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

positive results in TA 100 and TA98. Investigations with E. coli yielded positive and negative results (JETOC 1997).

In a study with deficiencies in the description of results, 1-chloro-2-nitrobenzene showed mutagenic acitivity in *Salmonella typhimurium* TA98 with metabolic activation and norharman (Suzuki et al. 1983). In summary, the available tests with *Salmonella typhimurium* showed mostly negative results without the addition of a metabolic activation system in different strains. Only in strain TA98 and TA1538 there were obtained mostly negative and one resp. 2 positive results. In the presence of a metabolic activation system positive and negative results were obtained in TA 98 and TA 100 mostly at high but not bacteriotoxic concentrations.

In an HPRT Test which was performed with Chinese Hamster V79 lung cells according to OECD Guideline 476 1-chloro-2-nitrobenzene does not induce gene mutations. The doses used were 100-1200 ug/ml in the presence of S9-mix and 100-900 ug/ml without S9-mix. Cytotoxicity was noted in the highest concentration (TNO 1989).

Conclusion

1-Chloro-2-nitrobenzene yielded positive results only in 2 tester strains of Salmonella typhimurium and mostly at high but not bacteriotoxic concentrations. Therefore it can be regarded as a weak mutagen in bacterial test systems. It showed no mutagenic activity in mammalian cell test systems in vitro.

(B) Cytogenicity

There is a study on cytogenicity using Chinese Hamster Ovary (CHO) cells and doses ranging from 10-100 µg/ml without addition of a metabolic activation system (S9-mix) and from 25-250 µg/ml in the presence of S9-mix. Harvest times were 8, 12, 21 hours. The study was performed according to OECD Guideline 473 and yielded negative results (Huntingdon 1988).

NTP (1993) reported additional cytogenetic tests with Chinese Hamster Ovary cells using different harvest times: Without metabolic activation an equivocal result at the highest concentration was obtained when the harvest time was 14 hours (doses: 16-160 ug/ml) and a negative result with a harvest time of 18.5 hours (dose: 47-216 ug/ml). In the presence of an activation system negative results were obtained after a harvest time of 14 hours (doses: 50-500 µg/ml) and weak positive results at the highest concentration when the harvest time was 13.6 hours (doses: 101-465 and 125-500 µg/ml).

Conclusion

1-Chloro-2-nitrobenzene showed weak clastogenic activity in CHO cells in vitro at high concentrations only.

(C) Indicator Tests

1-Chloro-2-nitrobenzene did not increase Unscheduled DNA repair in rat hepatocytes using a dose range from 1.0 to 100 µg/ml DMSO. Cytotoxicity was determined in preliminary results (Monsanto 1984).

An increase in Sister Chromatid Exchange (SCE) rate was found in Chinese Hamster Ovary cells treated with 1-chloro-2-nitrobenzene in doses ranging from 5 to 500 µg/ml (NTP 1993). The biological relevance of SCE is not yet clear.

Conclusion

1-Chloro-2-nitrobenzene did not induce Unscheduled DNA repair. It induced increased rates of Sister Chromatid Exchanges, whereas the biological relevance of this effect is not yet clear.

In vivo Studies

(A) Gene mutation

There are several Drosophila SLRL tests which are performed using different application routes: intraperitoneal injection, adult and larval feeding. Both dosing methods lead to negative results (Zimmering 1985, 1989).

Conclusion

1-Chloro-2-nitrobenzene showed no mutagenic activity in *Drosophila melanogaster*.

(B) Cytogenicity

Intraperitoneal injection of 60 mg 1-chloro-2-nitrobenzene/kg bw of unknown purity into CD-1 mice (n=8) induced single DNA strand breaks in liver and kidneys which were identified by alkaline elution technique (Cesarone et al. 1982). Intraperitoneal injection, however, is not the recommended exposure route of the respective OECD guideline because it could expose the organs directly rather than via the circulatory system.

Conclusion

Intraperitoneal injection of 1-chloro-2-nitrobenzene into mice resulted in DNA damage in the liver and kidney.

Conclusion

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.

3.1.7 Carcinogenicity

For evaluating carcinogenicity the only available studies in rats and mice don't meet the criteria of today (doses too high, number of animals too low, duration time too short) and are only reported in brief (Weisburger et al. 978).

25 male CD rats/group were given 1-chloro-2-nitrobenzene in the diet for 18 months (50 % of MTD, MTD): 0, 1000, 2000 mg/kg diet (approx. 0, 75, 150 mg/kg bw/day). After 6 months of treatment, dosage was reduced to 500, 1000 mg/kg diet (approx. 37.5, 75 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). Reduced doses were given for the remaining 12 months. Following the 6-month-observation period, necropsy was performed and male rats with tumours were recorded: 1/22 in the simultaneous control group (pooled control: 14/111) and 7/22 resp 1/19 in the low resp. the high dose group. These tumours of the low dose group usually included

pituitary adenomas along with either a stomach papilloma, a tumour of the adrenals, a thyroid adenocarcinoma, a lymphosarcoma, a bile duct carcinoma or a subcutaneous fibroma.

25 male and female CD1 HaM/ICR mice/group were given 1-chloro-2-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). After 8 months of treatment dosage was reduced to 1500, 3000 mg/kg diet (approx. 225, 450 mg/kg bw/day) which was given for the remaining 10 months (see above). Following the 3-month-observation period, necropsy was performed and mice with tumours were recorded: 3/18 (m), 0/20 (f) in the simultaneous control group (pooled control: (m) 7/99, (f) 1/102) and 7/17 (m), 5/22 (f) resp 3/16 (m), 5/19 (f) in the low resp. the high dose group, identified as hepatocellular carcinomas.

The objective of a subacute **feeding** study with B6C3F1 mice 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 in the diet 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/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.

The 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)). These marked changes in the carbohydrate metabolism were evaluated as possible promotion activity of 1-chloro-2-nitrobenzene (Bayer 1991, 1993).

Conclusion

1-Chloro-2-nitrobenzene induced tumours in different organs of rats and in the liver of mice.
Overall taking into consideration the results of the genotoxicity tests, the analogy to other nitroaromatics and the results of the available limited studies in rats and mice, there is a concern for a carcinogenic potential of 1-chloro-2-nitrobenzene.

3.1.8 Toxicity for Reproduction

Effects on Fertility

There are no specific studies on toxicity to reproduction using inhalative exposure, but there is a 13 week inhalation study which also evaluated the reproductive organs and can therefore be taken into account for this exposure route.

Male and female F344/N rats were exposed to 0, 1.1, 2.3, 4.5, 9, 18 ppm (0, 7, 14.7, 28.8, 57.6, 115.2 mg/m^3), 6 hours per day, 5 days per week over a period of 13 weeks (NTP, 1993; see also chapter 3.2.7). At the end of the study sperm morphology and vaginal cytology evaluations were performed of animals in the 0, 4.5, 9 and 18 ppm groups (reproductive organs of animals of the two lower exposure groups were not evaluated).

There were no clear clinical signs of toxicity. All rats survived till the end of the study. Concentration-related increase in methaemoglobinaemia and oxidative damage to red blood cells occurred from the first days of exposure and resulted in a regenerative anaemia; target organs were kidneys, spleen, liver, erythrocytes and nasal cavity respiratory epithelium (for details see chapter 3.2.7). Males of the 18 ppm group showed decreases in cauda epididymis weights and in the spermatid count and spermatid heads/testis (NOAEL $_{\text{reproductive organs}}$ = 9 ppm). Females reproductive system was not affected by treatment (NOAEL $_{\text{reproductive organs}}$ = 18 ppm).

Male and female B6C3F1 mice were exposed to 0, 1.1, 2.3, 4.5, 9, 18 ppm (0, 7, 14.7, 28.8, 57.6, 115.2 mg/m^3), 6 hours per day, 5 days per week over a period of 13 weeks (NTP 1993). At the end of the study sperm morphology and vaginal cytology evaluations were performed of animals in the 0, 4.5, 9 and 18 ppm group (reproductive organs of animals of the two lower exposure groups were not evaluated): There were no clinical signs of toxicity. 2/10 male mice exposed to 18 ppm died; target organs were kidneys, spleen and liver (for further details see also Chapter 3.2.7). Male mice in all evaluated dose groups demonstrated a decrease in sperm motility (a NOAEL $_{\text{reproductive organs}}$ for male mice was not determined); in females no effects were observed (NOAEL $_{\text{reproductive organs}}$ = 18 ppm).

In a 5 week feeding study 12 B6C3F1 mice/sex/dose received 0, 50, 500 or 5000 ppm 1-chloro-2-nitrobenzene. Males of the highest dose group showed decreased testis weight without histopathological changes (Bayer 1991, 1993; for further details on general toxicity see chapter 3.2.7).

There is a carefully performed study on toxicity to reproduction in mice using oral treatment (NTP 1992):

Male and female Swiss CD-1 mice were exposed to 1-chloro-2-nitrobenzene dissolved in corn oil by gavage to assess reproduction and fertility using the NTP continuous breeding protocol:

Groups of 20 breeding pairs received 40, 80 or 160 mg/kg bw per day 2-chloronitrotoluene 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 groups 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 second generation fertility.

Data from a 2-week dose-range-finding study were used to set exposure concentration. The highest dose used in the reproduction study was one-half of that caused mortality in the dose-range-finding study.

In the F0-generation mortality occurred in 2, 2, 2 and 3 mice in the control to the high dose groups, respectively, which was suggested not to be treatment related. There was a slight increase in male and post partum dam terminal weights. 3 females in the high dose group appeared cyanotic. No other clinical signs were observed. Necropsy of the high dose mice showed increased spleen weights by 50-100 % and 4-6 fold increased methemoglobin level. No other necropsy data were collected.

Reproductive performance and function of the F0-mice was not affected by treatment: number of litters, pup weight, and viability were all unchanged; live pups per litter and proportion of pups born alive were increased (15% resp. 10%) in the high dose group.

In the final litter of the holding period following the continuous breeding phase, pup weight gain during suckling was lower in the treated groups. At weaning, pups of the high dose group weighed 12% less than control. None of the pups showed clinical signs of toxicity.

Mating of the adult F1 mice (only control and high dose group) revealed no difference between the groups in terms of proportion of mated pairs, number of litters per group, number of live pups per litter and pup weight or viability. Treated F1 male and female mice had 3-fold increased methaemoglobin level compared to the control and were approximately 7 and 5 % heavier than their control counterparts. At necropsy, liver and spleen weights were increased by 40 to 60 %. In male mice, abs. right epididymis and kidney/adrenals weights were increased, seminal vesicle-to-body weight was reduced compared to controls. Sperm measured were unaffected by 1-chloro-2-nitrobenzene exposure (epididymal sperm motility, sperm count, percentage of abnormal sperm). In

females, oestrous cycle were unaffected by 1-chloro-2-nitrobenzene exposure. Thus, NOAEL for fertility is 160 mg/kg bw/day.

Conclusion

Following inhalational 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 weights and despite elevated methemoglobin levels. 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-chloro-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

25 female Sprague-Dawley rats per group received 0, 25, 75 or 150 mg/kg bw/day 1-chloro-2-nitrobenzene dissolved in corn oil by gavage from d6 to d15 of gestation. Due to severe toxicity and high mortality rate of the dams in the 150 mg/kg bw/day group, all females of the 150 mg-group were terminated prior to scheduled sacrifice. One year later, in another laboratory, a third dose group was examined together with a concurrent control group (see later).

No evidence of maternal toxicity was exhibited at the 25 mg/kg level.

For gestation d 6-10 a slight, but not significant reduction in maternal body weight gain at the 75 mg/kg level, urinary staining and alopecia were noted in some dams when compared to the respective control groups. The difference in maternal body weight gain was accompanied by reductions in food consumption for d 6-10. The reductions noted at 75 mg/kg were recovered later in gestation.

Maternal reproductive parameters and fetal body weight in the treatment groups were similar to the respective control groups except for the mean number of early resorptions and postimplantation loss at the 75 mg/kg level. However, postimplantation loss in the respective control group was very low compared to the historical control value.

No differences in the number of the litters exhibiting malformations were evident in the treatment groups compared to the control group. Increased incidences of variations were seen in the 25 and 75 mg/kg group: cervical #7 rib (sign. at 75 mg/kg); and 13 full pairs of ribs with lumbar #1 rudimentary rib; in the 25 mg/kg group: 12 full pair ribs with #13 unilateral full rib and/or rudimentary rib(s). No historical control data were given. Thus, $NOAEL_{maternal\ toxicty}$ is 25 mg/kg bw/day, a $NOAEL_{developmental\ toxicity}$ could not be conclusively derived (Monsanto 1990).

In an additional study which was performed in a different laboratory one year later and which was intended to clarify the observation of the first study, mated female rats received 0, or 100 mg 1-chloro-2-nitrobenzene/kg bw in corn oil by gavage from d6 to d15 of gestation. For gestation d 6-10 slight reduction in maternal body weight loss accompanied by reduction in food consumption for days 6-16 was noted. Maternal reproductive parameters and fetal body weights in the treatment group was comparable to the respective control group. No teratogenic effect nor statistically significant increase of skeletal variations like in the first experiment were observed (IRDC 1984).

Conclusion

Developmental toxicity was examined by two studies with Sprague Dawley rats which both have methodological deficiencies. In one study, due to high mortality rate at the highest dose level, only two doses could be evaluated: $NOAEL_{maternal\ toxicity}$ is 25 mg/kg bw/day, a $NOAEL_{developmental\ toxicity}$ 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: $NOAEL_{developmental\ toxicity}$ 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.

3.2 Initial Assessment for Human Health

All available reports relate to mixed exposure, frequently in combination with 4-chloronitrobenzene and/or nitrobenzene. A critical aspect in this context is that the chemical 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)

No allergenic potential had been indicated although 1-chloro-2-nitrobenzene has been used for decades (BUA 1985, BG-Chemie 2000)

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute and Chronic Toxicity Test Results

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

Acute toxicity to fish (*Brachydanio rerio*) has been tested in a flow through system according to OECD Guideline 203 with analytical monitoring. The 96 h-LC_{50} was determined to be 34.8 mg/l (Röderer 1990). In a semi static test with *Cyprinus carpio* according to OECD Guideline 203 as well, the 96 h-LC_{50} was determined to be 25.5 mg/l (no information about analytical monitoring) (Zhao 1997). An Early Life Stage Test was conducted in an analytically monitored flow through system with Pimephales promelas. In a first step 50 embryos were tested on hatchability and development after 4 - 5 days of incubation. In a second step 15 randomly selected frys from the initial egg cups where observed on their further development for 33 days. The 33 d-NOEC was determined by the authors Call & Geiger (1992) to be 0.264 mg/l based on the endpoint 'normal larvae' related to the hatched larvae. The review of the raw data of the study shows that at the next higher test concentration of 0.530 mg/l a statistically significant effect compared to the control could be observed, however, there is no dose-effect relation for this endpoint at higher test concentrations. The highest test concentration of 3.9 mg/l shows less normal larvae after hatch with a deviation of 7% compared to the control. Apart from that regarding the endpoint 'normal larvae related to initial embryos' no effect at any concentration can be seen. Regarding 'weight' and 'length' of the fry, at both endpoints a deviation to the control of > 5 % can be seen at a concentration of 2.04 mg/l. Also for this endpoint there is no dose-effect relationship seen at the next higher concentration. As statistically significant effects for the endpoint "normal larvae" were seen at concentrations above 0.264 mg/l, the NOEC derived by the authors is used for the hazard assessment for reasons of precaution.

With Daphnia three valid acute tests are available. A test according to a Dutch standard test showed a 48 h-EC_{50} of 23.9 mg/l for *Daphnia magna* (Deneer et al. 1989). A second test on *Daphnia carinata*, comparable to OECD guideline 202 part I, showed a 48 h-EC_{50} of 21.3 mg/l (Zhao 1997). For both tests there is no information about analytical monitoring given. The pretest to the reproduction test showed a lower 24 h-EC_{50} of 12 mg/l (nominal). The long-term study revealed a 21 d-NOEC of 3.0 mg/l (measured concentration) for reproduction of *Daphnia magna* (Kühn et al. 1988).

The lowest effect value for algae has been found for *Chlorella pyrenoidosa*. A 96 h-EC_{50} on biomass is reported with 6.9 mg/l (no information about analytical monitoring), but there is no EC_0 value given (Deneer 1989). With the green alga *Scenedesmus subspicatus* the following effect values were found:

48h-E_bC_{50}:	34 mg/l
48h-E_bC_{10}:	11 mg/l
48h-E_rC_{50}:	75 mg/l
48h-E_rC_{10}:	19 mg/l

The lowest available long-term test value without effects, a NOEC of 0.264 mg/l found in the early life stage test with *Pimephales promelas*, is used as basic value for the derivation of the PNECaqua. Since long-term tests with species from three trophic levels are available, an assessment factor of 10 is proposed.

Therefore: PNECaqua = 0.264 mg/l / 10 = 0.026 mg/l.

4.2 Terrestrial Effects

In a test according to OECD-Guideline 208 (Terrestrial plant growth test) 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 (Hulzebos 1993). The soil has an organic matter content of 1.8 %. In a second soil with an organic matter content of 1.4 % a 14d-EC50-value of 5.4 mg/kg soil dry weight was found. Both values are related to nominal concentrations.

With an assessment factor of 1000 a PNECsoil of 3.2 µg/kg dw can be derived from this test.

4.3 Other Environmental Effects

No data available.

5 CONCLUSIONS

Production and processing

The world wide production of 1-chloro-2-nitrobenzene amounted to 111,800 tons in 1995 by approximately 30 producers, excluding production in East European countries. 1-Chloro-2-nitrobenzene is a basic chemical for processing intermediates which are further processed mainly to dyestuffs, pigments, pesticides, and pharmaceuticals within the chemical industry. Direct use of 1-chloro-2-nitrobenzene is not known. Releases into the environment may occur during production and processing. Emission data are only available for Bayer AG. During normal operation no 1-chloro-2-nitrobenzene is emitted into the atmosphere. Following the Official German Emission Declaration in year 2000, less than 25 kg/a 1-chloro-2-nitrobenzene were emitted. Regular monitoring data at the industrial sewage treatment plant 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 taking the 10 percentile of the river flow into account. There is no information on releases into the environment from other production and processing sites. A significant exposure to the terrestrial compartment could not be identified.

Environmental behavior

The favourite target compartments for 1-chloro-2-nitrobenzene are water with 65.4 %, followed by air with 32.9 % according to a Mackay calculation level I. In air, the substance is indirectly photodegradable with $t_{1/2}$ = 187 days. 1-Chloro-2-nitrobenzene is not readily biodegradable. According to the model Simpletreat a removal in sewage treatment plants of 4.8 % can be estimated. Under the conditions of industrial waste water treatment plants removal to > 95 % was observed at one production/processing site. However, this removal 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.

Measured bioconcentration factors in fish are in the range of 7.0 - 22.3 at a 1-chloro-2-nitrobenzene concentration of 0.25 to 0.025 mg/l. A calculated Koc suggests the substance to have a medium geoaccumulation potential.

The lowest valid acute test results of aquatic testing were determined for fish (*Cyprinus carpio*) with a 96 h-LC_{50} of 25.5 mg/l, for *Daphnia magna* with a 24 h-EC_{50} of 12 mg/l and 48 h-EC_{50} of 23.9 mg/l, and for algae (*Chlorella pyrenoidosa*) with a 96 h-EC_{50} of 6.9 mg/l. With another algae species (*Scendesmus subspicatus*) a 48h-ErC50 of 75 mg/l and a 48h-ErC10 of 19 mg/l was found. Chronic toxicity has been tested for fish (*Pimephales promelas*) in an Early Live Stage Test with a 33 d-NOEC of 0.264 mg/l (endpoint number of normal larvae; measured concentration), and for *Daphnia magna* with a 21 d-NOEC of 3.0 mg/l on reproduction (measured concentration). A PNECaqua of 0.026 mg/l is derived from the fish NOEC of 0.264 mg/l using an assessment factor of 10.

In a test with terrestrial plants a 14 d-EC_{50} in the range of 3.2 - 10 mg/kg soil dry weight was determined for *Lactuca sativa* regarding the endpoint of growth. A PNECsoil of 3.2 µg/kg dw was derived from this test.

Human health

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). The acute inhalative toxicity and dermal toxicity is moderate (LC_{50} (rat) ca. 3200 mg/m³ (= 495 ppm, vapor/aerosol mixture) for 4 hours; LD_{50}, dermal, rat: male: 655 mg/kg bw, female: 1320 mg/kg bw; LD_{50} dermal rabbit: 400 mg/kg bw (male: 445 mg/kg bw, female: 355 mg/kg bw)).