16.07.2002 (3)

Memo : EPA Draft Report 4-Chloronitrobenzene

Flag : Critical study for SIDS endpoint
16.07.2002 (4)

2.1 MELTING POINT

Value : 83 °C

Flag : Critical study for SIDS endpoint
10.07.2002 (5)

Value : 83 °C
Sublimation :
Method :
Year : 1972
GLP :
Test substance :

06.05.2002 (6)

2.2 BOILING POINT

Value : 242 °C at 1010 hPa

Flag : Critical study for SIDS endpoint
10.07.2002 (5)

2.3 DENSITY

Type : density
Value : 1.52 g/cm³ at °C

Flag : Critical study for SIDS endpoint
10.07.2002 (7)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .085 hPa at 20 °C
Decomposition :
Method : Directive 84/449/EEC, A.4 "Vapour pressure"
Year : 1986
GLP : no
Test substance : other TS: no purity given

Remark : Vapour pressure calculated from regression curve with measured values
Flag : Critical study for SIDS endpoint
13.05.2002 (8)

Value : 8 hPa at 110 °C

(9)

Value : 40 hPa at 136 °C

(10)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : 2.39 at °C
pH value :
Method : other (measured)
Year : 1971
GLP :
Test substance :

Remark : Authors supplied very complete compilation of partition coefficients known at that time
Flag : Critical study for SIDS endpoint
27.11.2002 (11)

Partition coefficient : octanol-water
Log pow : 2.35 at °C
pH value :
Method : other (calculated)
Year : 1987
GLP :
Test substance :

Method : Calculation method not stated.
23.07.2002 (12)

Partition coefficient : octanol-water
Log pow : 2.4 at °C
pH value :
Method :
Year : 1984
GLP :
Test substance :

Remark : Authors do not specifically indicate whether octanol-water partition coefficient was taken from literature or experimentally determined
10.07.2002 (13)

Partition coefficient : octanol-water
Log pow : 2.46 at °C
pH value :
Method : other (calculated)
Year : 2002
GLP :
Test substance :

Method : The log Kow was determined via the computer program KOWWIN v1.66 (2000)
10.07.2002 (14)

Partition coefficient : octanol-water
Log pow : 2.59 at °C
pH value :
Method : other (calculated)
Year : 1986
GLP :
Test substance :

Method : Calculation method is not stated
10.07.2002 (15)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : 243 mg/l at 20 °C
pH value : ca. 6.4
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : Directive 84/449/EEC, A.6
Year : 1986
GLP :
Test substance : other TS: 99.7% purity

Remark : Solubility in distl. water
Result : Water solubility cited represents mean value of 4 measurements
Test condition : A mixture of p-Nitrochlorobenzene and water (6 g/l) was stirred during 5 days and finally filtrated to remove undissolved particles of the test substance.
For the analytical determination a HPLC instrument "Bayer AP 013-1269" with a RP 8 Merck column, length: 25 cm, at room temperature, pressure 165 bar and a flow of 2 ml/min was used.
Flag : Critical study for SIDS endpoint
29.04.2002 (16)

Solubility in : Water
Value : ca. .2 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :

13.03.2002 (9)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value : ca. 127 °C
Type : closed cup
Method : other: DIN 51758
Year :
GLP :
Test substance :

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
16.07.2002 (9)

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

Method : other: DIN 51794
Year :
GLP :
Test substance :

Remark : ignition temperature: approx. 515 °C
Flag : Critical study for SIDS endpoint
16.07.2002 (9)

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	.0000000000001714 cm³/(molecule*sec)
Degradation	:	50 % after 62 day(s)
Deg. product	:	
Method	:	other (calculated): with SRC-AOPWIN v1.90 (2000)
Year	:	
GLP	:	
Test substance	:	
Remark	:	The calculated half-life is based on a mean OH radical concentration of 1.5E+6 OH radicals/cm3, and 12 sunlight hours per day as suggested by U.S. EPA at AOPWIN
Reliability	:	(2) valid with restrictions accepted calculation method
Flag	:	Critical study for SIDS endpoint

15.07.2002 (14)

Type	:	water
Light source	:	other: high pressure mercury lamps
Light spectrum	:	> 290 nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	.09 mmol/l at °C
DIRECT PHOTOLYSIS		
Halflife t1/2	:	
Degradation	:	90 % after 120 minute(s)
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	water with additives
Conc. of sensitizer	:	500 mg/l
Rate constant	:	cm³/(molecule*sec)
Degradation	:	% after
Deg. product	:	
Method	:	other (measured)
Year	:	1987
GLP	:	no data
Test substance	:	
Method	:	Irradiation of TS in aqueous solution in the absence and in the presence of TiO2; HPLC analysis
Remark	:	The photocatalytic reaction of chloronitrobenzene takes also place in the presence of light (lambda > 230 nm) instead of TiO2 however a lower reaction velocity is expected.
Result	:	Quantitative degradation of TS was observed only in the presence of TiO2. Degradation products are carbon dioxide, water, hydrogen chloride and nitric acid.
Reliability	:	(2) valid with restrictions Basic data given

10.07.2002 (17)

Type	:	water

Light source	:	other: low pressure mercury vapor lamp
Light spectrum	:	253.7 nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	.0025 mmol/l at 16 °C
DIRECT PHOTOLYSIS		
Halflife t1/2	:	
Degradation	:	93 % after 4 minute(s)
Quantum yield	:	
Deg. product	:	
Method	:	other (measured): Oxidation of the TS in dilute aqueous solution in order to produce drinking water
Year	:	1990
GLP	:	no
Test substance	:	other TS: analytical grade
Remark	:	Analytical monitoring via HPLC and UV detection at lambda = 265 nm Laboratory study designed to evaluate the degradation of 1-chloro-4-nitrobenzene in water (pH=7.5) by ultraviolet radiation in combination with hydrogen peroxide or aqueous ozone at different concentrations of oxidants
Result	:	1-Chloro-4-nitrobenzene removal: 93% (test system: H2O2/UV, concentration of oxidant: 2x10E-5 mol/l) and 78% (test system: O3/UV, concentration of oxidant: 2x10E-5 mol/l
Reliability	:	(2) valid with restrictions Basic data given

10.07.2002 (18)

3.1.2 STABILITY IN WATER

Remark	:	Based on the chemical structure of the compound hydrolysis is not expected under environmental conditions
Flag	:	Critical study for SIDS endpoint

27.03.2002

Degradation	:	0 % after 8 day(s) at pH and °C
Deg. product	:	
Method	:	other: see remarks
Year	:	1985
GLP	:	no data
Test substance	:	other TS: > 99.5 % Purity (Origin: Riedel-de Haen AG)
Remark	:	- The decline of the concentration in nonaerated standardized medium was studied at room temperature. - The analyses were performed using gas chromatography or high-pressure liquid chomatography
Result	:	There was no variation on the concentration of the test compound in test medium during 8 days.
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

30.04.2002 (13)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

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

Method : Mackay Level 1, Version 2.11
Remark : Data used in the calculation:
Temperature (°C): 20
Molar Mass (g/mol): 157.56
Vapor Pressure (Pa): 8.5
Water Solubility (g/m3): 243
log Pow: 2.39

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 : Distribution:
air: 64.7 %
water: 33.4 %
soil: 1.3 %
sediment: 0.7 %
suspended sediment: < 0.01 %
fish: < 0.01 %
aerosol: < 0.01 %
Reliability : (2) valid with restrictions
accepted calculation method
Flag : Critical study for SIDS endpoint
22.08.2003 (19)

Media : water - air
Method : other (measurement)
Year : 1999

Result : The measured dimensionless Henry´s law constant is reported to be 0.0002, which corresponds to 0.5 Pa m3 mol-1
Test condition : The pure substances were dissolved in demineralised distilled water. The solutions were directly fed from the dosing funnel into the desorption column. The concentrations of test substances ranged between 10 and 200 mg/l depending on the aqueous solubities. Depending on the compound gas chromatography with different detection systems (ECD, FID, electrolytic conductivity detection) or HPLC with fluorescence detection was used. Identification and quantification were carried out using external standards. The experiments were performed at 25°C.
Reliability : (2) valid with restrictions
basic data given
Flag : Critical study for SIDS endpoint

27.11.2002 (20)

Media : water - air
Method : other (calculation): SRC-HENRYWIN version October 3, 2000
Year : 2002

Method : Bond Method
Result : Henry´s law constant = 1.6 Pa m3 mol-1
Test condition : Temperature: 25 °C
Reliability : (2) valid with restrictions
accepted calculation method in contrast to measured result
12.08.2003 (14)

Media : water - soil
Method : other (calculation): SRC-PCKOCWIN v 1.66
Year : 2002

Result : Koc=309
Reliability : (2) valid with restrictions
accepted calculation method
Flag : Critical study for SIDS endpoint
15.07.2002 (14)

Media : water - air
Method : other (calculation)
Year : 2002

Method : Estimation with US EPA software EPIWIN 3.10, Volatization from water
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
12.08.2003 (14)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : other: effluent of a model sewage treatment plant in accordance with OECD
Concentration : 2.4 mg/l related to Test substance
80 mg/l related to Test substance
Contact time :
Degradation : 0 (±) % after 20 day(s)
Result : under test conditions no biodegradation observed
Control substance : other: phenol
Kinetic : %
%
Deg. product :
Method : other: closed bottle test
Year : 1979
GLP : no
Test substance : other TS: purity 98.8 %
Test condition : - Closed bottle test in accordance with the later published method OECD 301 D (closed bottle test)

- 1-Chloro-4-nitrobenzene was the only source of organic carbon.
- Effluent of a (model) 3-l laboratory sewage treatment plant in accordance with OECD guideline, working with full mineral nutrient medium
- Initial concentrations of 1-chloro-4-nitrobenzene: 2.4, 8, 24, 80 mg/l, prepared from stock solution (1 g/l)
- Concentration of inoculum: 1000 - 1000000 bacteria/l
- Emulsifier W (CAS 68130-72-3) was used to emulsify the 1-chloro-4-nitrobenzene stock solution. The emulsifier was not degradable under these test conditions
- Incubation time was 0, 5, 10, 20 days at 20 °C +/- 1 °C
- Incubation in the dark

Reliability : (2) valid with restrictions
Basic data supplied

Flag : Critical study for SIDS endpoint

16.07.2002 (21)

Type : aerobic
Inoculum : other: activated sludge from waste water treatment plant for industrial and domestic influents, adapted
Concentration : 2.4 mg/l related to Test substance
related to
Contact time :
Degradation : 62 (±) % after 20 day(s)
Result : other: biodegradable with adapted microorganisms
Kinetic of testsubst. : 0 day(s) 0 %
5 day(s) 0 %
10 day(s) 0 %
20 day(s) 62 %
%
Control substance : other: phenol
Kinetic : %
%
Deg. product :
Method : other: closed bottle test
Year : 1979
GLP : no
Test substance : other TS: purity 98.8 %

Result : Other results:
8 mg/l 1-chloro-4-nitrobenzene after 5 d: 0 % degradation
8 mg/l 1-chloro-4-nitrobenzene after 10 d: 0 % degradation
8 mg/l 1-chloro-4-nitrobenzene after 20 d: 21 % degradation
24 mg/l 1-chloro-4-nitrobenzene after 5 d: 0 % degradation
24 mg/l 1-chloro-4-nitrobenzene after 10 d: 13 % degradation
24 mg/l 1-chloro-4-nitrobenzene after 20 d: emaciation
80 mg/l 1-chloro-4-nitrobenzene after 20 d: emaciation
The authours noted that toxic effects were seen at concentrations of 80 mg/l 1-chloro-4-nitrobenzene after 10 d.
However, also the low degree of degradation at 8 mg/l and the lag phases might indicate toxic effects.

Test condition : - Closed bottle test in accordance with the later published method OECD 301 D (closed bottle test)
- 1-Chloro-4-nitrobenzene was the only source of organic carbon.
Adaptation period was 2 weeks for activated sludge from a wastewater treatment plant for industrial and municipal wastewaters
- Initial concentrations of 1-chloro-4-nitrobenzene: 2.4, 8, 24, 80 mg/l, prepared from stock solution (1 g/l)
- Concentration of inoculum: 1000 - 1000000 bacteria/l
- Emulsifier W (CAS 68130-72-3) was used to emulsify the 1-chloro-4-nitrobenzene stock solution. The emulsifier was not degradable under

these test conditions
- Incubation time was 0, 5, 10, 20 days at 20 °C +/- 1 °C
- Incubation in the dark

Reliability : (2) valid with restrictions
Basic data supplied
Flag : Critical study for SIDS endpoint
23.07.2002 (21)

Type : aerobic
Inoculum : other fungi: Rhodosporidium sp.
Concentration : .1 mmol/l related to Test substance
related to
Contact time : 14 day(s)
Degradation : > 90 (±) % after 10 day(s)
Result : other: biotic reduction
Deg. product : yes
Method :
Year : 1981
GLP : no data
Test substance : no data
Deg. products : 4-chloro-2-hydroxyacetanilide
106-47-8 203-401-0 4-chloroaniline
539-03-7 208-707-8 4'-chloroacetanilide
932-98-9 213-263-3 4-chloronitrosobenzene

Result : 2 degradation routes postulated after analysis of metabolic products. Major final metabolites are 4-chloroacetanilide and 4-chloro-2-hydroxyacetanilide.
Test condition : - Rhodosporidium sp. is a basidiomycetous yeast previously described as Rhodotorula graminis <(ATCC 18159) and as Sporobolomyces coprophilus (CBS 5811)
- yeasts were cultured in complex nutrient medium which were continuously shaken aerobically
- after incubation for 14 days with various concentrations of 1-chloro-4-nitrobenzene (0.1, 0.25, 1 mM; ethanol as solubilizing agent), different sampling methods were employed depending on the metabolite to be analyzed
- 1-chloro-4-nitrobenzene and its degradation products were separated by HPLC and analyzed at 313 nm and 254 nm, respectively; TLC was also employed
Reliability : (2) valid with restrictions
study with acceptable limitations
Flag : Critical study for SIDS endpoint
16.07.2002 (22)

Type :
Inoculum : other: various cultures of microorganisms
Deg. product : yes
Method : other: degradation in adapted batch cultures
Year : 1999
GLP : no data
Test substance : other TS: monochloronitrobenzene

Test condition : It is stated that in a screening test no viable and degrading cultures could be isolated with chloronitrobenzenes as sole source of C and N. Under cometabolic conditions (additional C- and N-source present) cultures were able to degrade chloronitrobenzenes. The first step in degrading chloronitrobenzenes was the reduction to the corresponding chloroanilines. Two cultures (Fusarium solani (fungus) and Pseudomonas acidovorans CA50) were able to degrade monochloronitrobenzenes under

dechlorination. The reduction of monochloronitrobenzene by P. acidovorans CA50 was significantly accelerated with nitrate as an electron acceptor and under anaerobic conditions. In authentic wastewater the aerobic degradation of 1-chloro-4-nitrobenzene was slow. To improve the efficiency of the aerobic degradation a two step solid bed reactor was used. P. acidovorans CA50 was immobilised on Siran (unmodified sintered glass). The reactor conditions were stable during the observation period of 7 months. At a load of 42 mg/l x d and a residence time of 1.5 -1.8 d the elimination of monochloronitrobenzene and the metabolites 3- and 4-chloroaniline was 100%. Concomitantly, AOX was decreased by at least 80%.

Reliability : (2) valid with restrictions
Basic data given

Flag : Critical study for SIDS endpoint

08.08.2002 (23)

Type :
Inoculum : other: bacteria from soil, sewage, wastewater, rotting plant material, after adaptation
Contact time :
Degradation : > 60 (±) % after 7 day(s)
Result :
Deg. product :
Method : other: various adaptation technologies (see test condition)
Year : 1983
GLP : no data
Test substance : no data

Remark : Aerobic bacteria were adapted to Chloronitrobenzenes or other chemicals for up to 1 year with different methods. The adapted bacteria degraded more than 60 % of 1-chloro-4-nitrobenzene within 7 days.

Test condition : Forest soil, rotten bark and wood chips, river sediments, sewage and other environmental materials were screened for bacteria or other microorganisms with the potential to degrade various industrial chemicals. In a soil pot or liquid culture reactor aerobic bacteria were adapted to chloronitrobenzenes or other chemicals for up to 1 year with different methods:
- Isolation and adaptation of microorganisms in a minerals nutrient medium containing 1-Chloro-2-nitrobenzene, 1-chloro-3-nitrobenzene, and 1-chloro-4-nitrobenzene as the sole source of organic carbon
- Isolation of viable cultures on a minerals nutrient medium containing nitrobenzenes as the sole source of carbon, followed by adaptation of the isolates to the corresponding chloride-containing compounds
- Isolation and adaptation in a mixture of both compounds
- Isolation and adaptation in nutrient solution added with chloronitrobenzenes and ethanol as an additional source of carbon.
Degradation tests with 100 ml/l adapted inoculum were cultivated over 7 days. Concentrations of chloronitrobenzenes or other chlorocompounds were analysed with 3 different methods: GC, HPLC and EOX (extractable organic halogen compounds)

Reliability : (2) valid with restrictions
Basic data supplied, documentation very short

Flag : Critical study for SIDS endpoint

07.08.2002 (24)

Type : aerobic
Inoculum : activated sludge, domestic, adapted
Concentration : 14 mg/l related to Test substance
related to
Contact time : 1 day(s)

Degradation	:	> 99 (±) % after 1 day(s)
Result	:	
Deg. product	:	
Method	:	other: degradation in activated sludge reactor or in semi-immersed rotating disc reactor
Year	:	1984
GLP	:	no
Test substance	:	other TS: Mixture of up to 5 Nitrobenzene derivatives
Remark	:	Aerobic treatment by suspended activated sludge or by a biological film settled on a semi-immersed rotating disc was used to treat various solutions of up to 5 nitrocompounds (4 nitrobenzene derivates and 1-nitronaphthalene) or authentic wastewater from a nitrophenol production site. 1-Chloro-4-nitrobenzene was nearly completely degraded by adapted microorganisms. Simultanously, COD, colour, and toxicity of these liquids were significantly reduced.
Test condition	:	Domestic sewage was adapted to the compounds for 6 month. The adapted activated sludge was used in a activated sludge reactor to further adapt to operative conditions for another 8 month. This adapted sludge degraded 14 mg/l 1-chloro-4-chloronitrobenzene in a synthetic mixture containing 4 nitrocompounds by at least 99 % during a mean residence time of 1.02 days in the reactor. The process temperature was 34 +/- 2 °C. In additional experiments performed at 22 +/- 2 °C biodegradation of these compounds was observed but no detailed results reported by the authors. In a second examination the purification of industrial waste water containing up to 5 nitrocompounds was investigated. The total concentration of nitrocompounds was about 44 - 118 mg in the inflow. The mean residence time of wastewater in the reactor was 0.91 days. For the nitrocompounds in total a degradation rate of more than 90 % was achieved after 120 days. Additional studies were conducted using a semi-immersed rotating disc reactor at a process temperature of 22°C. An industrial wastewater containing up to 5 nitrocompounds was used. The mean residence time was 0.7 d and the concentration of the nitrocompounds was about 44 - 118 mg/l. In these case a degradation of the nitrocompounds of 99.5% was observed after 100 days.
Reliability	:	(2) valid with restrictions Extensive range of data given
Flag	:	Critical study for SIDS endpoint

07.08.2002 (25)

Type	:	anaerobic
Inoculum	:	other: anaerobic industrial microorganisms and domestic sewage
Concentration	:	40 mg/l related to Test substance related to
Contact time	:	1 day(s)
Degradation	:	> 99 (±) % after 1 day(s)
Result	:	
Kinetic of testsubst.	:	0 hour(s) 0 % 6 hour(s) ca. 94 % 12 hour(s) ca. 99 % 18 hour(s) > 99 % 24 hour(s) > 99 %
Deg. product	:	
Method	:	other: anaerobic flow-through apparatus
Year	:	1982
GLP	:	no
Test substance	:	
Test condition	:	Tube-shaped anaerobic apparatus was used in flow through modus. The

apparatus was inocculated with microorganisms from an industrial methane tank with a thermophilic regime of fermentation. The microorganisms were adapted for one month to 1-chloro-4-nitrobenzene, with a continuous supply of wastewater containing increasing concentrations of 1-chloro-4-nitrobenzene. Temperature was maintained at 50 - 53 °C.

Reliability : (2) valid with restrictions
Basic data supplied

Flag : Critical study for SIDS endpoint

15.05.2002 (26)

Type : aerobic
Inoculum : activated sludge
Concentration : 30 mg/l related to Test substance
related to
Contact time :
Degradation : 0 (±) % after 14 day(s)
Result : under test conditions no biodegradation observed
Deg. product :
Method : other: Japanese Guideline by MITI of 1974; corresponds to OECD 302C Modified MITI Test II
Year : 1992
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

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 "302C, Ready Biodegradability: Modified MITI Test II", stipulated in the OECD Guidelines for Testing of Chemicals (May 12, 1981).
According to other studies (Bayer AG 1979, Internal study 7900802-815: Biodegradation p-Nitrochlorobenzene, and Maas-Diepeveen, J.L. and van Leeuwen, C.J., Aquatic toxicity of aromatic nitro compounds and anilines to several freshwater species, Report 86-42 (1986, later published also in: Deneer, J.W. et al., QSAR study of the toxicity of nitrobenzene derivatives towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum, Aquatic Toxicology 15, 83-98 (1989)), the applied concentration of 1-chloro-4-nitrobenzene is inhibitory for the inocula.

Test condition : Sludge concentration: 100 mg/l
test substance concentration 30 mg/l.

Reliability : (3) invalid
Test procedure according to national standards. In general, good reliability but for 1-chloro-4-nitrobenzene, inhibitory test concentration used.

01.08.2002 (27)

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

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 (1976): Determination of biological degradability of organic substances, Water Res. 10, 231-235.)

Reliability : (3) invalid
Insufficient documentation: no details on origin and density of inoculum, and on tested concentrations and test conditions. No information on adaptation of the microorganisms.

16.07.2002 (13)

Type : aerobic
Inoculum : other: suspension of Niagara silt loam
Concentration : 10 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 :
Method : other: see test condition
Year : 1966
GLP : no data
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

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 283 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
- 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

10.07.2002 (28)

Type : aerobic
Inoculum : predominantly domestic sewage
Concentration : 100 mg/l related to Test substance
related to
Contact time :
Degradation : 6 (±) % after 28 day(s)
Result : other: not readily biodegradable
Kinetic of testsubst. : 0 day(s) 0 %
26 day(s) 0 %
28 day(s) 6 %

%
%

Control substance : Aniline
Kinetic : 8 day(s) 21 %
20 day(s) 78 %
Deg. product :
Method : other: Directive 84/449/EEC, C.3. Method was adapted to Assessement of Biodegradability of Chemicals in Water by Manometric Respirometry according to EU ring test
Year : 1986
GLP : no
Test substance : other TS: 1-chloro-4-nitrobenzene, no data further data given

Remark : related to BOD
Reliability : (2) valid with restrictions
Guideline study; basic data given

02.08.2002 (29)

Type : aerobic
Inoculum : activated sludge
Concentration : 200 mg/l related to Test substance
related to
Contact time :
Degradation : < 10 (±) % after 15 day(s)
Result :
Kinetic of testsubst. : 5 day(s) < 10 %
10 day(s) < 10 %
%
%
%
Deg. product :
Method : other: Respirometer test
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Source : Hoechst AG Frankfurt/Main
Reliability : (4) not assignable
Original reference not available

16.07.2002 (30)

Type : aerobic
Inoculum : activated sludge, industrial, non-adapted
Concentration : 200 mg/l related to Test substance
related to
Kinetic of testsubst. : 5 day(s) 80 %
10 day(s) > 90 %
%
%
%
Deg. product :
Method : OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Remark : - Elimination by stripping >50 %.
- The wastewater treatment plant which was presumably the source of the inoculum, was used to treat the Hoechst industrial wastewater which

contained chloronitrobenzenes at that time.
Source : Hoechst AG Frankfurt/Main
Reliability : (4) not assignable
Original reference not available
16.07.2002 (30)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 56 day(s) at °C
Concentration : .15 mg/l
BCF : 5.8 - 20.9
Elimination :
Method : other: see remarks
Year :
GLP : no data
Test substance :

Remark : 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).
Result : At a p-chloronitrobenzene concentration of 0.015 mg/l under the same test conditions as described, a BCF= 7.5 - 18.1 was determined.
Reliability : (1) valid without restriction
Test procedure according to national standards, comparable with guideline
Flag : Critical study for SIDS endpoint
03.05.2002 (27)

BCF : 39
Elimination :
Method : other: calculated
Year : 1985
GLP :
Test substance : as prescribed by 1.1 - 1.4

Remark : The log Pow-value (2.4) was used to calculate the bioaccumulation factor using the formula: log BCF = 0.76 logPow - 0.23 (Ross and Welch, 1979)
Reliability : (2) valid with restrictions
Acceptable calculation method
03.04.2002 (13)

Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 36 day(s) at 15 °C
Concentration : .78 µg/l
BCF : 84 - 112
Elimination :

Method	:	other: 30 fish exposed to 780+/-130 ng TS/l in a flow-through system; aceton used as solvent; samples of 6 fish each analysed at 5,12,20,28 and 36 days of exposure; duplicate water samples taken every 3 or 4 days; GC analysis
Year	:	1989
GLP	:	no data
Test substance	:	other TS: mono- to pentachloronitrobenzenes
Remark	:	Accepted new scientific name for Salmo gairdneri (Rainbow trout): Oncorhynchus mykiss
Result	:	The BCF were determined for 5 incubation periods: 84 +/- 8 for 5 days, 112 +/- 23 for 12 days, 96 +/- 14 for 20 days, 103 +/- 16 for 28 days, and 108 +/- 15 for 36 days. The mean BCF was 101 +/- 18 with significant differences (p </= 0.05) among the sample intervalls. Since the higher chlorinated nitrobenzenes are possibly dechlorinated by metabolism in fish, a BCF for p-chloronitrobenzene cannot be derived with this test design.
Reliability	:	(3) invalid Unsuitable test system (more than one substance tested in the same vessel)

16.07.2002 (31)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	14.36
Limit test	:	
Analytical monitoring	:	yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1990
GLP	:	no data
Test substance	:	other TS: no purity given
Remark	:	analytical monitoring: GC after extraction with toluene or hexane
Result	:	Furthermore the substance was tested according to the method OECD 204: "Fish, Prolonged toxicity test (14 days) under the same test conditions. As endpoint the following sub-lethal effects were observed: feeding behaviour, respiration, weight and malposition. The results of this test were: NOEC= 1.53 mg/l; LOEC=13.2 mg/l and NOLEC(no observed lethal effect concentration) = 3.03 mg/l Endpoints for LC50 were feeding behaviour and malposition.
Test condition	:	- Before starting the test fish was maintained at least 12 days in dilution water. - The stock solution of the test substance was prepared in aceton and finally diluted in water, so that a concentration of aceton of 0.2 ml/l was present in the test. - The test was conducted in 11 l aquarium filled with 10 l test solution and per concentration 10 fish were used. - The water flowthrough rate was 2.5 l/h, that means a renewal frequency of 6 times per day. - The dilution water was continuously produced and maintained at about 25°C and oxygen saturated. - The mean fish length was 2.0 +/- 1.0 cm; test temperature was 23 +/- 1 °C and pH = 8.15 +/- 0.2. - Fish was exposed to a cycle of 14 h light and 10 h darkness. - pH-values and oxygen-concentration were measured before introduction of fish, 2 times a week during the test and on the end of the test. - for LC50 determination, the following concentrations were used: 0, 3.62, 6.8, 13.1, 24.6 and 50 mg/l - for determination of prolonged toxicity to fish, the following concentrations were used: 0, 0.72, 1.53, 3.03, 6.15 and 13.2 mg/l
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint

27.11.2002 (32)

Type	:	other: not specified
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	1
LC50	:	2
LC100	:	2.5
Method	:	other: DIN-Standard 38412 L15 (Fish short-time test)
Year	:	1983
GLP	:	no
Test substance	:	other TS: no purity given

Method : Method of the German Standards Institution, Berlin, Germany
Reliability : (2) valid with restrictions
Test procedure according to national standard method
Flag : Critical study for SIDS endpoint
05.04.2002 (33)

Type : semistatic
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 14 day(s)
Unit : mg/l
NOLEC : .1
LOLEC : .22
Limit test :
Analytical monitoring : yes
Method : OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year : 1987
GLP : no
Test substance : other TS: no purity given

Remark : Since no TS concentration below 0.1 mg/l was tested it was not possible to determine the threshold concentration or the NOEC.
Result : - NOLEC = No observed lethal effect concentration
- The lowest observed lethal effect concentration (LOLEC) was 0.22 mg/l (geometric mean of measured values)
Test condition : - Test was performed under semistatic conditions in 8 l aquarium filled with 5 l of the test solution.
- The test solution were renewed after 2, 5, 7, 9, 12, 14, 16 and 19 days.
- Per concentration 10 fish (8 months old) with a length of 2.5 to 3.5 cm were introduced.
- The following nominal 1-chloro-4-nitrobenzene concentrations were tested: 0.1, 0.32, 1.0 and 3.2 mg/l.
- The concentrations of the test media were determined at the start of the incubation period and after 3 days. For the 0.32 mg/l nominal concentration, 0.255 mg/l and 0.19 mg/l, respectively, were determined (average during incubation period 0.22 mg/l). For the 3.2 mg/l nominal concentration, 2.68 and 1.59 mg/l, respectively, were determined.
Reliability : (2) valid with restrictions
Guideline study
16.07.2002 (34)

Type : semistatic
Species : Poecilia reticulata (Fish, fresh water)
Exposure period : 14 day(s)
Unit : mg/l
LC50 : 6.6
Limit test :
Analytical monitoring : yes
Method : other: Koenemann (1981)
Year : 1987
GLP : no data
Test substance : other TS: Purity >98 %

Result : Results are given in the original reference of Deneer, J.W. et al. (1987) as log LC50: log LC50 = 1.58 (LC50 µmol/l)
Test condition : temperature: 21-23 °C; pH=6.8-7.2
Reliability : (2) valid with restrictions
Basic data given.
16.07.2002 (12) (15)

Type	:	other: not specified
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	7 day(s)
Unit	:	mg/l
LC50	:	34 - 59
EC50	:	10 - 19
Limit test	:	no
Analytical monitoring	:	no data
Method	:	other: see Remarks
Year	:	1986
GLP	:	no data
Test substance	:	other TS: Purity >98 %
Remark	:	- The experiment was carried out in a constant temperature room at 25 +/-1 °C. - The test was conducted in 100 ml glass dishes to which 50 ml test solution was added. - The test medium consisted of standard water according to Alabaster and Abram (1965) with a hardness of 250 mg/l (as CaCO3) and a pH = 8.2 +/- 0.2. - Test were perfomed in singular with 25 eggs per jar. - Photoperiod during the test = 12h - Fishes were not fed during the test.
Test substance	:	Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO; Merck, Purity 99%)
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific priniciples, acceptable for assessment

16.07.2002 (15)

Type	:	semistatic
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	14.5
Limit test	:	
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	:	other TS: no purity given
Reliability	:	(1) valid without restriction Test procedure according to national standards

16.07.2002 (27)

Type	:	static
Species	:	Leuciscus idus melanotus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	10
LC50	:	16
LC100	:	25
Limit test	:	
Analytical monitoring	:	no
Method	:	other: see Test condition
Year	:	1980
GLP	:	no

Test substance	:	other TS: no purity given
Result	:	After 48 h the following concentrations were determined: LC0=10 mg/l LC50=20 mg/l LC100=40 mg/l
Source	:	Hoechst AG Frankfurt am Main
Test condition	:	10 fish per concentration; 8 concentrations tested; mean fish lenght: 5.8 cm; temperature: 20 °C; pH=8.1-8.3; 12 h light/12 h dark. The test substance was stirred for 5 minutes in water with an Ultra-Turrax. Before starting the test the fish were kept in dechlorinated water for 2 weeks
Reliability	:	(2) valid with restrictions GLP was not common pratice at the time

16.07.2002 (35)

Type	:	other: semistatic, renewal at 12 hours
Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	25.5
Method	:	other: comparable to OECD Guideline 203 (Fish: Acute Toxicity Test, 1992)
Year	:	1996
GLP	:	
Test substance	:	
Test condition	:	60 fish used in each test ; fish weight / length: 5 g / 5 cm; temperature: 20 °C
Reliability	:	(2) valid with restrictions According to guideline study with acceptable restrictions

29.04.2002 (36)

Type	:	static
Species	:	Poecilia reticulata (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	13
EC50	:	1.8
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 (Origin: Riedel-de Haen AG)
Remark	:	EC 50 measured behaviour
Reliability	:	(2) valid with restrictions Comparable to guideline study, without detailed documentation.

16.07.2002 (13)

Type	:	other: not specified
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	18
Method	:	
Year	:	1979
GLP	:	no
Test substance	:	other TS: no purity given

Test condition : 10 fish per concentration; 4 concentrations tested
Reliability : (4) not assignable
Documentation insufficient for assessment
02.08.2002 (37)

Type : other: not specified
Species : Leuciscus idus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : > 1
Limit test :
Analytical monitoring : no data
Method :
Year : 1986
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
Documentation insufficient for assessment.
16.07.2002 (38)

Type : other: not specified
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : > 2
Limit test :
Analytical monitoring : no data
Method :
Year : 1986
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
Documentation insufficient for assessment.
16.07.2002 (39)

Type : other: not specified
Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 10 hour(s)
Unit : mg/l
LC100 : 5
Method :
Year : 1971
GLP :
Test substance :

Remark : Accepted new scientific name for Salmo gairdneri (Rainbow trout): Oncorhynchus mykiss
Reliability : (4) not assignable
Original reference not available
03.05.2002 (40)

Type : other: not specified
Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : 6
Method :

Year : 1983
GLP : no data
Test substance :

Remark : Accepted new scientific name for Salmo gairdneri (Rainbow trout): Oncorhynchus mykiss
Reliability : (4) not assignable
Data are from an unknown industrial source which is cited as follows: Parris, G.E. 1983, Unpublished information on 4-chloronitrobenzenes and 2-chloronitrobenzenes from anonymous industry contacts, Memo to the files (June 1, 1983)

16.07.2002 (4)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : 3.3
EC50 : 15
Limit Test : no
Analytical monitoring : no
Method : other: DIN-Standard 38412 L11 (Daphnia short-time test)
Year : 1988
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : Method of the German Standards Institution, Berlin, Germany
Remark : - The test consists in four parallel test vessels per concentration level and at least four vessels for the control.
- Test System: Each vessel was filled with 24 h-old Daphnia (1 animal/50 ml). The total number of daphnias per concentration level was 20. Test temperature = 25 +/-1 °C.
- Test organism: Daphnia magna Straus, strain = IRCHA.
- Dilution water: Source = synthetic fresh water, Hardness = 2.5 mmol/l Ca + Mg, Na/K Ratio: 10:1, pH = 8.0 +/- 0.2
- pH-values and oxygen-concentration were measured during the test in two tests-vessels per concentration level.The detetected variation of these parameters had no negative influence on the organisms.
Reliability : (1) valid without restriction
Test procedure in accordance to national standard methods.
Flag : Critical study for SIDS endpoint
07.08.2002 (41) (42)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : 6.7
Limit Test : no
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

Remark : Publication later used by Deneer, J.W. et al., QSAR study of the toxicity of nitrobenzene derivatives towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum, Aquatic Toxicology 15, 83-98 (1989)

Test substance : - Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO; Merck, Purity 99%)
- During the tests daphnids were fed with Chlorella pyrenoidosa, which at the start of the experiments were present at a concentration of 1.0E+8 cells/l
- IC50 values were calculated according to Litchfield and Wilcoxon (1949) Deneer et al., who later apparently use the data but do not cite this publication (Deneer, J.W. et al., QSAR study of the toxicity of nitrobenzene derivatives towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum, Aquatic Toxicology 15, 83-98 (1989)) renamed the EC50 to IC50 and give results as log IC50: log IC50 = 1.63 (IC50 µmol/l). They state:
- The oxygen content of all solutions did not decrease below 7.9 mg/l (85%).
- Mortality in the controls never exceeded 10 %.

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

Flag : Critical study for SIDS endpoint
23.07.2002 (15)

Type : other: not specified
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : 2.7
LC50 : 8.9
Analytical monitoring : no
Method : OECD Guide-line 202
Year : 1985
GLP : no data
Test substance : other TS: > 99.5 % Purity (Origin: Riedel-de Haen AG)

Test condition : Analogue to OECD proposal for short-term toxicity tests perfomed on crustaceans (Daphnia magna) from 1979 (later OECD Guideline 202) EC50 was derived from immobilization

Reliability : (2) valid with restrictions
Comparable to guideline study, without detailed documentation.

Flag : Critical study for SIDS endpoint
23.07.2002 (13)

Type : other: not specified
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : 2
EC50 : 6
EC100 : 14
Limit Test : no
Analytical monitoring : no data
Method : other: DIN-Standard 38412 L11 (Daphnia short-time test)
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Method : Method of the German Standards Institution, Berlin, Germany
Reliability : (2) valid with restrictions

Test procedure in accordance with national standard methods. No details are given about the performance of the test.

03.04.2002 (33)

Type : static
Species : other: Daphnia carinata
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : 18.1
Method : other: comparable to OECD 202 part I (Daphnia, Acute Toxicity, 1984)
Year : 1996
GLP : no data
Test substance : other TS: purity not given

Reliability : (2) valid with restrictions
Comparable to guideline study, only basic data given
Flag : Critical study for SIDS endpoint

06.05.2002 (36)

Type : other: not specified
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
LC50 : 10
Limit Test : no
Analytical monitoring : no data
Method : other: not specified
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : LC50 = 13 mg/l (after 24 h)
NOEC = 3.2 mg/l (after 48h)
Reliability : (4) not assignable
Data are from an unknown industrial source which is labelled as follows: Parris, G.E. 1983, Unpublished information on 4-chloronitrobenzenes and 2-chloronitrobenzenes from anonymous industry contacts, Memo to the files (June 1, 1983)

16.07.2002 (4)

Type :
Species : Daphnia magna (Crustacea)
Exposure period :
Unit : mg/l
EC50 : 13
Limit Test : no
Analytical monitoring : no data
Method :
Year : 1982
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
Documentation insufficient for assessment

23.07.2002 (43)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC10	:	4.9
EC50	:	16
Method	:	other: DIN-Standard 38412 L9 (Algae, Cell multiplication inhibition test)
Year	:	1990
GLP	:	no data
Test substance	:	other TS: no purity given
Method	:	Method of the German Standards Institution, Berlin, Germany
Result	:	Effect levels determined the endpoint biomass and the results were the following: EC10= 2.2 mg/l ; EC50= 8 mg/l
Test condition	:	- Preliminary culture: - The cultivation of the preliminary cultures was undertaken 3 days prior to the preparation of the test solution. - The cell material was used after 72 h to inoculate the dilution preparation after the cell concentration had been fixed at 1.0E5/ml - Test preparations: - Wide-neck bottles of 250ml with ground-glass stoppers were used as the test vessels. - The test and control preparations were incubated under constant lighting and shaken daily. Before beginning the test pH was adjusted to 8
Reliability	:	(1) valid without restriction Test procedure in accordance to national standard methods.
Flag	:	Critical study for SIDS endpoint

03.04.2002 (44)

Species	:	Chlorella pyrenoidosa (Algae)
Endpoint	:	other: reduction of the maximum density (yield) of Chlorella pyrenoidosa
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC50	:	4.9
Limit test	:	no
Analytical monitoring	:	no data
Method	:	other: OECD Guideline 201 (1984) with slight modifications (Van Leeuwen et al. 1985b)
Year	:	1986
GLP	:	no data
Test substance	:	other TS: >98% Purity
Remark	:	In 1989, although this publication is not cited, the data were used by Deneer, J.W. et al., QSAR study of the toxicity of nitrobenzene derivatives towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum, Aquatic Toxicology 15, 83-98 (1989). These authors report the log EC50 in µmol/l to be 1.49.
Test substance	:	Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO; Merck, purity 99%) The concentrations causing 50 % reduction of the maximum density (yield) of Chlorella pyrenoidosa (EC50) during a 96-h period of exposure were calculated according to Kooyman et al., Parametric analysis of population growth in bio-assays, Water Research 17, 527 - 538 (1983). Results are given in the original reference as log EC50: log EC50 = 1.49

(EC50 µmol/l)
Reliability : (2) valid with restrictions
Guideline Study without detailed documentation.
Flag : Critical study for SIDS endpoint
23.07.2002 (15)

Species : Scenedesmus subspicatus (Algae)
Endpoint : growth rate
Exposure period : 7 day(s)
Unit : mg/l
TGK : 10
Limit test :
Analytical monitoring : no data
Method : other: UBA-Verfahrensvorschlag "Hemmung der Zellvermehrung bei der Gruenalge Scenedesmus subspicatus" (EC10; EC50; 72 Stunden; statisches System) (01.01.1984)
Year : 1982
GLP : no data
Test substance :

Remark : - Initial 1-chloro-4-nitrobenzene concentration was checked measuring the DOC (dissolved organic carbon)
- The concentration of the algae suspension was measured via turbidimetry, screening the scattered light
- The test was performed under static conditions
Result : TGK (toxische Grenzkonzentration) is the minimum inhibitory concentration (3% inhibition).
Reliability : (2) valid with restrictions
Test procedure in accordance with national standard methods
Flag : Critical study for SIDS endpoint
23.07.2002 (43)

Species : other algae: Scenedesmus obliquus
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : 15.4
Limit test :
Analytical monitoring : no data
Method : other: comparable to OECD Guideline 201 (Algae, Growth inhibition test, 1984)
Year : 1996
GLP :
Test substance : other TS: no purity given

Test condition : - The test was performed under the following conditions: Temperature 24 +/-1°C, in a schedule of 12 h light : 12 h dark
- Stock solution prepared in aceton
- Initial cell concentration was approximately 1E4 cells/l
- The growth of algae was monitored by measuring the cell density after 0, 24, 48, 72 and 96 hours and the optical density was determined at 96 hours at 650 nm.
Reliability : (2) valid with restrictions
Comparable to guideline study with acceptable restrictions
23.07.2002 (36)

Species : other algae: Scenedesmus obliquus
Endpoint : growth rate
Exposure period : 48 hour(s)

Unit : mg/l
EC50 : 15.4
Limit test :
Analytical monitoring : no data
Method : other: OECD 201(Algae, Growth inhibition test, 1981)
Year : 1995
GLP : no data
Test substance : other TS: no purity given

Test condition : - The test was peformed under the following conditions: Temperature 20 +/- 1 °C, pH 7.2 +/- 0.2, 3600 lux continuous light provided by white Neon lamps
- Stock solution prepared in aceton.
- Initial cell concentration was approx. 1E4 cells/l
Reliability : (2) valid with restrictions
Guideline study without detailed documentation

23.07.2002 (45)

Species : other algae: Scenedesmus obliquus
Endpoint : growth rate
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : 18.1
Limit test :
Analytical monitoring : yes
Method : other: OECD 201 (Algae, Growth inhibition test, 1981)
Year : 2000
GLP : no data
Test substance : other TS: no purity given

Test condition : - The test was peformed under the following conditions: Temperature 20 +/-1 °C, 4000 lux continuous light provided by fluorescent lamps
- Concentrations of TS tested in the interval: [1.17E-5 - 7.32E-5] mol/l
- Initial cell concentration was approx. 1E4 cells/l
- Algae growth monitored by hemocytometry for 48 hours
Reliability : (2) valid with restrictions
Guideline study without detailed documentation

23.07.2002 (46)

Species : Scenedesmus pannonicus (Algae)
Endpoint : other: not specified
Exposure period :
Unit : mg/l
EC50 : 5.5
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 (Origin: Riedel-de Haen AG)

Remark : The period of exposure is not specified.
Reliability : (2) valid with restrictions
Comparable to guideline study. No detailed data given about the performance of the test.

23.07.2002 (13)

Species : Haematococcus pluvialis (Algae)

Endpoint	:	other: effect on O2 production by the algae
Exposure period	:	4 hour(s)
Unit	:	mg/l
EC50	:	4 calculated
Method	:	other: Verfahren nach Tuempling, im Warburgapparat
Year	:	1983
GLP	:	no data
Test substance	:	other TS: no purity given
Remark	:	Test criteria: Inhibitory effect on O2 production by the algae
Test condition	:	Cell density: 80000 cells/ml
Reliability	:	(3) invalid Documentation insufficient for assessment

23.07.2002 (33)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	aquatic
Species	:	Pseudomonas putida (Bacteria)
Exposure period	:	30 minute(s)
Unit	:	mg/l
EC10	:	59
Method	:	other: Bewertung toxischer Wasserinhaltsstoffe aus ihrer Inhibitorwirkung auf die Substratoxydation von Pseudomonas Stamm Berlin mit Hilfe polarographischer Sauerstoff-Messungen. Robra, K.H. gwf Wasser/Abwasser 117 (2), 80-86 (1976)
Year	:	1983
GLP	:	no data
Test substance	:	other TS: no purity given
Remark	:	- Initial TS concentration was checked measuring the DOC (dissolved organic carbon).
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

04.04.2002 (33)

Type	:	aquatic
Species	:	Pseudomonas putida (Bacteria)
Exposure period	:	10 hour(s)
Unit	:	mg/l
EC10	:	47
Analytical monitoring	:	no data
Method	:	other: Bringmann and Kuehn
Year	:	1982
GLP	:	no data
Test substance	:	other TS: no purity given
Remark	:	- Initial TS concentration was checked measuring the DOC (dissolved organic carbon). - The concentration of the bacteria suspension was measured via turbidimetry, screening the scattered light.
Result	:	EC10= Effective concentration (10 % inhibition)
Test condition	:	pH=7
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods

23.07.2002 (43)

Type	:	aquatic
Species	:	Photobacterium phosphoreum (Bacteria)
Exposure period	:	15 minute(s)
Unit	:	mg/l
EC50	:	34
Analytical monitoring	:	no data
Method	:	other: Microtox
Year	:	1986
GLP	:	no data
Test substance	:	other TS: >98% Purity
Remark	:	These data were also reported by the two athours participationg in another publication (Deneer, J.W. et al., QSAR study of the toxicity of nitrobenzene derivatives towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum, Aquatic Toxicology 15, 83-98 (1989)).
Result	:	By Deneer et al. (Deneer, J.W. et al., QSAR study of the toxicity of nitrobenzene derivatives towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum, Aquatic Toxicology 15, 83-98 (1989)) the result is given as log EC50: log EC50 = 2.33 (EC50 µmol/l)
Test substance	:	Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO; Merck, Purity 99%)
Reliability	:	(3) invalid Unsuitable test system

23.07.2002 (15)

Type	:	aquatic
Species	:	anaerobic bact. from a domestic water treatment plant
Exposure period	:	24 hour(s)
Unit	:	mg/l
TT	:	ca. 70
Analytical monitoring	:	no
Method	:	ETAD Fermentation tube method "Determination of damage to effluent bacteria by the Fermentation Tube Method"
Year	:	1982
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	TT=toxicity threshold
Source	:	Hoechst AG Frankfurt/Main
Reliability	:	(4) not assignable Original reference not available

29.04.2002 (30)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	Daphnia magna (Crustacea)
Endpoint	:	reproduction rate
Exposure period	:	21 day(s)
Unit	:	mg/l
EC50	:	2.07
EC10	:	.103
Analytical monitoring	:	yes
Method	:	other: OECD Guideline 202, part 2 (1984)

Year : 1986
GLP : no
Test substance : other TS: 99.7% Purity

Result : Statistical EC10 effective = 0.103 mg/l (EC10 nominal = 0.15 mg/l).
The analytical recovery at a test concentration of 1.58 mg/l was 69.2 %.
This value was used to calculate the effective concentration.
Test condition : - Semistatic procedure: test medium was renewed 3 times a week
- The following TS concentrations were tested: 0.16, 0.5, 1.58 and 5.0 mg/l
- Test organism: Daphnia magna Straus
- Per concentration 10 trials were carried out in parallel starting with 1 Daphnia in each vessel. Control consisted of 20 animals
- pH value, oxygen concentration and mortality were observed at the beginning of the test and after each test medium exchange
- The temperature of the test was around 20 °C and pH value 8.3 +/- 1.
Reliability : (1) valid without restriction
Guideline Study
Flag : Critical study for SIDS endpoint
28.11.2002 (47)

Species : Daphnia magna (Crustacea)
Endpoint : reproduction rate
Exposure period : 21 day(s)
Unit : mg/l
NOEC : .19
Analytical monitoring : yes
Method : other: Provisional procedure proposed by the Federal Environmental Agency for extended toxicology with Daphnia magna (01.01.1984)
Year : 1988
GLP : no data
Test substance : no data

Method : Determination of NOEC for reproduction rate, mortality and the time of the first appearance of offspring; 21d
Remark : - Semistatic test
- There were four parallel test vessels per concentration level and at least four vessels for the control. Each vessel was filled with 24 h-old Daphnia (1 animal/50 ml). The total number of daphnias per concentration level was 20. Test temperature 25 +/- 1 °C.
- Test organism: Daphnia magna Straus, strain IRCHA.
- Dilution water: synthetic fresh water, Hardness 2.5 mmol/l Ca + Mg, Na/K-Ratio: 10:1, pH = 8.0 +/- 0.2
- pH-values and oxygen-concentration were measured during the test in two tests-vessels per concentration level. The detected variation of these parameters had no negative influence on the organisms.
Result : Tested concentration range: 0.32 - 5.0 mg/l
NOEC minimum value = 0.19 mg/l, related to analytical recovery of 60 % at test concentrations 0.63 and 1.25 mg/l, detection limit 0.3 mg/l
NOEC nominal value = 0.32 mg/l
Reliability : (1) valid without restriction
Test procedure according national standard method. Reported in sufficient detail.
Flag : Critical study for SIDS endpoint
22.08.2003 (48) (41)

Species : Daphnia magna (Crustacea)
Endpoint : other: reproduction rate
Exposure period : 21 day(s)
Unit : mg/l

LOEC	:	1.8
Analytical monitoring	:	no data
Method	:	other: NEN 6502: Determination of chronic toxicity to Daphnia magna (1980)
Year	:	1989
GLP	:	no data
Test substance	:	no data
Method	:	Method of the Dutch Standardization Organization, Rijswijk, The Netherlands
Remark	:	- 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. - During the test daphnids were fed with Chlorella pyrenoidosa 3E+8 cells/l/day. Later, Deneer et al. (Deneer, J.W. et al., QSAR study of the toxicity of nitrobenzene derivatives towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum, Aquatic Toxicology 15, 83-98 (1989)) using the data of Maas-Diepeveen and van Leeuwen, state: - 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. The medium was saturated with air prior to use. - All daphnids used were <24 h old at the start of the experiments. - The oxygen content of all solutions did not decrease below 7.9 mg/l (85%). Mortality in the controls never exceeded 10 %.
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) = 1.05 = 11 µmol/l...LRCT(Rm)= 1.8 mg/l 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) = 1.31 = 20 µmol/l....LRCT(L)= 3.2 mg/l 3. Lethal concentration: LC50 (21 days) = 4.6 (95 % confidence limits: 4.3 - 5.0) mg/l In another publication, Deneer et al. (Deneer, J.W. et al., QSAR study of the toxicity of nitrobenzene derivatives towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum, Aquatic Toxicology 15, 83-98 (1989)), two of the authors presented the fist two chronic effects but reported the third to be IC50 = 21 d immobilization concentration log IC50 = 1.46 = 28 µmol/l.....IC50 = 4.5 mg/l
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods. No information about an analytical monitoring

23.07.2002 (15)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species	:	Phaseolus aureus (Dicotyledon)
Endpoint	:	growth
Exposure period	:	6 day(s)
Unit	:	mg/l
EC50	:	91

Method	:	other: germination and growth of seedlings in sand
Year	:	1961
GLP	:	no
Test substance	:	other TS: recrystallized
Remark	:	The test solution was prepared by dissolving 1-chloro-4-nitrobenzene in Hoagland nutrient solution. A definite amount of test solution was added to sand. Three concentrations were tested (20, 50, and 100 ppm by weight). Although author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmoles / liter.
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

03.05.2002 (49)

Species	:	other terrestrial plant: Cucumis sativus var. National Pickling
Endpoint	:	growth
Exposure period	:	6 day(s)
Unit	:	mg/l
EC50	:	132
Method	:	other: germination and growth of seedlings in sand
Year	:	1961
GLP	:	no
Test substance	:	other TS: recrystallized
Remark	:	The test solution was prepared by dissolving 1-chloro-4-nitrobenzene in Hoagland nutrient solution. A definite amount of test solution was added to sand. Three concentrations were tested (20, 50, and 100 ppm by weight). Although author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmoles / liter.
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

05.12.2002 (49)

Species	:	other terrestrial plant: Lactuca sativa Ravel R2
Endpoint	:	growth
Exposure period	:	14 day(s)
Unit	:	mg/l
EC50	:	3 calculated
Method	:	OECD Guide-line 208 "Terrestrial Plants, Growth Test"
Year	:	1991
GLP	:	no data
Test substance	:	other TS: various Chloro(nitro)benzenes but not 1-chloro-4-nitrobenzene
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 % - 1-chloro-4-nitrobenzene was not tested but wide range of other chloronitrocompounds including 1-chloro-2-nitrobenzene and 1-chloro-3-nitrobenzene. - The authors derived an equation for the QSAR for the relationship between log EC50 (y, in µmol/l) and the log Kow (x) for chloro(nitro)benzenes: $y = -0.46 x + 2.38$
Reliability	:	(2) valid with restrictions Guideline study with acceptable restrictions
Flag	:	Critical study for SIDS endpoint

06.12.2002 (50)

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

25.03.2002

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo : In vivo
Type : Toxicokinetics
Species : rat
Number of animals
Males : 3
Females : 0
Doses
Males : 0, 0.65, 6.5, 65 mg/kg bw/day (0.0325, 0.325, 3.25 mg/cm2)
Females :
Vehicle : other: acetone
Route of administration : dermal
Exposure time : 72 hour(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 remark
Year : 1991
GLP : no data
Test substance : other TS: purity: 99 %

Method : Groups of 3 male rats received single topical applications of radiolabelled p-Nitrochlorobenzene (0.65, 6.5 or 65 mg/kg bw/day). The treated clipped area (back and shoulder, demarcated area of 4 cm2) was covered with a nonocclussive protective device to prevent ingestion during grooming. Urine and feces were collected until 72 hours post application (0-4, 4-8, 8-24, 24-48, 48-72, metabolites were not determined). Volatiles were collected in ethanol traps until 72 hours post application. Rats were sacrificed 72 hours post application.

Result : At the three dose levels, 51-62 % of the dose was absorbed from the skin within 72 hours. The balance of the dose (24-30 %) was recovered on the protective device and the organic trap. The absorbed radioactivity was excreted in urine (43-45 %) and feces (5-12 %). Only a negligible increase of dermal absorption was noted when the dose increased from 0.65-6.5 mg/kg/day, but greater absorption occurred at the high dose of 65 mg/kg. The extent of urinary excretion of radioactivity was not significantly affected by dose over the range studied. The extent of fecal excretion increased with dose. The initial rate of urinary excretion was similar over the 0.65 to 6.5 mg/kg dose range (26-28% in 24 hours); the rate of urinary excretion was much lower at the high dose (12% in 24 hours). The initial rate of fecal exrection increased with dose over the 0.65 to 6.5 mg/kg/day range, but decreased notably at the high dose

Flag : Critical study for SIDS endpoint
12.08.2002 (51) (52) (53)

In Vitro/in vivo :
Type :
Species : other: see also Chapter 5.11: additional remarks
Number of animals
Males :
Females :
Doses
Males :

Females :
Vehicle :

12.05.2002

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 294 mg/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals : 10
Vehicle : other: polyethylene glycol
Doses : 100, 200, 300, 350, 400, 500, 600 mg/kg bw
Method : other: 10 rats/dose, single oral appl. by gavage, observation time: 14 days, statistical analysis
Year : 1979
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Value: (232 - 349 mg/kg bw)

Dose (mg/kg bw)	time of death	dead	number of rats with symptoms	used
100	-	0	0	10
200	2d	3	10	10
300	2-3d	5	10	10
350	2-3d	5	10	10
400	2-4d	8	10	10
500	2-3d	8	10	10
600	3h-4d	10	10	10

Symptoms: reduced general condition, cyanotic appearance, diarrhea, increased excretion of urine

Reliability : (2) valid with restrictions
no pathologic examination performed, no individual animal data
Flag : Critical study for SIDS endpoint
02.04.2003 (54)

Type : LD50
Value : = 550 mg/kg bw
Species : rat
Strain : other: no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other: no information given
Year :
GLP :
Test substance :

Reliability : (4) not assignable
lack of information
16.07.2002 (55)

Type : LD50
Value : = 555 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle :
Doses :
Method : other: no further information given
Year : 1982
GLP : no data
Test substance : other TS: no data

Reliability : (4) not assignable
lack of information
16.07.2002 (56)

Type : LD50
Value : = 780 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :

Reliability : (4) not assignable
lack of information
16.07.2002 (57)

Type : LD50
Value : = 500 mg/kg bw
Species : rat
Strain : Fischer 344
Sex : male
Number of animals :
Vehicle :
Doses :
Method : other: no further information
Year :
GLP :
Test substance :

Reliability : (4) not assignable
lack of information
16.07.2002 (58)

Type : LD50
Value : = 530 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: no further information
Year :
GLP :
Test substance :

Reliability : (4) not assignable
lack of information
16.07.2002 (59)

Type : LD50
Value : = 420 mg/kg bw
Species : rat
Strain : no data
Sex : female
Number of animals :
Vehicle : other: gummi arabicum in water
Doses : 120 -2100 mg/kg bw as 10 % solution
Method : other: according to Deichmann-LeBlanc, J. industr. 25, 415 (1943), gross pathology, histol. examination, post exposure observation: 14 d, number of animals/group is missing
Year : 1959
GLP : no
Test substance : other TS: no data on purity; Smp: 84°C

Remark : signs of intoxication: decreased motility, sedation, atony, urinary stammering;
24 hours post appl. sign. increase in heinz bodies (60 %);
MetHb levels increased: 22%, 35%, 53% (0.5, 1 resp. 2 hrs) at 420 mg/kg bw; death occurred within 2-7 days and in the highest dose one day after appl.;
Gross Pathol: hyperemia of the liver; Histopathol. exam.: fatty degeneration of the liver, slight degeneration of the kidneys
Reliability : (3) invalid
insufficient for assessment: number of animals missing, TS given as 10 % solution
16.07.2002 (60)

Type : LD50
Value : = 830 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :

Reliability : (4) not assignable
lack of information
16.07.2002 (61)

Type : LD50
Value : = 810 mg/kg bw
Species : rat
Strain :
Sex : male
Number of animals :
Vehicle :
Doses :
Method : other: according to Smyth et al., Am. Ind. Hyg. Ass.J. 23: 95-107 (1962): no further details given
Year : 1977
GLP : no data
Test substance : other TS: no data on purity

Remark : Value: (510 - 1120 mg/kg bw)
Reliability : (4) not assignable
lack of information
16.07.2002 (62)

Type : LD50
Value : = 565 mg/kg bw
Species : rat
Strain : Wistar
Sex : female
Number of animals : 10
Vehicle : other: sesame oil
Doses : 250, 400, 500, 560, 630, 1000, 1600 mg/kg bw
Method : other: 10 rats/dose,TS dissolved in sesame oil, observation time 14 days, pathologic examination, statistical analysis
Year : 1975
GLP : no
Test substance : other TS: purity: 99.9 %

Remark : Value: (540 - 591 mg/kg bw)
Mortality:
no mortality in the 250 mg- and 400 mg-group,
500 mg-group: 1/10, 560 mg-group: 3/10,
630 mg-, 1000 mg-, 1600 mg-group: 10/10
clinical signs of intoxication:
imbalance and cyanosis, death occurred inabdominal position
pathologic examination:
decedents: pale lungs, liver brown dotted
survivors: viscera appeared normal
Source : Hoechst AG Frankfurt/Main
Reliability : (2) valid with restrictions
study meets the criteria of today, but information on GLP is missing, individual animal data of pathologic examination are missing
Flag : Critical study for SIDS endpoint
02.04.2003 (63)

Type : LD50
Value : = 694 mg/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals : 10
Vehicle : other: sesame oil
Doses : 400, 500, 630, 800, 1000 mg/kg bw
Method : other: 10 rats/dose, TS disssolved in sesame oil, observation time 14 days, pathologic examination, statistical analysis
Year : 1976
GLP : no
Test substance : other TS: purity: ca 99.3 % (techn. pure)

Remark : mortality:
400 mg-gr.: 0/10, 500 mg-gr.: 1/10, 630 mg-gr.: 5/10,
800 mg-gr.: 5/10, 1000 mg-gr.: 10/10
mortality occurred within 245 min to 4 d post application
clinical signs of intoxication:
imbalance, abdominal position, cyanosis, tremor, increasing weakness
pathologic examination:
decedents: brownisch colored lungs, darkbrown liver
survivors: viscera appeared normal

Source : Hoechst AG Frankfurt am Main
Reliability : (2) valid with restrictions
study meets the criteria of today, but information on GLP is missing, individual animal data of pathologic examination are missing
Flag : Critical study for SIDS endpoint
02.04.2003 (64)

Type : LD50
Value : = 664 mg/kg bw
Species : rat
Strain : Wistar
Sex : female
Number of animals : 10
Vehicle : other: sesame oil
Doses : 320, 500, 800 mg/kg bw
Method : other: 10 rats/dose, Ts dissolved in sesame oil, observation time: 14 days, pathological examination, statistical analysis
Year : 1976
GLP : no
Test substance : other TS: purity: ca. 99.3 % (techn. pure)

Remark : mortality:
320 mg-gr,: 0/10, 500 mg-gr.: 1/10, 800 mg-gr.: 8/10
death occurred within 2 to 4 days post treatment
clinical signs of intoxocation:
imbalance, abdominal position, cyanosis, weakness
pathological examination:
decedents: brownish colored lungs , dark brown liver
survivors: viscera appeared normal
Source : Hoechst AG Frankfurt/Main
Reliability : (2) valid with restrictions
study meets the criteria of today, but information on GLP and individual animal data are missing
Flag : Critical study for SIDS endpoint
19.08.2002 (65)

Type : other
Value :
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : 80, 100, 200, 400, 600 mg/kg bw
Method : other: no information
Year : 1944
GLP : no
Test substance : other TS: no data on purity

Remark : Number of animals: 3-5
Result : mortality:
80 mg/kg bw: 0/3; 100 mg/kg bw: 0/3; 200 mg/kg bw: 0/5;
400 mg/kg bw: 1/3; 600 mg/kg bw: 5/5
Reliability : (4) not assignable
lack of information
16.07.2002 (66)

Type : other: ALD (Approximate Lethal Dose)
Value : ca. 670 mg/kg bw

Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	peanut oil
Doses	:	up to 670 mg/kg bw
Method	:	other: TS administered as 20 % solution in peanut oil warmed to 50 °C, no further information
Year	:	1949
GLP	:	no
Test substance	:	other TS: no data on purity
Remark	:	Number of animals: no data
Result	:	All treated rats became cyanotic. In rats treated with sublethal doses it lasted 24 hours after treatment. The rat receiving 670 mg/kg bw lived nearly 48 hours after dosing. Pathological examination revealed necrosis and hemorrhage of the liver and incipient necrosis of the convoluted tubules of the kidneys. The bladder contained blood tinged urine.
Reliability	:	(4) not assignable lack of information

16.07.2002 (67)

Type	:	LD50
Value	:	= 650 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: no details given
Year	:	1966
GLP	:	no
Test substance	:	other TS: no data on purity
Remark	:	Value: (470-896 mg/kg bw) Signs of intoxication: lazy, reduced motility, lateral position, dyspeptic symtoms, cyanosis (paws, snout, belly), death occurred only within 3 days; color of blood: chocolate-brown
Reliability	:	(4) not assignable lack of information

16.07.2002 (68)

Type	:	LD50
Value	:	= 440 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: no further information given
Year	:	1982
GLP	:	no data
Test substance	:	other TS: no data on purity
Reliability	:	(4) not assignable

lack of information

16.07.2002 (56)

Type : LD50
Value : = 970 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :

Reliability : (4) not assignable
lack of information

16.07.2002 (61)

Type : LD50
Value : = 1410 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: according to Smyth et al., Am. Ind. Hyg. Ass. J. 23: 95-107 (1962): no further details given
Year : 1977
GLP : no data
Test substance : other TS: no data on purity

Reliability : (4) not assignable
lack of information

16.07.2002 (62)

Type : LD50
Value : = 520 mg/kg bw
Species : rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: no further information given
Year :
GLP :
Test substance :

Reliability : (4) not assignable
lack of information

16.07.2002 (69)

5.1.2 ACUTE INHALATION TOXICITY

Type : LCLo
Value : ca. 16100 mg/m³
Species : rat
Strain : other: Crl:CD
Sex : male

Number of animals	:	10
Vehicle	:	other: air
Doses	:	2.63, 2.84, 3.27, 3.35, 3.73, 6.12,9.47, 16.1 mg/l (approx. 2630,2840, 3270, 3350, 3730,6120, 9470, 16100 mg/m³)
Exposure time	:	4 hour(s)
Method	:	other: head-only exp., atmosph. contained vapor and micro-crystaline particles (particle size: 2.7 up to greater 90 u, respirable fraction: 6.8 to 92.3 %), 16100 mg/m³ was the highest practical concentration that could be generated
Year	:	1981
GLP	:	no data
Test substance	:	other TS: technical grade: 99.2 %
Remark	:	The purpose of the study was to determine an LC50
Result	:	Clinical signs of toxicity were related to dose. - during and immediately following exposure: cyanosis, corneal opacity, abnormal arched-back posture, lethargy, reddish-brown nasal and frothy mouth discharges,tachypnea, semi-prostration, weight loss: 6-13 % within the first 24 hours with normal gain thereafter - from 1 - 14 days post-exposure: pallor, lacrimation, alopecia, corneal opacity, stained perineal area, dermal irritation, death: 16100 mg/l: 1/10 3 days post exposure
Reliability	:	(2) valid with restrictions study meets the criteria of today, but information on GLP is missing and number of animals which show effects is missing
Flag	:	Critical study for SIDS endpoint

06.08.2002 (70)

Type	:	other: IRT
Value	:	
Species	:	rat
Strain	:	Wistar
Sex	:	male/female
Number of animals	:	12
Vehicle	:	other: air
Doses	:	
Exposure time	:	7 hour(s)
Method	:	other: 6 rats/sex, nose-only-exposition, 7 hours, post exposure observation time: 14 days, pathological examination
Year	:	1981
GLP	:	no
Test substance	:	no data
Remark	:	the analytical concentration in the chamber was 50 min. after the start of the test: 53 mg/m3 air 170 min after the start of the test: 74 mg/m3 air 290 min after the start of the test: 77 mg/m3 air
Result	:	The 7 hour inhalation period was tolerated without mortality. The only signs of intoxication during exposure were narrowed palpebral fissures and tachypnea. After termination of the exposure behaviour returned to normal. Pathologic examination after the 14 days-observation period revealed no remarkable findings.
Source	:	Hoechst AG Frankfurt/Main
Reliability	:	(2) valid with restrictions no information on GLP, no data on purity
Flag	:	Critical study for SIDS endpoint

12.08.2002 (71)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : = 750 mg/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals : 10
Vehicle : other: polyethylene glycol
Doses : 500, 600, 700, 900, 1000, 1200 mg/kg bw
Method : other: 10 rats/dose, single dermal appl., fixed with a gauze dressing for 24 hours, observation time: 14 days
Year : 1979
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Value: (670 - 830 mg/kg bw)

Dose (mg/kg bw)	time of death	dead	number of rats with symptoms	used
500	-	0	10	10
600	3d	2	10	10
700	3-4d	5	10	10
900	3-4d	8	10	10
1000	3-4d	8	10	10
1200	3h-2d	10	10	10

Symptoms: sedation, cyanotic appearance, unkempt fur, palmospasm, low body temperature
Reliability : (2) valid with restrictions
no pathologic examination was performed
Flag : Critical study for SIDS endpoint
19.08.2002 (72)

Type : LD50
Value : = 16000 mg/kg bw
Species : rat
Strain : no data
Sex : female
Number of animals :
Vehicle :
Doses : up to 16000 mg/kg bw
Method : other: no details of application mode given, pathologic (gross and histologic) evaluation, post appl. observation period: 14 d
Year : 1959
GLP : no data
Test substance : other TS: no data on purity; Smp: 82-84 C

Remark : signs of intoxication:
death occurred only at the highest dose of 16000 mg/kg bw within 48 hours,
all animals lost weight but recovered within the 14 day observation period (weight remained lower compared to controls), one rat with urinary stemmering, 30 min post application increasing methaemoglobinemia (10-70% single animals); mean MetHb level: 9%, 37%, 43% (0.5, 1 resp. 2 hrs) at a dose of 16,000 mg/kg bw; formation of Heinz bodies (10-30%)
gross pathologic examination:

hyperemia of the liver; Histopathol. exam.: marked degeneration of the kidneys, necrotic renal epithilial cells

Reliability : (4) not assignable
documentation insufficient for assessment

06.08.2002 (60)

Type : LD50
Value : = 1722 mg/kg bw
Species : rat
Strain : Wistar
Sex : female
Number of animals : 6
Vehicle : other: sesame oil
Doses : 1000, 1250, 1600, 2000 mg/kg bw
Method : other: 6 rats/dose, TS bloated in sesame oil, applied on the shaved back, fixed with alu foil and bandage for 24 hours, then washed, observation period: 14 day, pathologic examination, body weight, statistical analysis
Year : 1975
GLP : no data
Test substance : other TS: purity: 99.9 %

Remark : mortality:
1000 mg-gr.: 0/6, 1250 mg-gr.: 2/6, 1600 mg-gr.: 2/6,
2000 mg-gr.: 4/6
mortality occurred within 2 to 4 days
clinical signs of intoxication:
poor general condition, cyanosis,brown colored urin, treated skin grey-blue
pathologic examination:
decedents: not possible because autolysis
survivors: viscera appeared normal
Source : Hoechst AG Frankfurt/Main
Reliability : (2) valid with restrictions
study meets the criteria of today, but information on GLP is missing, pathologic examination was not possible
Flag : Critical study for SIDS endpoint
02.04.2003 (73)

Type : LD50
Value : 2000 - 3160 mg/kg bw
Species : rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: no further information
Year :
GLP :
Test substance :

Remark : exposure time: 84 h
Reliability : (4) not assignable
documentation insufficient for assessment

16.07.2002 (74)

Type : LD50
Value : > 3040 mg/kg bw
Species : rabbit
Strain :

Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Reliability	:	(4) not assignable lack of information

06.08.2002 (59)

Type	:	LD50
Value	:	ca. 3020 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	4
Vehicle	:	other: undiluted (warmed to make suitable for dosing)
Doses	:	2000, 2510, 3160, 3980, 5010 mg/kg bw
Method	:	other: 2 rabbits /sex/dose, single dermal application, exposure: 24 hours, 14 day post exposure observation, gross necropsy
Year	:	1983
GLP	:	no data
Test substance	:	other TS: no data on purity

Remark :

Dosage: mg/kg bw	m: mort.//day of death	f: mort.//day of death
2000	0/2 // -	0/2 // -
2510	0/2 // -	2/2 // 4-5
3160	1/2 // 5	1/2 // 6
3980	2/2 // 4-5	2/2 // 4
5010	1/2 // 4	2/2 // 4

observations: males and females
lethargy (lasting up to one day),increasing weakness, collapse, and death
gross necropsy: decedents (males and females) hemorrhagic areas of the lungs, liver and kidney discoloration, spleen darkened, gastrointestinal inflammation survivors (males and females) viscera appeared normal
LD50(male) : 3550 mg/kg bw (2910 - 4330 mg/kg bw)
LD50(female): 2510 mg/kg bw (2030 - 3090 mg/kg bw)
LD50(combined): 3020 mg/kg bw (2540-3560 mg/kg bw)

Reliability	:	(2) valid with restrictions study meets the criteria of today, but information on GLP and on purity of TS are missing, no individual animal data
Flag	:	Critical study for SIDS endpoint

02.04.2003 (75)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD50
Value	:	= 420 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Remark	:	Formation of MetHb: 12%, 21%, 34% (0.5, 1 resp. 2 hrs) at a dose of 420

mg/kg bw; symptoms (1.5 h post application of 420 mg/kg bw resp. 20 min post application of higher doses): ataxie, tonic and clonic spasm, foamy discharge from nose, lateral position, brown-blue discoloration of skin and mucous membrane

02.08.2002 (60)

Type	:	LD50
Value	:	ca. 740 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	s.c.
Exposure time	:	
Remark	:	LD50 = 4.69 mM/kg bw

(76)

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	500 mg
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	water
PDII	:	
Result	:	slightly irritating
Classification	:	
Method	:	other: according to: Code of Federal Regulations, Title 16, Section 1500.41, dee also freetexr ME
Year	:	1980
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	TS was mixed with 1 or 2 drops of water, skin was shaved (intact and sarified) , 24 hours application time, reading times, 24,48 72 hours and day 8 post application, control-reading 48 hours and day 8 post appl. was not included in the evaluation
Remark	:	intact skin: reading: erythema: 24hs/score 0, 72hs/score 0, reading: oedema: 24hs/score 2.17/4 (max. score), 72hs/score 1.0 /4 scar. skin: reading: erythema: 24hs/score 0.17/4 (max. score) 72hs/score 0 reading: oedema: 24hs/score 1,67/4 (max score) 72hs/score 1,0 /4
Reliability	:	(2) valid with restrictions study meets the criteria of today, but information on GLP is missing
Flag	:	Critical study for SIDS endpoint

27.11.2002 (77)

Species	:	rabbit
Concentration	:	
Exposure	:	

Exposure time	:	
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: no data
Year	:	
GLP	:	
Test substance	:	

Reliability : (4) not assignable
Documentation insufficient for assessment
16.07.2002 (59)

Species	:	rabbit
Concentration	:	20 mg
Exposure	:	no data
Exposure time	:	24 hour(s)
Number of animals	:	
Vehicle	:	no data
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: according to Draize et al., J. Pharm.(Lond.) 82, 377 (1944), observation up to 96 hours, no further details of the study available4
Year	:	1959
GLP	:	no
Test substance	:	other TS: no data on purity, Smp.: 82-84°C

Remark : no signs of skin irritation were observable
Reliability : (4) not assignable
Documentation insufficient for assessment
16.07.2002 (60)

Species	:	rabbit
Concentration	:	other: undissolved
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	other: none
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: according to: Code of Federal Regulations, Title 16, Section 1500.41 see also freetext ME
Year	:	1976
GLP	:	no
Test substance	:	other TS: purity: ca. 99.3 % (techn. pure)
Method	:	skin was shaved (intact and sarified) , 500 mg TS 24 hours application time, reading times: 24, 48 72 hours post application
Remark	:	Two rabbits showed slightly irritation (irritation index: 0.1, max. 8.0). One rabbit showed slight cyanotic appearance after 24 hours which was judged as significant resorption through the skin.
Source	:	Hoechst AG Frankfurt/Main
Reliability	:	(2) valid with restrictions study meets the criteria of today, but information on GLP is missing, no individual animal data

Flag	:	Critical study for SIDS endpoint
02.04.2003		(78)
Species	:	rat
Concentration	:	other: 1% and 5 % solution
Exposure	:	no data
Exposure time	:	no data
Number of animals	:	
Vehicle	:	other: ethanol
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: ear: 11 exposures of 1% TS in 95% ethanol; 12 exposures of 5% TS in pure ethanol; abdomen: 12 exposures of 5% TS in 95% ethanol, bandaged
Year	:	
GLP	:	
Test substance	:	
Remark	:	The animal receiving 5% solution on the abdomen showed cyanotic appearance.
Reliability	:	(3) invalid Study doesn't meet the criteria of today
16.07.2002		(66)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	
Dose	:	.1 ml
Exposure time	:	
Comment	:	
Number of animals	:	6
Vehicle	:	other: no data
Result	:	not irritating
Classification	:	
Method	:	other: according to: Code of Federal Regulations, Title 16, Section 1500.42 , see also freetext ME
Year	:	1980
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	100 ul polymorphic TS into 1 eye of each rabbit, no rinsing, reading times: 24, 48, 72 hours and day 8 d after application
Remark	:	Dose: (polymorphic substance) reading: summary of the effects (cornea, iris, conjunctiva, only conjunctiva affected): 24 hs: score 2/110 in 6/6 rabbits; 48 hs: score 2/110 in 4/6 rabbits; 72 hs: score 2/110 in 2/6 rabbits; 8d: score 0/110 in 6/6 rabbits (not included for calculation of irritation index) primary eye irritation index: 1.3
Reliability	:	(2) valid with restrictions study meets the criteria of today, but information on GLP is missing
Flag	:	Critical study for SIDS endpoint
19.08.2002		(79)

Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	not irritating
Classification	:	
Method	:	other: no data
Year	:	
GLP	:	
Test substance	:	

Reliability : (4) not assignable
Documentation insufficient for assessment
16.07.2002 (59)

Species	:	rabbit
Concentration	:	other: undissolved
Dose	:	20 other: mg
Exposure time	:	
Comment	:	
Number of animals	:	2
Vehicle	:	
Result	:	slightly irritating
Classification	:	
Method	:	other: according to Draize et al., J. Pharm.(Lond.) 82, 377 (1944), observation period: at least 96 h
Year	:	1959
GLP	:	no
Test substance	:	other TS: no data on purity, Smp.: 82-84 C

Remark : dose: 20 mg
effects: maximal effects at 1 h and 24 h after application of the test substance (Score: 9), after 48 hs Score: 1 and after 96 hs score: 0; no lesions of the cornea observable,
(no further data)

Reliability : (4) not assignable
Documentation insufficient for assessment
06.08.2002 (60)

Species	:	rabbit
Concentration	:	other: undissolved
Dose	:	100 other: mg
Exposure time	:	
Comment	:	
Number of animals	:	6
Vehicle	:	other: none
Result	:	not irritating
Classification	:	
Method	:	other: according to: Code of Federal Regulations, Title 16, Section 1500.42 see also freetext ME
Year	:	1977
GLP	:	no
Test substance	:	other TS: purity: ca. 99.3 % (techn. pure)
Method	:	100 mg undissolved TS into 1 eye of each rabbit, reading times: 1, 7, 24, 48, 72 hours and day 8 after application, rinsing 24 hours after application

Remark	:	Irritation index 6, maximum 110
Source	:	Hoechst AG Frankfurt/Main
Reliability	:	(2) valid with restrictions study meets the criteria of today, but information on GLP is missing, no individual animal data

02.04.2003 (78)

Species	:	rabbit
Concentration	:	other: undissolved
Dose	:	10 other: mg
Exposure time	:	
Comment	:	
Number of animals	:	2
Vehicle	:	none
Result	:	slightly irritating
Classification	:	
Method	:	other: 10 mg TS was placed into the right conjunctival sac of each of 2 rabbits: after 20 sec. the treated eye of 1/2 rabbits was washed with tap water for 1 min., the other was not washed; observation: at 1 and 4 hours, at 1, 2, 3 day
Year	:	1982
GLP	:	no data
Test substance	:	other TS: purity: 99.5 %

Result	:	dose: 10 mg unwashed rabbit eye: no corneal, irritic or conjunctival effects washed rabbit eye: small area od transient slight corneal cloudiness (normal 4 hours after treatment), no conjunctival or irritic effects (scores not available)
Reliability	:	(2) valid with restrictions study meets the criteria of today, but information on GLP is missing
Flag	:	Critical study for SIDS endpoint

12.08.2002 (80)

5.3 SENSITIZATION

Type	:	Draize Test
Species	:	guinea pig
Concentration	:	1st: Induction 3 % other: no data 2nd: Challenge .3 % other: no data 3rd:
Number of animals	:	
Vehicle	:	other: acetone
Result	:	not sensitizing
Classification	:	
Method	:	other: according to Draize et al., J. Pharm.(Lond.) 82, 377 (1944): induction exposure by skin painting with a 3 % solution in acetone, challenge exposure by dermal application of a 0.3 % solution in acetone
Year	:	1959
GLP	:	no
Test substance	:	other TS: no data on purity, Smp.: 82-84°C

Reliability	:	(4) not assignable Documentation insufficient for assessment

06.08.2002 (60)

Type	:	no data

Species	:	guinea pig
Number of animals	:	
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	
Year	:	1935
GLP	:	
Test substance	:	
Remark	:	in the case of p-chloronitrobenzene (among other chloro- and nitro-substitution products of benzene) no details concerning the experimental design are given; other test compounds which are described in the study were tested by intracutaneous or dermal induction exposure and by intracutaneous or dermal challenge exposure
Reliability	:	(3) invalid Documentation insufficient for assessment

16.07.2002 (81) (82)

Type	:	other
Species	:	mouse
Concentration	:	1st: Induction .05 other: M 2nd: Challenge .016 other: M 3rd:
Number of animals	:	5
Vehicle	:	other: acetone
Result	:	not sensitizing
Classification	:	
Method	:	other: 5 mice,induction made on shaved back, non irritant conc. was chosen for challenge and was applied to the surface of one ear, reading after 24, 48, 72 h, no further details given
Year	:	1991
GLP	:	no data
Test substance	:	other TS
Remark	:	Concentration: Induction: 0.05 M; Challenge: 0.016 M
Reliability	:	(4) not assignable Documentation insufficient for assessment

12.08.2002 (83)

Type	:	other: modified Draize test
Species	:	guinea pig
Concentration	:	1st: Induction 1 % 2nd: Challenge 1 % 3rd:
Number of animals	:	10
Vehicle	:	other: acetone
Result	:	not sensitizing
Classification	:	
Method	:	other: 3 drops of a 1 % solution to the clipped area of the skin for 5 d; on d7 1 drop of the 1 % solution to an untreated area of the skin; reading time not mentioned
Year	:	1973
GLP	:	no
Test substance	:	other TS: no data
Reliability	:	(3) invalid the study documentation is incomplete and the methodology employed is no longer in use

02.04.2003 (84)

Type : other: modified Freunds complete adjuvant test
Species : guinea pig
Concentration : 1st: Induction 10 %
2nd: Challenge 10 %
3rd:
Number of animals : 10
Vehicle : other: acetone
Result : sensitizing
Classification :
Method : other: 3 drops (10 % sol.) to the clipped area of the skin; 22nd day inj. of Freund-adjuvans and TS into the hind paw (0.5 mg/kg bw), d28 1 drop (10 % sol.) to an untreated clipped area of the skin; reading time not mentioned
Year : 1973
GLP : no
Test substance : other TS: no data

Remark : the allergenic activity of p-chloronitrobenzene is less marked than that of 2,4-dinitrochloroben-zene but p-chloronitrobenzene provokes stronger sensitisation effects than o-chloronitrobenzene
Reliability : (3) invalid
the study documentation is incomplete and the methodology employed is no longer in use

02.04.2003 (84)

Type : other: rats were exposed via inhalation for 5 months
Species : rat
Number of animals :
Vehicle :
Result : sensitizing
Classification :
Method : other: the rats were exposed via inhalation to p-chloronitrobenzene for 5 months; test concentration: 0.000008 mg/l (a concentration which has a minimal toxic effect), control group
Year :
GLP :
Test substance :

Reliability : (3) invalid
the study documentation is incomplete and the methodology employed is no longer in use

02.04.2003 (84)

5.4 REPEATED DOSE TOXICITY

Type : Sub-acute
Species : rat
Sex : male
Strain : other: Crl:CD
Route of admin. : inhalation
Exposure period : 2 w
Frequency of treatm. : 6 h/d, 5 d/w (10 exposures)
Post exposure period : 14 d
Doses : 0, 0.05, 0.29 or 0.64 mg/l (approx. 50, 290, 640 mg/m³)
Control group : no
LOAEL : = 50 mg/m³

Method : other: 16 rats/dose, head-only exposure, controls to air only
additional information: see freetxt ME
Year : 1984
GLP : no data
Test substance : other TS: purity: 99.2 %

Method : Concentration measured every hour, body weight and observation daily (except weekends, during exposure period and 14-day post exposure), urinalysis: 10 rats/group following the nineth exposure, 5 rats per group on the thirteenth day of recovery, blood collecting from 10 rats every 2nd day inclusive recovery period, 6rats/exposure group exclusively used for methemoglobin- and Hb-determination throughout the exposure and recovery phases, 5 rats sacrificed after 10 exp. and 5 rats sacrificed after recovery period for clin. and pathol. examination, organ weights, statistical methods: LSD, Dunnett's test, Bartlett's test, and several other tests if applicable
Remark : see also chapter 5.8.3. for reproductive organ evaluation
Result : mortality: 1/16 in the o.64 mg/l-gr. due to Corynebacteria kutscheri-infection
clinical signs of toxicity:
Control, 0.05, 0.64 mg/l: 12-20 % of the rats displayed lung rales during 2-3 d in the 2nd week of exposure
0.29,0.64 mg/l: stained fur, pallor, allopecia
0.64 mg/l: hyperactivity during exposure and recovery period
Clinical pathology:
During exposure phase, methemoglobin levels reached peaks of 19, 38 and 52 %, respectively but returned to normal values during recovery; treatment related [control versus low/mid/high dose] hemolytic effects: decrease in erys [7.05 versus 5.44/4.44/ 3.71 MM/MM], hemoglobin [15.2 versus 13.9/14.1/12.7 g/dl] and hematocrit [40 versus 37/37/34 %], increase in white blood cell count [11.5 versus 13.9/29.8/41.8 M/cm]; compensatory increase in red blood cell proliferation: increase in MCV [57 versus 68/85/92 FL], MCH [22 versus 26/32/34 pg], polychromasia, nucleated red blood cells [0.1 versus 0.4/1.6/2.4 NRBC/100 WBC], increase in serum activity of AST [66 versus 88/99/124 IU], increase in urobilinogen excretion [0.9 versus 1.0/1.8/2.7 mg/dl], increase in serum urea nitrogen concentration (16.8 versus 19.2/20.2/21.9 mg%); recovery period: decreased RBC counts and increased MCV and MCH persisted (magnitude less than following 10 exposures), hematocrit values higher, MCHC, platelet, leucocyt and monocyt counts were lower than these of controls
Pathology:
all exposure levels: dark brown and enlarged spleen, ncreased spleen weight (mean spleen weights were 260, 381, and 505 % of the control spleen), microscopicallyconfirmed as hyperplastic red pulp (congestion, increase in erythroheopoiesis and hemosiderin,
0.29 mg/l- and 0.64 mg/l-group: dose-dependant decrease in mean body weight, hyperplastic bone marrow with decrease of the M/E ratio; mild kidney changes (hyaline droplet degeneration), not present following the 14 d-recovery period;
0.29 and 0.64 mg/l: slight increase in relative liver and kidney weight
Reliability : (2) valid with restrictions
no data on GLP, there are more relevant studies using a longer exposure time and therefore more relevant for the assessment.

06.08.2002 (85)

Type : Sub-chronic
Species : rat
Sex : male/female
Strain : other: F344/N
Route of admin. : inhalation

Exposure period	:	13 w
Frequency of treatm.	:	6 h/d, 5 d/w
Post exposure period	:	no
Doses	:	0, 1.5, 3, 6, 12 or 24 ppm (= ca. 0.0, 9.81, 19.62, 39.24, 78.48 or 156.96 mg/m³ air)
Control group	:	yes
LOAEL	:	ca. 9.81 mg/m³
Method	:	other: see freetext Me
Year	:	1993
GLP	:	yes
Test substance	:	other TS: purity: approx. 99 %

Method :
10 rats/sex/group, whole body exposure,
Clin. Chem., hematol., bw., org. weight, compl. necropsies on all animals, compl. histopathol. in all control rats and 24 ppm gr. and rats that died, gross lesions and selec. organs of rats < 24 ppm-grps,
add. 10 rats/sex/conc: clin pathol. ad d3, d23,
statistical methods: parametric multiple comparisons procedures of Williams or Dunnett, nonparametric multiple comparisons methods of Shirley or Dunn, Jonckheere's test or trend-sensitive test, outlier test of Dixon and Massey

Remark :
although a no-observed-effect level was not found for histopathology, most effects at the lower exposure levels were minimal.
see also chapter 5.8.3 for reproductive organ evaluation

Result :
no clear clinical signs of toxicity,
no rat died, 3 ppm-gr.: 1 female killed moribund due to malocclusion,
mean body weight gain similar to those of the respective controls.
haematology, male(m) and female(f):
concentration-related increase in methaemoglobinaemia (m: significant (sign.) from 1.5 ppm at day(d)3 at all time points with max. of 4.13 g/dl at d3 in 24 ppm-gr; f: sign. from 1.5 ppm at d3 at all time points with max. of 5.90 g/dl at d3 in 24 ppm-gr), reticulocyte count (m: sign. d3: 1.5 and 24 ppm, d23 >= 6 ppm, week(w)13 >= 1.5 ppm; f: sign. d3 24 ppm, d23 >= 3 ppm, w13 >= 3 ppm), nucleated erythrocytes and leucocyte count (predominantly at the highest dose groups in males and females); concentration-related decrease in haematocrit, haemoglobin and RBC at all time points; concentration and time-dependent alterations of RBC morphology, severity decreased with decreasing concentration. RBC indices (MCV, MCH, MCHC) were increased in males and females exposed to 12 ppm and above at d23 and exposed to 6 ppm and above at w13. MCV and MCHc were increased in females exposed to 1.5 ppm and above at w13, MCHC in males and females exposed to 24 ppm at d3
clinical chemistry, males and females:
Changes in alanine aminotransferase (decrease), sorbitol dehydrogenase (increase) and alkaline phospatase activities (decrease)
bile acid levels increase (m: >=3 ppm, all almost every time points, f:>=6 ppm d3, d23 >=12 ppm). Increase in serum activities of sorbitol dehydrogenase in various exposure groups at different time points
pathology:
increase in abs. and rel. spleen weight (m >=3 ppm, f >=6 ppm), liver weight (m: 24 ppm, f >=6 ppm), kidney (m and f: 24 ppm), decrease in abs. and rel. testes weight (24 ppm); incidence of enlarged and darkened spleens in male and female rats increased with increasing concentrations; 12, 24 ppm: kidneys darkened (female only), mediastinal lymph nodes enlarged (males and females)
histopathology:
Increasing in severity (average severity is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked) and incidences with increasing concentration (contr., low to high dose): bone marrow:

haematopoietic cell proliferation (m: = 0/10, 0/3/10/10/10(2.8), f: 0/10, 0/9/10/10/10(3.8)) hadrian gland: chronic inflammation (m: 2(1), 1/1/3/1/8(2.2), f: 1/10(1.0), 2/4/5/8/10(3.0)), kidney: hyaline droplet nephropathy (m: 0/10, 8/9/10/10/10(3.0)) and tubule pigment (m: 0/10, 0/0/0/8/10(1.0), f: 0/10, 0/0/10/10/10(3.0)) liver: haemosiderin (m: 0/10, 0/0/0/9/10(1.0), f: 0/10, 0/7/10/10/10(2.4)), mediastinal lymph nodes: histiocytic hyperplasia (m: 0/10, 0/0/0/4/9(2.3), f: 0/10, 0/0/0/6/10(2.7)), spleen: congestion (m:0, 10/10/10/10/10(3.0), f: 0/10, 10/10/10/10/10(3.0)), haemosiderin (m:0/10, 10/10/10/10/10(1.0), f: 3/10(1.0), 10/9/10/10/10(1.0)) haematopoietic cell proliferation m: 0/10,0/10/9/10/10(1.0)), f: 0/10, 0/9/10/9/10(2.0)) capsular fibrosis (m: 0/10, 0/4/8/10/10(2.1), f: 0/10, 0/2/10/10/10(2.3)), testis atrophy (m: 1/10(4.0), 2/1/0/1/10(1.6))

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

12.08.2002 (86) (53) (87)

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	4 w
Frequency of treatm.	:	6 h/d, 5 d/w
Post exposure period	:	no
Doses	:	0, 5, 15 or 45 mg/m³ (see freetext RM)
Control group	:	yes, concurrent no treatment
LOAEL	:	= 5 mg/m³
Method	:	other: 10 rats/sex/dose exposed to an aerosol (see RM), for further information see freetext ME
Year	:	1986
GLP	:	yes
Test substance	:	other TS: purity: 99.43 %

Method : records of clin. signs of toxicity, body weight, ophthal. exam., hematology at week 2 and 4, clin. chemistry, gross (all groups) and histopathol. changes (control and high- dose group, spleen, all groups), statistical analysis: Bartlett's test, one-way analysis of variance followed by Dunnett's test, Kruskal-Wallis test, Dunn's Summed Rank test, Jonckheere's test, standard linear regression

Remark : for generation of an aerosol, p-chloronitrobenzene(PNCB) was dissolved in ethylene glycol monoethyl ether (EGME) at concentrations of 0.33, 1.10, 3.3 % (weight/volume). The test solution was then atomized and conducted into the inhalation chambers; analytical concentrations: 0, 6, 19, 46 mg/m³

Result : all concentration levels:
no mortality, mean weekly body weights comparable to control groups, no ocular abnormalities identifiable during the ophthalmoscopic examination at study termination, concentration-dependent increasing degree of cyanosis clinical chemistry:
according to the authors, changes at 4 w not related to treatment because they were within historical control limits, and exhibit no consistent dose-reponse relationship (no details available)
significant hematological findings:
increase in methemoglobin: males: w 2 at 45 mg/m³ (14.0 versus 3.2 % in controls(c)) and w 4: at 5, 15, 45 mg/m³ (3.1, 3.1, 7.7 % versus 0.9 % in c), females: week 2 at 15, 45 mg/m³ (11.5, 14.9 % versus 2.7 % in c) and week 4 at 15, 45 mg/m³ (5.0,12.3% versus 1.8 % in c); increase in WBC: (week 2, males, 45 mg/m³, females, 15, 45 mg/m³), (week 4, males and females, 45 mg/m³);

decrease in RBC: (week 2, males, 45 mg/m³ and females, 15, 45 mg/m³), (week 4: males, females, 15, 45 mg/m³); decrease in hemoglobin: (week 2, males, females, 15, 45 mg/m³), (week 4, males, females, 45 mg/m³); decrease in hematocrit: (week 2, females, 15, 45 mg/m³), week 4, males, 45 mg/m³, females, 5, 15, 45 mg/m³);
significant gross and histopathological changes:
liver: increase in weights at 45 mg/m3 (male, absolute and relative, females relative)
spleen: increase in weights at 45 mg/m³ (males, females, absolute and relative), increases in splenic congestion, extramedullary hematopoiesis, hemosiderosis, at 15 and 5 mg/m³: greater amounts of iron-positive pigments than in controls

Reliability : (2) valid with restrictions
histopathologic evaluation was not performed from all rats

Flag : Critical study for SIDS endpoint

12.08.2002 (88) (89)

Type : Sub-acute
Species : rat
Sex : male/female
Strain : other: F344/N
Route of admin. : inhalation
Exposure period : 2 weeks
Frequency of treatm. : 6 hours/day, 5 d/week
Post exposure period : no
Doses : 0, 1.5, 3, 6, 12, or 24 ppm (= ca. 0.0, 9.81, 19.62, 39.24, 78.48, or 156.96 mg/m³ air)
Control group : yes
LOAEL : = 9.81 mg/m³
Method : other: 5 rats/sex/group, whole body exposure, for further information see freetext ME
Year : 1993
GLP : yes
Test substance : other TS: purity: approx. 99 %

Method : rats were observed twice daily, weighted on d1, d8 and at necropsy, clinical observations recorded daily, complete necropsies on all rats, histopathologic evaluation of all rats in the controls and the 24 ppm group, gross lesions in all lower exposure groups, target organs identified and examined until a NOAEL was determined (kidney, liver, spleen)

Result : all rats survived,
clinical signs of toxicity: 24 ppm, males and females: hypoactivity and pale skin; 12 ppm males and females: hypoactivity
final body weight gains of the exposed groups were similiar to those of the controls, exposure related increase in absolute and relative liver and spleen weight in males and females, decreased absolute and relative thymus weight in males, 24 ppm, males and females: increased relative und absolute heart weights and relative kidny weights
pathology:
spleen: 12,24 ppm (all males and females), 6 ppm (3/5 males, 1/5 females): darkened and enlarged; microscopic changes with increasing concentration congestion, haemosiderin deposition, increase haematopoietic cell prolifertation
kidneys: 12, 24 ppm: male: hyaline droplet nephropathy, minimal haemosiderin deposition; female: cells stippled with small brown pigment granules
liver: >= 6 ppm females and 24 ppm males: minimal haemosiderin deposites in the sinusoidal Kupffer cells

Reliability : (2) valid with restrictions

dose-finding study

06.08.2002 (53)

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	90 d
Frequency of treatm.	:	daily
Post exposure period	:	no data
Doses	:	0, 3, 10 or 30 mg/kg bw/day dissolved in corn oil
Control group	:	yes
LOAEL	:	ca. 3 mg/kg bw
Method	:	other: according to OECD Guide-line 408, see also freetext ME
Year	:	1979
GLP	:	yes
Test substance	:	other TS: 99.12 %
Method	:	blood and urine samples were analyzed twice during the study (d42/43, d83/84), statistical methods: Dunnett's and Bartlett's test,modified Mann-Witney test(Bonferroni inequality), Kolmogorov-Smirnov one-tailed test
Result	:	one control female died due to physical trauma during dosing. clinical observation: 30 mg-group, males and females, 10 mg-group, females: general paleness immediately after dosing, no statistically significant differences in body weight gain when compared to control rats, food consumption was sign. higher: females: mid dose, 1 of 13 w, high dose, 5 of 13 w, males: mid dose, 9 of 13 w, high dose, 10 of 13 w hematological changes: significant increased methemoglobin levels at 3, 10, 30 mg/kg bw: male/female: day 45: 4.2/4.6, 7.8/9.2, 15.0/18.1 %, resp., versus 0.5/0.9 % of controls and day 90: 4.5/4.9, 9.0/9.8, 14.2/18.2 % resp., versus 0.9/1.0 % in controls; dose-related increase in WBC in males and females (d 45): up to 44.21 % versus 11.67 %(control), (d 90): up to 12.95 % (control 10.24 %); dose related increase in reticulocyte count in males and females: (d 90): up to 39.8 % versus 0.6 % in controls, and in MCV, MCH values in males and females; significant dose related decrease in erythrocyte count (d45): up to 5.51 versus 8.49 (control),(d 90): up to 5.58 versus 9.07 in controls, in HGB up to 14.45 % versus 18.78 % in controls and in HCT, and MCHC values in males and females clinical chemistry: total protein sign. reduced with increasing dosing d45: females 10 and 30 mg/kg bw; d90: males 10 and 30 mg/kg bw and SGPT reduced: males 30 mg/kg bw urinalysis: At week 13 (= d 90) qualitative increases in levels of urinary urobilinogen were found in all male and female rats receiving TS. gross and histopathology: spleen: (both sexes, all dosages, dose dependent in incidence and severity): abnormal coloration, enlargement increased relative and/or absolute spleen weights, excessive hemosiderin, excessive hemopoiesis, congestion, vacuolization of the congested red pulp liver: male and female, 30 mg: enlargement, hemosiderosis and excessive hemopoiesis kidneys: both sexes, dose-dependent: discoloration, enlargement, hemosiderosis in kidney tubules 30 mg-group: enlargement of the hearts in females, in both sexes: hyperplasia of bone marrow
Reliability	:	(1) valid without restriction

Flag : Critical study for SIDS endpoint
12.08.2002 (90) (91)

Type : Chronic
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : 24 months
Frequency of treatm. : daily
Post exposure period : no
Doses : 0, 0.1, 0.7 or 5.0 mg/kg bw/day dissolved in corn oil
Control group : yes, concurrent vehicle
Adverse Effect Level : = .7 mg/kg bw
Method : other: according OECD Guideline 453, see also freetext ME
Year : 1983
GLP : yes
Test substance : other TS: purity: 99.33 %

Method : deviations: no satellite group (high and control), hematology: 10 rats/sex/group, time interval for interim examinations: month: 6, 10, 12, 18, 24
60 rats/sex/group
tissues examined histopathologically: all rats in control and high dose group regardless of their time point of death, as well as gross lesions, testes, epididymides and spleens for all low and mid dose animals
statistical methods: Bartlett's test, ANOVA, Summed Rank Test (Dunn), Regression Analysis, Jonckheere's Statistics

Remark : see also Chapter 5.7 Carcinogenicity

Result : Two year survival ranged from 33 to 43 % for males and 48 to 60 % for females; additional death of rats were attributed to intubation accidents as confirmed by grosss or microscopic postmortem observations.
Physical abnormalities were seen in all dose groups including control groups. Slightly higher incidences in high dosed rats than in control rats were seen in the 2nd year of the study: in males between week 60 and week 104: yellow staining of the anogenital area, in females during the last months: excessive lacrimation, chromodacryorrhea, alopecia
Ophthalmology:
no evidence of any dose-or test-substance-related abnormalities
Hematology:
0.7 and 5.0 mg/kg bw/day:
significant increases in methaemoglobin levels in males 1.9/3.9 to 1.5/6.0 % (month 6 to 24) versus pretest value (pt) 1.2 % and in females 1.9/4.0 to 1.5/5.6 % (month 6 to 24) versus pt 0.0 %; mean values for mid-dose group were approx. 3 to 4 times higher than control means. No increase in the magnitude of the differences was apparent over the course of the study.
5.0 mg/kg bw/day:
slight anaemia as demonstrated by slightly decreased haemoglobin with min. in males/females mo 18: 12.7/11.9 g/dl versus pt 14.4/14.1 g/dl, haematocrit with min. in males mo 24: 38 % and in females mo 12: 35 % versus pt 44/43 % in males/females, erythrocyte values with min in males/females in mo 18: 6.07/5.26 x 10[exp.6]/mm³ versus pt 6.43/6.15 x 10[exp6]/mm³ and concomitant slight increases in numbers of reticulocytes with max in males mo 24: 10.9 % and in females mo 18: 5.6 % versus control at month 6 of 1.0/0.9 %; platelet values of high dose females were generally higher than those of control females (12th and 18th month stat. sign.)
Clinical chemistry:
Evaluations revealed no differences between values for control and treated groups

Urinalysis:
values for controls and treated rats were considered comparable.
pathology and histology:
absolute and relative spleen weights sign. increased for high-dose male and female rats, consistent with the increases in the incidence and/or severity of accumulation of brown pigment (possibly haemosiderin) in the reticuloendothelial cells of the spleen (m: 46/60, 44/60, 50/60, 58/60; f: 54/60, 56/60, 57/60, 59/60, control and low- to high-dose-group); liver: proliferation of biliary ducts (m: 4/60, 3/31, 6/29, 11/60; f: 7/60, 2/29, 1/28, 12/60, control and low- to high-dose-group), sclerosing cholangitis (m: 3/60, 3/31, 4/29, 11/60), hepatocellular vacuolation (m: 12/60, 5/31, 11/29, 9/60; f: 7/60, 9/29, 5/28, 9/60); spleen: extramedullary hematopoiesis (m: 15/60, 21/60, 20/60, 26/60), stomach: necrosis of glandular mucosa (m: 4/60, 2/9, 6/15, 4/60; f: 2/60, 3/12, 1/13, 3/60), kidneys: uni- and bilateral hydronephrosis (f: 7/60, 6/14, 6/20, 2/60); uterus: endometrial cysts (5/60, 5/11, 5/14, 7/60), endometrial polyps (2/60, 2/11, 7/14, 3/60). testes: bilateral degeneration of germinal epithelium (6/60, 14/59, 12/60, 11/60), bilateral periarteritis nodosa (44/60, 11/59, 8/60, 3/60); epididymides: uni- and bilateral oligospermie (10/60, 22/59, 10/59, 12/60); testes: slightly elevated weights (absolute and relative), pulmonary discolorations and pleural adhesions.

Results concerning neoplasms: see chapter 5.7

Reliability : (2) valid with restrictions
Histopathological examination was not performed of all rats.

Flag : Critical study for SIDS endpoint

19.08.2002 (92) (93) (94)

Type :
Species : rat
Sex : no data
Strain : no data
Route of admin. : oral unspecified
Exposure period : 20 d
Frequency of treatm. : daily
Post exposure period : no data
Doses : 110 mg/kg bw/d
Control group : no data specified
Method : other: no further data
Year :
GLP :
Test substance :

Result : 7/20 rats perished (thus, the test substance was found to exert cumulative effects)

Reliability : (3) invalid
only 1 dose used and lack of relevant information

02.04.2003 (55)

Type :
Species : rat
Sex : no data
Strain : no data
Route of admin. : oral unspecified
Exposure period : 7 months
Frequency of treatm. : daily
Post exposure period : no data
Doses : 0.0025, 0.005, 0.025 or 5 mg/kg bw/d
Control group : yes
LOAEL : ca. 5 mg/kg bw

Method	:	other: 42 rats (no details given)
Year	:	1967
GLP	:	no
Test substance	:	other TS: no data on purity
Remark	:	o-, m- and p-chloronitrobenzene were tested: the para-isomer was found to be most haemotoxic and the ortho-isomer exerted the least effect on the peripheral blood
Result	:	dose level(s) not specified: increase in the methaemoglobin content in the blood during the first month of the experiment, decrease of the haemoglobin content towards the second month of the experiment (from 12.3% to 10.7%), increase in the reticulocyte count and presence of Heinz bodies in the erythrocytes (up to 113 % reticulocytes and 242 % Heinz bodies in the blood) 5 mg/kg bw/d: liver function tests: increase in the blood alkaline phosphatase activity, rise in the level of bilirubin in the urine; effects on CNS function: some slowing down of fixation of the positive conditioned reaction and of the development of the differentiation reaction
Reliability	:	(4) not assignable lack of relevant information

30.07.2002 (55)

Type	:	
Species	:	rat
Sex	:	male
Strain	:	no data
Route of admin.	:	oral unspecified
Exposure period	:	30 d
Frequency of treatm.	:	daily
Post exposure period	:	no data
Doses	:	78 mg/kg bw/d
Control group	:	yes
Result	:	increase in the aerobic and anaerobic metabolic rate of carbohydrates as well as increased concentrations of ATP and (or) creatine phosphate in the brain and liver; coupling of oxidative phosphorylation increased in the brain and decreased in the liver; increased anaerobic glycolysis rates in the brain and liver
Reliability	:	(4) not assignable lack of relevant information

30.07.2002 (57)

Type	:	
Species	:	rat
Sex	:	no data
Strain	:	no data
Route of admin.	:	s.c.
Exposure period	:	10 d
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	100 mg/kg bw/d
Control group	:	yes
Result	:	methaemoglobinaemia, anaemia, marked loss of body

weight, lowering of the blood pressure, decrease in the rhythm of cardiac contractions, increase in the weight coefficient of the heart

Reliability : (4) not assignable
lack of relevant information

30.07.2002 (95)

Type :
Species : rat
Sex : male
Strain : no data
Route of admin. : s.c.
Exposure period : 10 d
Frequency of treatm. : daily
Post exposure period : 14 d
Doses : 100 mg/kg bw/d
Control group : no data specified

Result : methaemoglobinaemia and nitroxyhaemoglobinaemia (both completely reversible within 14 d after the end of the exposure period), sulfhaemoglobinaemia (observable till end of the observation period), anaemia (lowered level of haemoglobin) and formation of Heinz bodies (both normalised until 7 d after the end of the exposure period)

Reliability : (4) not assignable
lack of relevant information

30.07.2002 (96)

Type : Sub-acute
Species : rat
Sex : no data
Strain : no data
Route of admin. : oral unspecified
Exposure period : until death occurred, max. up to 20 times
Frequency of treatm. : daily
Post exposure period : no
Doses : 1, 10, 100 mg/kg bw
Control group : no data specified
LOAEL : ca. 1 mg/kg bw
Method : other: daily application of TS until death occurred, but max. 20 doses, evaluation of blood chemistry
Year : 1944
GLP : no
Test substance : other TS: purity no data

Result : 100 mg/kg bw could be applied 7 times: marked decrease in erythrocytes, marked increase in neutrocytes
10 mg/kg bw could be applied 20 times: similar but less marked effects
1 mg/kg bw could be applied 20 times: might have produced a slight decrease in erythrocytes

Reliability : (3) invalid
Method meets not the criteria of today

30.07.2002 (66)

Type : Sub-chronic
Species : mouse
Sex : male/female
Strain : B6C3F1

Route of admin.	:	inhalation
Exposure period	:	13 w
Frequency of treatm.	:	6 h/d, 5 d/w
Post exposure period	:	no
Doses	:	0, 1.5, 3, 6, 12 or 24 ppm (ca. 9.81, 19.62, 39.24, 78.48 or 156.96 mg/m³ air)
Control group	:	yes
NOAEL	:	ca. 6 ppm
Method	:	other: 10 mice/sex/group, whole body exposure, see also freetext ME
Year	:	1993
GLP	:	yes
Test substance	:	other TS: purity: approx. 99 %
Method	:	body/organ weight, compl. necropsies on all mice, compl. histopathol. in all control mice and 24 ppm-gr. and mice that died, gross lesions and selected organs of mice <24 ppm-grps, statistical methods:parametric multiple comparisons procedures of Williams or Dunnett, nonparametric multiple comparisons methods of Shirley or Dunn, Jonckheere's test or trend-sensitive test, outlier test of Dixon and Massey histopathologic evaluation on reproductive organs: see chapter 5.8.3
Remark	:	the NOAEL for histopathological changes was 6 ppm;
Result	:	no clinical signs of toxicity related to exposure; death of 1 male in the 6 ppm group during week 8, mean body weight gain of exposed mice greater or equal than in the controls the following organ weights were sign. increased: spleen(m,f): 12,24 ppm, liver(m,f) >=12 ppm resp. 6, right kidney (m,f) >=1.5 ppm resp. >=3 ppm; histopathology: bone marrow: hyperplasia, males:12 and 24 ppm: 3/10 and 9/10, females: 24 ppm: 10/10; hemosiderin, red blood cell fragments, males: 24 ppm: 10/10, females, 24 ppm 8/10 and 9/10; forestomach, hyperplasia, 24 ppm, males: 1/10 and females 7/10 liver: hemosiderin, 24 ppm: 10/10 males and 10/10 females, necrosis, males, 12 ppm: 1/10, 24 ppm: 5/10, cytoplasmatic basophilia, males, 24 ppm: 4/10 Spleen: congestion, males,12 ppm: 1/10, 24 ppm: 10/10, females, 24 ppm: 10/10, hemosiderin, 12, 24 ppm: all males and females (increasing severity with increasing dose), hematopoietic cell proliferation, males, 12 ppm 7/10, 24 ppm 10/10, females: all dose groups, increasing incidences and severity with increasing doses
Reliability	:	(2) valid with restrictions Haematology was not performed
Flag	:	Critical study for SIDS endpoint

30.07.2002 (86) (53) (87)

Type	:	Sub-acute
Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	inhalation
Exposure period	:	2 weeks
Frequency of treatm.	:	6 hours/day, 5 days/week
Post exposure period	:	no
Doses	:	0, 1.5, 3, 6, 12 or 24 ppm (ca. 0, 9.81, 19.62, 39.24, 78.48 or 156.96 mg/m³ air)
Control group	:	yes
NOAEL	:	ca. 3 ppm

Method	:	other: 5 mice/sex/group, whole body exposure, see also freetext ME
Year	:	1993
GLP	:	yes
Test substance	:	other TS: purity: approx. 99 %
Method	:	animals were observed twice daily, weighted on d1, d8 and at necropsy, clinical observations recorded daily, complete necropsies on all mice, histopathologic evaluation of all mice in controls and the 24 ppm-group, gross lesions in all lower exposure groups, target organs identified and examined until a NOAEL was determined (kidney, liver, spleen)
Result	:	no death occurred, body weight gain of the exposed mice similar to respective controls, except in males of the 24 ppm-group which was 111 % of the concurrent control mice. Clinical signs of toxicity: 12 ppm, males: hypoactivity; 24 ppm, males and females: hypoactivity and dyspnea pathology: spleen: increased weight (relative and absolute), enlarged and dark in 3/5 males and 4/5 females of the 24 ppm-group, 12,24 ppm, male and female and some of the 6 ppm-group: minimal increases in primarily erythropoietic activity, minimal increase in hemosiderin pigment liver: increased weight(relative and absolute), subtile increasing amounts of hemosiderin pigments in the Kupffer cells kidneys: subtile increasing amounts of hemosiderin pigments in the cortical tubule epithelial cells
Reliability	:	(2) valid with restrictions dose range finding study

06.08.2002 (53)

Type	:	
Species	:	mouse
Sex	:	male/female
Strain	:	other: Swiss CD-1
Route of admin.	:	gavage
Exposure period	:	14 d
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	0, 40, 80, 160, 320 or 640 mg/kg bw/day dissolved in corn oil
Control group	:	yes
Method	:	other: Continuous breeding protocol, task 1: dose finding study, 8 mice/sex/group, clin. signs of tox., bw., water consumption
Year	:	1991
GLP	:	yes
Test substance	:	other TS:>99 %
Result	:	mortality: attributed to gavage trauma, females: 4/8 in controls, 40 mg-gr.: 1/8, 60 mg/gr.: 1/8, 160 mg-gr.: 1/8, 320 mg-gr.: 2/8; 640 mg-gr.: males/females all mice : By d4 8/16 had died or been sacrificed for human reasons. the remaining mice: were hunched, cyanotic, inactive, rough fur and therefore sacrificed <= 160 mg-gr.: bw change similiar to control values 320 mg-gr.:,females bw increased by 18 % 40 mg/kg bw/d: water consumption values signifi- cantly decreased for females during week 2 160 and 320 mg/kg bw/d: cyanosis (especially vis- ible near the nasal area, feet and tail) 160 and 320 mg/kg bw/d: water consumption values significantly decreased for females during week 2 resp.1

640 mg/kg bw/d: water consumption values were significantly decreased for all animals
conclusion: MTD approx. 250 mg/kg bw, doses for the main study: 62.5, 125, 250 mg/kg bw

Reliability : (2) valid with restrictions
dose finding sudy for the study using continuous breeding protocol, see chapter: 5.8.1

12.08.2002 (97) (53)

Type :
Species : rabbit
Sex : no data
Strain : no data
Route of admin. : oral unspecified
Exposure period : 6 months
Frequency of treatm. : daily
Post exposure period : no data
Doses : 0.005, 0.05 or 0.5 mg/kg bw/d
Control group : yes
NOAEL : ca. .05 mg/kg bw
Method : other: 6 rabbits/dose, investigation of the effect on immunological reactivity; in addition to these tests, the effects on the blood were studied
Year : 1967
GLP : no
Test substance : other TS: no data on purity

Result : 0.005 and 0.05 mg/kg bw/d: no effect on the production of antibodies by the test animals and on blood parameters
0.5 mg/kg bw/d: a not very marked inhibition of a fall in the agglutinin titer, only towards the end of experimental period (in the fifth month); increased red cell count, changes in the differential white count and increased serum SH content (no changes in the haemoglobin content, the total white count, or in the serum protein fraction ratios)

Reliability : (4) not assignable
Documentation insufficient for assessment

30.07.2002 (55)

Type :
Species : cat
Sex : no data
Strain : no data
Route of admin. : inhalation
Exposure period : all together 17.5 h during 3 consecutive d
Frequency of treatm. : no data
Post exposure period : no data
Doses : 0.2 mg/l (ca. 200 mg/m³)
Control group : no data specified

Result : sickness, drowsiness (no further data)
Reliability : (4) not assignable
Documentation insufficient for assessment

30.07.2002 (98)

Type :
Species : cat
Sex : no data

Strain	:	no data
Route of admin.	:	inhalation
Exposure period	:	no data
Frequency of treatm.	:	7 h/d, 5 d/w
Post exposure period	:	no data
Doses	:	average concentrations ranging from 2.8 to 25.9 ppm
Control group	:	no data specified
Result	:	at 25 ppm (= ca. 0.1633 mg/l) one cat died after 7 h and one cat survived; at 6.5 ppm (= ca. 0.0425 mg/l), one cat died after 74 h of exposure; at 3.3 ppm (= ca. 0.0216 mg/l), one cat died after 24 h of exposure; at 2.8 ppm (= ca. 0.0183 mg/l), one cat survived after 198 h of exposure; all the animals lost weight, all presented gross methaemoglobinaemia at various times, and the two which had long exposures developed anaemia; sections of tissue from these two animals showed deposits of blood pigment, with damage to the liver and kidney (no further data)
Reliability	:	(4) not assignable Documentation insufficient for assessment

30.07.2002 (99)

Type	:	
Species	:	cat
Sex	:	no data
Strain	:	no data
Route of admin.	:	inhalation
Exposure period	:	6 w
Frequency of treatm.	:	8 h/d
Post exposure period	:	no data
Doses	:	0.087 mg/l (ca. 87 mg/m³)
Control group	:	no data specified
Remark	:	two cats were exposed to the vapour of p-chloronitrobenzene
Result	:	methaemoglobinaemia, slight anaemia
Reliability	:	(4) not assignable Documentation insufficient for assessment

30.07.2002 (100)

Type	:	
Species	:	guinea pig
Sex	:	no data
Strain	:	no data
Route of admin.	:	inhalation
Exposure period	:	6 w
Frequency of treatm.	:	8 h/d
Post exposure period	:	no data
Doses	:	0.087 mg/l (ca. 87 mg/m³)
Control group	:	no data specified
Remark	:	two guinea pigs were exposed to the vapour of p-chloronitrobenzene
Result	:	methaemoglobinaemia, slight anaemia
Reliability	:	(4) not assignable Documentation insufficient for assessment

30.07.2002 (100)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	DNA damage and repair assay
System of testing	:	rat hepatocytes
Test concentration	:	0, 5, 50 uM in DMSO
Cycotoxic concentr.	:	not mentioned
Metabolic activation	:	no data
Result	:	
Method	:	other: alkaline elution assay, (1) cell culture 1.5 hrs, then 3 hrs treatm.(5,50 uM), determ. of elution rate (ER) 3, 24 and 48 hrs post treatm.; (2) cell culture: 24 hrs, then 3 hr- treatm.(50uM), ER 3, 24 and 48 hrs post treatm.
Year	:	1984
GLP	:	no data
Test substance	:	other TS: no data on purity
Remark	:	A 50% cytocidal action was observed after treatment with 50x10-3 M (3 hrs exposure time) (1) a dose-dependent increase of DNA damage was obtained by exposure of cultured hepatocytes to 5 and 50 uM p-chloronitrobenzene, increase of time after treatment diminish the elution rate. (2) Prolonging the culture time before treatment from 1.5 hrs to 24 hrs: DNA was not affected by treatment with the test compound
Reliability	:	(3) invalid cytotoxic concentration not mentioned, no data on purity, no information about GLP

01.08.2002 (101)

Type	:	Ames test
System of testing	:	S. typhimurium TA 100
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	with
Result	:	positive
Method	:	other: no further details given
Year	:	
GLP	:	
Test substance	:	
Remark	:	in this assay, p-chloronitrobenzene was a weak mutagen compared to o-chloronitrobenzene
Reliability	:	(4) not assignable Documentation insufficient for assessment

06.08.2002 (102)

Type	:	Ames test
System of testing	:	S. typhimurium TA 98, TA 100, TA 1530, TA 1532, TA 1535, TA 1537, TA 1538, TA 1950, TA 1975, TA 1978, G 46
Test concentration	:	1-2000 ug/plate
Cycotoxic concentr.	:	the toxicity was evaluated by determing the bacterial survival.
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: (1) plate incorporation method, incubation 48 hours, in aerobic and in anaerobic conditions, solvent: ethanol abs., duplicates (2) bacterial fluctuation test, incubation 72 hours, solvent: ethanol abs., triplicates
Year	:	1980

GLP : no data
Test substance : other TS: the purest grade available

Reliability : (3) invalid
no details given, special study
16.07.2002 (103)

Type : Ames test
System of testing : S. typhimurium TA 1535, TA 1537, TA 1538
Test concentration :
Cycotoxic concentr. :
Metabolic activation : with and without
Result :
Method : other: no further details given
Year :
GLP :
Test substance :

Remark : 9 Salmonella bacterial assays of p-chloronitrobenzene or mixtures of p-chloronitrobenzene with o-chloronitrobenzene, m-chloronitrobenzene and other chloronitrobenzenes were reported; only one of these studies with a sample >99 % pure p-chloronitrobenzene gave the result "not mutagenic to strains TA 1535, TA 1537, TA 1538 both in the presence and absence of metabolic activation"; the other eight assays were weakly positive, the purity of the p-chloronitrobenzene in these positive tests ranged from greater than 99.99 % to a mixture of p-chloronitrobenzene (46.5 %), o-chloronitrobenzene (36.7 %) and other chloronitrobenzenes (no further data)
Reliability : (4) not assignable
Documentation insufficient for assessment
06.08.2002 (104)

Type : HGPRT assay
System of testing : Chinese Hamster Ovary (CHO) cells
Test concentration : 0, 1.59, 1.9, 2.22, 2.38 mM (approx. 0, 250, 299, 350, 375 ug/ml) dissolved in Acetone
Cycotoxic concentr. : preliminary cytotoxicity evaluation, conc. not given
Metabolic activation : with and without
Result : negative
Method : other: according OECD guideline 476, prelim. cytotox. evaluation to select conc. for mutag. assay; solvent acetone, incubation time: with TS and with S9-mix: 5 hrs, with TS without S9-mix: 18 hrs, solvent and positive control (DMN)
Year : 1979
GLP : no data
Test substance : other TS: 99.53%

Remark : The higher concentrations tested (no details) +/- S9 were soluble in acetone and precipitated when added to the treatment medium. Toxicity was observed at higher concentrations (2.38 mM +S9)
Reliability : (2) valid with restrictions
no information about GLP
Flag : Critical study for SIDS endpoint
06.08.2002 (105) (104)

Type : Ames test

System of testing	:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537
Test concentration	:	all strains, +/-S9, PM: 0.0, 30, 100, 300, 1000, 3000 ug/plate; add. only TA100,+/-S9, PM: 0.0, 62.5, 250, 500, 1500, 2000 ug/plate only TA 100,+/-S9, SPM: 0, 62.5, 250, 500, 1000, 1500, 2000 ug/plate
Cycotoxic concentr.	:	preliminary check for toxicity to TA100 up to 10 mg/plate or limit of solubility
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: see remark
Year	:	1983
GLP	:	no data
Test substance	:	other TS: purity: > 99 %
Method	:	preincubation methodology (PM), add. TA100: standard plate methodology (SPM), rat liver S9-mix and hamster liver S9 mix, solvent: DMSO, positive control: 2-AA, NOPD, 9-AAD, solvent control, performed in triplicate and repeated twice, statistical method according to Margolin et al. 1981
Remark	:	The test substance was mutagenic only in strain TA 100 with metabolic activation using preincubation methology as well as standard plate methodology (mutagenicity with hamster liver S9 mix stronger than with rat liver S9 mix), revertants/plate up to 3.8 times higher than control
Reliability	:	(2) valid with restrictions only four strains used, no information about GLP
Flag	:	Critical study for SIDS endpoint
02.04.2003		(106) (53)

Type	:	Chromosomal aberration test
System of testing	:	Chinese hamster ovary cells
Test concentration	:	- S9: (1) 0, 50, 167, 500 ug/ml, harvest time (ht): 10.5 hrs; (2) 0, 700, 800, 900 ug/ml, ht: 10.6 hrs; (3)0, 500, 600, 700 ug/ml, ht: 19.0 hrs; + S9: (4) 0, 50, 167, 500, 5000 ug/ml, ht: 10.5 hrs; (5) 0, 600, 800, 900 ug/ml, ht: 19 hrs
Cycotoxic concentr.	:	dose selection based on preliminary growth inhibition test, or based on observations of cell confluence and mitotic cell availability in the SCE test, performed in the same laboratory
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: see remark
Year	:	1987
GLP	:	no data
Test substance	:	other TS: purity > 99 %
Method	:	solvent: DMSO, harvest time: (1) 10.5 hrs, (2) 10.6 hrs, (3) 19.5 hrs, (4) 10.5 hrs, (5) 19.5 hrs, harvest times depends on information on the extend of cell cycle delay seen in the SCE test performed in the same laboratory, little or no delay: 8-12 hrs, delay: 18-26 hrs
Result	:	(1) neg. (2) weak pos. (3) weak pos. (4) neg. (5) pos. The aberration test without activation gave a high frequency of breaks in two of the three tests and at an extremely toxic dose. The high point was based on only 33 and 22 metaphases. Positive results were obtained in the second of the two tests with activation by using closely spaced, high doses. Cells with aberrations up to 31% increased compared to solvent control
Reliability	:	(2) valid with restrictions no data on GLP
Flag	:	Critical study for SIDS endpoint
02.04.2003		(107) (53)

Type	:	Sister chromatid exchange assay
System of testing	:	Chinese hamster ovary cells
Test concentration	:	-S9: (1) 0, 100, 150 ug/ml; +S9: (2)0, 250, 400, 500 ug/ml; (3) 0, 300, 400, 500 ug/ml
Cycotoxic concentr.	:	dose selection based on preliminary growth inhibition test
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: see remark
Year	:	1987
GLP	:	no data
Test substance	:	other TS: purity > 99 %
Method	:	other: TS dissolved in DMSO, treatment period: -S9: 25 or 30 hrs, +S9: 2 hrs; total incubation time with BrdU: 25-26 or 28,5 hrs, colcemid during the last 2-3 hrs, harvest times delayed because of cell cycle delay, positive control cyclophosphamide
Remark	:	(1) neg. (2) pos. (3) (pos) The SCE test with S9 was positive; the positive result was repeatable. SCEs/chromosome up to 85% increased compared to solvent control
Reliability	:	(2) valid with restrictions no information about GLP
Flag	:	Critical study for SIDS endpoint

02.04.2003 (107) (53)

Type	:	other: Bacterial mutation assay (no further data)
System of testing	:	no data
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	no data
Result	:	positive
Method	:	other: no further information given
Year	:	
GLP	:	
Test substance	:	
Reliability	:	(4) not assignable Documentation insufficient for assessment

01.08.2002 (108)

Type	:	Ames test
System of testing	:	S. typhimurium TA 98, TA 100
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	
Year	:	1987
GLP	:	
Test substance	:	
Remark	:	the test result was negative with TA 100 without metabolic activation and with TA 98 with and without S9 mix; the test result was "pseudo positive" with TA 100 with metabolic activation, i.e., p-chloronitrobenzene showed mutagenic activity as pseudomutagen which showed dose-response relationship but did not give revertant colonies more than twice of that of control

Reliability : (4) not assignable
abstract only
01.08.2002 (109)

Type : Unscheduled DNA synthesis
System of testing : rat hepatocytes
Test concentration : 0, 0.33, 1.0, 3.33, 10.0, 33.33, 100, 333.33, 1000, 3333.33, 10000 ug/well dissolved in DMSO
Cycotoxic concentr. : >= 1000 ug/well dissolved in DMSO
Metabolic activation : without
Result : negative
Method : other: according to OECD Guide-line 482, negative, vehicle and positive control (2AAF)
Year : 1982
GLP : yes
Test substance : other TS: purity not mentioned

Reliability : (2) valid with restrictions
no data on purity
Flag : Critical study for SIDS endpoint
01.08.2002 (110) (111)

Type : Chromosomal aberration test
System of testing : Chinese hamster ovary cells
Test concentration :
Cycotoxic concentr. :
Metabolic activation : no data
Result : ambiguous
Method : other: no further information available
Year :
GLP :
Test substance :

Reliability : (4) not assignable
documentation insufficient for assessment
01.08.2002 (112)

Type : Sister chromatid exchange assay
System of testing : Chinese hamster ovary cells
Test concentration :
Cycotoxic concentr. :
Metabolic activation : no data
Result : positive
Method : other: no further information
Year :
GLP :
Test substance :

Reliability : (4) not assignable
Documentation insufficient for assessment
01.08.2002 (112)

Type : Ames test
System of testing : S. typhimurium (no further data)
Test concentration :
Cycotoxic concentr. :
Metabolic activation : no data
Result : positive
Method :

Year :
GLP :
Test substance :

Remark : two separate tests were performed
Reliability : (4) not assignable
Documentation insufficient for assessment
01.08.2002 (113)

Type : Ames test
System of testing : S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Test concentration : 25.6, 51.2, 102.4, 204.8, 409.6, 819.2, 1638.4, 3276.8 ug/plate
Cycotoxic concentr. : 3276.8 ug/plate
Metabolic activation : without
Result : positive
Method : other: According to Ames et al., Proc. Natl. Acad. Sci (USA) 70: 782 (1973), pour-plate method, solvent: DMSO, performed in duplicate and repeated at least 3 times, solvent control, pos. Controls (ENNG, 2-NF, 9-AA)
Year : 1983
GLP : no data
Test substance : other TS: purity: 99 %

Remark : the test substance exhibited mutagenic activity
only in strains TA 100 (1.9 times over control value) and TA 1535 (4 times over control value)
Reliability : (2) valid with restrictions
study meets the criteria of today, but is only performed without metabolic activation and information on GLP is missing
Flag : Critical study for SIDS endpoint
02.04.2003 (114)

Type : Ames test
System of testing : S. typhimurium TA 98, TA 100
Test concentration : 0 - 100 ug/plate in DMSO
Cycotoxic concentr. : not determined
Metabolic activation : with and without
Result : positive
Method : other: preincubation method;
TA98, TA100 without S9-mix; TA98 with S9 mix; TA98 with S9 mix + norharman (200 ug/plate),
4 replicate plates for each dose level
Year : 1983
GLP : no data
Test substance : other TS: chromatographically pure

Remark : TA98 TA100 (without S9-mix) : negative
TA98 (with S9-mix) : negative
TA98 (with S9-mix and norharman): positive
Reliability : (3) invalid
only two strains used, cytotox concentration not determined, no information about GLP, no exact data about purity
01.08.2002 (115)

Type : Ames test
System of testing : S. typhimurium TA 98, TA 98NR (nitroreductase deficient strain) and TA 98/1,8-DNP6 (esterifying enzyme-deficient strain)
Test concentration : 0, 100, 200, 300 ug/plate dissolved in DMSO
Cycotoxic concentr. : not determined

Metabolic activation	:	with
Result	:	positive
Method	:	other: preincubation method, in the presence of rat liver S9 and norharman, 3 replicate plates at each dose level
Year	:	1982
GLP	:	no data
Test substance	:	other TS: chromatographically pure

Remark : p-chloronitrobenzene exhibited mutagenicity towards all 3 strains; the mutagenic activity towards S. typhimurium TA 98NR was liable to be lower than that towards S. typhimurium TA 98; however the difference in mutagenicity between the two strains was not regarded as statistically significant; the test substance tended to exhibit higher mutagenicity towards TA 98/1,8-DNP6 than towards TA 98, but again the difference in mutagenic activity was not significant

Reliability : (3) invalid
special study, only performed in the presence of metabolic activation, cytotox concentration not determined, no information about GLP, no exact data about purity

01.08.2002 (116) (117)

Type	:	other: SOS chromotest
System of testing	:	E. coli PQ 37
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: no details given
Year	:	1988
GLP	:	
Test substance	:	other TS: no data on purity

Remark : Metabolic activation: (S9 mix of rat and hamster); p-chloronitrobenzene did not induce SOS-repair in the chromotest with and without S9 mix (without norharman); it was tried to increase the sensitivity of the SOS chromotest by addition of norharman to the S9 mix: a negative result was obtained again with the test substance

Reliability : (4) not assignable
Documentation insufficient for assessment

01.08.2002 (118)

Type	:	Ames test
System of testing	:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537
Test concentration	:	0, 0.01, 0.04, 0.2, 1.0, 3.0, 10.0 mg/plate dissolved in DMSO
Cycotoxic concentr.	:	>= 3 mg/plate (with S9-mix), >= 10 mg/kg (without S9-mix)
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: see remark
Year	:	1980
GLP	:	no data
Test substance	:	other TS: purity not mentioned

Method : According to Ames et al., Mutat. Res. 31: 347 (1975), plate incorporation methodology, solvent: DMSO, preliminary cytotoxicity test using TA100,

solvent-, negative- and positive control (2-Nitrofluorene, benzo(a)pyrene, NaNO2, 2-AA, 9AA), statistical. analysis: Bartlett's test, t-test, fit-test, Grubb's analysis

Result : positive only in TA1535 without S9-mix (increase of revertants/plate up to 2.7fold higher than control)
Source : Monsanto
Reliability : (2) valid with restrictions
only four strains used, no information about GLP
Flag : Critical study for SIDS endpoint
02.04.2003 (110) (119)

Type : HGPRT assay
System of testing : Chinese Hamster Ovary (CHO) cells
Test concentration : with S9-mix: 0, 100, 200, 300,350, 400 ug/ml dissolved in DMSO
without S9-mix:0, 100, 300, 500, 700, 900 ug/ml dissolved in DMSO
Cycotoxic concentr. : 1000 ug/ml; preliminary cytotoxicity test
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 476
Year : 1982
GLP : yes
Test substance : other TS: no data on purity

Method : negative, vehicle and positive controls (EMS, DMN), cloudiness with a slight precipitate was noted, when TS was added to the media (+/- S9 >= 300 ug/ml); triplicates, incubation time +/- S9: 5 hrs.
Source : Monsanto
Reliability : (2) valid with restrictions
no data on purity
Flag : Critical study for SIDS endpoint
01.08.2002 (110) (120)

Type : Mouse lymphoma assay
System of testing : mouse lymphoma cells L5178Y TK+/-
Test concentration : without S9: 0, 25, 60, 100, 150, 300, 402, 504, 600 ug/ml
with S9: (1) 0, 42, 77, 105, 140, 175, 203, 252, 350 ug/ml
(2) 0, 21, 42, 77, 105, 140, 175, 203, 252, 350 ug/ml
Cycotoxic concentr. : >= 300 ug/ml; preliminary test
Metabolic activation : with and without
Result : positive
Method : other: according to OECD Guide-line 476, see also freetext ME
Year : 1983
GLP : yes
Test substance : other TS: purity > 99 %

Method : solvent: DMSO
no differentiation between small and large colonies, positive controls (EMS, 3-MCA), incubation time: 4 hrs, cultivation time: 11 d, statistical methods: student's t-test, dose-response analysis of variance by Irr and Sace
Remark : positive in presence and absence of metabolic activation, average mutation frequency solvent control: 38x10-6, treated cells (60-402 µg/ml): 106-164x10-6
Source : Monsanto
Reliability : (2) valid with restrictions
no differentiation between small and large colonies
Flag : Critical study for SIDS endpoint
02.04.2003 (110) (121)

Type : Chromosomal aberration test

System of testing	:	human lymphocytes
Test concentration	:	0, 0.05, 0.10, 0.50, 1.0 and 1000 mmol/l
Cycotoxic concentr.	:	no data
Metabolic activation	:	no data
Result	:	negative
Method	:	other: cultures of whole blood were incubated for 48 h, then add. of TS in 10 ul DMSO for 24 h; 2h before termination colchicine was added, staining with giemsa, 100 cells/culture were recorded, 5 duplicate cultures
Year	:	1995
GLP	:	no data
Test substance	:	other TS: no data on purity

Reliability : (4) not assignable
documentation insufficient for assessment

01.08.2002 (122) (123) (124)

Type	:	other: UMU test
System of testing	:	Salmonella typhimurium TA1535/pSK1002
Test concentration	:	100 ug/ml
Cycotoxic concentr.	:	no information
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: the culture with the test strain was incubated with the test compound for 4 hours at 37 deg.C. and then ß-galactose activity in the cells was assayed
Year	:	1992
GLP	:	no data
Test substance	:	other TS: no data on purity

Reliability : (4) not assignable
Documentation insufficient for assessment

01.08.2002 (125)

Type	:	other: spot test
System of testing	:	Salmonella typhimurium TA98, TA100, TA1535, TA1537
Test concentration	:	0, 25 mg/spot dissolved in DMSO
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: according to Ames et al., Mutat. Res. 31: 347 (1975) and MRC's Standard Operating Procedure for S. typhimurium, Rat S9-mix, Mouse S9-mix
Year	:	1979
GLP	:	no data
Test substance	:	other TS: purity not mentioned

Remark : Under all test conditions, a cyrstalline formation was noted throughout the top agar when 25 mg of sample was added per spot

Reliability : (3) invalid
only 4 strains used, only one dose given, no data on purity and no information about GLP

06.08.2002 (119)

Type	:	Chromosomal aberration test
System of testing	:	Chinese hamster Lung (CHL) cells
Test concentration	:	1): 0, 0.05, 0.1, 0.2, 0.4, 0.6 mg/ml DMSO, treatment time: 24/48 hrs, without S9-mix 2): 0, 0.2, 0.3, 0.4, 0.5, 0.6 mg/ml DMSO, each with/without S9-mix

Cycotoxic concentr. : ad 1): precipitation: 0.4, 0.6 mg/ml, no metaphase at 0.6 and 0.8 mg/ml
ad 2): precipitation: 0.4, 0.5, 0.6 mg/ml, no metaphase at 0.7 mg/ml
Metabolic activation : with and without
Result : positive
Method : other: according OECD Guide-line 473, see test conc., cytotoxicity was determined prior to testing, treatment time +S9: 6 hrs, 18 hrs further incubation after change of medium, positive control: MMC, BaP
Year : 1996
GLP : no data
Test substance : other TS: no data on purity, melting point: 82-84 °C

Remark : ad 1): negative
ad 2): without S9-mix: negative;
with S9-mix: 0.5 mg/ml: questionable (cells with structural chromosomal aberrations: 18fold increased compared to solvent control); 0.6 mg/ml: positive (34fold increase)
increased % of polyploid cells (dose-dependent >= 0.1, 48 hrs treatment without S9)
Reliability : (2) valid with restrictions
no information about GLP, instead of purity range of melting given
Flag : Critical study for SIDS endpoint
02.04.2003 (126)

Type : Ames test
System of testing : Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538
Test concentration : 0, 600, 1200, 1800, 2400, 3000 ug/plate dissolved in DMSO
Cycotoxic concentr. : > 3000 ug/plate
Metabolic activation : with and without
Result : positive
Method : other: according to Ames et al., Mutat. Res. 31: 347 (1975), cytotoxicity was determined prior to testing, solvent: DMSO, negative- and positive controls (2-AA), incubation time: 48 hrs
Year : 1977
GLP : no data
Test substance : other TS: purity not mentioned

Remark : positive only in TA1535 in the presence of metabolic activation, (4.1fold induced increase over the spontanous mutant frequency)
Reliability : (2) valid with restrictions
no data on purity and no information about GLP
Flag : Critical study for SIDS endpoint
02.04.2003 (127)

Type : Ames test
System of testing : Salmonella typhimurium TA1535, TA1537, TA 1538
Test concentration : with S9-mix: 0, 50, 100, 250, 500, 750, 1000 ug/plate dissolved in DMSO
without S9-mix: 0, 100, 250, 500, 750, 1000, 1500 ug/plate dissolved in DMSO
Cycotoxic concentr. : with S9-mix: >1000 ug/plate, without S9-mix: >1500 ug/plate
Metabolic activation : with and without
Result : negative
Method : other: in accordance with Ames et al., Mutat. Res. 31: 347 (1975), negative, positive controls (2-AA, MNNG, 2-NF, 9-AA), incubation time: 48 hrs
Year : 1975
GLP : no
Test substance : other TS: purity not mentioned

Reliability : (2) valid with restrictions

only 3 strains used, no data on purity and no information about GLP
01.08.2002 (128)

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA98, TA100, TA1535, TA1537
Test concentration	:	Trial 1: all strains a: with S9-mix: 0, 250, 500, 1000, 2500, 5000 ug/plate; b: without S9-mix: 0, 100, 250, 500, 1000, 2500 ug/plate Trial 2: TA 1535, TA100, with and without S9-mix: 50, 100, 250, 500, 1000 ug/plate
Cycotoxic concentr.	:	determined by preliminary exp. with TA1535, conc. not given
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: according to Ames et al., Mutat. Res. 31: 347 (1975), negative and positive controls (2-AA, MNNG, 9-AA, 2NF), incubation time: 48 hrs
Year	:	1979
GLP	:	no data
Test substance	:	other TS: purity 99.99 %
Remark	:	positive only in TA100 und TA1535, with and without metabolic activation, -S9: TA1535: 1.7-2.3 times the spontanous reversion frequency, TA100: 1.6-1.7 times +S9: TA1535: 3.7-4.7 times the spontanous reversion frequency, TA100: 2.1-3.0 times
Reliability	:	(2) valid with restrictions only 4 strains used, no information about GLP
Flag	:	Critical study for SIDS endpoint

02.04.2003 (129)

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538
Test concentration	:	all strains, with S9-mix: (1): 0, 500, 1000, 2500, 5000, 10000 ug; (2) and (3): 0, 100, 250, 500, 1000, 2500 ug/plate; all strains without S9-mix: (1) and (2) and (3: TA1535 only): 0, 100, 250, 500, 1000, 2500 ug/plate;
Cycotoxic concentr.	:	determined in preliminary tests with TA 1535 with and without S9-mix, conc. not mentioned
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: according to Ames et al., Mutat. Res. 31: 347 (1975), negative and positive controls (2AA, MNNG, 2NF, 9-AA)
Year	:	1978
GLP	:	no data
Test substance	:	other TS: purity not mentioned
Remark	:	positive only in TA 100 and TA1535 in the presence of an activation system, TA1535: 2.4-3.9 times the spontanous revertant frequency, TA100: 2.0-2.4 times
Reliability	:	(2) valid with restrictions no data on purity and no information about GLP
Flag	:	Critical study for SIDS endpoint

02.04.2003 (130)

Type	:	Unscheduled DNA synthesis
System of testing	:	Rat hepatocytes
Test concentration	:	0.1, 0.5, 1.0, 5.0, 10.0, 50.0, 75.0, 100.0, 500.0 ug/ml, dissolved in acetone
Cycotoxic concentr.	:	100.0, 500.0 ug/ml
Metabolic activation	:	without
Result	:	negative
Method	:	other: see remark
Year	:	1985

GLP	:	yes
Test substance	:	other TS: purity: commercial grade
Method	:	According to OECD Guide-line 482 with one deviation: extent of 3H-TdR incorporation in the cytoplasm and the number of grains found over cell nucleus were not reported separately; negative and positive controls (2-AA), limit of solubility: 500 ug/ml (solubility test, not observed when tested in experiment), incubation time: 20 hrs, triplicate, 2 experiments (2nd <= 75 ug/ml)
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

12.07.2002 (131)

Type	:	Ames test
System of testing	:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537
Test concentration	:	0, 3.3, 33.3, 100, 333.3, 666.7, 1000, 3333.3 ug/pl.
Cycotoxic concentr.	:	preliminary check for toxicity to TA100 up to 10 mg/plate or limit of solubility
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: preincubation methodology (PM), rat liver S9-mix and hamster liver S9 mix, solvent: DMSO, solvent and positive controls (2-AA, 4-nitro-o-phenylenediamine, sodium azide, 9-AAD)
Year	:	1983
GLP	:	no data
Test substance	:	other TS: purity: > 99 %
Remark	:	positive only in TA 100 and TA1535 in the presence of an activation system. A positive response was also obtained with TA1535 in the absence of metabolic activation. +S9: TA100 up to 3.3 times the spontanous revertant frequency (hamster), 2.2 times (rat); TA1535 up to 6.4 times (hamster), 7.1 (rat), -S9: TA1535 up to 3.1
Reliability	:	(2) valid with restrictions only four strains used, no information about GLP
Flag	:	Critical study for SIDS endpoint

02.04.2003 (106) (53)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Drosophila SLRL test
Species	:	Drosophila melanogaster
Sex	:	male
Strain	:	other: Canton-S wild
Route of admin.	:	other: feeding or i.p. injection
Exposure period	:	feeding solution: 72 h injection: once
Doses	:	100 ppm (feeding: in EtOH/5 % sucrose or injection: in EtOH/saline)
Result	:	negative
Method	:	other: feeding: 4 day old males (24 hrs old for feeding, 72 hrs exposure) were mated; injection: 1-3 d old; 24 hrs after exposure mated, primarily post-meiotic germ cells were tested.
Year	:	1985
GLP	:	no data
Test substance	:	other TS: purity: > 99 %
Remark	:	p-chloronitrobenzene was first assayed in the SLRL test in an adult feeding experiment; the result was negative and the test substance was retested by injecting adults
Reliability	:	(2) valid with restrictions

no information about GLP
Flag : Critical study for SIDS endpoint
19.08.2002 (53) (132)

Type : Drosophila SLRL test
Species : Drosophila melanogaster
Sex : male
Strain :
Route of admin. : unspecified
Exposure period : no data
Doses : no data
Result : negative
Method : other: no further information
Year :
GLP :
Test substance :

Reliability : (4) not assignable
Documentation is insufficient for assessment
16.07.2002 (133)

Type : Drosophila SLRL test
Species : Drosophila melanogaster
Sex : male
Strain : other: Canton S-wild
Route of admin. : oral feed
Exposure period : from larvae to adult
Doses : (1) 0, 77 ppm, (2) 0, 79 ppm, dissolved in EtOH and added to the food
Result : negative
Method : other: see remark
Year : 1989
GLP : no data
Test substance : other TS: purity: 99 %

Method : In order to obtain individuals for larval treatment Canton-S females and males were mated and eggs exposed in vials with standard cornmeal food containing the chemical plus solvent alone. Adult males emerging from the treatment were mated at approximately 24 hours of age with two successive harems of three to five Basc females to establish two single day broods. Males were then discarded and the conventional SLRL assay was carried out.
Remark : the test compound was tested in the SLRL mutation assay after being fed to Drosophila melanogaster larvae
Reliability : (2) valid with restrictions
no information about GLP
Flag : Critical study for SIDS endpoint
01.08.2002 (53) (134)

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : NMRI
Route of admin. : i.p.
Exposure period : single application
Doses : 0, 500 mg/kg bw/day dissolved in corn oil based on a range finding study (100, 500, 750, 1000 mg/kg bw/day)
Result : positive
Method : OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"

Year	:	1990
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	positive control: cyclophosphamide, preparation times: 24, 48, 72 hrs post application, 5 animals/sex/group, statistical methods: Wilcoxon's non-parametric rank sum test, one-sided chi-square-test
Remark	:	3/40 treated animals died before the end of the test. Treated animals showed lasting symptoms of toxicity after administration (apathy, sounds, roughened fur, cyanosis, spasm, staggering gait, twitching, shivery, difficulty in breathing), the ratio of polychromatic to normochromatic erythrocytes was altered by treatment with p-chloro-nitrobenzene (1000:1012 (control), 1000:1807 (24 h), 1000:995 (48 h), 1000:742 (72 h)) incidences of micronucleated cells: 1.5/1000 (control), 3.7/1000 (24 h), 9.1/1000 (48 h), 3.6/1000 (72 h)
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

02.04.2003 (135)

Type	:	Sister chromatid exchange assay
Species	:	other: Chinese Hamster bone marrow
Sex	:	male/female
Strain	:	
Route of admin.	:	i.p.
Exposure period	:	single treatment
Doses	:	0, 65, 130 or 260 mg/kg bw/day dissolved in corn oil based on a range finding study (125, 250, 300, 400, 500, 1000, 2000 mg/kg bw/day)
Result	:	
Method	:	EPA OTS 798.5915
Year	:	1990
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	positive control: cyclophosphamide, 5 animals/sex/group, sacrifice: 24 hrs post administration
Remark	:	the DNA-modifying effects of p-chloronitrobenzene were evaluated in vivo in the bone marrow of Chi-nese hamsters
Result	:	weakly positive: frequency of SCEs per metaphase: 1.62, 2.31**, 2.17, 2.20* (control, 65, 130 resp. 260 mg/kg bw/day) *,** = statistical significant (but data within the range of the historical controls); animals showed no symptoms after <= 130 mg/kg bw/day; 260 mg/kg bw/day (up to 5 hrs): apathy, reduced reflexes, extension, spasms, thereafter unaffected; 1 animal died
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

19.08.2002 (136)

Type	:	other: Chromosome aberration
Species	:	other: rat bone marrow
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	once
Doses	:	0, 30.0, 100.0, 300.0 mg/kg bw dissolved in corn oil
Result	:	negative
Method	:	OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"

Year : 1984
GLP : yes
Test substance : other TS: purity: 99.3 %

Method : positive control: cyclophosphamide, 5 animals/sex/group (high dose: 6); dose range finding study performed (200, 400, 600 mg/kg bw)
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.08.2002 (110) (137)

Type : other: DNA damage assay: single-strand DNA-breaks in brain
Species : mouse
Sex : male
Strain : Swiss
Route of admin. : i.p.
Exposure period : single application
Doses : 0, 60 or 1000 mg/kg bw/day in DMSO
Result : positive
Method : other: other: 4 hrs post appl. mice (number not mentioned) were sacrificed, DNA damage was evaluated by the alkaline elution technique coupled with a microfluorometric method for DNA assay
Year : 1980
GLP : no data
Test substance : other TS: no data on purity

Result : effects: DNA damage was recognizable, i.e., an increased elution rate in alkali of DNA from brain was obtained, 60 mg/kg: 2.6 fold increase in elution rate, 1000 mg/kg: 3.1 fold increase
Reliability : (2) valid with restrictions
study meets the criteria of today, but information on GLP and on purity of TS are missing
Flag : Critical study for SIDS endpoint
02.04.2003 (138)

Type : other: DNA damage assay: single-strand DNA-breaks in brain, liver and kidney
Species : mouse
Sex : male
Strain : Swiss
Route of admin. : i.p.
Exposure period : single application
Doses : 0, 30, 60, 180 or 1000 mg/kg/day bw in DMSO
Result : positive
Method : other: 12 mice/group, 4 hrs post injection mice were killed, brain, liver and kidney removed: alkaline elution procedure coupled with a microfluorometric method for DNA assay
Year : 1983
GLP : no data
Test substance : other TS: no data on purity

Result : effects: DNA damage was recognizable 4 h after administration in vivo, i.e., an increased elution rate in alkali of DNA from brain (6.8 fold increase in elution rate), liver (up to 6.2 fold) and kidney (up to 7.9 fold) was obtained
Reliability : (2) valid with restrictions
study meets the criteria of today, but information on GLP and on purity of TS are missing
Flag : Critical study for SIDS endpoint

02.04.2003 (139)

5.7 CARCINOGENICITY

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	24 months
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	0, 0.1, 0.7 or 5.0 mg/kg bw/day dissolved in corn oil
Result	:	
Control group	:	yes, concurrent vehicle
Method	:	other: according OECD Guideline 453, see also freetext ME
Year	:	1983
GLP	:	yes
Test substance	:	other TS: purity: 99.33 %
Method	:	deviations: no satellite group (high and control), hematology: 10 rats/sex/group, time interval for interim examinations: month: 6, 10, 12, 18, 24 60 rats/sex/group tissues examined histopathologically: all rats in control and high dose group regardless of their time point of death, as well as gross lesions, testes, epididymides and spleens for all low and mid dose animals statistical methods: Bartlett's test, ANOVA, Summed Rank Test (Dunn), Regression Analysis, Jonckheere's Statistics
Remark	:	see also chapter 5.4 Repeated Dose Toxicity
Result	:	Neoplasms were seen in both control and treated rats (low to high dose) pituitary gland ademomas: m: 25/60, 13/16,14/15, 22/60; f: 43/60, 39/49, 42/45, 43/59 phaeochromocytoma: m: 14/60, 6/15, 11/15,14/60 m: liver adenoma: f: 3/60, 8/29, 4/28, 2/60 bening and malign neoplasms of the skin, mammary glands and adrenal cortical or medullary neoplasms were similiar in control and treated groups of rats. Unilateral and bilateral interstititial cell tumors of the testes occurred in controls (1.7 %) and in the low (6.8 %), the mid (8.3%) and high(10.0 %) dosed male rats: incidences of rats that died prior to term, control, low to high dose: 0/39, 2/38, 1/43, 2/39 incidences of rats sacrificed at term, control, low to high dose: 1/21, 2/21 4/17, 4/21 Historical control data compiled from 14 long-term studies of the same institute using the same strain showed incidence of 9.8 % interstititial cell tumors of testes.
Reliability	:	(2) valid with restrictions Histopathological examination was not performed of all rats.
Flag	:	Critical study for SIDS endpoint

12.08.2002 (92) (93) (94)

Species	:	rat
Sex	:	male
Strain	:	CD-1
Route of admin.	:	oral feed
Exposure period	:	18 months

Frequency of treatm.	:	daily
Post exposure period	:	6 months
Doses	:	0, 250, 500, 1000, 2000 or 4000 ppm (= approx. 0, 18.75, 37.5, 75, 150 or 300 mg/kg bw/day)
Result	:	negative
Control group	:	yes, concurrent no treatment
Method	:	other: 25 rats/group, see also freetext ME
Year	:	1978
GLP	:	no data
Test substance	:	other TS: purity: 97-99 %
Method	:	200 or 400 ppm for 3 mo., 250 or 500 for the following 2 mo, 500 or 1000 ppm for another 12 mo; complete gross necropsy and histology on certain organs, on all grossly abnormal organs and tumour masses; statistical methods: Fisher Exact Test, Bonferroni correction
Remark	:	rats which died during the first 6 months of the study were discarded without necropsy
Result	:	1-Chloro-4-nitrobenzene was ineffective in male rats
Reliability	:	(2) valid with restrictions Study doesn't meet the criteria of today and reported in brief
Flag	:	Critical study for SIDS endpoint

12.08.2002 (140)

Species	:	mouse
Sex	:	male/female
Strain	:	CD-1
Route of admin.	:	oral feed
Exposure period	:	18 months
Frequency of treatm.	:	daily
Post exposure period	:	3 months
Doses	:	0, 3000 or 6000 ppm (= ca. 450 or 900 mg/kg bw/day)
Result	:	
Control group	:	yes, concurrent no treatment
Method	:	other: 25 mice/sex/group, see also freetext ME
Year	:	1978
GLP	:	no data
Test substance	:	other TS: purity: 97-99 %
Method	:	300 or 600 ppm for 18 months, complete gross necropsy, histology on certain organs, on all grossly abnormal organs and tumour masses, pathological examination was not performed on mice which died within the first 6 months, statistical methods: Fisher Exact Test , Bonferroni correction
Result	:	both male and female mice showed a significant increase in vascular tumors at the high dose level, (localisation of the vascular tumors was not specified) incidences of vascular tumors: male mice: low dose level: 2/14, high dose level: 4/14, simultaneous control: 0/14, pooled control: 5/99; female mice: low dose level: 2/20, high dose level: 7/18, simultaneous control: 0/15, pooled control: 9/102; male mice also had some elevation in liver tumors (hepatomas) at the low dose level incidences of liver tumors in male mice: low dose level: 4/14, high dose level: 0/14, simultaneous control: 1/14, pooled control: 7/99
Reliability	:	(2) valid with restrictions Study doesn't meet the criteria of today and reported in brief

Flag : Critical study for SIDS endpoint
12.08.2002 (140)

5.8.1 TOXICITY TO FERTILITY

Type	:	Two generation study
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	see type and remarks
Frequency of treatm.	:	daily
Premating exposure period		
Male	:	F0: 100 d prior to mating , F1 120 d prior to mating
Female	:	F0: 100 d prior to mating , F1 120 d prior to mating
Duration of test	:	55 weeks
No. of generation studies	:	2
Doses	:	0, 0.1, 0.7 or 5.0 mg/kg bw/day dissolved in corn oil
Control group	:	yes, concurrent vehicle
other: LOAEL(general tox., adult and offspring)	:	= .1 mg/kg bw
Method	:	other: according to OECD Guide-line 416, see also freetext ME
Year	:	1981
GLP	:	yes
Test substance	:	other TS: 99.43 %

Method : premating period prolonged, 20 pregnant females were not achieved in every group, testes of males in the low and mid-dose group were not examined histopathologically, hematology was not performed, esp. methemoglobin levels were not measured, statistical methods: ANOVA, Dunnett's test, Kruskal-Wallistest, Dunn's Rank Sum, Chi-Square, Fisher Exact test, Armitage's test

Result : F0:
Mortality: none of control and treated males, females: 1, 0, 3, 2 (control-high dose, mostly attributed to dosing-related injuries); 1 low-dose female was sacrificed in moribund condition; body weight gain and food consumption did not reveal an adverse effect of treatment,
Mating indices for F0 females were comparable between control (86.7 %) and low-dose-gr.(80.0 %) but not statistically significant lower in the mid-(71.4 %) and high-(71.4 %)-dose- group; for F0 males mating indices were 93.3 % 86.7 %, 80.0 % and 93.3 % (control to high dose), respectively; fertility indices of males (contr. to high dose): 92.9 %, 100 %, 91.7 % 71.4 % and pregnancy rate (control to high dose): 80.8 % 83.3 %, 80.0 %, 70.0 % were (not statistically significant) lowered in the high dose-groups. Gestation length, parturitions data, litter size data during lactation, mean pup weight data, pup sex distribution data were comparable between control and treated groups. Litter survival indices slightly lower than control at the mid and the high-dose levels (93.8% 85.7% resp. 100% control). 1 female of mid-dose group delivered 1 litter of 6 live pups, no live pup remained in the litter by d6 of lactation; pup survival index was significantly reduced in the high dose group (d0-4: 85.6 % (control: 94.4 %), d4-21: 91.6 % (control: 98.7%) because 2 high dose females experienced complete pup mortality within their litters.
No external malformations, or histopathological changes of tissues from selected organs were seen in the dead pups recovered at birth or during lactation in control, mid- and high-dose groups. 1 dead pup of the low dose

group, recovered at birth, had no tail.
F1-adult:
mortality (control to high dose): male: 6.7 %, 13.3 % 33.3 %, 6.7 %, respectively and female: 3.3 %, 3.3 %, 3.3 %, 3.3 %, rspectively which was attributed to dosing-related injuries; body weight gain and food consumption did not reveal an adverse effect of treatment,
Female mating indices were (not stat. sign., not dose-related) lower than the respective control data (contr. to high dose: 86.2 %, 76.7 %, 65.5 %, 72.4 %), Male mating (contr. to high dose: 92.9%, 91.7%, 84.6%, 86.7%) and fertility indices (contr. to high dose: 92.3%, 91.7%, 100%, 92.3%) and female pregnant rates (contr. to high dose: 68%, 73.9%, 84.2%, 85.7%) were comparable between the control and the treated groups. No adverse effect was indicated in regard to gestation length, partiturition data, litter size data during lactation, litter survival indices (2 low- and 1 high-dose females failed to wean litters), mean pup weight data or pup sex distribution data. No external malformations, or histopathological changes of tissues from selected organs were seen in the dead pups recovered at birth or during lactation in control, low-, mid- and high-dose groups.
Histopathology:
in F0 males, high dose: histological changes in testes (bilateral degeneration/atrophy of epithelium in 2/15 animals and bilateral maturation arrest of the germinal epithelium in 1/15 animals), epididymal observations (oligospermia) also noted in these same F0 males which did not mate (males in the low- and mid-dose group were not examined);
F1 adult rats: histopathological evaluation of tissues revealed extramedullary hematopoiesis and reticuloendothelial cells containing brown pigment, in the spleens of all rats in all groups, appeared more pronounced in males and females of the high dose group.

Reliability : (2) valid with restrictions
testes of males in the low and mid-dose group were not examined histopathologically, hematology was not performed esp. methemoglobin levels were not measured

Flag : Critical study for SIDS endpoint

27.11.2002 (141) (93)

Type : other: NTP Reproduction and Fertility Assessment using continuous breeding protocoll with evaluation of fertility of the F1 from the final litter
Species : mouse
Sex : male/female
Strain : other: Swiss CD-1
Route of admin. : gavage
Exposure period : see type and remarks
Frequency of treatm. : daily
Premating exposure period
Male : 7 d
Female : 7 d
Duration of test : 34 weeks
No. of generation studies :
Doses : 0, 62.5, 125 or 250 mg/kg bw/day dissolved in corn oil
Control group : yes, concurrent vehicle
other: NOAEL(fertility) : = 125 mg/kg bw
other: NOAEL (adult general toxicity) : = 125 mg/kg bw
other: LOAEL (offspring general toxicity) : = 62.5 mg/kg bw
Method : other: NTP continuous Breeding Protocol, see also ME
Year : 1989
GLP : yes

Test substance : other TS: purity >99 %

Method : NTP Continuous Breeding Protocol: 20 ps/group, 40 ps control, exposure period: F0: 7d prior to cohousing, 98d of continuous breeding. Last litter from F0, control and high dose group were rared, weaned and kept until mating. Siblings received the same treatment as their parents. At sexual maturity, 20 non-sibling males and females were cohabited for 7 days and housed singly through delivery, until sacrifice. Exam.: symptoms, body wweight gain, water consumption; F1: contr, 250 mg-gr.: liver, spleen weight; F0: No. of litters per pair, No. of live pups per litter, proportion of pups born alive, sex ratio of live pups; F0, F1: fertility indices; F1(m): testes, epididymis, epididymal sperm motility, sperm morphology, sperm count, F1(f): vaginal cytology, estrual cyclisity;
statistical methods: Wilcoxon's test, F-test, multivariate analysis of variance, Cochron-armitage test, Chi-Square test, Shirley's test, Jonckheere's test, Dunn's test

Remark : see also chapter 5.4: task 1: dose finding

Result : F0-mice:
mortality: 3,3,1,4 control to high dose, but not evaluated as treatment related; all groups: body weight gain throughout the study, reduced water consumption, none of the mice cyanotic, high dose: reduced food consumption
F0 fertility:
control, 62.5, 125 mg-pairs:
100 resp. 95 % of the pairs delivered at least four litters
250 mg-gr.: 100 % of the pairs delivered the 1rst and 93 % the 2nd litter and only 86 % delivered the 3rd and 79 % delivered the 4th litter, for the 2nd through the 5th litters significant dose-dependent decreasing trend of percent of pairs delivering, proportion of born alive of the final litter significantly reduced;
in all groups: average number of live pups per litter was comparable, sex ratio of pups born alive was not affected
125 mg- and 250 mg-gr.: compared to the control group dam weights were increased at delivery of the final litter (113% resp. 110% compared to the control) and 21 days after the delivery of the final litter (112% resp. 118%). A trend test was positive.
F1-pups:
none of the pups were noted as being cyanotic,
125, 250 mg-gr (male, female and combined), 62.5 mg-gr (male and combined): reduced live pup weights at birth;
125, 250 mg-gr.: pup weights adjusted for litter size sign. reduced, dose-related response, in the final litter of the continuous breeding phase, the F1 weight gain was adversely affected
250 mg-gr.: in the final litter of the continuous breeding phase, the F1 pup survival were adversely affected, proportion of born pups alive sign. decreased;
F1-adult (control and 250 mg-gr):
water consumption comparable, at mating most of the 250 mg/kg bw/d group F1 animals were cyanotic (eyes,skin: blue tint, urine color: amber); fertility, mating and pregnancy indices were comparable, number of live pups delivered by 250 mg-dosed pairs was lower than from conrol pairs, the proportion of F2 pups born alive and live F2 pups weights at birth were significantly reduced in the 250 mg-group;
sperm parameters and vaginal cytology (F1, contr. and 250 mg-gr.):
the average estrous cycle length in the F1 females was
significantly increased whereas epididymal sperm motility,
sperm count and sperm morphology were not affected in the
F1 males by 4-chloronitrobenzene treatment;
gross pathology, F1, 250 mg-gr.:
in both F1 sexes: at terminal sacrifice, absolute liver weight and liver-to-

body weight ratios were increased and spleens were extremely enlarged and darkened while body weights were unchanged;
in males, absolute seminal vesicle weights were significantly decreased and seminal vesicle-to-body weight ratios were similarly affected at 250 mg/kg bw/d;
there was no evidence of an androgen deficiency lesion in the testes of 5 high-dose males examined

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
02.04.2003 (142) (97)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	days 6-19 of gestation
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	0, 5, 15 or 45 mg/kg bw/day, dissolved in corn oil
Control group	:	yes, concurrent vehicle
NOAEL teratogen.	:	ca. 15 mg/kg bw
LOAEL Maternal Toxicity	:	ca. 5 mg/kg bw
Result	:	see freetext RS
Method	:	other: according to OECD Guide-line 414: 24 mated rats per group, see also freetoxt ME
Year	:	1980
GLP	:	yes
Test substance	:	other TS: purity: > 99 %

Method : body determination not weekly but on d 0,d6, d20, food consumption not monitored, stat. methods: Kruskal-Wallis non-parametric procedure, Dunnett's test, ANOVA, Bartlett's test, Chi-Square analyses, Fisher Exact test, Bonferroni

Result : Maternal data:
Pregnancy rates were similar between the groups. No mortality occurred in the control or treated groups during d6-20. Terminal body weight and body weight gain (d6-20) was comparable between control, low- and mid-dose females, but sign. reduced in high-dose females (71 g versus 118 g of controls). Several high-dose females were reported to have pale eye color during dosing interval. Comparable between control, low- mid- and high-dose groups were the mean numbers of implantations (13.3,13.9,14.1,13.6), but mean number of resorptions were sign. increased in the high-dose group: 5.6 versus 0.5 in controls and mean number of live fetuses were significant decreased (8.0 versus 12.8 in controls) in this dose-group, 7/22 high-dose females (31.8%) had uterine implantation sites comprised entirely of resorption sites.
Maternal gross postmortem observations: mean spleen weights sign. higher than control in each treated group, mean spleen to body weight ratio higher in all treated groups, sign. in the mid- and high-dose group (dose-related increase), from low-dose onwards significant dose-dependent lesions of the spleen: enlargement (up to 4 x normal size in high-dosed females), dark coloration and/or pitted surface.
Fetal data:
The mean number of male and female fetuses and mean fetal weights were comparable between the control, low- and mid-dose groups. In the

high dose group, the mean number of male and female fetuses (male: 4.2 versus 6.2 in control, female 3.8 versus 5.5 in contr.) and the respective mean weights (male: 3.33 g versus 3.95 g, female: 3.11 g versus 3.76 g in contr.) were markedly lowered. In this dose-group the incidence of ossification variations (i.e., assymetrical/unossified sternebrae, incompletely ossified cervical vertebral transverse processes, rudimentary structures) was sign. increased when compared to controls (95.7 % versus 85.5 % in contr.).
Fetal evaluations at low- and mid-dose levels did not reveal a teratogenic response. At high-dose level, a significant increase in the incidence of skeletal malformations (30.4 % versus 1.3 % in controls), predominantly angulated ribs alone or associated with misshapen and/or shortend bones of the forelimbs (i.e. humerus, radius, ulna) was noted.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.08.2002 (143) (144)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : gestation d 6-19
Frequency of treatm. : daily
Duration of test :
Doses : 0, 2, 9, 35, 135 or 550 mg/kg bw dissolved in corn oil
Control group : yes
Result : other: see freetext RS
Method : other: pilot study to set dose levels, 5 mated rats/group, the rats were sacrified on d 20 of gestation
Year :
GLP :
Test substance : other TS: Purity > 99 %

Result : all dose levels: signs of cyanosis exhibited by all dams
at and above 9 mg/kg bw: enlarged spleens of the dams
at and above 35 mg/kg bw: increased incidence of resorptions
235 and 550 mg/kg bw: maternal deaths (mortality: 2/5 at 135 mg-group and 5/5 at 550 mg-gr.; reduced body weight gain of the dams
Reliability : (2) valid with restrictions
Dose Finding Study
31.07.2002 (144)

Species : rabbit
Sex : female
Strain : New Zealand white
Route of admin. : gavage
Exposure period : days 7-19 of gestation
Frequency of treatm. : daily
Duration of test :
Doses : 0, 5, 15 or 40 mg/kg bw/day dissolved in corn oil
Control group : yes, concurrent vehicle
LOAEL Maternal Toxicity : = 5 mg/kg bw
other: LOAEL (Developmental Toxicity) : = 5 mg/kg bw
Method : other: according to OECD Guide-line 414 see also freetext ME
Year : 1982
GLP : yes
Test substance : other TS: purity: >99%

Method : 18 females/group were used.
In the high-dose group, mortality rate was so high (8 females during d18-22) and two females had aborted their pregnancies that the decision was made to terminate that group. No gross post mortem, reproduction or fetal evaluation data were taken for the high-dose females killed wth the decision to terminate the group.
Post treatment, surviving rabbits were observed until d 30
statistical methods: Kruskal-Wallis non-parametric procedure, Dunnett's test, ANOVA, Bartlett's test, Chi-Square analyses, Fisher Exact test, Boniferroni correction

Result : Maternal data:
Mortality rate (control to 40mg-gr): 1/18, 1/18, 1/18, 8/18
During treatment interval mean body weight loss in all groups, control and treated rabbits; mean body weight change during post-treatment interval comparable between control, low- and mid-dose groups.
In the 15-mg-group, anogenital staining was observed in a few dams at d 19; the 5-, 15- and 40-mg-dosed rabbits suffered from soft stool at d 19 of gestation; and the 40 mg-rabbits females had grayish/pale appearing eyes at d 10, d15 and d19.
Pregnancy rates: 94.4, 88.9, 88.9 % (control, 5 and 15 mg/kg bw)
Abortion occurred in 1 control female on d25 and 2 high-dosed females on d18 and d20, respectively. Premature delivery occurred in the low dose group on d30, d27, d29 and in the mid dose group on d26
In all other surviving dams there were no significant changes in reproductive parameters (i.e. mean number of implantations, resorptions and fetuses)
At necropsy of the dams, there were no adverse effects on maternal spleen weights. Although mortality was notably increased in the high dose level, no consistent treatment-related morphologic alterations were identified among the animals that died prior to the decision to terminate the group.

fetal data:
Between the control, the low- and the mid-dose groups, mean fetal weights (male: 40.6gr, 39.4gr, 41.5gr, female: 39.0gr, 38.6gr, 39.0gr) mean number of fetuses (male: 3.5, 4.3, 3.6, female: 4.4, 4.1, 4.1) were comparable.
Some variability in the sex distribution ratio (male/female) between control (53/66), the low-dose (51/49) and the mid-dose (51/57) was considered not to be treatment related.
No treatment related effect was evident in fetal ossification data, in external and soft tissues evaluation.
During the skeletal evaluations, the incidence of fetuses with malformations (predominantly fused sternebrae) was (not significantly) increased in the low and the mid dose levels, the number of affected litters were not dose-dependingly increased: contol: 1/63 fetus (1.6 %) in 1/15 litters, 5 mg-gr.: 3/54 fetus (5.9 %) in 2/12 litters, 15 mg-gr.: 4/58 fetus (6.9 %) in 2/14 litters. (According to the authors, this finding is seen historically at low incidence with this strain of rabbit)

Reliability : (2) valid with restrictions
Due to high mortality in the 40 mg-gr and termination of this group, only two dose groups were used to evaluate developmental toxic potency

Flag : Critical study for SIDS endpoint
12.08.2002 (145) (144)

Species : rabbit
Sex : female
Strain : other: New Zealand White
Route of admin. : gavage
Exposure period : gestation d7-19
Frequency of treatm. : daily
Duration of test :

Doses	:	0, 5,15,45 or 135 mg/kg bw
Control group	:	yes
Result	:	other: see freetext RS
Method	:	other: pilot study to set dose levels, post exposure period: 11 d, 5 mated rabbits/group, the rabbits were sacrificed on d30 of gestation
Year	:	
GLP	:	
Test substance	:	
Result	:	all dose levels: possible signs of cyanosis in one or more dams in each of the treated test groups 5 and 15 mg-group: no significant maternal toxicity at or below 45 mg-groups: no adverse fetal effects 45 mg-group: death of 1/5 dams by gestation d19 135 mg-group: death of all 5 dams by gestation d19
Reliability	:	(2) valid with restrictions Dose-Finding Study

07.08.2002 (144)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type	:	other: subacute toxicity
In vitro/in vivo	:	In vivo
Species	:	rat
Sex	:	male
Strain	:	other: CrCl:CD
Route of admin.	:	inhalation
Exposure period	:	2 weeks
Frequency of treatm.	:	6 h/d, 5 d/w (10 expsures)
Duration of test	:	4 weeks
Doses	:	0, 0.05, 0.29 or 0.64 mg/l (approx. 0, 50, 290, 640 mg/m³)
Control group	:	yes, concurrent no treatment
Result	:	0.29 and 0.64 mg/l: decreased testes weight after 10 exp. (ca.78 resp. 79 % of contr), seminiferous tubule degeneration (3/5, 4/5), abnormal spermatic contents of epididymides (4/5 in 0.64mg/l-gr. (not reversible)
Method	:	other: 16 rats/dose, head-only exposure, blood collecting from 10 rats every second day inclusive recovery period,5 rats sacrificed after 10 exp. and 5 rats sacrifieced after recovery period for clin. and pathol. examination
Year	:	1984
GLP	:	no data
Test substance	:	other TS: purity: 99.2 %
Remark	:	see also Chapter 5.4 for general toxicity
Reliability	:	(2) valid with restrictions no data on GLP, there are more relevant studies using a longer exposure time and therefore more relevant for the assessment.

31.07.2002 (85)

Type	:	other: subchronic
In vitro/in vivo	:	In vivo
Species	:	rat
Sex	:	male/female
Strain	:	other: F344/N
Route of admin.	:	inhalation
Exposure period	:	13 w
Frequency of treatm.	:	6 h/d, 5 d/w
Duration of test	:	13 w

Doses	:	0, 1.5, 3, 6, 12, or 24 ppm (= ca. 0.0, 9.81, 19.62, 39.24, 78.48,or 156.96 mg/m³ air)
Control group	:	yes, concurrent vehicle
Result	:	see freetext RS
Method	:	other: 10 rats/sex/group of the 0,6, 12, 24 ppm groups, whole body exposure, f: necropsy bw, estrous cycle length, percent of cycle spent in the various stages; m: necropy bw, reproduct. tissue weights, spermatozoal data, spermatogenes
Year	:	1993
GLP	:	yes
Test substance	:	other TS: purity: 98.8 %
Remark	:	see also Chapter 5.4 for general toxicity
Result	:	6, 12 and 24 ppm: among females, average estrous cycle length significantly decreased 24 ppm: among males, left caudal epididymal and testicular weights, epididymal sperm (spermatozoa) count per gram of caudal tissue and total spermatid head count per testis significantly decreased
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

31.07.2002 (86) (53)

Type	:	other: subchronic
In vitro/in vivo	:	In vivo
Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	inhalation
Exposure period	:	13 w
Frequency of treatm.	:	6 h/d, 5d/w
Duration of test	:	13 w
Doses	:	0, 1.5, 3, 6, 12, or 24 ppm (=ca. 0, 9.81, 19.62, 39.24, 78.48 or 156.96 mg/m³ air)
Control group	:	yes, concurrent no treatment
Result	:	24 ppm, females: significantly increased oestruous cycle length; no significant changes in sperm morphology in males
Method	:	other: 10 mice/sex/group of the 0, 6, 12, 24 ppm groups, whole body exp., f: necropsy bw, estrous cycle length, percent of cycle spent in the various stages; m: necropsy bw, reproduct. tissue weights, spermatozoal data, spermatogenesis
Year	:	1993
GLP	:	yes
Test substance	:	other TS: purity: 98.8 %
Remark	:	see also chapter 5.4 for general toxicity
Reliability	:	(2) valid with restrictions Haematology was not performed
Flag	:	Critical study for SIDS endpoint

06.08.2002 (53)

Type	:	other: testicular toxicity
In vitro/in vivo	:	In vivo
Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	oral unspecified
Exposure period	:	single application
Frequency of treatm.	:	once

Duration of test	:	26 d
Doses	:	250 mg/kg bw
Control group	:	
Result	:	no effect on testicular histopathoilogy (at d1) or testes weight and daily sperm production (up to d25)
Method	:	other: no rats: 5 or 6
Year	:	
GLP	:	
Test substance	:	

Reliability : (4) not assignable
abstract only

31.07.2002 (58)

5.9 SPECIFIC INVESTIGATIONS

Endpoint	:	Immunotoxicity
Study descr. in chapter	:	5.9 Specific Investigations
Reference	:	
Type	:	other
Species	:	mouse
Sex	:	male
Strain	:	other: BDF1
Route of admin.	:	i.p. or s.c.
No. of animals	:	24
Vehicle	:	other: olive oil
Exposure period	:	
Frequency of treatm.	:	once
Doses	:	0, 300 mg/kg bw
Control group	:	yes, concurrent vehicle
Observation period	:	up to 10 days
Result	:	see remark
Method	:	other: see remark
Year	:	1999
GLP	:	no data
Test substance	:	other TS: no data on purity

Method : 24 males as exp. group and 24 males as control, single inj., on day 3, 5, 7 and 10 after inj. spleens were weighed and splenocytes were harvested (6 mice/gr/harvest time), staining of the cells and flow cytometric analysis; d5: s.c. injection was given to two other groups of mice (n=?, control and exposure group); statistical methods: F-test, unpaired t-test, Mann-Whitney-U test

Result : Spleen weight sign. greater in exposed animals
Indication of immunotoxic effect: Reduction of natural killer cells, T cells and B cells in the spleen, and increase in macrophages and nucleated erythrocythes and dead cells (after i.p.) in the spleen
comparison of i.p. and s.c. application: sign. difference in macrophages and nucleated erythrocytes, i.p. more macrophages, s.c. more nucleated erythrocytes

Reliability : (2) valid with restrictions
no data on purity and non information on GLP

31.07.2002 (146)

Endpoint	:	Immunotoxicity
Study descr. in chapter	:	5.9 Specific Investigations
Reference	:	
Type	:	other

Species	:	mouse
Sex	:	male
Strain	:	other:BDF1
Route of admin.	:	i.p.
No. of animals	:	20
Vehicle	:	other: olive oil
Exposure period	:	
Frequency of treatm.	:	once
Doses	:	0, 300 mg/kg bw/day
Control group	:	yes, concurrent vehicle
Observation period	:	up to 14 days
Result	:	NK cell activity inhibited (3rd d>14th d), CTL activity weaker on d3 (returned to contr. level on d7), LPS-stimulated lymphocyte proliferation weaker on d3, d7 (recovery up to d 14), no. of splenocytes increased, no diff. in Hb conc. and bw
Method	:	other: see remark
Year	:	1998
GLP	:	no data
Test substance	:	other TS: no data on purity

Method	:	other: 20 as exp. gr., 20 as controls, single inj., harvest of splenocytes on d1,3,7,14 after inj., determ. of cell viability, activity of natural killer(NK) cells, cytotox. T-lymphocytes(CTL), LPS-stim. splenocyte prolif., Hb-Conc.; statistical methods: F-test, paired and unpaired t-test, Mann-Whitney U-test
Reliability	:	(2) valid with restrictions no data on purity and no information on GLP

12.08.2002 (147)

Endpoint	:	Immunotoxicity
Study descr. in chapter	:	5.9 Specific Investigations
Reference	:	
Type	:	other
Species	:	mouse
Sex	:	male
Strain	:	other: BDF1
Route of admin.	:	i.p.
No. of animals	:	15
Vehicle	:	other: olive oil
Exposure period	:	28 day(s)
Frequency of treatm.	:	3 times/week, 4 weeks
Doses	:	0, 30 mg/kg bw/day
Control group	:	yes, concurrent vehicle
Observation period	:	no
Result	:	NK cell activity lowered, CTL activity weaker from the 3rd week onward, LPS-stimulated lymphocyte proliferation weaker (sign. at the 3rd week), no. of splenocytes less at week 4, no diff. in Hb conc. but sign. decreased bw
Method	:	other: see freetext ME
Year	:	1998
GLP	:	no data
Test substance	:	other TS: no data on purity

Method	:	other: 15 mice/group, harvest of splenocytes 2, 3, 4 weeks after init. inj. (5 mice for each harvest), determ. of cell viability, activity of natural killer(NK) cells, cytotox. T-lymphocytes(CTL), LPS-stim. splenocyte prolif., Hb-Conc., bw-gain; statistical methods: F-test, paired and unpaired t-test, Mann-Whitney U-test
Reliability	:	(2) valid with restrictions no data on purity and non information on GLP

12.08.2002 (147)

5.10 EXPOSURE EXPERIENCE

Remark : in man, p-chloronitrobenzene may be absorbed through the lungs and skin giving rise to methaemoglobin

(148)

Remark : clinical observations on 12 workers (from 21 to 44 years old) exposed to p-chloronitrobenzene in a chemical factory, are reported: haematological tests revealed moderately decreased erythrocyte counts, haemoglobin levels, and haematocrit values; slight increases in reticulocytes and formation of Heinz bodies were observable; the workers often complained of dizziness, headache, nausea and other symptoms and had a pale appearance (the workmen had been in service for 4 months to 17 years)

12.07.2002 (149)

Remark : p-chloronitrobenzene causes severe toxic effects in persons, who are exposed to the substance via inhalation at a concentration of 10 ppm (= ca. 0.066 mg/l) for 1 minute (no further data)

(150)

Remark : case report: a 3 year old boy who had put p-chloronitrobenzene into his mouth, developed signs of toxicity: cyanosis of lips, ears and of mucous membranes of mouth and eyes; paroxysms of dyspnoea, headache, temporarily running pulse, superactivity, ataxia; blood tests 1 d after the intoxication revealed brownish discoloration of the blood and the haemoglobin level of the blood: 80 %. Leucocytes: 20,000. 10 d after intoxication: Hb level 60%, leucocytes 18,000

12.07.2002 (151)

Remark : case report on late effects of toxic methaemoglobinaemia: a psychological testing rendered it possible to reveal some disorders of the central nervous system in a 52 year old chemical industry worker who had suffered from a serious intoxication with p-chloronitrobenzene a year ago; such symptoms of acute toxicity as loss of consciousness, convulsions and serious methaemoglobinaemia (MetHb > 60%) had been observed (in this case, the symptoms occurred after dermal exposure to p-chloronitrobenzene and after subsequent consumption of alcoholic beverages); this case indicated a possibility of the development of late effects of poisoning

12.07.2002 (152)

Remark : based on clinical and laboratory evaluation of cyanosis cases during a 10-year period a number of cyanogenic aromatic nitro compounds were ranked in descending order of relative hazard relating to the chemical cyanosis anaemia syndrome seen in exposed industrial workers (rank 1 = most potent, rank 13 = least potent): p-chloronitrobenzene was classified in rank 7 relating to its cyanogenic potential, in rank 2 relating to the anaemiagenic potential and in rank 5 relating to the overall po-

tential; laboratory evaluation showed that total oxygenatable haemoglobin in some cases, notably after exposure to the chloronitrobenzenes, was less than would be expected from methaemoglobin analysis (unspecified route of absorption)

Flag : Critical study for SIDS endpoint

(153)

Remark : a number of the more important aromatic nitro-compounds were ranked showing their comparative hazard ratings for cyanosis, anaemia and over-all toxicity (the degree of hazard ranges from 1 = slight hazard to 6 = severe hazard): for p-chloronitrobenzene, the degree of hazard is 4 concerning cyanosis hazard, 2 concerning anaemia hazard and 3 concerning over-all toxic hazard (no further data)

(154)

Remark : in chloronitrobenzene poisoning cardiac complications appear to be more frequent and more serious than in aniline poisoning and gastrointestinal irregularities (anacidity) also appear to be quite common (no further data, isomer(s) of chloronitrobenzene not specified)

(155) (156)

Remark : several workers who were employed in factories producing aromatic nitro compounds were examined; the workmen were exposed to p-chloronitrobenzene at concentrations of 0.0086-0.0223 mg/l air: these workers developed cyanosis, headache and vertigo; haematological tests showed decreased haemoglobin levels and decreased erythrocyte counts, increased blood methaemoglobin and increases in Heinz bodies

(157)

Remark : four workmen were reported who were hospitalized as the result of exposure to a mixture of o- and p-chloronitrobenzene; these cases resulted from 2 to 3 exposures of 2 days each and all were cyanotic; headache and weakness accompanied the cyanoses

Flag : Critical study for SIDS endpoint
12.07.2002

(158)

Remark : all 325 records of industrial chemical cyanosis poisoning in Britain notified to the inspectorate from 1961 to 1980 were scrutinised: the cases occurred mainly during chemical or dyestuff manufacture; a total of 50 cases of chemical cyanosis syndrome due to chloronitrobenzene were reported; 23 (46 %) cases were "early cases", i.e., the symptoms developed while at work on the same day of exposure, and 27 (54 %) cases were "delayed cases", i.e., the symptoms developed insidiously or some definite time after the "working" day on which the poisoning occurred (the route of absorption is not described in detail for each test compound, the most cases resulted from skin absorption and/or inhalation; in this study, the isomer(s) of chloronitrobenzene is (are)

not clearly specified)

Flag : Critical study for SIDS endpoint

(159)

Remark : the methaemoglobin and diazo substances in urine of stevedores with acute p-chloronitrobenzene poisoning in Osaka, Japan were determined daily; the poisoning was mostly via skin absorption; the diazo substances in urine increased with increasing exposure to p-chloronitrobenzene and decreased exponentially in daily determination; a high correlation was observed between diazo substances and methaemoglobin (no further data)

(160)

Remark : 12 chemical operators who were exposed to vapours of p-chloronitrobenzene at an average concentration of 13.7 ppm (51 air samples taken during the survey showed concentrations varying between 1 and 61 ppm) did not show more definite symptoms or signs of toxicity, there were no medical complaints, and no worker showed any methaemoglobinaemia

12.07.2002 (99)

Remark : ten male workers handling p-chloronitrobenzene in a dye-producing factory were monitored for 1 w in autumn, in winter and in summer; the workmen were engaged for 8 h/d
in a day shift under a 5-d workweek system and the concentrations of p-chloronitrobenzene in air near workers breathing zone were 0.000306 mg/l (autumn), 0.000158 mg/l (winter) and 0.00038 mg/l (summer); urine samples were collected from the workers both before and after each shift: it was found that the urinary diazo-positive metabolites levels reflect the p-chloronitrobenzene levels in personal ambient air in autumn or winter and that the ambient p-chloronitrobenzene level can be estimated from the urinary level in these seasons; however, in summer, most of the workers showed higher values of the urinary diazo-positive metabolites irrespective of the ambient exposure level and the ambient level cannot be estimated from the urinary level; both the excretion of p-chloronitrobenzene metabolites into the sweat and the contact of the substance with the skin may have increased in summer

(161)

Remark : eight longshore-men were poisoned with p-chloronitrobenzene; headache, faintness and anaemia due to methaemo-globinaemia were observed as characteristic symptoms of p-chloronitrobenzene poisoning

(162)

Remark : Urinary metabolites from human subjects acutely poisoned with p-Chloronitrobenzene were identified:
large amounts of: N-acetyl-S-(4-nitrophenyl)-l-cysteine, p-chloroaniline, 2-chloro-5-nitrophenol and

p-chloroformanilide produced by pyrolysis of
p-chloro-oxanilic acid originating from p-CNB
small amounts of: 2-amino-5-chlorophenol,
2,4-dichloroaniline and traces of p-chloroacetanilide and
4-chloro-2-hydroxyacetanilide.
All of the absorbed p-CNB was metabolized prior to
excretion, as the parent compound was not found in urine.

Flag : Critical study for SIDS endpoint
12.02.1998 (163) (164) (165)

Remark : case report
The exposition against a mixture of 2-chloro- and
4-chloronitrobenzene caused severe intoxications which
exceeds the signs of intoxication during repair of a unit
for isolation of the isomers. As symptoms cyanotic
appearance and collapse were described. Hb-content was
decreased up to 65 % of the normal value. During recovery
period the patient suffered from difficulty in breathing and
sensation of dizziness. Within 7 weeks Hb content increased
to 80 % of the normal value.

Flag : Critical study for SIDS endpoint
21.11.2001 (166)

Remark : Accidental inhalation of large quantities of nitrochlorobenzene vapours caused marked cyanosis, transient
methemoglobinemia, a large number of erythrocytes with Heinz
bodies and persistent sulphohemoglobinemia, hemolytic anemia.
Sideremia was high in the first days after the accident, then decreased to
normal values. Within 21 days after the accident
reticulocytosis was noted.
12.07.2002 (167)

Remark : Metabolism of p-NCB in 53 individuals [43 exposed Chinese workers (38 post-shift, 5 pre-shift), 10 non-exposed controls, additional 8 controls (non matched)] determined by HPLC-UV analysis of urine samples before and after acid hydrolysis.
Result: There were no metabolites in the control samples within the detection limits.
Pre- and post-shift: the mercapturic acid, N-acetyl-S-(4-nitrophenyl)-L-cysteine(NANPC), was the only metabolite detected in the pre-hydrolysed urine.
Pre- and post-shift: p-Chloroaniline (p-CA) and 2-chloro-5-nitrophenol(CNP) were detected following acid hydrolysis.
The acetylated metabolites, 2-acetamino-5-chlorophenol and p-chloroacetanilide and the de-acetylated metabolite, 2-amino-5-chlorophenol, were not detected in free or hydrolysed urine respectively.
In conclusion: The metabolites detected were excreted following phase 2 conjugation with glutathione, glucorunide or sulphate. p-CA was released only following acid hydrolysis indicating that N-conjugation had occurred.
12.07.2002 (168)

5.11 ADDITIONAL REMARKS

Type : Cytotoxicity

Remark : the cytotoxicity of p-chloronitrobenzene (among other nitrobenzene compounds) towards isolated rat hepato-

cytes under aerobic or hypoxic conditions has been determined; the effectiveness of p-chloronitrobenzene at causing cytotoxicity under aerobic conditions was markedly increased if hepatocyte catalase was inhibited with azide, whereas addition of azide only slightly increased the susceptibility of hepatocytes to p-chloronitrobenzene under hypoxic conditions; p-chloronitrobenzene at cytotoxic concentrations induced cyanide-resistant respiration in the hepatocytes; in the absence of azide, the test substance was more cytotoxic under hypoxic than aerobic conditions

13.02.2002 (169)

Type : Metabolism

Remark : metabolism in vitro: radiolabelled (14 C) p-chloronitrobenzene (concentration not specified) was incubated with isolated rat hepatocytes for up to 90 min.: after 90 min., 76 % of the p-chloronitrobenzene had been metabolized; p-chloronitrobenzene was reduced and p-chloroaniline accounted for 15.4 % of the radioactivity after 90 min.; the major metabolite formed from p-chloronitrobenzene was p-chloroacetanilide, which represented 16.3 % of the total radioactivity after 90 min. incubation; p-chloronitrobenzene was also conjugated with glutathione

(170)

Type : Metabolism

Remark : in order to identify the specific enzymes involved in the metabolism of p-chloronitrobenzene by isolated rat hepatocytes, hepatic subcellular fractions were isolated from rats; microsomes incubated with radiolabelled (14 C) p-chloronitrobenzene in the presence of NADPH, produced p-chloroaniline under aerobic conditions and SKF 525 A and metyrapone had no effect on the metabolism to p-chloroaniline: these findings suggest that cytochrome P-450 reductase is responsible for p-chloronitrobenzene reduction; radiolabelled p-chloronitrobenzene was also incubated with or without microsomes, cytosol and/or glutathione: p-chloronitrobenzene was converted to S-(4-nitrophenyl)glutathione in the presence of cytosol and glutathione suggesting that cytosolic glutathione transferase is involved in this conjugation (concentration of the test substance unspecified)

(170)

Type : Metabolism

Remark : metabolism of p-chloronitrobenzene by hepatic subcellular fractions from rats: to determine the enzyme systems involved in the metabolism of p-chloronitrobenzene by rat isolated hepatocytes, radiolabelled (14 C) p-chloronitrobenzene (100 uM) was incubated with hepatic microsomes (incubation mixture containing microsomes and NADPH, some incubations also containing UDP-glucuronic acid) or with cytosol (incubation mixture containing GSH and cytosolic protein): reduction of p-chloronitrobenzene

to p-chloroaniline occurred readily in microsomal incubations; substitution of NADH for NADPH or incubation of microsomes under a carbon monoxide atmosphere significantly inhibited nitroreduction, boiling the microsomes completely abolished reduction of p-chloronitrobenzene; addition of SKF 525-A or metyrapone significantly inhibited the microsomal reduction of p-chloronitrobenzene to p-chloroaniline (the inhibition of nitroreduction by carbon monoxide, SKF 525-A and metyrapone suggests that cytochrome P-450 catalyzes this reaction); incubation of p-chloronitrobenzene with rat hepatic cytosol and glutathione resulted in the formation of S-(4-nitrophenyl)glutathione

(171)

Type : Metabolism

Remark : in vitro study of metabolism: after 90 min. incubation of isolated rat hepatocytes with radiolabelled (14 C) p-chloronitrobenzene (100 uM final concentration), 42.1 % of the added p-chloronitrobenzene was metabolized; the calculated half-life for disappearance of p-chloronitrobenzene from the incubations was 91 min.; a major metabolic pathway for p-chloronitrobenzene was reduction to p-chloroaniline (15.4 % of the total radioactivity after 90 min. incubation); another major metabolite was 4-chloroacetanilide accounting for 16.3 % of the total radioactivity; p-chloronitrobenzene was conjugated with glutathione and S-(4-nitrophenyl)glutathione accounted for 10.4 % of the total radioactivity

(171)

Type : Metabolism

Remark : the urinary metabolites of p-chloronitrobenzene were analyzed 8-24 h following i.p. injection of 100 mg/kg bw of p-chloronitrobenzene to rats: p-chloroaniline, 2,4-dichloroaniline, p-nitrothiophenol, and trace amounts of p-chloroacetonitrile as well as intact p-chloronitrobenzene were identified in urine samples; in addition, metabolites possibly associated with 2-chloro-5-nitrophenol, 2-amino-5-chlorophenol, p-chloroformanilide, and 4-chloro-2-hydroxyacetoanilide were also observed

(172)

Type : Metabolism

Remark : a single dose of 100 mg/kg bw of p-chloronitrobenzene (dissolved in olive oil) was administered i.p. to male Sprague-Dawley rats and urine samples were collected from the 8th to 24th hour after the administration; nine urinary metabolites were identified: p-chloroaniline; 2,4-dichloroaniline; p-nitrothiophenol; 2-chloro-5-nitrophenol; 2-amino-5-chlorophenol; p-chloroformanilide; 4-chloro-2-hydroxy-acetanilide; a small amount of p-chloroacetanilide and traces of unchanged p-chloronitrobenzene

Flag : Critical study for SIDS endpoint

(173)

Type : Metabolism

Remark : Comparison of the urinary metabolites in human subjects following exposure to p-CNB with those of the rats (p-chloroaniline, 2,4-dichloroaniline, 2-amino-5-chlorophenol, p-chloracetanilide, 4-chloro-2-hydroxyacetanilide, p-chloro- oxanilic acid) suggest p-CNB to be excreted in urine through the same metabolic pathway.

12.02.1998 (163) (174) (175) (176)

Type : Toxicokinetics

Remark : 48 h after a single oral administration of 200 mg/kg bw of p-chloronitrobenzene to rabbits, 2.8 % of the administered dose was found in faeces as unabsorbed material which consisted of approximately 1 part of p-chloronitrobenzene and 2 parts of p-chloroaniline; in the urines collected each 24 h for up to 96 h the following metabolites of p-chloronitrobenzene were detectable (expressed as percentages of the administered dose): ether glucuronide (19 %), ethereal sulphate (21 %), mercapturic acid (3-7 %), free chloroaniline (9 %) and conjugated chloroaniline (4 %) (total accounted for: 63 %)

Flag : Critical study for SIDS endpoint

12.05.2002 (177)

Type : Toxicokinetics

Remark : 14C-p-chloronitrobenzene was administered by gavage to groups of 8 male F344 rats at 2, 20 or 200 mg/kg bw (single administration); radioactivity was determined in urine and faeces up to 72 h and in tissues at 24 and 72 h: at all dose levels 68-74 % of the dose was excreted in urine and 10-12 % in faeces; 14C was excreted more slowly at 200 mg/kg bw, such that 35 % was in the 24-h and 7 % in the 72-h tissues, versus 25 % in the 24-h and 5 % in the 72-h tissues at the lower doses; in all tissues except fat, p-chloronitrobenzene equivalent concentrations were proportional to dose, but at 200 mg/kg bw concentrations in fat were disproportionately higher; unlike other tissues, concentrations in blood cells and spleen were the same at 72 h as at 24 h; at 24 h after all doses the highest concentrations were in fat, followed by blood cells, kidney, liver and skeletal muscle and at 72 h they were in blood cells, followed by fat and skeletal muscle at least 12 metabolites were in urine

Flag : Critical study for SIDS endpoint

06.08.2002 (53) (178)

Type : Toxicokinetics

Remark : p-chloronitrobenzene was administered by gavage to adult and geriatric rats at 65 mg/kg bw/d for 11 d; 14C-p-chloronitrobenzene was administered on days 1, 5 and 9; 14C was determined in urine and faeces up to 96 h after each 14C-dose and in tissues at 72 h after the day 9 dose: in adult rats, at all treat-

ment intervals, 61-81 % of each dose was excreted in urine and 7-15 % in faeces and the urinary excretion of 14C was marginally more rapid and extensive with pretreatment; 2 % of the day 9 dose was in tissues, the highest concentrations were in blood cells and spleen; of the 25 urinary metabolites 5 were = or > 5 % of the dose; the rates of urinary and faecal excretion of 14C varied between individual geriatric rats, such that 5-30 % of the day 9 dose remained in the tissues at 72 h, overall excretion was slower than in adult rats, but the pattern and extent of excretion and the tissue distribution of 14C were similar in the faster excreters and adult rats

Flag : Critical study for SIDS endpoint
12.07.2002 (53) (179)

Type : other: Haematotoxizitaet

Remark : result: In cat, 50 mg/kg bw lead to marked signs of intoxications, marked increase in heinz bodies and Methaemoglobin formation; 1/2 animals died 3 hours post application.
In cat, 5 mg/kg bw lead to slight methaemoglobin formation (0.1 %), no signs of intoxication were observed and no death occurred.

Source : Hoechst AG Frankfurt/Main
06.08.2002 (180)

Type : other: transplacental hematol. effect

Remark : abstract only
Single i.p. administration of 0.2 mg/kg bw p-NCB to pregnant ICR mice on day 16 of gestation resulted 24 h after administration in Heinz body containing erythrocytes (94.8 %, highest value), leading consecutively to an ncrease in reticulocytes and basophilic stippled erys (BSE).
In fetus, after additional 24 h, Heinz body containing erythrocytes, BSE and reticulocytes were observed.
14.01.2002 (181)

Remark : p-chloronitrobenzene undergoes three major types of transformation in vivo in mammals: nitro group reduction, displacement of the chloride in glutathione conjugation, and ring hydroxylation; conjugates of hydroxyl and amino groups can form
(182)

Remark : after a single s.c. administration of 0.5 g/kg bw of p-chloronitrobenzene to rabbits, increased levels of methaemoglobin, formation of Heinz bodies, decreased blood oxygen (increased affinity of haemoglobin for oxygen), decreased RBC count and a decrease in catalyse activity in blood were observed
(183)

Remark : after a single s.c. administration of 0.5 g/kg bw to rabbits, an increase in methaemoglobin levels and formation of Heinz bodies were observed
(149)

Remark : germ-free or conventional mice were injected i.p. with

p-chloronitrobenzene (dose not specified) and blood samples were drawn at half-hour intervals for determination of methaemoglobin: p-chloronitrobenzene produced measurable amounts of methaemoglobin only in the presence of intestinal flora

(184)

Remark : after a single s.c. administration of 4.69 mM/kg bw (= ca. 740 mg/kg bw) of p-chloronitrobenzene to rats, a significant decrease in the activity of the cerebral succinatedehydrogenase was observable 24 h after the administration; the internal respiration of the brain tissue and the activity of the cerebral cytochromeoxidase were not significantly effected by p-chloronitrobenzene

(76)

Remark : p-chloronitrobenzene, among other nitrobenzene derivatives, has been tested in vitro for its effects on cellular oxygen utilization in Ehrlich ascites tumor cells: p-chloronitrobenzene inhibited cellular oxidation in the presence and absence of glucose; inhibition was greater with diamide (glutathione oxidant)-treated cells; the test substance did not stimulate cellular oxidation by KCN- or antimycin A-inhibited cells, nor did it alter microsomal oxidations

(185)

Remark : after intragastric administration of 150 mg/kg bw of p-chloronitrobenzene to rats, blood concentration reached a maximal concentration of 25.9 ug/cc after 1.5 h and dropped to zero after 7 h; the highest concentration of methaemoglobin (75.5 %) was observed after 3 h, and within 30 h decreased to physiological levels

10.10.2001 (186)

Remark : the toxic effects of single oral (gavage) doses of p-chloronitrobenzene in rabbits were aggravated by subsequent oral administration of single doses of alcohol; signs of toxicity after administration of both p-chloronitrobenzene and alcohol: convulsions, fibrillary contractions of the whole muscular apparatus, nystagmus, cyanosis, tracheal hyperaemia

(187)

Remark : after a single intragastric administration of 78 mg/kg bw of p-chloronitrobenzene to rats, an increased content of glycogen, a decreased activity of lactate dehydrogenase and a decreased content of ADP were determined in the liver

(57)

Remark : an in vitro assay under physiological conditions was undertaken to obtain the non-enzymic rate of reaction with glutathione; p-chloronitrobenzene was incubated with glutathione overnight at 37 degrees centigrade, in sodium phosphate buffer (0.1 M) at pH 7.5; the con-

centrations of p-chloronitrobenzene and of glutathione were 0.1 mM and 10.0 mM, respectively: the test substance gave no detectable reaction, the relative reactivity of p-chloronitrobenzene compared with 1-chloro-2,4-dinitrobenzene, expressed as a percentage (i.e. 1-chloro-2,4-dinitrobenzene = 100 %), was < 0.1 %

(188)

Remark : p-chloronitrobenzene was injected s.c. into 15 dogs (dose: 400 mg/kg bw as 50 % acetone solution) and observed for up to 45 days. 24 hours post application methemoglobinemia ranged between 25-86 %. Dogs with methemoglobin concentrations of 63-86 % died within 3 days whereas dogs with a methemoglobin content ranging up to 43-47 % survived and recovered within 25 days.

11.02.2002 (189)

Remark : in an in vitro assay, p-chloronitrobenzene was found to inhibit type B monoamine oxidase from rabbit liver mitochondria

(190)

Remark : the effects of p-chloronitrobenzene in rabbits were studied by s.c. injection of 1.5 g and by dermal application: the methaemoglobin level increased in several h, decreased after about 12 h, then increased again 18 to 24 h following exposure; Heinz bodies formed during the second period of increasing methaemoglobin; clinical symptoms, such as anaemia, haematuria and haemoglobinuria were noted (no further data)

(191) (191)

Remark : following a single i.p. administration of 0.5 g/kg bw of p-chloronitrobenzene to rabbits, the blood pressure and the myocardial glycogen level were reduced

(95)

Remark : in vitro assay: the effect of p-chloronitrobenzene on microtubule organization in Swiss 3T3 murine fibroblasts and normal diploid human foreskin fibroblasts (strain AG1522) was studied; the assay was carried out by exposing mouse 3T3 fibroblasts for 3 h to 100 uM of p-chloronitrobenzene and by incubating human AG1522 fibroblasts for 3 h with 40 uM of the test substance: p-chloronitrobenzene had no discernible effect on the organization of microtubules of both cell types, i.e., it did not cause microtubule disassembly (these studies represent an approach to investigating the mechanisms of contact sensitivity induced by simple chemicals; the authors think that the results of the studies may provide the basis for developing an in vitro screening assay for identifying other potential halogenated nitrobenzene sensitizers)

(192)

Remark : effects of single and repeated subcutaneous injections of p-chloronitrobenzene on the osmotic fragility of red cell membrane in rabbits were studied, methaemo-

globin levels and appearance of Heinz bodies were also determined: in the single injection experiment, the osmotic fragility was increased immediately after the s.c. injection (doses: 50, 100 or 200 mg/kg bw); the increased methaemoglobin levels were observed first, and secondly the maximum changes of the osmotic fragility and appearance of Heinz bodies were observed nearly simultaneously after the injection of the compound; in the repeated injection experiment, haemolysis starting point was shifted toward higher osmotic pressure immediately after the injection of p-chloronitrobenzene (doses: 5 or 10 mg/kg bw/d), while it was shifted toward lower osmotic pressure after the last doses of the compound; haemolysis ending point was shifted toward higher osmotic pressure after the injection of 10 mg/kg bw/d, while it was shifted toward lower osmotic pressure after the injection of 5 mg/kg bw/d; the repeated injection of p-chloronitrobenzene induced continuously the increased methaemoglobin levels and appearance of Heinz bodies (no further data)

(193)

Remark : a single oral dose of 200 mg/kg bw of 14C-p-chloronitrobenzene was administered to male rats and the disposition of the test substance was investigated: within 72 h after dosing, 74.6 % and 20.5 % of the radioactivity was recovered in the urine and faeces respectively, and the body retained 2.9 % of the dose; the highest concentrations of the compound or its metabolites retained in the body were detected in the blood and spleen; two major early metabolites were identified in the urine: the sulfate or glucuronide conjugates of a nitrochlorophenol, and the N-acetylcysteine conjugate of nitrobenzene; two other metabolites were tentatively identified as aminochlorophenol and its N-acetylated derivative; at the dose level used in this study, male rats developed significant levels of methaemoglobin (14.2 %) compared with the control (1.04 %), 6 h after dosing; peak levels of methaemoglobin (41.1 %) were reached at 24 h after dosing with recovery apparent at 72 h (12.1 %)

01.08.2002 (194) (195)

Remark : p-chloronitrobenzene was administered i.p. (dose: 0.42 g/kg bw), orally (dose: 0.42 g/kg bw) or dermally (dose: 16.0 g/kg bw) to rats: methaemoglobinaemia was observed; 30 min. after administration of the test substance, the lowest methaemoglobin level (9.0 %) was seen in the case of dermal application; after i.p. and oral administration, the methaemoglobin levels were 12.0 and 22.0 %, respectively; 24 h after application of the test substance, a marked formation of Heinz bodies (40-60 %) was seen in the rats treated orally or i.p. and a moderate formation of Heinz bodies (10-30 %) was observed in the animals treated dermally

(60)

Remark : in vitro assay: the reduction of chloronitrobenzenes was investigated in purified milk xanthine oxidase-

xanthine system: reduction rate of chloronitrobenzene was highest with the para isomer

(196)

Remark : in vitro methaemoglobin formation was studied by incubating haemolyzate (containing 0.1 umole of haemoglobin, obtained from rats) with 0.5 umole of p-chloronitrobenzene at pH 6.6 and 37 degrees centigrade for 5 h: 5.6 % methaemoglobin was formed (increase over the control value weak, but statistically significant)

(197)

Remark : in an in vivo study, 100 umoles/kg bw (= 15.7 mg/kg bw) of p-chloronitrobenzene was given i.p. to male rats, the animals were killed 5 h after the injection to examine methaemoglobin levels: formation of methaemoglobin was observable (methaemoglobin level: 16.3 %)

(197)

Remark : a single dose of 100 mg/kg bw of p-chloronitrobenzene was injected i.p. to male rats; plasma samples containing various concentrations (0.075-35.24 ug/ml) of p-chloronitrobenzene were prepared by collection of blood at suitable times (no further data) after the dosage of the test substance in the rats

(198)

Remark : male rats were i.p. injected with p-chloronitrobenzene at 1.0 mmol/kg bw (= ca. 158 mg/kg bw): the urinary enzyme activities of N-acetyl-beta-D-glucosaminidase and of gamma-glutamyltranspeptidase did not show significant changes compared to those of the controls (no further data)

(199)

Remark : a single dose of 1.0 mmol/kg bw (= ca. 158 mg/kg bw) of p-chloronitrobenzene was administered i.p. to male rats; the urine was collected for 24 h after the injection, the blood was collected and the kidney was removed at 48 h after the injection: p-chloronitrobenzene caused a significant increase in urine volume (polyuria) but the urinary creatinine excretion was not changed; a significant elevation of urinary N-acetyl-beta-D-glucosaminidase activity, but not of urinary gamma-glutamyltranspeptidase activity was observed; p-chloronitrobenzene induced swelling of the renal tubular epithelial cells in 4/5 rats (severity of this pathological change: "intermediate half grade") but did not cause necrosis in the renal sections of the rats; the transaminase (aspartate aminotransferase and alanine aminotransferase) activities and urea nitrogen levels in plasma of the rats were not significantly changed; the methaemoglobin level was increased (5.4 % methaemoglobin)

(200)

Remark : Single oral administration of 0.1 ml/100 g bw of a 0.5 M tricaprylin solution of 1-chloro-4-nitrobenzene (p-CNB) to female Wistar rats resulted in hemoglobin binding: 215.4 (mmol TS/mol HB)/(mmol TS/kg bw)

23.02.1998 (201) (202)

Remark : p-Nitrochlorobenzene was evaluated as not being an endocrine disrupter using the yeast two-hybrid assay, a screening test method.

15.02.2002 (203)

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