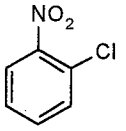
SIDS INITIAL ASSESSMENT PROFILE

CAS No.	88-73-3
Chemical Name	1-Chloro-2-nitrobenzene
Structural Formula	

RECOMMENDATIONS

The chemical is a candidate for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

After single oral application 1-chloro-2-nitrobenzene is toxic to moderate toxic (LD_{50}, oral: rat, male: 144, 251or 560 mg/kg bw, rat, female: 263 mg/kg bw); the acute inhalative and dermal toxicity is moderate (LC_{50}, rat: 3200 mg/m^3 (= 495 ppm, vapor/aerosol mixture); LD_{50}, dermal, rat: female: 1320 mg/kg bw, male: 655 mg/kg bw; LD_{50}, dermal, rabbit: 400 mg/kg bw (male: 455 mg/kg bw, female: 355 mg/kg bw): Cyanotic appearance was the predominant symptom for all routes of application.

The documentation of the available studies on skin irritation is incomplete in one case and in two other cases the test substance was applied undissolved or respectively diluted. However, the studies gave no evidence of a skin irritating potential. 1-Chloro-2-nitrobenzene caused slight irritation effects to the eyes of rabbits, which were reversible within 24 hours. 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.

Target organs of repeated dose toxicity in rats and mice are blood, liver, kidney and spleen with methemogobinaemia as the most sensitive parameter. The repeated dose toxicity was examined in rats and in mice for a period of 13 weeks via whole body inhalation. The NOAEL in rats was not achieved, the LOAEL is 1.1 ppm (7 mg/m^3). In mice, increased liver and kidney weights were observed even at 1.1 ppm and respectively 2.3 ppm. The NOAEL for histopathological injury in mice is 4.5 ppm (28.8 mg/m^3). In a subacute feeding study with mice the NOAEL was 50 ppm (males: 16 mg/kg bw/day; females: 24 mg/kg bw/day).

1-Chloro-2-nitrobenzene showed weak mutagenic activity in bacterial test systems but not in mammalian cell test systems *in vitro*. It was not mutagenic in *Drosophila melanogaster*. In mammalian cells *in vitro*, it showed weak clastogenic activity. The substance induced increased rates of Sister Chromatid Exchanges, whereas the biological relevance of this effect is not yet clear. Intraperitoneal injection into mice resulted in DNA damage in the liver and kidney. The inconsistent results of the available genotoxic studies are typical for nitroaromatics. As a whole 1-chloro-2-nitrobenzene is suspected of being genotoxic, at least a weak clastogen.

1-Chloro-2-nitrobenzene induced tumours in different organs of rats and in the liver of mice. Based on the available studies, which have methodological deficiencies, there is a concern for a carcinogenic potential of 1-chloro-2-nitrobenzene. Following inhalative exposure of F344/N rats and B6C3F1 mice for 13 weeks, only in males 1-chloro-2-nitrobenzene affects the reproductive organs. Performance of a specific study on toxicity to reproduction (NTP continuous breeding protocol) reveals that 1-chloro-2-nitrobenzene was without reproductive toxicity in a different mice strain following oral treatment by gavage despite of significant changes in liver and spleen weight and despite of elevated methaemoglobin levels. Thus, the $NOAEL_{fertility}$ in Swiss CD-1 mice after oral application is 160 mg/kg bw/day whereas the dams showed general toxicity effects at this concentration. Because 1-choro-2-nitrobenzene affected the reproductive organs in systemic toxic doses in male rats and in males of one strain of mice

after subchronic inhalation there is a concern for a reproductive toxicity potential, even if an impairment of reproduction after oral administration in males of a second strain of mice could not be detected.

Developmental toxicity was examined by two studies with Sprague-Dawley rats which have methodology deficiencies. In one study, due to high mortality rate at the highest dose level, only two doses could be evaluated. NOAEL $_{\text{maternal toxicity}}$ is 25 mg/kg bw/day, a NOAEL $_{\text{developmental toxicity}}$ could not be conclusively derived since there was an increase in the number of litters exhibiting specific skeletal variations. In the second study only one dose was applied: NOAEL $_{\text{developmental toxicity}}$ is 100 mg/kg bw/day, a NOAEL maternal toxicity could not be derived. Based on the available studies the overall conclusion is, that there is no indication of developmental toxicity, although there are some limitations within the studies.

Environment

1-Chloro-2-nitrobenzene has a melting point of 32 °C, a solubility in water of 441 mg/l at 20 °C, and a vapour pressure of 4.0 Pa at 20°C. The log Kow was measured to 2.24.

According to Mackay fugacity model level I the main target compartments for 1-chloro-2-nitrobenzene are water (65.4 %) followed by air (32.9 %). 1-Chloro-2-nitrobenzene shows no ready biodegradation in aquatic compartments (OECD 301 C: 8.2% after 14d) but under the conditions of industrial waste water treatment plants removal to > 95 % was observed at one production/processing site. However, this elimination cannot be transferred to other sewage treatment plants. Special tests showed adapted cultures to be able to degrade 1-chloro-2-nitrobenzene in a cometabolic pathway. Bioconcentration factors determined for fish were in the range of 7.0 – 22.3 and thus indicate no significant bioaccumulation potential of 1-chloro-2-nitrobenzene. A calculated Koc suggests the substance to have a medium geoaccumulation potential. In the atmosphere the substance is photodegradable indirectly with a calculated half-life of 187 d.

The acute toxicity has been determined for: fish (*Cyprinus carpio*) with a 96 h-LC_{50} of 25.5 mg/l; daphnia (*Daphnia magna*) with a 24 h-$EC_{50}$of 12 mg/l and a 48 h-EC_{50} of 23.9 mg/l, and *Daphnia carinata* with a 48 h-EC_{50} of 21.3 mg/l; algae (*Chlorella pyrenoidosa*) with a 96 h-EbC_{50} of 6.9 mg/l. With another alga species (*Secendesmus subspicatus*) a 48h-ErC50 of 75 mg/l and a 48h-ErC10 of 19 mg/l was found.

Chronic toxicity has been tested for *Daphnia magna* with a 21 d-NOEC of 3 mg/l on reproduction (measured concentration) and for fish (*Pimephales promelas*) in an Early Life Stage Test with a 33 d-NOEC of 0.264 mg/l concerning the endpoint normal larvae (measured concentration). A PNECaqua of 0.026 mg/l is derived using an assessment factor of 10.

In a test with terrestrial plants a 14 d-EC50 in the range of 3.2 - 10 mg/kg soil dry weight was determined for Lactuca sativa regarding the endpoint of growth. APNECsoil of 3.2 µg/kg bw was derived from this value using an assessment factor of 1000.

Exposure

About 111,800 t/a 1-chloro-2-nitrobenzene are produced by about 30 producers worldwide. 1-Chloro-2-nitrobenzene is a basic chemical which is processed chemically to other intermediates in different fields of application. There is currently no information that there is consumer use.

NATURE OF FURTHER WORK RECOMMENDED

Human Health: The substance is a candidate for further work. Due to possible hazards (haemotoxicity, reproductive toxicity, genotoxicty, and carcinogenicity) the exposure situation in occupational settings and consumer settings should be clarified and, if then indicated, a risk assessment should be performed.

Environment: The substance is a candidate for further work. Environmental exposure at the sponsor company is adequately controlled. However, as there are no information on environmental releases from other production / processing sites, exposure assessment should be conducted and, if then indicated, a risk assessment may need to be considered. This is justified because the substance is not readily biodegradable and has a PNECaqua of 26 µg/l.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 88-73-3
IUPAC Name: 1-Chloro-2-nitrobenzene
Molecular Formula: $C_6H_4ClNO_2$

1.2 Purity/Impurities/Additives

The purity of the substance is given with > 99 % w/w.

1.3 Physico-Chemical properties

1-Chloro-2-nitrobenzene is a yellowish substance with a melting point of about 32 °C (Bayer AG 1989). With a density of 1.37 g/cm³ at 22 °C . 1-chloro-2-nitrobenzene is heavier than water (Ullmann 1991). The substance is soluble in water with 441 mg/l at 20 °C (Eckert 1962). The vapour pressure has been tested to 4.0 Pa at 20 °C (Bayer AG 2001a). Log K_{ow} is measured with 2.24 (Leo et al. 1971).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

The world wide (excluding East Europe) production of 1-chloro-2-nitrobenzene amounted to 111,800 tons in 1995 (about 27,000 in West Europe, 19,000 t in USA, 9,000 t in Japan, 39,000 t in China, 15,500 t in India, and 2,300 t in South Korea) by approximately 30 producers. There is no information about production in East European countries (Bayer AG 2001).

1-Chloro-2-nitrobenzene is a basic chemical, used industrially for manufacturing of further intermediates by chlorination, nitration, sulfonation, reduction, and substitution. In the following an overview of further processing products and their percentage is given:

- 2-nitroaniline (31 %), an intermediate mainly for pesticides

- dichlorobenzidine (26 %), 2-nitroanisole (23 %), and 2-chloroaniline (8 %), processed mainly to dyestuffs and pigments

- others (12 %), including the manufacturing of nitrochlorobenzenesulphonic acid, dinitrodiphenyldisulphide, and nitrophenetole which are processed mainly to dyestuffs and pigments, of o-fluoronitrobenzene which is processed mainly to pharmaceuticals, and of nitrophenol an intermediate mainly for pesticides.

These data relate to the above cited world wide production demand in 1995 (Bayer AG 2001).

A direct use of 1-chloro-2-nitrobenzene is not known (Bayer AG 2001).

Production of 1-chloro-2-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-2-nitrobenzene with a purity above 99 % (Bayer AG 2001).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Releases into the environment may occur during production and processing.

Readily available information on exposure from production and processing to the chemical in the Sponsor country at Bayer AG is available.

The exhausts from production and processing of 1-chloro-2-nitrobenzene are connected to air washing units and thermal exhaust purification plants. Thus during normal operation no 1-chloro-2-nitrobenzene is emitted. Following the Official German Emission Declaration in year 2000, less than 25 kg/a 1-chloro-2-nitrobenzene were emitted into the atmosphere (Bayer AG 2001).

Waste water leaving the production and processing facilities are pretreated before reaching the industrial waste water treatment plant. 1-Chloro-2-nitrobenzene is monitored daily at the influent and the effluent of the waste water treatment plant.

Weekly, at changing days, the effluent is monitored on a fine analysis scale. All values of the fine analysis scale from January 2000 to May 2001 showed the substance to be eliminated to less than 5 µg/l. As worst case for the receiving water a PEC of <0.007 µg/l is calculated from this effluent concentration taking the 10 perzentil of the river flow into account (Bayer AG 2001).

There is no information on releases into the environment from other production and processing sites.

Significant environmental releases from biological reformation of 1-chloro-2-nitrobenzene from end-products are not likely to occur. This is supported by monitoring data from German surface waters for the years 1991 – 2000. These data show that the environmental concentration of 1-chloro-2-nitrobenzene (90%ile) is in the range of < 0.005 µg/l to 0.58 µg/l.

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

2.2.2 Other Information on Environmental Fate

With regard to its chemical structure 1-chloro-2-nitrobenzene is not expected to hydrolyze under environmental conditions. According to the Mackay Fugacity Model Level I (1991), the main target compartments for 1-chloro-2-nitrobenzene are the hydrosphere with 65.4 %, followed by air with 32.9 %. The Henry constant is calculated to be 1.43 Pa m^3 mol^{-1}.

Based on the available experimental data 1-chloro-2-nitrobenzene is not readily biodegradable. In a modified MITI I test according to OECD guideline 301 C a non adapted mixed microbial inoculum mineralized 8.2 % of the initial test substance concentration within 14 days (MITI 1992).

Using the model Simpletreat 3.0 the following distribution/elimination in sewage treatment plants can be estimated using a degradation rate constant of 0 h^{-1} (not readily biodegradable), a Henry constant of 1.43 Pa m^3 mol^{-1} and a log Kow of 2.24:

% to air	2.7
% to water	95.2
% to sludge	2.1
% degraded	0
% removal	4.8

The comparison of influent and effluent concentrations of an industrial sewage treatment plant showed the substance to be removed to > 95 % [Bayer AG 2001]. However, this elimination cannot be transferred to other sewage treatment plants due to possible different waste water composition and adaptation processes. .

Examination of the degradation pathway of chloronitrobenzenes, showed these substances only to be biodegraded by isolated bacteria and adapted mixed sludge as long as the chloronitrobenzenes are not the only sole source for carbon and nitrogen (Kuhlmann 1999).

The indirect photochemical degradation in air by hydroxyl radicals is calculated with a half-life of 187.2 days.

Measured bioconcentration factors (BCF) determined for fish (Cyprinus carpio) according to OECD guideline 305 C, were in the range of 7.0 – 22.3. 1-Chloro-2-nitrobenzene concentrations of 0.25 and 0.025 mg/l had been tested. Thus no significant potential for bioaccumulation of 1-chloro-2-nitrobenzene in aquatic organisms is indicated (MITI 1992).

There is no test on geoaccumulation available. Binding to soil organic matter has been calculated with Koc = 315.5 [SRC-PcKocWIN v1.66, 2000]. According to Blume [1990] 1-chloro-2-nitrobenzene can be regarded as a substance with medium geoaccumulation properties.

2.3 Human Exposure

Note: In Germany/Europe no workplace limit concentration is laid down for 1-chloro-2-nitrobenzene as the substance is classified in Germany in Cancerogenicity Category 3 and Fertility Category 3. A technical limit concentration (TRK-Wert) is planned by German authorities according to "Bundesministerium für Arbeit und Sozialordnung: Übernahme von Luftgrenzwerten in die TRGS 900 Bundesarbeitsblatt 7-8/1998; S. 70-71".

2.3.1 Occupational Exposure

From information from the Swiss (July 2001) and Swedish product register (September 2001) there is no other use pattern of 1-chloro-2-nitrobenzene than intermediate confirmed. To protect workers from exposure to 1-chloro-2-nitrobenzene at workplace, several different precautionary and protective measures are undertaken.

Workplace monitoring is carried out periodically and appropriate personal protection equipment is prescribed in detail for different work situations.

During the past five years (1997 - 2001) 31 8-hour shift samples were taken. Thereof 25 measurements were < 0.05 mg/m³. One measurement was < 0.32 mg/m³, the higher determination limit was due to a smaller air volume taken. Four measurements, taken during filling operations showed values between 0.032 and < 0.6 mg/m³. Here masks were worn to protect the workers from inhalation of 1-chloro-2-nitrobenzene. One value of 0.11 mg/m³ was caused by not appropriate sampling within the production process. This source of exposure has been put right immediately [Bayer AG 2001].

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

1-Chloro-2-nitrobenzene, under appropriate conditions of exposure, is absorbed by the body both via the skin and the gastrointestinal tract as well as via the respiratory tract. Rat studies with labelled chemical show that 1-chloro-2-nitrobenzene absorption is 80 % following oral administration and at least 40 % after open dermal application. On 11 consecutive days, 65 mg 1-chloro-2-nitrobenzene/kg bw was administered by gavage to adult and to old rats. On d 1, 5, and 9 applied substance was labelled and urine and faeces were collected in the following 96 hours. The adult rats excreted 71-74 % of the dose in the urine and 20-27 % of the dose in the faeces. Excretion rate increased with the duration of treatment. Urinary excretion rate in the old rats consisted 71-85 % of the dose and did not increase with the duration of treatment. The radioactivity level in the tissues were determined 72 hours after d9-treatment and shown to be found 5 % of the dose in adult rats and 8 % in the old rats. At very high doses, e.g. 200 mg/kg bw given orally, urinary excretion is delayed and faecal excretion is markedly suppressed. There is evidence to suggest involvement of the enterohepatic cycle, but there are no signs of accumulation of 1-chloro-2-nitrobenzene or one of its metabolites (BG-Chemie 2000, Nomeir et al. 1992).

After oral administration of 100 mg 1-chloro-2-nitrobenzene/kg bw to rabbits 42 % of the dose was excreted in the urine as glucuronides, 24 % as sulfates, 7 % as mercapturic acids and 9 % as free 2-chloroaniline. Only 2-Chloroaniline (0.3%) could be detected in the faeces. 48 hours after administration elimination was complete (Bray et al. 1956).

In tissue, only a very small fraction of the administered radioactivity is recovered (BG-Chemie 2000).

The main metabolic routes for 1-chloro-2-nitrobenzene in the body consist in reduction of the nitro group to an amino group and hydroxylation of the benzene ring. Apart from 2-Chloroaniline, the corresponding nitrophenols and aminophenols are formed, which are excreted as conjugates of glucuronic acid and sulfuric acid. 2-Chloroaniline also appears in the urine and faeces in the unconjugated form (BG-Chemie 2000, Bray et al. 1956, Sabbioni 1994, Rickert and Held 1990).

During reduction of the nitro group to the amino group, the hydroxylamine compound is formed as a highly reactive intermediate which has been detected both in vivo in rats, and in vitro (BG-Chemie 2000, Sabbioni 1994)

3.1.2 Acute Toxicity

Inhalation

There are no studies according to the current OECD guideline but there are study reports with rats which give sufficient information to evaluate this endpoint: (Haskell Laboratory, 1992) LC_{50} ca. 3200 mg/m^3 for 4 hours (= 495 ppm, vapor/aerosol mixture) . Signs of intoxication during exposure were lethargy, slight to moderate cyanosis, slight to moderate corneal opacity, semi-prostration or prostration, reddish brown nasal discharge and tachypnoe. Signs of intoxication post exposure were pallor, reddish brown nasal discharge, semi-prostration and lethargy, corneal opacity.

Death occurred within 7 days but not dose-dependently. Thus LC_{50} value was calculated from statistically not significant regression.

Conclusion

The acute inhalative toxicity is moderate: LC_{50} (rat) ca. 3200 mg/m³ (= 495 ppm, vapor/aerosol mixture) for 4 hours. Cyanotic appearance was the predominant symptom.

Dermal

There are no studies according to the current OECD guideline but there are study reports with rats and rabbits which give sufficient information to evaluate this endpoint: (Bayer 1976): The dermal LD_{50} following a 24-hour occlusive application of the test material to the skin of rats is determined to be 1320 mg/kg bw in females and 655 mg/kg bw in males. The test material was applied as emulsion with the vehicle polyethylene glycole 400. Reduced general condition, difficulties in breathing and cyanotic appearance were the signs of intoxication starting 18 hours post application. Skin irritation was not reported. Deaths occurred within 4 days (males), and 7 days (females), respectively. A section was not performed. In rabbits (2/sex/dose, undissolved substance but warmed to make suitable for dosing, no further information on application procedure, 5 doses, exposure time: 24 hours, observation time: 14 d; Younger Labs. Inc. 1992) the LD_{50} was 400 mg/kg bw (male: 445 mg/kg bw; female: 355 mg/kg bw). Lethargy for up to three days, increasing weakness, collapse and deaths were reported. At gross autopsy, decedents showed haemorrhagic areas in the lungs, liver-, kidneys- and spleen-discoloration, gastrointestinal inflammation and enlarged gall bladder whereas in survivors the viscera appeared normal.

A further investigation on acute dermal toxicity with rabbits yielded a similar result (LD_{50} = 450 mg/kg bw, 5/dose). The sex of the animals used was not mentioned and a section was not performed (United States Testing Company 1976).

Conclusion

The acute dermal toxicity is moderate (LD_{50} (rat, male) = 655 mg/kg bw, LD_{50} (rat, female) = 1320 mg/kg bw; LD_{50}(rabbit) = 400 mg/kg bw (male: 445 mg/kg bw, female: 355 mg/kg bw)). Cyanotic appearance was the predominant symptom.

Oral

There are no studies according to the current OECD guideline but there are study reports with rats which give sufficient information to evaluate this endpoint: (Bayer, 1982 a; b) LD_{50} (Wistar, male) 251 mg/kg bw; LD_{50} (Wistar, female) 263 mg/kg bw. As signs of intoxication rats displayed reduced general condition, cyanotic appearance, rough fur, sedation, narcosis and females showed paralysis of the hind limb. Death occurred within 3 days. No macroscopic findings were recorded from decedents and from survivors 14 days post application. In another study the LD_{50} of male and female Sprague-Dawley rats was determined to be 560 mg/kg bw (Younger Labs 1991). As signs of intoxication reduced appetite and reduced activity (in survivors for at least 2-3 days), increasing weakness, ocular discharge, collapse and death were noted. Death occurred within one to four days post application of 1-chloro-2-nitrobenzene, with most death within 2 days. Hemorrhagic lungs, jaundiced liver, darkened kidneys and spleen and gastrointestinal inflammation were seen at gross autopsy of decedents. From survivors 7 days post application, lung congestion and darkened kidneys and spleen were reported.

An older study on male Wistar rats (Hoechst 1975) yielded an LD_{50} of 144 mg/kg bw. As signs of intoxication rats showed imbalance, tremor, rough fur and diarrhea. Section of the rats, that had died, could not be performed because of ongoing autolytic changes.

Conclusion

After single oral application 1-chloro-2-nitrobenzene is toxic to moderate toxic (LD_{50}, oral: rat, male: 144, 251 or 560 mg/kg bw; rat, female: 263 or 560 mg/kg bw). Cyanotic appearance was the predominant symptom.

3.1.3 Irritation

Skin Irritation

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

In an older study, 0.5 ml of a 10 % sesame oil solution of 1-chloro-2-nitrobenzene was applied to the shaved (intact and abraded) skin of six rabbits for 24 hours covered by semi-occlusive dressing. When the dressing was removed (24 hour-reading) only mild erythema (score 1/0-4) was noted in both, intact and abraded skin of 4/6 rabbits. Erythema were not observed at 48 hour- and at 72 hour-reading. According to Fed. Reg. 38, No 187, p. 27109, §1500.41, 1973, the compound was evaluated as no irritant (Hoechst 1975).

In another study, 500 mg 1-chloro-2-nitrobenzene was applied undissolved to the inner surface of one ear of each of two rabbits for 24 hours covered by cellulose pads and plaster. To fix the plaster tightly a rolled gauze pad was put on it. Ear, substance, pad, plaster and rolled pad were then bandaged. No signs of irritation (sore 0/4) were observed neither when the pad, plaster, rolled pad were removed nor during the 7 day post exposure observation period (Bayer 1976). In addition, in the same report, the results of acute dermal testing in rats with the substance formulated in polyethylene glycole 400 are mentioned. Signs of irritation were not reported.

0.5 ml of warmed, undiluted 1-chloro-2-nitrobenzene was applied to the skin of six rabbits for 24 hours. No erythema or edema was observed till 168 hours after application (no information about the type of application and pretreatment of the skin) (Younger Labs. 1991).

No skin irritation was reported in an acute dermal toxicity study (see chapter 3.2.3; Bayer 1976).

Conclusion

The study documentation of the available studies is incomplete in one case and in the two other cases the test substance was applied undissolved or respectively diluted. However, the studies gave no evidence of a skin irritating potential of 1-chloro-2-nitrobenzene.

Eye Irritation

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

In an older study, performed as described in Fed. Reg, Vol. 38, No.187, §1500.42, 1973, 100 mg of 1-chloro-2-nitrobenzene was applied undissolved into one eye of each of 6 rabbits (the other eye served as control). One hour post application slight conjunctival injections (score 1-2/0-3) were noted in the eyes of 6/6 rabbits, 7 hours post application in the eyes of only 2/6 rabbits (score 1/0-3) and 24 hours post application no irritational effects were observed. The compound was evaluated to be a mild irritant (Hoechst 1975).

In another study in the same report, a 10 % solution was applied into one eye of each of 6 rabbits which leads to slight irritational effects (score 1/0-3) in the eyes of 3/6 rabbits one hour post application. These effects had disappeared after 7 hours. The compound was evaluated as slightly irritating (Hoechst 1975).

In another study 50 mg 1-chloro-2-nitrobenzene was applied into the right eye of each of two rabbits. Slight redness (score 1/3) was observed in the eye of one rabbit, which disappeared within 24 hours. No signs of irritation were observed in cornea neither on the application day nor during the 7 day post exposure observation period (Bayer 1976).

Conclusion

1-Chloro-2-nitrobenzene caused slight irritational effects to the eyes of rabbits which were reversible within 24 hours.

3.1.4 Sensitisation

Skin

Skin sensitization potency was examined in tests with 10 guinea pigs using test methods which are no longer in use and which are incompletely documented (Rusakov 1973): In a modified Draize test induction was performed with an 1 % aceton-solution of the compound on the shaved back 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-2-nitrobenzene at day 22: 0.2 ml Freunds Adjuvans together with 0.5 mg 1-chloro-2-nitrobenzene/kg bw was injected into the hind paw. 6 days later one drop of a 10 % solution of 1-chloro-2-nitrobenzene was applied on the shaved untreated skin as challenge. The author reported that 50 % of the treated guinea pigs showed a positive reaction. Rats exposed via inhalation to 0.008 mg/m^3 for 5 months showed also positive reactions (see above; Rusakov et al. 1973).

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

The repeated dose toxicity was examined in male and female Fischer 344/N rats and in male and female B6C3F1 mice for a period of 13 weeks via whole body inhalation of vapor (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 (kidney, liver, nasal cavity, and spleen (rats); liver and spleen (mice)) and on reproductive organs (see also chapter 3.2.10) were performed on all animals in the control and 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 10 female rats were exposed to 0, 1.1, 2.3, 4.5, 9, 18 ppm (approx. 0, 7, 14.7, 28.8, 57.6, or 115.2 mg/m^3), 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 at d 1 (only methaemoglobin - data not shown), d 4, and d 23 consisting of hematology and clinical chemistry evaluations. Animals in the base study were evaluated at the end of the study. There were no clear clinical signs of toxicity. All rats survived till the end of the study. Body weight gain was similar to the respective controls. At necropsy, males of the 18 ppm group had significant increased spleen (absol. and rel.) and from 9 ppm increased right kidney (rel.) weights. Absolute liver weights were increased from 1.1 ppm and the relative liver weight from 2.3 ppm. In males exposed to 18 ppm, abs. and rel. lung weights were significant decreased. 2/10 males in the 18 ppm group showed

darkened spleen. Histopathologic evaluation of the kidney showed tubule pigments from 4.5 ppm and tubule regeneration from 1.1 ppm. In the liver, cytoplasmic basophilia was noted from 9 ppm. Spleenic congestion was observed in all exposed and in the control male rats with dose-dependent slight increase in severity. Females, at necropsy, had increased right kidney (absol. and rel.) in the 18 ppm-group and increased absolute liver weights from 2.3 ppm and increased relative liver weights from 4.5 ppm. Significant increased spleen weights (absol. and rel.) were noted from 4.5 ppm. 1/10 females in the 18 ppm group showed darkened spleen. Histopathologic evaluation yielded in the kidney tubule pigment and cytoplasmic basophilia of the liver from 9 ppm. Spleenic congestion was noted in exposed and in the control females with dose-dependent slightly increased incidences. Hyperplasia of the nasal cavity respiratory epithelium in all exposed male and female rats was considered as a toxic effect due to 1-chloro-2-nitrobenzene exposure.

Concentration-related increase in methaemoglobinaemia (males: significant from 1.1 ppm at d 23 and from 2.3 ppm at all time points with max. of 1.14 g/dl at 18 ppm; females: significant from 1.1 ppm at week 13 and from 2.3 ppm at all time points with max. of 1.04 g/dl at 18 ppm; data from d1 not shown) and oxidative damage to red blood cells occurred from the first days of exposure (males: significant at 1.1 ppm (d23), at 4.5 ppm (week 13), at 9 ppm (d4, week13), at 18 ppm (at all time points) when compared to the control values at the respective time point; females: significant in every exposure group at week 13 when compared to the control values at the respective time point). Decrease in haematokrit, haemoglobin and increase in leukocytes predominantly in the highest dose groups of male and female rats was recorded. The beginning regeneration could be recognized in the increase in reticulocyte count at all dose groups of male and female rats at week 13. Serum activities of alanine aminotransferase and sorbitol dehydrogenase were mildly increased in different male and female exposure groups at various time points. A NOAEL was not achieved, the LOAEL is 1.1 ppm (7 mg/m³).

Male and female mice were exposed to 0, 1.1, 2.3 , 9, 4.5, 18 ppm, 6 hours per day, 5 days per week over a period of 13 weeks. There were no clinical signs of toxicity. 2/10 male mice exposed to 18 ppm died. In females from 1.1 ppm body weight gain was greater than in the concurrent control females; in males, body weight gain was similar to the respective control. Exposed mice had treatment-related increased liver and kidney weights (males: abs. and rel. right kidney weights, rel. liver weights sign. increased from 2.3 ppm, abs. liver weights from 9 ppm; females: abs. right kidney weight from 2.3 ppm, abs. liver weights in all exposed groups, rel. liver weight from 9ppm) . Pale discoloration in the liver was noted in 6/10 males and 1/10 females in the 18 ppm group. The spleen was grossly enlarged in 3 females in the 9 ppm group and 4 females in the 18 ppm group. Hepatocellular necrosis, cytomegaly, mineralization and chronic inflammation were seen in the liver, primarily in mice in the 18 ppm group but also in the 9 ppm-group. In addition, increased haematopoetic activity of the spleen was seen in both sexes of mice, particularly in females at 9 ppm and greater. The NOAEL for histopathologic injury is 4.5 ppm (28.8 mg/m³).

Oral

The repeated dose toxicity was also examined in a subacute feeding study with B6C3F1 mice according OECD Guideline 407 (Bayer 1991, 1993). The objective of the study was to recognize possible prae-neoplastic lesions by means of enzyme histochemistry.

12 mice/sex/dose received 0, 50, 500, 5000 ppm 1-chloro-2-nitrobenzene for 5 weeks. Additional 6 mice/sex/dose were used for interim kill and examination after one week of treatment. The calculated feed intake was 0, 16, 167, 1120 mg 1-chloro-2-nitrobenzene/kg bw/day for males and 0, 24, 220, 1310 mg/kg bw/day for females. Except of one male in the lowest dose group, no animal died during treatment. No clinical signs of toxicity up to and including 500 ppm were observed. At 5000 ppm narrowed palpebral fissures and corneal opacity in males were reported. From 5000 ppm reduced body weight gain and reduced food intake in both sexes and additionally in females from 500 ppm.

From 5000 ppm in both sexes reduced number of erythrocytes (change in morphology: anisocytosis, poikilocytosis and polychromasie), haematokrit- and haemoglobin-content and increased bilirubin-, methaemoglobin-(f: 2.8 %; m:1,7 %) MCV-, MCH- and MCHC-values. Increased spleen weights, dark red discoloration of the spleen and increased haemosiderin deposition could be seen.

No treatment related changes in the kidneys were observed.

From 500 ppm increase in cholesterin content in the blood, increased liver weights (differences of up to 89 % were noted in females) accompanied by hypertrophy of the centrolobular hepatocytes. From 5000 ppm gross changes in the liver, increase in the activity of ASAT and ALAT and alkaline phosphatase (male) was noted. In males, blood-urea was decreased.

Additional investigations demonstrate from 500 ppm increase in liver enzyme activities (EOD, ALD, EH, GSH-T,GLU-T) and disturbance of carbohydrate metabolism (decreased gluconeogenesis and glycogen, activated pentose phosphate cycle (at 5000 ppm), increased glycolysis (at 5000 ppm)).

At 5000 ppm males showed decreased testis weight without histopathological changes.

No other treatment-related functional disturbances or impairment of other organs were observed.

Thus, the NOAEL of 50 ppm (16 mg/kg bw/day for males and 24 mg/kg bw/day for females) could be derived.

Also in several other studies on rats and mice with oral or inhalational exposure for 2 and 4 weeks or 7 months, spleen, liver and kidneys were identified as target organs.

Effects on CNS function in rats were reported in a subchronic oral study with poor reliability (Davydova SG 1967). These effects cannot be evaluated because of the incomplete description of the results and the method used.

Conclusion

The repeated dose toxic ity was examined in rats and in mice for a period of 13 weeks via whole body inhalation. As target organs liver, kidney and spleen were identified in both species, and furthermore, in rats erythrocytes and the nasal cavity respiratory epithelium. The NOAEL in rats was not achieved, the LOAEL is 1.1 ppm (7 mg/m³). In mice, increased liver and kidney weights were observed even at 1.1 and 2.3 ppm, respectively. The NOAEL for histopathological injury in mice is 4.5 ppm (28.8 mg/m³).

In a subacute feeding study with mice target organs were blood, spleen and liver. The NOAEL was 50 ppm (males: 16 mg/kg bw/day ; females 24 mg/kg bw/day).

3.1.6 Mutagenicity

In vitro Studies

(A) Gene mutation

There are several Ames-tests which are mostly performed according to OECD Guideline 471 with and without metabolic activation. In every study at least the highest dose levels exhibit 100 % toxicity. For example 1-chloro-2-nitrobenzene was evaluated as mutagenic in the tests reported by Haworth et al. (1983) (doses: 6-600 resp. 10-1000 µg/plate) and by Bayer (1984) (doses: 833.3-2073.6 µg/plate). An additional Ames test, which was reported in JETOC (1996) (doses: 10-1000 µg/plate), yielded negative results. A repetition of the study (doses: 39.1-10000 µg/plate) showed