

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 activity 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 µg/ml in the presence of S9-mix and 100-900 µg/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 µg/ml) and a negative result with a harvest time of 18.5 hours (dose: 47-216 µg/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. 1978).

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 pre-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/m³), 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_{reproductive organs} = 9 ppm). Females reproductive system was not affected by treatment (NOAEL_{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/m³), 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_{reproductive organs} for male mice was not determined); in females no effects were observed (NOAEL_{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 2week 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 toxicity} 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-LC₅₀ 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-LC₅₀ 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 fry from the initial egg cups were 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-EC₅₀ 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-EC₅₀ 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-EC₅₀ 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-EC₅₀ on biomass is reported with 6.9 mg/l (no information about analytical monitoring), but there is no EC₀ value given (Deneer 1989). With the green alga *Scenedesmus subspicatus* the following effect values were found:

48h-E ₃ C ₅₀ :	34 mg/l
48h-E ₆ C ₁₀ :	11 mg/l
48h-E ₇ C ₅₀ :	75 mg/l
48h-E ₇ C 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: $PNECaqua = 0.264 \text{ mg/l} / 10 = 0.026 \text{ mg/l}$.

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 $\mu\text{g/kg dw}$ 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-LC₅₀ of 25.5 mg/l, for *Daphnia magna* with a 24 h-EC₅₀ of 12 mg/l and 48 h-EC₅₀ of 23.9 mg/l, and for algae (*Chlorella pyrenoidosa*) with a 96 h-EC₅₀ of 6.9 mg/l. With another algae species (*Scenedesmus 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-EC₅₀ 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 (LD₅₀, 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 (LC₅₀ (rat) ca. 3200 mg/m³ (= 495 ppm, vapor/aerosol mixture) for 4 hours; LD₅₀, dermal, rat: male: 655 mg/kg bw, female: 1320 mg/kg bw; LD₅₀ dermal rabbit: 400 mg/kg bw (male: 445 mg/kg bw, female: 355 mg/kg bw)).

Cyanotic appearance was the predominant appearance for all three routes of application.

The documentation of the available studies on skin irritation 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.

1-Chloro-2-nitrobenzene caused slight irritational 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.

The repeated dose toxicity 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 ppm 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)

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 genotoxic tests as described above are typical for nitroaromatics. As a whole 1-chloro-2-nitrobenzene is suspected of being genotoxic, or at least a weak clastogen.

1-Chloro-2-nitrobenzene showed tumours in different organs of rats and in the liver of mice. Overall taking into consideration the results of the genotoxicity tests, 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.

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 methemoglobin 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-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 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_{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.

6 RECOMMENDATIONS

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, national or regional exposure information gathering and 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.

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

7 REFERENCES

- Bayer AG data, Report No. 5800, 2-Chlor-1-nitrobenzol: Untersuchungen zur akuten Toxizität, 1976/jan./05.
- Bayer AG data, Löser E, o-Nitrochlorbenzol, Untersuchungen zur akuten oralen Toxizität an männlichen Wistar-Ratten, 1982 a/April/02
- Bayer AG data, Löser E, o-Nitrochlorbenzol, Untersuchungen zur akuten oralen Toxizität an weiblichen Wistar-Ratten, 1982 b/April/01
- Bayer AG data, Report No. 12848, o-Nitrochlorbenzol, Salmonella/Mikrosomen-Test zur Untersuchung auf punktmutagene Wirkung, 1984/aug/09
- Bayer AG, Internal Study: Identity and Material Balance of 1-Chloro-2-nitrobenzene (1989)
- Bayer AG data, Report No. 20209 (F), Enzymhistochemisch darstellbare Veränderungen des Kohlenhydratstoffwechsels der Mausleber nach Gabe von o-Chlornitrobenzol, 1991/mai/05.
- Bayer AG data, Report No. 22240, o-Chlornitrobenzol- subakute Toxizitätsstudie an B6C3F1-Mäusen - Schwerpunkt Leberdiagnostik-(Verabreichung im Futter bis zu 5 Wochen), 1993/mai/05 (at the request of BG-Chemie)
- Bayer AG 2001, Letter of Bayer AG dated 16.07.2001
- Bayer AG 2001a, Internal Study: Test on vapour pressure (2001a)
- BG-Chemie, Toxicological Evaluation: Report No. 73, o-Chlornitrobenzol, 11/2000
- Blume, H.-P. 1990 (ed.), Handbuch des Bodenschutzes, ecomed-Verlag Lands-berg/Lech, p.581
- Bray H.G., et al., The metabolism of the monochloronitrobenzenes in, the rabbit *Biochem. J.* 64, 38-44 (1956)
- BUA (1985), BUA Report No. 2, VCH Weinheim, October 1985
- Call, D.J. and Geiger, D.L., Subchronic toxicities of industrial and agricultural chemicals to Fathead Minnows (*Pimephales promelas*) Vol. I, Center for Lake Superior Environmental Studies, Lake Superior Research Institute, University of Wisconsin-Superior, USA (1992)
- Cesarone CF, Bolognesi C, Santi L. Evaluation of damage to DNA after in vivo exposure to different classes of chemicals. *Arch Toxicol, Suppl* 5, 355--359 (1982)
- Davydova SG (1967) A comparison of the properties of nitrochlorobenzene isomers for the determination of their permissible concentrations in water bodies. *Hyg and Sanit* 32, 161-166
- 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)
- Eckert, J.W., Fungistatic and Phytotoxic Properties of Some Derivatives of Nitrobenzene, *Phytopathology*, 52, 642-649 (1962)
- Gerbis H., Nitrochlorbenzol-Vergiftung, gewerbliche, Reparative Hyperglobulie, cited in: Fühner H. (ed.), *Sammlung von Vergiftungsfällen*, Bd3, 125-126 Verlag von F.W.C. Vogel, Berlin, 1932
- Haskell Laboratory, Inhalation of medial lethal concentration toxicity study with ortho-chloronitrobenzene in rats (at the request of Dupontde Nemour), OTS0540655, 1992