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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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m-Chloro-nitrobenzene

No. 74

CAS No. 121-73-3

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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m-Chloronitrobenzene

A Toxicological Evaluation on o-chloronitrobenzene is also available in volume 17 and can be consulted for comparison.

1 Summary and assessment

Under appropriate conditions of exposure, m-chloronitrobenzene is absorbed by the body both via the skin and the gastrointestinal tract as well as via the respiratory tract. As demonstrated by a study in which rabbits were given a single oral m-chloronitrobenzene dose of 200 mg/kg body weight, the chemical is metabolised by the body, undergoing hydroxylation to phenolic compounds on the one hand, and reduction to amino compounds on the other. Within 48 hours, 33% of the administered dose was excreted in urine as glucuronide, 18% as sulfate, 1% as nitrophenylmercapturic acid and 11% as free m-chloroaniline. Only 0.6% of the m-chloronitrobenzene dose administered was excreted as free m-chloroaniline in the faeces within 48 hours. The formation of m-chloroaniline has also been demonstrated in rats after oral administration of 78.8 mg m-chloronitrobenzene/kg body weight. Reduction of the nitro group to the amino group proceeds via the hydroxylamine compound as a highly reactive intermediate. It has also been demonstrated in vitro in rat hepatocytes and the xanthine oxidase-xanthine enzyme system that m-chloronitrobenzene is reduced to m-chloroaniline.

Upon acute single oral administration and dermal application, m-chloronitrobenzene proves to be harmful. The oral LD_{50} values for rats and mice range between 300 and 470 mg/kg body weight, while the dermal LD_{50} for rats is between 800 and 1700 mg/kg body weight. An inhalation hazard test showed no serious toxic effects following exposure to m-chloronitrobenzene. A typical sign of intoxication, apart from other, rather unspecific effects, is cyanosis. On treatment with a single dose of m-chloronitrobenzene, rats and cats are observed to develop methaemoglobinaemia, which is marked in rats and very severe in cats.

Studies in the rabbit show that m-chloronitrobenzene has no irritant effect on the skin and causes only mild eye irritation. From a Magnusson and

Kligman maximisation test, there is no indication that m-chloronitrobenzene has a skin-sensitising potential.

A number of studies in which rats were exposed to o-chloronitrobenzene by oral administration or inhalation for 8, 20, 28 or 100 days or 7 months show that the haematopoietic system, including the spleen, is the main target organ for the chemical's toxicity. In addition, methaemoglobin formation plays an important role. Effects on the liver were weaker. No other organs were affected. Independently of the route of administration, m-chloronitrobenzene administration resulted in markedly increased blood methaemoglobin levels and reduced haemoglobin levels in all of the studies. Further changes included decreased red and white blood cell counts and haematocrit, increased lymphocyte, platelet and reticulocyte counts and a marked increase in the formation of Heinz bodies. Where investigated, the spleen was enlarged, spleen weight was increased and liver weight was increased after longer treatment. The spleen displayed slight to moderately increased haematopoiesis and minimal hyperplasia of the red pulp. There was minor impairment of hepatic function, and histopathologically, centrilobular hypertrophy of the liver was observed. In the 28-day oral toxicity study in rats, the animals exhibited a relevant effect on blood composition even at the lowest dose level of 1 mg/kg body weight so that it was not possible to establish a *no effect level* in this study.

There are numerous in-vitro studies investigating the genotoxicity of m-chloronitrobenzene, nearly all of which gave negative results. Of the numerous tests carried out in *Salmonella typhimurium* and *Escherichia coli* only one test on *Salmonella typhimurium* strain TA 100 was positive. However, when the same investigators repeated the only positive test, which was inadequately documented, one year later they were unable to confirm their previous result. In two cases, test results obtained with *Salmonella typhimurium* strain TA 100 were considered questionable by the authors, one test being carried out with metabolic activation, the other without metabolic activation. The corresponding tests which were carried out in parallel with and without metabolic activation were clearly negative. To summarise, it can be said that m-chloronitrobenzene most probably has no genotoxic potential in the bacterial test systems. Similarly, studies conducted in vitro using mammalian cells gave predominantly negative results, but there are also some clearly positive findings. In Chinese hamster ovary cells, the results of a chromosome aberration assay carried out with and without meta-

bolic activation were negative. When cells from the lung of the Chinese hamster were used, the chromosome aberration test was positive at the highest m-chloronitrobenzene concentration of 400 µg/ml in the presence of metabolic activation, whereas it was negative in the absence of metabolic activation. The result of a chromosome aberration test in human lymphocytes, which was only conducted without metabolic activation, was clearly positive. A sister chromatid exchange test in Chinese hamster ovary cells was negative in the presence of metabolic activation but the result was questionable in the absence of activation. A gene mutation test (HPRT test) carried out in Chinese hamster lung cells gave negative results both with and without metabolic activation. Therefore, it remains unclear whether or not m-chloronitrobenzene is capable of inducing mutations in mammalian cells in vitro. In vivo, two genotoxicity studies of m-chloronitrobenzene have been conducted in mice. One test for chromosome aberrations in bone marrow cells produced negative results up to the highest dose tolerated by the animals. In a micronucleus test, there was only a slight, though dose-dependent, increase in the number of micronucleated polychromatic erythrocytes, a finding which should be evaluated as a clastogenic effect of the test substance.

Reproductive toxicity has been investigated in rat studies. Pregnant female rats receiving daily oral treatment with m-chloronitrobenzene at dose levels of 5, 15 or 50 mg/kg body weight on days 6 to 15 of gestation showed dose-dependent changes in the spleen in all dose groups, the top dose group exhibiting reduced food consumption and body weight gain (maternal *no effect level* < 5 mg/kg body weight). m-Chloronitrobenzene causes no reproductive toxicity at the maternally toxic doses of 5 or 15 mg/kg body weight (foetal *no effect level* 15 mg/kg body weight). The chemical appeared to induce minimal retardation in skeletal development only at the highest test dose of 50 mg/kg body weight. When a systemically toxic oral dose of 25 mg/kg body weight was administered for 28-days, reduced testicular weight and slight to moderate degeneration of testicular tissue, in addition to other toxic effects (haematopoietic system), resulted at the end of treatment in 4 out of 5 males, compared with controls. Single oral administration of m-chloronitrobenzene at 200 mg/kg body weight (equivalent to half the LD_{50}) led to signs of degenerative changes in the testes after only one day. After 25 days, testicular weight was markedly lower than in the controls, and sperm production was markedly reduced.

There are inadequately documented studies which report that m-chloronitrobenzene affects the conditioned reflexes and chronaxy.

It has long since been known that m-chloronitrobenzene is dangerous to humans. However, the data reported in the literature pertains nearly exclusively to mixed exposure with other aromatic nitro and amino compounds. In this context, it is considered critical that m-chloronitrobenzene is rapidly absorbed via the skin and the respiratory tract. The signs of acute intoxication include methaemoglobinaemia, cyanosis, vomiting, headache and, in severe cases, collapse.

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") has assigned m-chloronitrobenzene to Section IIb of the List of MAK and BAT Values, the section covering "substances for which no MAK value can be established at present". Because of the danger of cutaneous absorption, the chemical has been designated with "H".

2 Name of substance

2.1	Usual name	m-Chloronitrobenzene
2.2	IUPAC name	1-Chloro-3-nitrobenzene
2.3	CAS No.	121-73-3
2.4	EINECS No.	204-496-1

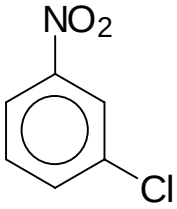
3 Synonyms, common and trade names

1-Chlor-3-nitrobenzol
3-Chlor-1-nitrobenzol
Chlor-m-nitrobenzol
3-Chlornitrobenzol
3-Chloro-1-nitrobenzene
3-Chloronitrobenzene
m-Chloronitrobenzene
MCNB
Metachloronitrobenzene

3-Nitrochlorbenzol
m-Nitrochlorbenzol
1-Nitro-3-chlorobenzene
3-Nitrochlorobenzene
m-Nitrochlorobenzene

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_6H_4ClNO_2$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	157.56	
5.2	Melting point, °C	46	(Budavari et al., 1989; Booth, 1991; Falbe and Regitz, 1996)
		44.4	(Lide and Frederikse, 1996)
5.3	Boiling point, °C	236	(Budavari et al., 1989; Falbe and Regitz, 1996)
		235.5 (at 1010 hPa)	(Booth, 1991)
		235 (at 1013 hPa)	(Bayer, 1995)
5.4	Vapour pressure, hPa	< 1	(Bayer, 1995)
		0.0112 (at 20 °C)	(Bayer, 1987)
		0.0185 (at 25 °C)	(Bayer, 1987)
5.5	Density, g/cm³	1.534 (at 20 °C)	(Budavari et al., 1989; Booth, 1991; Bayer, 1995; Falbe and Regitz, 1996)
		1.343 (at 50 °C)	(Lide and Frederikse, 1996)
5.6	Solubility in water	Insoluble	(Budavari et al., 1989; Booth, 1991; Falbe and Regitz, 1996; Lide and Frederikse, 1996)
		Ca. 0.39 g/l (at 20 °C)	(Bayer, 1995)

5.7	Solubility in organic solvents	Dissolves well in benzene and diethyl ether, soluble in acetone and hot ethanol (Booth, 1991) Dissolves well in hot alcohol, chloroform, diethyl ether, carbon disulfide and glacial acetic acid (Budavari et al., 1989) Soluble in ethanol, diethyl ether and benzene (Lide and Frederikse, 1996)
5.8	Solubility in fat	No information available
5.9	pH value	Neutral (Bayer, 1995)
5.10	Conversion factor	1 ml/m³ (ppm) $\triangleq$ 6.54 mg/m³ 1 mg/m³ $\triangleq$ 0.153 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

m-Chloronitrobenzene is mainly reduced to m-chloroaniline and further processed to dyestuffs. In addition, complete chlorination yields pentachloronitrobenzene, which is used as a fungicide (Booth, 1991).

7 Experimental results

7.1 Toxicokinetics and metabolism

In a study on the metabolism of m-chloronitrobenzene, female rabbits (weighing 2.3 kg, number of animals not given) received a single oral dose of 200 mg/kg body weight of the chemical. Urine and faeces were collected for the first 24 hours and for another 24 hours and analysed for nitro and amino compounds. Excretion was complete at 48 hours. Female rabbits receiving only water served as controls. The administered dose of m-chloronitrobenzene was recovered as urinary metabolites: glucuronide (33%), sulfate (18%), nitrophenylmercapturic acid (1%) and free m-chloroaniline (9%). Only 0.6% of the administered m-chloronitrobenzene underwent faecal excretion in the form of m-chloroaniline. Detailed analysis of the urinary metabolites revealed that m-chloronitrobenzene was, on the one hand, hydroxylated to phenolic compounds while on the other hand it was reduced to amino compounds. Appreciable amounts of m-chloro-

aniline, 2-amino-4-chlorophenol, 4-amino-2-chlorophenol and 2-chloro-4-nitrophenol were detected in urine. The phenols occurred predominantly as conjugates of glucuronic acid or sulfuric acid (Bray et al., 1956).

The in-vivo formation of m-chloroaniline from m-chloronitrobenzene was also demonstrated in a study conducted in rats. In a first reaction step N-hydroxy-m-chloroaniline was formed, which was isolated from the blood of the treated animals as the haemoglobin adduct. The same haemoglobin adduct was also found following administration of m-chloroaniline, which was oxidised to N-hydroxy-m-chloroaniline in the body. In this study, groups of 2 female Wistar rats (weighing 200 to 225 g) were treated with a single dose of 0.5 mmol m-chloronitrobenzene/kg body weight (equivalent to 78.8 mg/kg body weight) or 0.5 mmol m-chloroaniline/kg body weight (equivalent to 63.8 mg/kg body weight), administered by oral gavage. Both chemicals were 99% pure and given as a solution in tricaprylin (1 ml/kg body weight). After 24 hours, the animals were sacrificed, blood was taken by heart puncture, and the red blood cells were isolated. The haemoglobin was isolated from the red blood cells, the adduct hydrolysed with 0.1 M sodium hydroxide, and m-chloroaniline liberated from N-hydroxy-m-chloroaniline. The treatment of rats with m-chloronitrobenzene yielded about 108.4 mmol of haemoglobin adduct per mol haemoglobin, whereas with m-chloroaniline under the same conditions only 25 mmol/mol were formed. The investigators concluded from this result that N-hydroxy-m-chloroaniline was available in the body and that more N-hydroxy-m-chloroaniline was formed from m-chloronitrobenzene than was from m-chloroaniline. In the author's opinion, the highly reactive N-hydroxy compound could be responsible for potential cytotoxic, mutagenic and carcinogenic effects of o-chloronitrobenzene (Sabbioni, 1994).

Furthermore, when 6 male Wistar rats (weighing 150 to 220 g) were given m-chloronitrobenzene intraperitoneally at 100 µmol/kg body weight (equivalent to 15.8 mg/kg body weight) as a suspension in propylene glycol and their urine was subsequently collected for 5 hours, appreciable amounts of one or several metabolites were found which were identified as diazo-positive by means of the Bratton-Marshall reaction (no details of the chemical structures or absolute amounts of metabolites; Watanabe et al., 1976).

The metabolism of m-chloronitrobenzene was studied in vitro in primary hepatocytes from the livers of male Fischer rats (CDF[F-344]CrlBR,

weighing 200 to 250 g) as well as in microsomes and cytoplasm isolated from the cells. Hepatocytes (6 x 10^6) were incubated with ^{14}C-labelled m-chloronitrobenzene (1 µCi, 100 µmol in 3 ml, equivalent to 5.3 mg/ml) in 3 ml Krebs' bicarbonate buffer at 37 °C for 90 minutes. Subsequently, the reactions were terminated by adding aliquots of the reaction mixtures to cold methanol, the metabolites formed and any unconverted m-chloronitrobenzene were isolated by HPLC, the radioactivity was determined in the individual fractions and the structures of the isolated radioactive substances were elucidated by GC/MS. After the 90-minute incubation, 54.3% of the administered radioactivity was detectable in the form of three major metabolites. m-Chloroaniline, the N-glucuronide of m-chloroaniline and m-chloroacetanilide represented 30.9%, 6.7% and 16.7% of the radioactivity, respectively. Incubation of radioactive m-chloronitrobenzene (0.33 µCi/ml and 100 µmol/ml, equivalent to 15.8 mg/ml) with isolated microsomes (2 mg protein/ml) in phosphate buffer pH 7.4 in the presence of NADPH resulted in the formation of m-chloroaniline. Substitution of NADH for NADPH, incubation under a carbon monoxide atmosphere or addition of the known inhibitors of cytochrome P450-dependent reactions, SKF 525-A and metyrapone, significantly inhibited m-chloroaniline formation. It can therefore be presumed that reduction of the nitro group to the amino group is P450-dependent. In this system, addition of α-naphthoflavone enhanced the reduction of m-chloronitrobenzene to m-chloroaniline about 2-fold, indicating that the involved cytochrome P450 can be induced by phenobarbital. Incubation of radiolabelled m-chloronitrobenzene (0.33 µCi/ml and 50 µmol/ml, equivalent to 7.9 mg/ml) with hepatic cytosol (2 mg protein/ml) in phosphate buffer pH 7.4 in the presence of glutathione did not result in the replacement of the chlorine atom by glutathione. There was no formation of S-(2-nitrophenyl)glutathione (Rickert and Held, 1990).

The reduction of m-chloronitrobenzene was also studied in vitro in the xanthine oxidase-xanthine enzyme system. The reduction rate was determined as the amount of uric acid formed from xanthine per milligram enzyme protein per minute. In the absence of m-chloronitrobenzene, the system produced no uric acid, because it lacked an electron acceptor. The reduction product was identified by thin-layer chromatography and UV spectrophotometry. m-Chloronitrobenzene was reduced to the hydroxylamino compound in this system, in which the nitroso compound was believed to be formed as an intermediate. The reduction rate of the meta iso-

mer was markedly lower than that of the para isomer, a finding which was attributed steric hindrance (Tatsumi et al., 1978).

7.2 Acute and subacute toxicity

Acute toxicity

The acute toxicity data for m-chloronitrobenzene are summarised in Table 1.

Beginning of Table 1

Table 1. Acute toxicity of m-chloronitrobenzene

Species, strain, sex*	Route	Dose (mg/kg b. w.)	Effect	Observation period	Reference
Rat	oral	300	LD_{50}	no information	Davydova, 1967
Rat	oral	470	LD_{50}	no information	Vasilenko and Zvezdai, 1981
Rat	oral	420	LD_{50}	no information	Izmerov et al., 1982
Rat	oral	400	LD_{50}	no information	Mohr and Working, 1988
Mouse	oral	390	LD_{50}; deaths mainly within the first 3 days, exhaustion and sluggishness, lying on the side, cyanosis, rough coat	1 month	Alisev and Osipov, 1966
Mouse	oral	380	LD_{50}	no information	Vasilenko and Zvezdai, 1981
Rat, Wistar, male	dermal	890	LD_{50}; cyanosis, impaired movement, respiration, consciousness and reflexes; discoloration of various organs, pulmonary congestion	14 days	Hoechst, 1987 a
Rat, Wistar, female	dermal	800–1250	LD_{50}; cyanosis, impaired movement, respiration, consciousness and reflexes; discoloration of various organs, pulmonary congestion	14 days	Hoechst, 1987 a

Table 1. Acute toxicity of m-chloronitrobenzene					
Species, strain, sex*	**Route**	**Dose (mg/kg b. w.)**	**Effect**	**Observation period**	**Reference**
Rat, Wistar, male	dermal	1354	LD_{50}; sedation, spasms, latero-abdominal position, ruffled fur; red to dark-red discoloration of the lungs and intestines	14 days	RCC, 1988 a
Rat, Wistar, female	dermal	1700	LD_{50}; sedation, spasms, latero-abdominal position, ruffled fur; red to dark-red discoloration of the lungs and intestines	14 days	RCC, 1988 a
Rat, Wistar	inhalation (inhalation hazard test in 12 animals)	7-hour exposure to atmosphere enriched with max. 0.4 mg/l at 20 °C, nose exposure	no deaths; no remarkable findings at necropsy; narrow palpebral fissures, increased respiratory rate and bloody lacrimation during exposure	16 days	Hoechst, 1981
* where specified					

End of Table 1

The LD_{50} values found after oral administration were between 300 and 470 mg/kg body weight, which is in very good agreement both within the individual species as well as between rats and mice as experimental animals. LD_{50} values for dermal application are available only for rats. They vary between 800 and 1700 mg/kg body weight. In the inhalation hazard test in rats there were no deaths. Based on the data available, m-chloronitrobenzene is to be considered harmful after single-dose administration via different routes. The typical sign of intoxication was cyanosis. The concomitant clinical signs of toxicity tended to be unspecific.

A dose-finding study for a subacute toxicity study was carried out in a group of 5 male and 5 female Charles River rats (Crl:CD(SD)BR strain) which received m-chloronitrobenzene orally at a daily dose of 100 mg/kg body weight for 2 days and at 200 mg/kg body weight for another 2 days. A 2-day observation period followed. Body weight gain and food consumption were slightly depressed during treatment, but returned to normal levels within the 2-day observation period. The clinical observations included pi-

loerection, increased salivation, pallor and ataxia, and dark urine was noted with a few animals. No necropsy was performed (Hazleton, 1988).

The formation of methaemoglobin following administration of m-chloronitrobenzene was demonstrated in vivo in 6 male rats. They received 100 µmol m-chloronitrobenzene/kg body weight (equivalent to 15.8 mg/kg body weight), as a solution in propylene glycol, by the intraperitoneal route. The blood which was collected from the rats sacrificed after 5 hours contained 31.9% methaemoglobin. When rat-blood haemolysate was mixed with m-chloronitrobenzene, which was dissolved in propylene glycol in combination with phosphate buffer (0.5 µmol m-chloronitrobenzene, 0.1 µmol haemoglobin in 5 ml), and incubated for 5 hours at 37 °C, there was only 6.5% methaemoglobin formation. From this the investigators concluded that the agent responsible for methaemoglobin formation was not m-chloronitrobenzene itself but the m-chloroaniline which was formed in the body by reduction (Watanabe et al., 1976).

In a study conducted in 18 cats in total, single intraperitoneal doses of m-chloronitrobenzene were administered at dose levels 5, 7, 7.5 or 10 mg/kg body weight. Observation of the animals and determination of blood methaemoglobin levels for a period of 60 hours gave the following results. Methaemoglobin formation was variable, but on the whole very extensive (up to 75%) at dose levels of 7 to 10 mg/kg body weight. At 5 mg/kg body weight, methaemoglobin formation was markedly lower, and was not detectable in 2 out of 5 animals. On average, methaemoglobin formation reached maximum levels after 10 hours. The effect was reversible after 40 hours. Formation of Heinz bodies was increased up to 90%, the maximum being reached after 20 hours, and there was no decrease during the period of measurement. About one hour after injection, observations included vomiting in half of the animals and strong salivation in other cases. The animals gradually became weak, with very wide pupils, and died without any further concomitant effects. Out of the 13 animals treated with 7 to 10 mg/kg body weight, only 2 survived. In contrast, 3 out of 5 cats treated with 5 mg/kg body weight survived (no further details; Jung, 1947).

Subacute toxicity

In a dose-finding study for a 28-day study, m-chloronitrobenzene was investigated following oral administration to rats. Groups of 5 male and 5 fe-

male Charles River rats (Crl:CD(SD)BR strain) were given m-chloronitrobenzene for 8 days at daily doses of 6, 30 or 150 mg/kg body weight as a solution in corn oil, while the controls received corn oil alone (5 ml/kg body weight). All animals of the high dose group died in the course of the first week of treatment. All other animals survived the treatment. Clinical signs of toxicity observed in the top dose group included slightly blue extremities as of day 2, and hyperactivity, high gait, ataxia, lethargy, reduced body temperature and salivation as of day 4. Additional signs recorded only prior to sacrifice included tremors, pallor, prostration and respiratory distress as well as hair loss. The animals of the intermediate dose group had slight blue extremities post dose on one occasion, but this was no longer observed the next morning. There were no other clinical observations in that dose group and the low dose group. Lower body weight gain was only found for the males of the high dose group. Food consumption for the low and intermediate dose groups was unchanged compared with controls. The haematology results for the intermediate dose group showed markedly elevated methaemoglobin levels, accompanied by lower haemoglobin, erythrocyte count, packed cell volume and mean cell haemoglobin concentrations. Platelet and white cell counts were minimally higher than controls. The animals of the low dose group had slightly higher methaemoglobin levels than controls with minimally higher total white cell counts for occasional females of this group. Necropsy of the high dose group animals revealed dark and inflated lungs and enlarged spleen in a portion of the animals. There were no substance-related changes in the animals of the intermediate and low dose groups. No histopathological examination was performed (Hazleton, 1988).

The 28-day study was performed using rats of the same strain and the same number of animals per group as in the dose finding study. m-Chloronitrobenzene, in corn oil, was administered by daily oral gavage at dose levels of 1, 5 or 25 mg/kg body weight. A control group received corn oil only (5 ml/kg body weight). There were no deaths during the study. The clinical signs noted in the high dose group included pallor from treatment week 2 until study termination, and fur staining. Body weight gains of the intermediate and high dose animals were retarded, whereas food consumption was not affected in any of the treatment groups. The haematology results showed a dose-related increase in methaemoglobin levels in all treatment groups, although the effect was minimal at the low dose level.

For high dose animals, slightly lower erythrocyte counts, haemoglobin concentrations and packed cell volumes were recorded than for controls. The clinical chemistry findings revealed slightly elevated glucose and decreased triglyceride levels in the blood of the high dose males only. At terminal necropsy of all animals, there were changes only in the high dose group. General visceral pallor was observed, with half of the animals exhibiting pale and/or enlarged spleens. In addition, increased spleen and liver weights were recorded. Slight centrilobular hypertrophy was seen in the livers of all high dose animals and in one intermediate dose male. There was slight to moderate haemopoiesis in the spleen in all high and intermediate dose animals and in one male and 4 females of the low dose group. Minimal red pulp hyperplasia of the spleen was seen in all animals of the high dose group and in all intermediate dose females and one low dose female. The males of the high dose group showed reduced testis weights. Histopathological examination revealed slight to moderate degeneration of the testis in 4 out of 5 animals (see also Section 7.8). In this 28-day study, toxic changes were detectable in the blood of the animals even at the low dose of 1 mg/kg body weight, and hence a *no observable effect level* could not be deduced (Hazleton, 1993).

In an older study, 20 albino rats received a daily oral m-chloronitrobenzene dose of 60 mg/kg body weight (equivalent to 1/5 of the LD_{50}) for 20 days. Four of the animals died during treatment, an outcome which led the author to conclude that the chemical possessed cumulative properties to some extent (no further details; Davydova, 1967).

The effect of m-chloronitrobenzene was studied in 11 cats with respect to survival and methaemoglobin formation in the blood following dermal application. Except on Sundays, the animals received daily applications of the melted chemical (44 °C) to a previously depilated 4 cm x 5 cm area of skin which was covered with a cotton wool-gauze dressing. Doses of 34, 166 and 376 mg/kg body weight were administered. Mean survival of the cats treated with 166 (4 cats) or 376 mg/kg body weight (3 cats) was 3 or 4 days. The animals of the low dose group survived treatment for 25 days on average (19 to 32 days). Signs of intoxication included refusal of food, exhaustion and motor disorders, serous nasal discharge and, immediately prior to death, clonic convulsions. In the 4 animals of the low dose group, body weight dropped to about 80% of initial weight in the course of 20 days, while erythrocytes fell to about 60%. Methaemoglobin levels in the

blood reached over 80% during this period. The authors concluded from these findings that m-chloronitrobenzene is well absorbed via the skin and is a potent methaemoglobin former (no further details; Alisev and Osipov, 1966).

7.3 Skin and mucous membrane effects

The dermatological actions of o-chloronitrobenzene were studied in 3 New Zealand rabbits which received a 4-hour application of 500 mg (99% pure) undiluted chemical to the shorn intact skin. m-Chloronitrobenzene was applied as a paste made with 0.3 ml propylene glycol, and covered with a semi-occlusive bandage which was affixed to the application site with adhesive plaster. Following the exposure period, the chemical was removed from the skin with lukewarm water. Assessments of the treated area of skin after 30 and 60 minutes revealed mild, hardly noticeable erythema in 2 animals. After 24 hours, the animals no longer showed any signs of irritation. Therefore, m-chloronitrobenzene was not irritating to the rabbit skin (Hoechst, 1987 b).

A further study of the same experimental design was conducted as described above, the only difference being that the first assessment of the treated area of skin took place one hour, instead of 30 minutes, after removal of the dressing. m-Chloronitrobenzene was not observed to cause any skin effects (RCC, 1988 b).

The effects of m-chloronitrobenzene on the eye were studied in 3 New Zealand rabbits which had a single 100 mg dose of the chemical (99% pure) instilled into the conjunctival sac of the left eye. Twenty-four hours prior to the beginning of the experiment, and 72 hours after administration, one drop of sodium fluorescein solution was instilled into the treated eye of each animal, and damage to the cornea was assessed under ultraviolet light. Inspection of the treated eyes at 1, 24, 48 and 72 hours after instillation gave the following results. At 1 and 24 hours after treatment, mild to moderate swelling and severe reddening of the conjunctivae occurred. Two animals exhibited slight cloudiness of the cornea. At 48 hours after treatment, one animal showed marked hyperaemia of the conjunctival blood vessels. The iris of that animal was reddened, while the cornea was slightly clouded. At 72 hours after treatment, 2 animals were still observed to have

marked hyperaemia of the conjunctival blood vessels. After 7 days, all signs of irritation were reversible. Therefore, m-chloronitrobenzene was evaluated as slightly irritating to the eye (Hoechst, 1987 c).

7.4 Sensitisation

The skin-sensitising potential of m-chloronitrobenzene was investigated in Dunkin-Hartley albino guinea pigs (DUHA KFM strain, 7 to 8 weeks old) in the Magnusson and Kligman maximisation test. Groups of 20 animals were given intradermal injections of 0.1 ml Freunds' adjuvant, diluted with ethanol, and 0.1-percent m-chloronitrobenzene or just the adjuvant without test substance (control). One week thereafter, the animals which had been exposed to m-chloronitrobenzene were treated epidermally with a 25-percent ethanol solution of the chemical by affixing a soaked filter paper to the skin for 48 hours by means of a semi-occlusive dressing. The controls received ethanol alone. In order to assess the skin sensitising potential, a challenge treatment with 25-percent m-chloronitrobenzene or ethanol was carried out again after 2 weeks, but in this case the dressing was only left on for 24 hours. A second challenge was carried out in the same way after another 2 weeks. No reactions were observed in any of the cases so that m-chloronitrobenzene was evaluated as nonsensitising in the guinea pig (RCC, 1987).

7.5 Subchronic and chronic toxicity

A total of 42 rats received oral treatment with m-chloronitrobenzene at dose levels of 0.0025, 0.005, 0.025 or 5 mg/kg body weight for 7 months (no further details). At the low dose levels, the chemical showed no effect on the treated animals. Daily administration to rats of 5 mg m-chloronitrobenzene/kg body weight resulted in marked changes in peripheral blood count which started even during the first month of treatment. There was a marked rise in the methaemoglobin content while the haemoglobin content dropped, accompanied by an increase in reticulocyte counts and very marked formation of Heinz bodies. These findings indicated the presence of haemolytic anaemia in the experimental animals with stimulation of the haematopoietic system. Alkaline phosphatase activity levels in the blood and urinary bilirubin concentrations were slightly elevated. Furthermore, in

the highest dose group, there were weak effects on the central nervous system – measured as the chronological establishment of conditioned reflexes under the influence of m-chloronitrobenzene (see also Section 7.10; no further details; Davydova, 1967). Insufficient documentation renders the study suitable only to a limited extent for the assessment of the toxic effects of m-chloronitrobenzene.

Groups of 15 to 18 rats continuously inhaled m-chloronitrobenzene vapours at concentration levels of 0.004, 0.008 and 0.08 mg/m³ for 100 days. The study investigated the chronaxy of antagonistic muscles and the animals' blood with respect to erythrocytes, reticulocytes and Heinz bodies, total haemoglobin, oxyhaemoglobin, methaemoglobin and sulfhaemoglobin and cholinesterase and catalase activities (no further details of the experimental setup and conduct of the study). The following results were reported. Administration of m-chloronitrobenzene at 0.08 mg/m³ resulted in a shift in the chronaxial ratio of extensors and flexors as of day 22, whereas at 0.008 mg/m³ a shift was seen as of day 54 (increase in the chronaxy of flexors). After 20 days without treatment, values were back to normal. Total blood haemoglobin level was decreased in the animals treated with m-chloronitrobenzene at 0.08 mg/m³. No relevant amounts of methaemoglobin were found even at the highest concentration level of 0.08 mg/m³. In contrast, at the 0.08 and 0.008 mg/m³ levels, pronounced amounts of sulfhaemoglobin were observed from treatment day 40. The investigator's conclusion was that in longer-term administration of m-chloronitrobenzene it is not methaemoglobin but sulfhaemoglobin formation that is predominant (see also Section 7.10; no further details; Andreeshcheva, 1970). The results of the study must be regarded with great scepticism seeing that methodological details and proof of results in the form of numerical data are lacking (except in the case of chronaxy).

7.6 Genotoxicity

7.6.1 In vitro

The available data on the in-vitro genotoxicity of m-chloronitrobenzene are summarised in Table 2.

Beginning of Table 2

Table 2. In-vitro genotoxicity tests with m-chloronitrobenzene					
Test system	Concentration range tested* (µg/plate or µg/ml)	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
1. Tests in *Salmonella typhimurium* and *Escherichia coli*					
Salmonella/microsome test, *Salmonella typhimurium* TA 100 (no further details)	no information	no information	positive		Tardiff et al., 1976
Salmonella/microsome test, *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98, TA 100, standard-plate incorporation test	no information	S9 mix from Aroclor-induced rat liver	negative	negative	Simmon et al., 1977
Salmonella/microsome assay	no information	no information	negative		D'Addario and Jagannath, 1981
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, preincubation test	3.3–666, bacteriotoxic at 666 (99% pure)	S9 mix from Aroclor-induced rat and hamster liver	negative	negative, TA 100 equivocal	Haworth et al., 1983
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1537, TA 1538, preincubation test	25.6–3276.8, bacteriotoxic at the highest concentration (99% pure)	S9 mix from PCB-induced rat liver	negative	negative	Shimizu et al., 1983
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, preincubation test	5–100, no bacteriotoxicity	S9 mix from PCB-induced rat liver	negative	negative	Suzuki et al., 1983
Salmonella/microsome test, *Salmonella typhimurium* TA 97, TA 98, TA 100, TA 102, TA 1535, TA 1537, TA 1538	no information	S9 mix (no further details)	negative		Koch et al., 1985
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, preincubation test	100–5000	S9 mix from Kanechlor-induced rat liver	TA 98 negative, TA 100 equivocal	negative	Kawai et al., 1987
SOS chromotest, *Escherichia coli* PQ 37	up to 1576	S9 mix from Aroclor-induced rat liver	negative	negative	von der Hude et al., 1988
umu Test, *Salmonella typhimurium* TA 1535/pSK1002	100	S9 mix from phenobarbital- and 5,6-benzoflavone-induced rat liver	negative	negative	Ono et al., 1992
2. Tests in mammalian cells					
Chromosome aberration test, Chinese hamster ovary cells (CHO), 100 metaphases examined per concentration	50–500	S9 mix from Aroclor-induced rat liver	negative	negative	Galloway et al., 1987

Table 2. In-vitro genotoxicity tests with m-chloronitrobenzene					
Test system	Concentration range tested* (µg/plate or µg/ml)	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
Chromosome aberration test, Chinese hamster lung cells (V79), 400 metaphases examined per concentration	15–275 (–S9), 30–400 (+S9); bacteriotoxic at the highest concentrations	S9 mix from Aroclor-induced rat liver	positive at 400 µg/ml after an 18-hour interval	negative	LMP, 1987 a
Chromosome aberration test, human lymphocyte, 100 metaphases examined per concentration	7.9–236.4	–	–	positive	Huang et al., 1995
Sister-chromatid exchange test (SCE test), Chinese hamster ovary cells (CHO), 50 metaphases examined per concentration	5–50 µg/ml (–S9), 1.6–160 µg/ml (+S9)	S9 mix from Aroclor-induced rat liver	negative	equivocal	Galloway et al., 1987
HPRT test (gene mutation test), Chinese hamster lung cells (V79)	27.5–275 µg/ml (–S9), 40-400 µg/ml (+S9)	S9 mix from Aroclor-induced rat liver	negative	negative	LMP, 1987 b
* unless stated otherwise, publications give no details of bacteriotoxic or cytotoxic effects or of the purity of the m-chloronitrobenzene used and/or any impurities it may have contained					

End of Table 2

In different studies in *Salmonella typhimurium*, which were carried out both as standard plate incorporation and preincubation tests, results with m-chloronitrobenzene were negative. When the same investigators repeated the only positive test (Tardiff et al., 1976), which was inadequately documented, one year later they were unable to confirm their previous result (Simmon et al., 1977). In two instances, the result obtained with strain TA 100 was equivocal; in one case the study was carried out in the presence of S9 mix (Kawai et al., 1987), while in the other, the result was obtained in the absence of S9 mix (Haworth et al., 1983). In both cases, the complementary test which was carried out in parallel in the absence and presence of S9 mix, respectively, gave an unequivocally negative result. A umu test on *Salmonella typhimurium* (Ono et al., 1992) and an SOS chromotest on *Escherichia coli* (von der Hude et al., 1988) were also negative. Therefore, it can be said that m-chloronitrobenzene most probably has no genotoxic potential in the bacterial test systems.

In mammalian cells, the result of a gene mutation test (HPRT test) was also negative (LMP, 1987 b). Of three chromosome aberration tests, one test on CHO cells of the hamster gave a clearly negative result (Galloway et al., 1987), whereas another test, which was carried out in human lymphocytes, was clearly positive (Huang et al., 1995). The third test, which employed V79 hamster cells, was negative in the absence of metabolic activation with S9 mix, while in the presence of S9 mix the result was dose-dependent and clearly positive at the highest dose level (LMP, 1987 a). In this case, the question as to whether m-chloronitrobenzene has the potential to induce chromosome aberrations must remain unanswered. A study of sister chromatid exchange in treated CHO cells of the hamster gave an equivocal positive result without S9 mix and a negative one with S9 mix (Galloway et al., 1987).

7.6.2 In vivo

The ability of m-chloronitrobenzene to induce chromosome aberrations in bone marrow cells of the mouse was investigated in vivo. Groups of 5 male and 5 female mice (NMRI strain, weighing about 30 g) were treated with a single oral dose of m-chloronitrobenzene at 0 (controls), 15, 50 and 150 mg/kg body weight. The highest dose tolerated by the animals was 150 mg/kg body weight. At 6, 24 and 48 hours after dosing, the bone marrow was prepared and 50 metaphase cells per animal were examined. There was no case in which the number of chromosome aberrations was increased in comparison with the control (CCR, 1989).

For the purpose of a micronucleus test, groups of 5 male and 5 female mice (Bor:NMRI strain, weighing 28 to 43 g) were treated intraperitoneally with a single m-chloronitrobenzene dose of 300 mg/kg body weight. At 24, 48 and 72 hours after dosing one dose group at a time was sacrificed, and the femoral bone marrow was extracted and examined. Cyclophosphamide (20 mg/kg body weight) and corn oil served as the positive and negative controls, respectively, sacrifice being at 24 hours in both cases. The animals receiving m-chloronitrobenzene treatment exhibited clear signs of toxicity, mortality being 12%. No change was detected with respect to the polychromatic/normochromatic erythrocyte ratio. A weak clastogenic effect was observed in the group of animals which were treated with m-chloronitrobenzene and sacrificed 48 later. In that group, there was a significant

increase in the number of micronuclei/1000 polychromatic erythrocytes (3.9) relative to the control (1.2). At the other time points of sacrifice, there was a nonsignificant trend in the same direction (Bayer, 1990).

7.7 Carcinogenicity

No information available.

7.8 Reproductive toxicity

The reproductive toxicology of m-chloronitrobenzene was investigated in a study in which groups of 28 pregnant female rats (Charles River Crl:CD BR) were treated by oral gavage with daily m-chloronitrobenzene doses of 0 (controls), 5, 15 or 50 mg/kg body weight from days 6 to 15 of gestation. The chemical was dissolved in an aqueous carboxymethylcellulose/Tween 80 mixture (CMC), which was used as the control treatment in a further group of animals. The administered m-chloronitrobenzene was 99.5% pure. On day 20 of gestation, all dams were killed, and their reproductive organs including the foetuses were examined. Evaluated parameters included pregnancy rate, numbers of corpora lutea, implantations and resorptions, litter size, placental weight, pre- and postimplantation loss as well as foetal weight, appearance and viability and the sex ratio in each litter. From each litter, 50% of foetuses was examined for organ changes, the remainder for skeletal variations and malformations. Throughout the study, maternal body weight gain and food consumption were measured, and the dams were kept under careful observation. Upon sacrifice, the dams were dissected, and the spleens were weighed and examined histopathologically. No other organs showed visible effects. None of the animals died or showed clinical signs of intoxication while under treatment. In the highest dose group, body temperature and food consumption were reduced. The animals treated with m-chloronitrobenzene at 15 and 50 mg/kg body weight had an enlarged spleen. The spleen-to-body weight ratio was markedly increased relative to controls. In all 3 dose groups, histopathological changes occurred in the spleens, such as increased incidences of extramedullary haematopoietic foci and haemosiderosis, and in the high dose group there were also signs of visceral peritonitis of the spleen. Thus, maternal toxicity was seen in all dose groups tested (maternal *no effect level* < 5 mg/kg body weight). No

adverse effects were seen up to the highest dose of m-chloronitrobenzene on the maternal reproductive parameters investigated. In the foetuses, adverse effects of m-chloronitrobenzene treatment were observed only at the highest, clearly maternally toxic dose in the form of skeletal variations (wavy ribs) and delayed ossification of a few skeletal elements. All other parameters did not differ from the controls. m-Chloronitrobenzene caused no reproductive toxicity at maternally toxic doses of 5 or 15 mg/kg body weight (foetal *no effect level* 15 mg/kg body weight). The chemical appeared to induce minimal retardation in skeletal development only at the highest test dose of 50 mg/kg body weight (Miles, 1991).

The potential testicular toxicity of m-chloronitrobenzene was investigated in male rats (Fischer 344) which received a single oral administration of the chemical at 200 mg/kg body weight (half of the LD_{50}). The rats were killed 1 or 25 days after administration, and testicular weights, daily sperm production and histopathological changes in the testes were determined. After only one day, signs of early degenerative change were observed in the testes of the 6 males which were treated. By day 25 after m-chloronitrobenzene administration, testis weights were markedly reduced relative to controls (1.9 g versus 2.6 g), as was daily sperm production (6.8×10^6 versus 17.8×10^6/g testis). Therefore, m-chloronitrobenzene exhibited marked testicular toxicity under the exposure conditions described when administered as a single dose at a level which corresponded to half of the LD_{50} (no further details; Mohr and Working, 1988).

The potential toxicity of m-chloronitrobenzene to the rat testis at systemically toxic doses is also suggested by the result of a subacute study (see also Section 7.2). Male rats which received daily treatment with m-chloronitrobenzene at 25 mg/kg body weight by oral gavage for 28 days, in addition to other toxic effects (on the haematopoietic system), exhibited reduced testis weights and slight to moderate degeneration of the testis in 4 out of 5 animals at the end of treatment (no further details; Hazleton, 1993).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

In a chronic toxicity study, in which rats were treated with a daily oral m-chloronitrobenzene dose of 5 mg/kg body weight for 7 months (see also Section 7.5), the chemical showed a weak effect on the central nervous system. Measurements were performed to assess establishment of conditioned reflexes, the time required for their appearance and their latent period, magnitude and frequency of occurrence. In 4 out of 6 animals, m-chloronitrobenzene caused some slowing down of fixation of the positive conditioned reaction and of the development of the differentiation reaction. At a lower dose level of 0.025 mg/kg body weight, m-chloronitrobenzene had no effect in this test (no further details; Davydova, 1967). Insufficient documentation renders the study suitable only to a limited extent for the assessment of the potential neurotoxic effects of m-chloronitrobenzene.

In a further study, groups of 15 to 18 rats continuously inhaled m-chloronitrobenzene vapours at concentration levels of 0.004, 0.008 and 0.08 mg/m³ for 100 days (see also Section 7.5). In addition to other parameters, the chronaxy of antagonistic muscles was also investigated at the end of treatment. In animals treated with m-chloronitrobenzene at 0.08 mg/m³, the chronaxial ratio of extensors and flexors was < 1 from day 22, but returned to normal after a treatment-free recovery period of 20 days. The animals treated with 0.008 mg/m³ did not show this effect until treatment day 54 and thereafter, and again, the effect was reversible during the recovery period. In both cases, the chronaxy of flexors was increased (no further details; Andreeshcheva, 1970).

7.11 Other effects

No information available.

8 Experience in humans

Although it has long since been known that m-chloronitrobenzene is dangerous to humans, the relevant literature contains no data on the pure chemical. The available reports relating to intoxication deal exclusively with mixed exposure, frequently involving p-chloronitrobenzene and/or nitrobenzene.

The principal manifestations of human poisoning with chloronitrobenzenes are reported to include methaemoglobin formation, skin sensitisation, mild skin irritation, kidney and liver damage, slight depression of the central nervous system and hyperthermia. No distinction was made between the different isomers (Dreisbach, 1969).

Linch (1974) attempted classification according to a system which was based on the experience of 10 years and 187 cases of cyanosis, ranking potency from “1: most potent” to “13: least potent”. m-Chloronitrobenzene was assigned to potency rank 7, but was not distinguished from p-chloronitrobenzene or a mixture of chloronitrobenzenes. The assumption was made that exposure always involved parallel absorption via the skin and by inhalation (Linch, 1974).

The olfactory threshold of m-chloronitrobenzene in water was reported as 0.02 mg/l. However, no distinction was made between the isomers of chloronitrobenzene (Davydova, 1967).

The following summary of the toxic properties of m-chloronitrobenzene was given in the handbook entitled “Dangerous properties of industrial materials”. The principal manifestations of poisoning are methaemoglobin formation, cyanosis and blood changes. The effects are cumulative. m-Chloronitrobenzene is probably converted in the body to m-chloroaniline, which is also poisonous (no further details; Sax, 1995).

There are a number of reports on cases of poisoning in the workplace with considerable involvement of chloronitrobenzenes. As early as 1902, one case of lethal poisoning with “nitrochlorobenzene” was described together with cases of severe intoxication in 4 workers at a plant producing chlorobenzene. The 4 workers had been exposed to nitrochlorobenzene before losing consciousness after alcohol consumption, and they did not regain consciousness until 8 to 10 hours later. They showed clinical signs typical of cyanosis (Leymann, 1904).

Two cases of severe “nitrochlorobenzene” poisoning with unconsciousness and severe cyanosis were placed under observation for several months in order to assess late injury. In one case, headaches and feelings of weakness still occurred after 2 months, and slight secondary anaemia was detectable. After 3 months, recovery was complete. In the second case, headache, stomach trouble and lymphocytosis were still observed even

after 9 months. However, alcohol abuse was suspected in this patient (Bonzanigo, 1931).

In a survey of 325 cases of industrial chemical cyanosis which were registered in Great Britain during the 1961–1980 period, “nitrochlorobenzenes” were listed as responsible agents. With these chemicals (not broken down by isomer), 50 cases of poisoning were registered in the period covered by the report, 23 of which were “early” cases, meaning that symptoms developed while at work on the same day, and 27 were “delayed” cases, which became manifest only after some time. Absorption took place either via the skin, by inhalation or due to mixed exposure through the skin and the lungs (Sekimpi and Jones, 1986).

9 Classifications and threshold limit values

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (“MAK-Kommission”) has assigned m-chloronitrobenzene to Section IIb of the List of MAK and BAT Values, the section covering “substances for which no MAK value can be established at present”. Because of the danger of cutaneous absorption, the chemical has been designated with “H” (DFG, 2000, Greim, 2000).

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