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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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o-Chloro-nitrobenzene

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o-Chloronitrobenzene

A Toxicological Evaluation on m-chloronitrobenzene is also available and can be consulted for comparison.

1 Summary and assessment

o-Chloronitrobenzene, 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 the ^{14}C-labelled chemical show that o-chloronitrobenzene absorption is 80% following oral administration and at least 40% after open dermal application. Essentially, most of the compound is excreted within the first 24 hours in the form of metabolites which are found in the urine and faeces (60 to 80% and 20 to 30% of the absorbed amount, respectively). At very high doses, e.g. 200 mg/kg body weight 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 o-chloronitrobenzene or one of its metabolites. In tissue, only a very small fraction of the administered radioactivity is recovered. The main metabolic routes for o-chloronitrobenzene in the body consist in reduction of the nitro group to an amino group and hydroxylation of the benzene ring. Apart from o-chloroaniline, the corresponding nitrophenols and aminophenols are formed, which are excreted as conjugates of glucuronic acid and sulfuric acid. o-Chloroaniline also appears in the urine and faeces in the unconjugated form. During reduction of the nitro group to the amino group, the hydroxylamine compound forms as a highly reactive intermediate which has been detected both in vivo in rats, and in vitro.

Upon acute oral administration and dermal application, o-chloronitrobenzene proves to be harmful to toxic. The oral LD_{50} ranges for the rat and the mouse are 144 to 560 mg/kg body weight and 135 to 440 mg/kg body weight, respectively. For rabbits, the oral LD_{50} is 280 mg/kg body weight and the dermal LD_{50} is in the range between 355 and 445 mg/kg body weight. In male rats, the dermal LD_{50} is 655 mg/kg body weight and in females it is in the range between 1320 and 1796 mg/kg body weight. A typical sign of intoxication is cyanosis. Following single treatment with low do-

ses of o-chloronitrobenzene, rats and cats are observed to develop methaemoglobinaemia. The inhalation toxicity of o-chloronitrobenzene was determined at an ambient temperature of 31.8 °C because of the chemical's low vapour pressure, and the resulting LC_{50} was found to be approximately 3200 mg/m³ for rats.

In rabbit studies, o-chloronitrobenzene has no irritant effect on the skin or eye. Studies conducted in rats and guinea pigs indicate a sensitising potential but because study documentation is incomplete and the methodology employed is no longer in use, they can not serve as a basis for final evaluation of the chemical in this respect.

A series of studies in which rats or mice were exposed to o-chloronitrobenzene by oral administration or inhalation for 14 days, 4 weeks, 3 months and 7 months shows that because of the marked methaemoglobin formation which leads to haemolytic anaemia, the haematopoietic system is the main target organ for the chemical's toxicity. Independently of species and route of administration, o-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 showed dark discoloration, enlargement and increased organ weight. Haemosiderin deposits were found in the spleen, as were slight to moderate haematopoiesis and minimal hyperplasia of the red pulp. The effects on the liver were weaker. Liver weight was increased after treatment, hepatic function was slightly impaired and histologically, centrilobular hypertrophy was noted. Kidney weights were slightly increased but no dysfunction was observed. No other organs were affected. It is not possible on the basis of the available data to predict the toxic effects of o-chloronitrobenzene after longer periods of exposure. In the 13-week inhalation studies carried out in the mouse and the rat, even the lowest dose of 7 mg/m³ was effective.

Numerous studies on the in-vitro genotoxicity of o-chloronitrobenzene have produced contradictory results. In tests on *Salmonella typhimurium*, most of the strains investigated gave clearly negative results. Out of 12 assays and 13 assays carried out in strains TA 98 and TA 100, respectively, with metabolic activation by addition of S9 mix, half of the tests were positive (5 and

7 tests, respectively), the other half negative (7 and 6 tests, respectively). In the absence of metabolic activation, the results are practically all reported to be negative. Only in two cases were positive test results obtained with *Salmonella typhimurium* strain TA 1538. These are counterbalanced by two negative results from comparable tests. With regard to DNA-damaging activity, negative results were also obtained with several tests on *Escherichia coli* and one umu test on *Salmonella typhimurium*. Contradictory results were also obtained with tests on mammalian cells. Of two reported studies on chromosome aberrations in CHO cells, one gave clearly negative, the other weakly positive results. A test for sister chromatid exchange which was conducted in the same cells was also weakly positive. A gene mutation test on V79 cells and a UDS test on primary rat hepatocytes were clearly negative. Only few data are available on the in-vivo genotoxicity of o-chloronitrobenzene. Two sex-linked recessive lethal tests in *Drosophila melanogaster* show that the chemical is not genotoxic. Single intraperitoneal administration of 60 mg o-chloronitrobenzene/kg body weight to mice leads to an increase in the frequency of DNA fragments upon isolation of hepatic and renal cell nuclei from the mice and subsequent elution of the DNA. According to the studies available, o-chloronitrobenzene can not be evaluated as genotoxic nor has it been legally classified.

For the assessment of the carcinogenicity of o-chloronitrobenzene, the only available results are those from a long-term study involving 18-month treatment of rats and mice, and a 4-week mouse study investigating the formation of preneoplastic liver foci and the effects of o-chloronitrobenzene on hepatocellular metabolism. In the long-term study, which was not conducted according to current requirements for study conduct and documentation, administration of 100 mg o-chloronitrobenzene/kg body weight (later on, 50 mg/kg body weight) in the feed of rats for 18 months, followed by a rest period of 6 months, resulted in an increase in tumour incidence, compared with controls. A great variety of different tumours were found in different tissues. Following treatment at a higher dose level of o-chloronitrobenzene (200 mg and, later on, 100 mg/kg body weight), the numbers of tumours were not increased over controls. Mice receiving 18-month treatment with o-chloronitrobenzene (initially 300 mg and, later on, 150 mg/kg body weight) followed by another 3 months on a diet of normal feed showed an increase in the number of hepatomas relative to controls. In this case too, the findings in males treated with a higher dose of 600 mg and, la-

ter on, 300 mg/kg body weight were not confirmed. Only the female mice showed the same effect at the high dose level as they did at the lower dose level. In the 4-week study in mice, o-chloronitrobenzene administrations of 5, 50 and 500 mg/kg body weight in the feed did not result in the development of typical liver foci. It was demonstrated, however, that the carbohydrate metabolism of hepatocytes was altered in a dose-dependent manner. Particularly at the mid and high doses, there was a marked decrease in hepatocyte gluconeogenesis and glycogen content and an increase in glycolysis and pentose phosphate pathway activity. Thus, a metabolic situation was induced in hepatocytes which, according to the authors of the study, may have contributed to tumorigenesis. The studies referred to above suggest that the chemical may have a carcinogenic potential.

When female rats were treated with daily o-chloronitrobenzene doses of up to 100 mg/kg body weight by oral gavage on days 6 to 15 of gestation, there was no change, compared with controls, in fertility rate, mean numbers of live and dead foetuses, total implantations, late resorptions and corpora lutea or preimplantation losses, despite the body weight loss and reduced food consumption seen in the dams of the high dose group. Similarly, there were no biologically relevant differences from the controls with respect to the number of pups with visceral or skeletal malformations. Hence, in this study o-chloronitrobenzene was found not to cause teratogenicity, nor were embryotoxic or foetotoxic effects detectable. In a study in which rats and mice inhaled o-chloronitrobenzene with the air they breathed (at concentrations up to 115.2 mg/m³) for 13 weeks, no differences were noted relative to controls in the females' oestrus cycles as determined by vaginal cytology. In the male rats, reduced spermatogenesis and testicular weight occurred at the highest concentration of 115.2 mg/m³. Epididymal weight as well as the number of spermatid heads/testis and the number of spermatids were lower than controls. Concentration levels of 57.6 mg/m³ have no effect on the genitals of male rats, however, even at this concentration level, there are considerable toxic effects on the haematopoietic system. In male mice, reduced sperm motility was the only observation to be made. These findings could not be confirmed in studies with o-chloronitrobenzene treatment of shorter duration as summarised in the following. Upon single oral administration of 150 mg o-chloronitrobenzene/kg body weight to male rats, no effects whatsoever were seen on the testes, and 4-week treatment with 1120 mg o-chloronitrobenzene/kg body weight administered in feed gave

no other result in male mice than a slight reduction in testicular weight without any histopathological correlate. From a comprehensive, two-generation reproductive toxicity study in mice treated with oral doses of up to 160 mg o-chloronitrobenzene/kg body weight, there is, finally, no indication that reproduction in these mice was impaired, even though at the highest dose level marked toxic effects (methaemoglobinaemia, enlargement of the spleen) were noted. Neither the number of pairs with offspring nor the number of litters/pair and viable offspring, nor their sex ratio or body weight were affected in the first or second generation by the o-chloronitrobenzene treatment in comparison with untreated controls. At the end of the study, the treated males were found to be unaffected in terms of sperm numbers, abnormal sperm cells and sperm motility.

It has long since been known that o-chloronitrobenzene is dangerous to humans; however, all reports relate to mixed exposure, frequently in combination with p-chloronitrobenzene and/or nitrobenzene. A critical aspect in this context is that the chemical 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.

In the Federal Republic of Germany, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") of the Deutsche Forschungsgemeinschaft has assigned o-chloronitrobenzene to category 3 of carcinogenic substances in the List of MAK and BAT Values (i.e. "substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data") and the chemical has been designated with "H" because of the danger of cutaneous absorption. Furthermore, o-chloronitrobenzene has been legally classified in the TRGS 905 and placed into carcinogenicity category C3 of "substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment" and in category R_F3 of substances toxic to reproduction, the category comprising "substances which cause concern for human fertility", in accordance with the EU classification criteria.

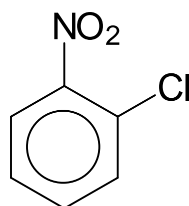
2 Name of substance

2.1	Usual name	o-Chloronitrobenzene
2.2	IUPAC name	1-Chloro-2-nitrobenzene
2.3	CAS No.	88-73-3
2.4	EINECS No.	201-854-9

3 Synonyms, common and trade names

Benzene, 1-chloro-2-nitro-
1,2-Chlornitrobenzol
2-Chlornitrobenzol
o-Chlornitrobenzol
Chlor-o-nitrobenzol
2-Chlor-1-nitrobenzol
1,2-Chloronitrobenzene
2-Chloronitrobenzene
Chloro-o-nitrobenzene
1-Chloro-2-nitrobenzene
2-Chloro-1-nitrobenzene
o-Nitrochlorbenzol
1,2-Nitrochlorbenzol
2-Nitrochlorbenzol
o-Nitrochlorbenzoyl TTR
1-Nitro-2-chlorbenzol
2-Nitro-1-chlorbenzol
o-Nitrochlorobenzene
2-Nitrochlorobenzene
1-Nitro-2-chlorobenzene

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	31.8	(Monsanto, 1990)
		32	(Bayer, 1998)
		32.5	(Lide and Frederikse, 1996)
		32–33	(Budavari et al., 1989)
		33	(Booth, 1991)
		33–35	(Falbe and Regitz, 1996)
5.3	Boiling point, °C	243	(Bayer, 1998)
		245.5	(Booth, 1991; Lide and Frederikse, 1996)
		245–246	(Budavari et al., 1989)
		246	(Monsanto, 1990; Falbe and Regitz, 1996)
5.4	Vapour pressure, hPa	0.0575 (at 20 °C)	(Bayer, 1998)
		0.532 (at 25 °C)	(Monsanto, 1990)
		49.8 (at 150 °C)	(Hoechst, 1997)
5.5	Density, g/cm³	1.35 (at 40 °C)	(Bayer, 1998)
		1.368 (at 22 °C)	(Booth, 1991; Lide and Frederikse, 1996)
		1.49 (at 15 °C)	(Bayer, 1998)
5.6	Solubility in water	Insoluble	(Budavari et al., 1989; Booth, 1991; Lide and Frederikse, 1996)
		280 mg/l (at 20 °C)	(Monsanto, 1990)
		580 mg/l	(Bayer, 1998)
		590 mg/l (at 20 °C)	(BUA, 1985)
5.7	Solubility in organic solvents	Soluble in alcohol, benzene and ether	(Budavari et al., 1989; Booth, 1991)
		Soluble in ethanol, diethyl ether and acetone	(Lide and Frederikse, 1996)

5.8	Solubility in fat	No information available
5.9	pH value	Ca. 6 (at 400 mg/l water) (Bayer, 1998)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 6.54 mg/m³ 1 mg/m³ ≙ 0.153 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

Used in the chemical industry for large-scale production of intermediates in the manufacture of dyestuffs, plant protectants, rubber chemicals and pharmaceuticals (BUA, 1985).

7 Experimental results

7.1 Toxicokinetics and metabolism

In a study on absorption, distribution and metabolism of o-chloronitrobenzene, the chemical was administered to groups of 8 male rats (Fischer 344, 11 weeks old) in single doses of 2, 20 and 200 mg/kg body weight by oral gavage. The o-chloronitrobenzene used was labelled with ^{14}C (51.17 mCi/mmol). In each group, the urine and faeces of 4 animals was collected from 0 to 24 hours after o-chloronitrobenzene administration. The animals were then sacrificed, their livers, kidneys, hearts, lungs and brains were removed and samples of muscle, fat and blood were taken. All tissue, urine, blood and faeces samples were analysed for radioactivity. The same procedure was used for the other 4 animals of each group, except that the urine and faeces were collected from 0 to 72 hours prior to sacrifice. The radioactive urinary metabolites were quantitatively determined by HPLC analysis. In total, 23 metabolites were detected, but their structures were not elucidated. Only 3 of the metabolites, those referred to as XXI, XIX and XI by the investigators, were important in terms of quantity. The results of the study are shown in Table 1 and are discussed together with those of two other studies performed by the same laboratory, which are summarised in Table 2 below (Arthur D. Little, 1989 a).

Table 1. Absorption, distribution, excretion and metabolism of o-chloronitrobenzene as the percentage of radioactivity recovered after single oral administration of ^{14}C-labelled test substance to rats				
Parameter		Dose (mg/kg b. w.)		
		2	20	200
Excreted in urine	0 to 24 hours	56.4	53.0	39.2
	0 to 72 hours	59.6	57.7	73.5
Excreted in faeces	0 to 24 hours	21.9	19.8	0.0
	0 to 72 hours	28.2	26.3	6.9
Total in tissue	after 24 hours	5.42	6.08	20.85
	after 72 hours	2.64	2.75	3.90
Minimum extent of absorption in 72 hours		62	61	77
Most important urinary metabolites in terms of quantity	0 to 48 hours			
XI		2.9	3.2	21.1
XIX		8.2	8.4	5.9
XXI		27.3	26.4	23.2
All other metabolites, each representing ≤ 6%		20.1	21.5	23.6
All data represent mean values of 4, in some cases 3 treated animals.				

In one study, o-chloronitrobenzene was administered to 4 male rats (Fischer 344, 9 weeks old) by oral gavage at daily doses of 65 mg/kg body weight for 11 days. On days 1, 5 and 9, the administered o-chloronitrobenzene was labelled with ^{14}C (51.17 mCi/mmol). After every dose of labelled o-chloronitrobenzene, urine and faeces were collected as in the first study, commencing on days 1 and 5 and also for up to 96 hours. One day before treatment was commenced and on days 4, 8 and 12, blood samples were taken from the orbital sinus and assayed for methaemoglobin levels (see also Section 7.2). Subsequently, the animals were sacrificed on day 12, and blood and tissue samples were collected as described above. The urinary metabolites were determined as in the study discussed above. The results are shown in Table 2 together with those from the study to be discussed in the following (Arthur D. Little, 1989 b).

In a third study, which was carried out to investigate the kinetics and metabolism of the chemical in geriatric rats and was designed and conducted in the same way, 4 males (Fischer 344) aged approximately 19 months were treated with daily doses of 65 mg/kg body weight by oral gavage for 11 days. Administration of radioactively labelled chemical, collection and analysis of urine and faeces and collection and preparation of blood and tissue samp-

les followed the same procedures as described above (see Arthur D. Little, 1989 b). The results of this study, together with those from the study described above, are presented and discussed in Table 2 (Arthur D. Little, 1990).

Table 2. Absorption, distribution, excretion and metabolism of o-chloronitrobenzene as the percentage of radioactivity recovered after oral administration of ^{14}C-labelled test substance (65 mg/kg body weight) to rats

Parameter		Age	
		9 weeks	19 months
Urinary excretion following a single dose	0 to 24 hours	60.6	63.5
	0 to 96 hours	70.9	85.1
Urinary excretion following 5 doses	0 to 24 hours	70.8	61.3
	0 to 96 hours	74.4	75.1
Urinary excretion following 9 doses	0 to 24 hours	69.6	52.2
	0 to 72 hours	73.5	70.8
Faecal excretion following a single dose	0 to 24 hours	5.4	1.4
	0 to 96 hours	19.9	22.4
Faecal excretion following 5 doses	0 to 24 hours	18.1	7.0
	0 to 96 hours	23.0	21.7
Faecal excretion following 9 doses	0 to 24 hours	21.4	7.5
	0 to 72 hours	26.6	18.9
Overall recovery from tissues following 9 doses		4.67	8.21
Minimum extent of absorption in 72 hours following a single dose		70.9	85.1
Minimum extent of absorption in 72 hours following 5 doses		74.4	75.1
Minimum extent of absorption in 72 hours following 9 doses		78.2	79.0
Urinary metabolites following a single dose	0 to 72 hours		
XI		12.6	5.6
XIX		6.4	7.3
XXI		21.1	24.8
All other metabolites, each representing ≤ 6%		30.8	46.0
Urinary metabolites following 5 doses	0 to 72 hours		
XI		10.7	8.7
XIX		4.2	3.5
XXI		18.1	12.8
All other metabolites, each representing ≤ 6%		40.8	48.4
Urinary metabolites following 9 doses	0 to 72 hours		
XI		8.0	10.1
XIX		4.7	2.6
XXI		20.1	10.6
All other metabolites, each representing ≤ 6%		40.7	47.5
Methaemoglobin levels in blood (%)	one day prior to dosing	0.9	0.7
	on day 4	6.4	–*
	on day 8	5.8	6.1
	on day 12	4.6	6.2

* no data available
All data represent mean values of 4, in some cases 3 treated animals.

The data summarised in Tables 1 and 2 demonstrate that upon oral administration of o-chloronitrobenzene at least 60% to over 80% of the dose was absorbed by the body. However, absorption was in fact presumably higher, seeing that the calculation did not take into account the radioactivity recovered in the faeces, which can be assumed to have comprised not only unabsorbed o-chloronitrobenzene, but also its metabolites and unchanged parent compound which had been absorbed but then excreted back into the intestines without undergoing change.

The radioactivity administered as ^{14}C-labelled o-chloronitrobenzene was primarily excreted in urine in the form of metabolites of the test substance. At the low doses of up to 65 mg/kg body weight, excretion essentially took place within the first 24 hours. At a dose level of 200 mg/kg body weight, urinary excretion was delayed and was not complete until 72 hours after administration. In the young animals, the amount of metabolites excreted in urine rose dose-dependently from approx. 60% to over 70% of the administered radioactivity. The amount of radioactivity excreted in faeces and the rate of excretion were clearly dose-dependent. At the lower dose levels of 2 and 20 mg/kg body weight, excretion within the first 24 hours amounted to approx. 20% of the administered radioactivity, increasing by only a few percent during the next 48 hours. When the 65 mg/kg body weight dose was given, only a few percent of the administered radioactivity underwent faecal excretion in the first 24 hours. After another 48 hours, approx. 20% excretion was reached, which meant, however, that excretion was lower at this dose than it was at the lower doses. At 200 mg/kg body weight, no radioactivity was excreted in faeces in the first 24 hours, and only 7% of the administered radioactivity was excreted in the next 48 hours. This shift in urinary and faecal excretion seen at higher dose levels was considered by the investigators to indicate that the radioactive compounds derived from ^{14}C-labelled o-chloronitrobenzene entered the intestinal tract by biliary secretion (Arthur D. Little, 1989 a).

Following single administration of radioactively labelled o-chloronitrobenzene, all examined tissues from the young animals were found to contain only small amounts of radioactive material, which by 72 hours had clearly dropped below the 24-hour values. The highest dose group was the only one to show a high recovered fraction of 21% of the administered radioactivity after 24 hours, but that value also dropped to 3.9% after another 48 hours. Furthermore, the radioactivity was spread over the tissues, higher levels

being reached only in the liver and, at the highest dose level, the adipose tissue (in the first 24 hours; Arthur D. Little, 1989 a).

In the fraction of radioactivity excreted in urine over 72 hours after administration of ^{14}C-labelled o-chloronitrobenzene, 23 metabolites of the parent compound were identified by HPLC analysis. Twenty of the metabolites, each representing no more than 6% of the administered radioactivity, played a minor role. The major product of metabolism was metabolite XXI, which represented more than 20% of the radioactivity. Metabolites XIX and XI also played a certain role. For metabolite XI, in particular, excretion was more extensive with increasing doses of o-chloronitrobenzene in the young animals, an excreted fraction of up to 21% of the administered radioactivity being reached at 200 mg/kg body weight. This observation could, in the investigators' opinion, be indicative of saturation of the metabolic pathways preferred at lower doses, or be attributable to biliary secretion of o-chloronitrobenzene metabolites (Arthur D. Little, 1989 a).

When o-chloronitrobenzene was administered once daily to 9-week-old rats at 65 mg/kg body weight for 11 days and urine and faeces were collected from the time of administration of the ^{14}C-labelled chemical on day 9 until day 11 and tissues were examined on day 12, no appreciable differences in results were observed compared with the single-dose treatment. As regards dose-dependency of the excretion parameters, the 65 mg/kg body weight dose (see Table 2) fitted in well with the data shown in Table 1. Compared with the single dose of 65 mg/kg body weight, the rate of faecal excretion was found to be increased following repeated dosing and was already high after an observation period of 24 hours. In this study, there was also a slight shift in the metabolite pattern because more radioactivity was recovered in the form of various minor metabolites (I: 5.7%, XVI: 5.7%; Arthur D. Little, 1989 b).

When the same investigations were carried out as described above in male rats which were 19 months old instead of 9 to 11 weeks, no appreciable differences in study parameters were noted in comparison with the young rats treated with the same dose. This applies to both the single-dose treatment and treatments lasting for up to 11 days. In the geriatric rats, the latter treatment resulted in slightly lower urinary and faecal excretion, compared with single dosing. After 9 days' treatment, the young animals' metabolite pattern was altered, though not quite in the same way. Strikingly, excretion

of the major metabolite XXI was down to 10% (25% after single-dose treatment), an effect which did not occur in the young animals. Apart from this, recovery rates for quantitatively unimportant metabolites were elevated (I: 5.2%, VI: 5.1%, VII: 4.8%, XIII: 4.3%). The total radioactivity recovered in the tissues, measured at the end of the treatment period, rose to 8% in geriatric rats and, again, the highest percentage was found in liver (Arthur D. Little, 1990).

The percutaneous absorption of o-chloronitrobenzene was studied in 10 to 12-week-old male rats (Fischer 344, weighing 200 to 225 g). Groups of 3 animals had acetone solutions containing [ring-^{14}C]-labelled o-chloronitrobenzene applied to an area of 4 cm² of the shaved dorsal skin at dose levels of 0.65, 6.5 and 65 mg/kg body weight. In order to simulate actual conditions of exposure encountered in practice, no occlusive cover was used, the treated area of skin only being lightly covered with a nonocclusive protective device. Urine and faeces were collected at 4-hour intervals up to 72 hours after application, and the excreted radioactivity was determined. Irrespective of the dose applied, under the experimental conditions described above, at least 33 to 40% of the o-chloronitrobenzene was absorbed (as calculated from urinary and faecal excretion within 72 hours, not taking into account the amount of chemical remaining in the body, which was not determined). Excretion was measured as total radioactivity without identifying the chemical structure of the metabolites, and of the excreted radioactivity about ⅔ appeared in urine (21 to 28% of the nominal dose) and about ⅓ in faeces (11 to 15% of the nominal dose). Excretion largely took place during the initial 24 hours, but because application was maintained for the entire duration of the study, it was not possible to calculate kinetics (Nomeir et al., 1992).

When 6 male Wistar rats (weighing 150 to 220 g) were given o-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).

In a study on the metabolism of o-chloronitrobenzene, female rabbits (weighing 2 to 3 kg, number of animals not given) received a single oral do-

se of 100 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 o-chloronitrobenzene was recovered as urinary metabolites: glucuronide (42%), sulfate (24%), nitrophenylmercapturic acid (7%) and free o-chloroaniline (9%). Only 0.3% of the administered o-chloronitrobenzene underwent faecal excretion in the form of o-chloroaniline. Detailed analysis of the urinary metabolites revealed that o-chloronitrobenzene was, on the one hand, hydroxylated to phenolic compounds while on the other hand it was reduced to amino compounds. Appreciable amounts of o-chloroaniline, 3-amino-4-chlorophenol, 4-amino-3-chlorophenol and 3-chloro-4-nitrophenol were detected in urine. The phenols were predominantly found as conjugates of glucuronic acid or sulfuric acid. Nitrophenylmercapturic acid formation was considered by the investigators to be a minor metabolic pathway (Bray et al., 1956).

The in-vivo formation of o-chloroaniline from o-chloronitrobenzene was also demonstrated in a study conducted in rats. In a first, reductive step N-hydroxy-o-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 o-chloroaniline, which was oxidised to N-hydroxy-o-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 o-chloronitrobenzene/kg body weight (equivalent to 78.8 mg/kg body weight) or 0.5 mmol o-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 o-chloroaniline liberated from N-hydroxy-o-chloroaniline. The treatment of rats with o-chloronitrobenzene yielded about 4 mmol of haemoglobin adduct per mol haemoglobin, whereas with o-chloroaniline only about 1.6 mmol/mol were formed under the same conditions. The investigator concluded from this result that N-hydroxy-o-chloroaniline was available in the body and that more N-hydroxy-o-chloroaniline was formed from o-chloronitrobenzene than from o-chloroaniline. The author discussed the possi-

bility that the highly reactive N-hydroxy compound could be responsible for the cytotoxic, mutagenic and carcinogenic effects of o-chloronitrobenzene. However, the concentrations detected were very low in comparison with other compounds investigated in the study (p-chloronitrobenzene, nitrobenzene; Sabbioni, 1994).

The metabolism of o-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 o-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 o-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, 46.7% of the administered radioactivity was detectable in the form of three major metabolites: o-chloroaniline, the N-glucuronide of o-chloroaniline and S-(2-nitrophenyl)glutathione, representing 19.2%, 14.2% and 13.3% of the radioactivity, respectively. Incubation of radioactive o-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 o-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 o-chloroaniline formation. It can therefore be presumed that reduction of the nitro group to the amino group is P450-dependent. However, there was a measurable rate of conversion of the nitro compound to the amine (about 14% of normal conversion) via cytochrome b5 reductase, as demonstrated by the experiment which used NADH instead of NADPH. Incubation of radiolabelled o-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 resulted in the formation of S-(2-nitrophenyl)glutathione. The chlorine had undergone displacement by glutathione. The investigators suggested that this type of conversion also played a role in the metabolism of o-chloronitrobenzene (Rickert and Held, 1990).

The reduction of o-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 o-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. o-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 ortho isomer 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 oral and dermal toxicity data for o-chloronitrobenzene are summarised in Table 3.

Beginning of Table 3

Table 3. Acute oral and dermal toxicity data for o-chloronitrobenzene

Species, strain, sex*	Route	Dose (mg/kg b. w.)	Effects	Observation period	Reference
Rat	oral	350	LD_{50}, no further data	no information	Davydova, 1967
Rat	oral	268	LD_{50}, no further data	no information	Back et al., 1972
Rat, Sprague-Dawley, male and female	oral	560	LD_{50}; reduced appetite and activity, weakness, ocular discharge, collapse; necropsy of deceased animals: gastrointestinal inflammation, dark kidneys and spleen, icteric liver, pulmonary haemorrhages	7 days	Younger Laboratories, 1973
Rat, Wistar, male	oral	144	LD_{50}; disturbances of balance, slight tremor, diarrhoea	14 days	Hoechst, 1975 a
Rat, Wistar II, male	oral	219	LD_{50}; cyanosis, poor general condition	14 days	Bayer, 1976
Rat, Wistar II, female	oral	457	LD_{50}; cyanosis, poor general condition	14 days	Bayer, 1976
Rat, Sprague-Dawley, male	oral	270	LD_{50}, no further data	no information	Vernot et al., 1977

Table 3. Acute oral and dermal toxicity data for o-chloronitrobenzene					
Species, strain, sex*	Route	Dose (mg/kg b. w.)	Effects	Obser-vation period	Reference
Rat	oral	510	LD_{50}, no further data	no information	Vasilenko and Zvezdai, 1981
Rat	oral	339	LD_{50}, no further data	no information	Izmerov et al., 1982
Rat, Wistar TNO W74, female	oral	263	LD_{50}; narcosis, sedation, rough coat, cyanosis, poor general condition, hindlimb paralysis; no necropsy findings	14 days	Bayer, 1982 a
Rat, Wistar TNO W74, male	oral	251	LD_{50}; sedation, narcosis, poor general condition, cyanosis, rough coat; no necropsy findings	14 days	Bayer, 1982 b
Mouse	oral	135	LD_{50}, no further data	no information	Back et al., 1972
Mouse, CF-1, male	oral	140	LD_{50}, no further data	no information	Vernot et al., 1977
Mouse	oral	340	LD_{50}, no further data	no information	Vasilenko and Zvezdai, 1981
Mouse	oral	440	LD_{50}, no further data	no information	Izmerov et al., 1982
Rabbit	oral	280	LD_{50}, no further data	no information	Izmerov et al., 1982
Rat, Wistar, female	dermal (24 hours, occlusive)	1796	LD_{50}; no appreciable signs of toxicity	14 days	Hoechst, 1975 b
Rat, Wistar II, female	dermal (24 hours, occlusive)	1320	LD_{50}; dyspnoea, cyanosis, poor general condition	14 days	Bayer, 1976
Rat, Wistar II, male	dermal (24 hours, occlusive)	655	LD_{50}; dyspnoea, cyanosis, poor general condition	14 days	Bayer, 1976
Rabbit, New Zealand	dermal (24 hours, occlusive)	450	LD_{50}; lethargy, loss of motor co-ordination; methaemoglobinaemia	14 days	Harton and Rawl, 1976
Rabbit, New Zealand, female	dermal	355	LD_{50}; lethargy, increasing weakness; necropsy of deceased animals: haemorrhages in the lungs, discoloration of the liver, kidneys and spleen, gastrointestinal inflammation	14 days	Younger Laboratories, 1983
Rabbit, New Zealand, male	dermal	445	LD_{50}; lethargy, increasing weakness; necropsy of deceased animals: discoloration of the liver, kidneys and spleen, gastrointestinal inflammation	14 days	Younger Laboratories, 1983
* where indicated					

End of Table 3

The LD_{50} values show considerable variability, irrespective of the route of administration and the species studied. For the rat and the mouse, the oral LD_{50} ranges were 144 to 560 mg/kg body weight and 135 to 440 mg/kg body weight, respectively. The oral LD_{50} value found for the rabbit, 280 mg/kg body weight, was within this range. The dermal LD_{50} values found for the rabbit ranged between 355 and 458 mg/kg body weight, while the values found for the rat varied greatly between 655 and 1796 mg/kg body weight. A typical sign of acute intoxication with o-chloronitrobenzene was cyanosis. Therefore, o-chloronitrobenzene must be considered harmful to toxic both upon oral administration and upon dermal application.

In a study investigating methaemoglobin formation, 6 male Wistar rats were treated with a single o-chloronitrobenzene dose of 100 µmol/kg body weight (equivalent to 15.8 mg/kg body weight), given intraperitoneally as a solution in propylene glycol. The blood which was collected at sacrifice 5 hours after administration contained 20.6% methaemoglobin. When rat-blood haemolysate was mixed with o-chloronitrobenzene, which was dissolved in propylene glycol in combination with phosphate buffer (0.5 µmol o-chloronitrobenzene, 0.1 µmol haemoglobin in 5 ml), and incubated for 5 hours at 37 °C, there was no methaemoglobin formation. From this the investigators concluded that the agent responsible for methaemoglobin formation was not o-chloronitrobenzene itself but the o-chloroaniline which was formed in the body by reduction (Watanabe et al., 1976).

Following single oral administration of 10 mg o-chloronitrobenzene/kg body weight to 2 cats, methaemoglobin formation resulted within 7 hours. It was very slight in one cat and slight in the other and was no longer detectable after 48 hours. Blood counts were altered indicating neutrophilia, leukocytosis and lymphocytopenia. Up to 14.5% Heinz bodies were found. The effects were also reversible after 48 hours (Hoechst, 1975 c).

Results on the acute inhalation toxicity of o-chloronitrobenzene are available from one study in which rats were placed in restrainers which allowed head-only exposure to a stream of air containing the test substance. The air and the entire surroundings were kept at a temperature of at least 31.8 °C so that a mixture of vapour and liquid o-chloronitrobenzene aerosol formed in the stream of air. Groups of 10 male Cr1:CD rats (8 weeks old, weighing 230 to 260 g) underwent a single 4-hour exposure, followed by a 14-day observation period. An approximate LC_{50} of 3200 mg/m^3 was ascer-

tained, indicating that inhalation of o-chloronitrobenzene must also be considered harmful. The little specific clinical signs of toxicity were reported to include lethargy, prostration, corneal opacity, cyanosis and partial hind-leg paralysis (Haskell, 1982).

Subacute toxicity

In an older study, 20 albino rats received a daily oral o-chloronitrobenzene dose of 70 mg/kg body weight for 20 days. All animals survived the treatment, an outcome which led the author to conclude that the chemical lacked marked cumulative properties (no further details; Davydova, 1967).

In two studies investigating the toxicokinetics and metabolism of o-chloronitrobenzene (see also Section 7.1) by identical methods, daily doses of 65 mg/kg body weight were administered by oral gavage to groups of 4 9-week-old or 19-month-old rats (Fischer 344) for 11 days. One day prior to treatment initiation, on treatment days 4 and 8 and one day after completion of treatment, blood samples were taken from the orbital sinus of the rats and assayed for methaemoglobin levels. The data, which are also shown in Table 2, demonstrated that there was a marked increase in blood methaemoglobin levels from 0.7–0.9% (before treatment initiation) to 6.1–6.4% after only 4 days of treatment with o-chloronitrobenzene. The remaining treatment up to day 11 did not result in any further increase in methaemoglobin levels. The animals' age did not influence the results (Arthur D. Little, 1989 b, 1990).

In a subacute toxicity study conducted in B6C3F1 mice (5 weeks old, weighing 21 to 25 g) in accordance with OECD guideline No. 407, groups of 12 males and 12 females received dietary treatment with o-chloronitrobenzene (99.7% pure) for 4 to 5 weeks. In order to expand the scope of the study beyond the OECD guideline, comprehensive additional enzyme and histochemical investigations were carried out to detect potential preneoplastic changes in hepatocytes. Additional groups of 6 males and females were included in the study and sacrificed after one week's treatment. Dose levels of 0 (controls), 50, 500 and 5000 ppm in the feed were chosen. The actually ingested dose levels, as calculated from individual body weights and food consumption, were found to be 0, 16, 167 and 1120 mg/kg body weight for the males and 0, 24, 220 and 1310 mg/kg body weight for the fe-

males. At study termination, the sacrificed animals underwent very thorough examination, including histochemical and enzyme tests on liver samples. A complete analysis of blood and urine was performed, and gross and histopathological examination was carried out. The animals for the interim study were autopsied after one week and their livers were examined histochemically and studied for enzyme activity, but no histopathological examination was performed. The findings are summarised in Table 4.

Beginning of Table 4

Table 4. Findings following administration of o-chloronitrobenzene in the feed of mice for 1 week and for 4 to 5 weeks

	50 ppm	500 ppm	5000 ppm
Clinical signs	–	–	narrow palpebral fissures and ocular opacity in males
Mortality	–	–	–
Food consumption	–	11% lower in females	50% lower in females, 38% lower in males
Water consumption	–	–	–
Body weight development	–	–	4% (females) and 8% (males) lower after 4 weeks
Haematology and histopathology of the haematopoietic organs	–	–	reduced red blood cell count, haematocrit and haemoglobin levels, increased bilirubin and methaemoglobin levels; increased spleen weight, reddish-black discoloration of the spleen, haemosiderin deposits in the spleen, no bone-marrow findings
Liver changes after one week	–	increased liver weights	increased liver weights
Liver changes after 4 to 5 weeks	slightly increased liver weights (without histopathological correlates)	increased liver weights, moderate hypertrophy of centrilobular hepatocytes	increased liver weights, markedly enlarged livers; severe hypertrophy of centrilobular hepatocytes; elevated cholesterol levels, elevated ASAT and ALAT transaminase activity and elevated alkaline phosphatase activity in blood, decreased urea levels in plasma
Enzyme levels in liver samples (homogenates) after one week	no elevated enzyme activities except for a slight increase in GSH-T in females	EOD, ALD, EH, GSH-T and GLU-T were induced 1.5 to 3.8-fold, EOR was only slightly elevated	EOD, EH and GLU-T were increased 1.5 to 2.3-fold, ALD was increased 5-fold and GSH-T was increased 6-fold in females and 2.3-fold in males, EOR was only slightly elevated

Table 4. Findings following administration of o-chloronitrobenzene in the feed of mice for 1 week and for 4 to 5 weeks			
	50 ppm	500 ppm	5000 ppm
Enzyme levels in liver samples (homogenates) after 4 to 5 weeks	no elevated enzyme activities except for a slight increase in GSH-T in males and females	EOD, EOR, EH, GLU-T and ALD (only in males) were increased 1.5 to 3-fold, ALD was not elevated in females, GSH-T was increased 7-fold in females and 2.3-fold in males	EH and GLU-T were increased 1.9 to 2.4-fold, in males EOD was increased 3-fold, ALD 1.8-fold and GSH-T 2-fold, in females ALD was not elevated, EOD was only slightly elevated and GSH-T showed a great, 11-fold increase, EOR was only marginally elevated
Histochemical analysis of liver samples after one week	–	decrease in hepatocytic glycogen storage (only females) and decrease in gluconeogenesis in the “perivenular region”	decrease in glycogen storage and gluconeogenesis in the hepatocytes of the “perivenular region”, activation of the pentose phosphate pathway at that site and increase in “perivenular” glycolysis
Histochemical analysis of liver sections after 4 to 5 weeks	–	decrease in hepatocytic glycogen storage and decrease in gluconeogenesis in the “perivenular region”	decrease in glycogen storage and gluconeogenesis in hepatocytes, increase in glycolysis and activation of the pentose phosphate pathway in the “perivenular region”

– no changes compared with controls
ALAT alanine aminotransferase
ALD aldrine epoxidase
ASAT aspartate aminotransferase
EH epoxide hydrolase
EOD 7-ethoxycoumarin deethylase
EOR 7-ethoxyresorufin deethylase
GSH-T glutathione S-transferase
GLU-T uridine diphosphate glucuronyl transferase

End of Table 4

Apart from minor enzyme changes in the liver and a slight increase in liver weight without a histopathological correlate seen after 4 weeks’ treatment, a o-chloronitrobenzene level of 50 ppm was tolerated without findings. At the 500 ppm level, the only observation apart from liver changes (see below) was that food consumption in the females was lower than in the controls. However, 5000 ppm o-chloronitrobenzene, when administered in the feed for up to 5 weeks, resulted in clear findings which included lower food consumption, narrow palpebral fissures and ocular opacity in the males, delayed body weight development, marked blood count changes accompanied by methaemoglobin formation and discoloration of the spleen with haemosiderin deposits and increase in spleen weight. With the exception of the liver, no other organs exhibited substance-related dysfunctions or changes in respect of clinical chemistry parameters, pathological anatomy, organ weights and histopathology findings. Marked, dose-dependent liver

changes were observed in the 500 ppm and 5000 ppm dose groups and included increased liver weights and moderate hypertrophy of centrilobular hepatocytes. The liver weights exhibited a dose-dependent increase after only one week's treatment. In addition, the concentration levels of cholesterol and the activity levels of transaminases and alkaline phosphatase in the blood were increased. Enzyme studies on liver homogenates demonstrated that after only one week of treatment, both sexes had elevated activity levels of the cytochrome P450-dependent mono-oxygenases, 7-ethoxycoumarin deethylase and aldrine epoxidase, and the phase-II enzymes, epoxide hydrolase, glutathione S-transferase and uridine diphosphate glucuronyl transferase, at and above a dose level of 500 ppm in feed. The 6-fold increase in glutathione S-transferase seen in the females of the 5000 ppm group after one week was particularly striking. There were no other marked differences between the sexes. The picture clearly changed after 4 weeks' treatment. In the females, hardly any increase in 7-ethoxycoumarin deethylase activity and no increase in aldrine epoxidase activity was found, but instead there was an 11-fold rise in glutathione S-transferase activity. In the males, 1.8 to 3-fold increases were seen in all enzyme activities when they were given 5000 ppm o-chloronitrobenzene in their feed for 4 weeks. Histochemical staining of liver sections revealed that the animals treated with 500 ppm had reduced glycogen storage (females only) and decreased gluconeogenesis (measured as glucose 6-phosphatase activity) after one week. At the 5000 ppm level, activation of the pentose phosphate pathway (measured as glucose 6-phosphate dehydrogenase activity), enhancement of glycolysis (measured as glycerine 3-phosphate dehydrogenase activity) and a decrease in glycogen storage were additionally seen after one week in the males as well. The same effects were observed in treatment week 5. The findings were essentially restricted to the "perivenular" region. No focal lesions of the liver were observed. Because activation of the pentose phosphate pathway and enhancement of glycolysis in conjunction with a decrease in gluconeogenesis are considered to be potentially significant changes in the transformation process of mouse hepatocytes, the investigators suggested that o-chloronitrobenzene induces a metabolic situation in the parenchymal cells of the liver which may promote tumorigenesis (see also Section 7.7). This suggestion was supported by the elevated enzyme activities found in the liver homogenates (for an account of the results of the macroscopic and histopathological examination of the testes, see Section 7.8; Bayer, 1991, 1993).

In a dose-finding study for a reproductive toxicity study, groups of 8 male and 8 female mice (CD-1 Swiss) per dose were treated orally with o-chloronitrobenzene (> 99% pure, in corn oil) at daily dose levels of 0 (controls), 20, 40, 80, 160 and 320 mg/kg body weight, administered by gavage for 14 days. Endpoints of investigation were death, clinical signs of toxicity, body weight and water consumption. All animals in the group receiving 320 mg/kg body weight died within the first 2 days of treatment. Those receiving 160 mg/kg body weight were slightly weak and inactive in the first week and became slightly cyanotic in the second week, but remained active. After dosing with 80 mg/kg body weight, animals were inactive only on the first two days of treatment, but later appeared normal. All dose groups up to 160 mg/kg body weight showed no body weight changes compared with controls. Water consumption was significantly increased for females in the groups dosed with 20 and 160 mg/kg body weight during week 1 and for males and females receiving 40 mg/kg body weight in week 2 (NTP, 1992).

The subacute inhalation toxicity of o-chloronitrobenzene was investigated in a study conducted in male rats (Cr1:CD[SD]BR strain, 8 to 10 weeks old, weighing 218 to 235 g). Groups of 16 animals were exposed to an atmosphere containing o-chloronitrobenzene (99.8% pure) vapour 6 hours/day, 5 days/week for 2 weeks. The chemical was heated to 80 to 100 °C and nitrogen was passed over it. The resulting vapour was diluted with air in a chamber and administered to the animals by head-only exposure. The respirable portion of o-chloronitrobenzene contained in the exposure atmosphere was 74%, equivalent to a mean particle size of 6.8 µm. The concentration levels selected for inhalation exposure were 0 (controls), 30, 160 and 530 mg/m³. Following the 9th exposure, urinalyses (16-hour urine) were performed and after the 10th exposure, haematology and clinical chemistry parameters were determined for 10 animals per group. Subsequently, 5 of these animals were immediately sacrificed for gross and histopathological examination. The remaining 5 animals were left to recover without further exposures, urinalysis was performed on day 12 of the observation period and haematology and clinical chemistry parameters were determined prior to sacrifice for gross and histopathological examination on recovery day 13. From the other six animals in each group, blood samples were taken at regular intervals during the exposure and observation periods, on study days 1, 3, 5, 8, 10, 12, 17, 19, 23 and 25, and the samples were analysed for methaemoglobin and haemoglobin only. Marked clinical signs of

toxicity were only seen in isolated cases in the highest concentration group (cyanosis was observed in 6 rats, hyperaemia of the ears in one rat). In the mid and top concentration groups, one death each occurred during the 7th and 9th exposures, respectively, but the cause of death could not be determined. Ten exposures to the lowest concentration of o-chloronitrobenzene (30 mg/m³) produced no effects, compared with the controls. At the high concentration a slight decrease in mean body weight was observed, which was reversible during the recovery period. Urinalyses showed no substance-related effects. In the groups treated with 160 or 530 mg/m³ haemolytic anaemia, methaemoglobinaemia and a compensatory haematopoietic response were seen. Haemoglobin concentration was decreased after only one day of treatment. This finding persisted in a dose-dependent manner until the 10th exposure (day 12). Blood methaemoglobin levels showed a nonsignificant maximum increase of 3% at an o-chloronitrobenzene concentration of 160 mg/m³. At 530 mg/m³, they rose to 21% on the 3rd day of treatment and remained at that level until the end of the treatment period. Treatment with 530 mg o-chloronitrobenzene/m³ also caused a drop in red blood cell count. In the animals of the top concentration group which were sacrificed immediately after the 10th exposure, spleen weights were slightly increased, and macroscopically the spleens were enlarged and of a darker colour, while histopathologically splenic congestion and haemosiderin deposition were observed. With the exception of the splenic haemosiderin deposits and the decreases in red blood cell count, all effects were reversible by the end of the 13-day observation period. At the mid and top concentrations, slight degenerative and regenerative liver changes were noted. Liver weight was slightly increased, which was consistent with slight enlargement of the liver. Histopathologically, hepatocytes were observed to have cytoplasmic lipid vacuolation, pale centrilobular cytoplasm and an increased number of mitotic figures. The effects were reversible after the recovery phase. Equally, the elevated serum levels of alanine aminotransferase activity, urea, total protein and cholesterol seen in the top dose group were reversible. There were no further substance-related findings. The results of the study showed that the haematopoietic system and the liver were the targets for the toxic actions of o-chloronitrobenzene. The *no effect level* was 30 mg/m³ (Haskell, 1984).

In a comprehensive subacute inhalation toxicity study, Sprague-Dawley rats underwent whole-body exposure to air enriched with o-chloronitroben-

zene (99.7% pure) 6 hours/day, 5 days/week for 4 weeks. Dose groups consisted of 15 males (weighing 216 to 283 g) and 15 females (weighing 150 to 183 g). A control group of 15 males and 15 females was included, which were treated with air in the inhalation chamber. The o-chloronitrobenzene concentrations were 10, 30 and 60 mg/m³. In order to achieve these concentrations, nitrogen was passed through o-chloronitrobenzene which was preheated to 40 to 45 °C, and the vapour phase thus obtained was mixed with air appropriately. On completion of the exposure regimen, the animals were sacrificed after blood samples had been taken by venipuncture of the orbital sinus. A gross post-mortem was performed on all animals, followed by histopathological examination of a total of 35 organs and tissues from 10 animals/sex from the control and top concentration groups. The spleen was histopathologically examined in all concentration groups. Blood for methaemoglobin determination was taken from the orbital sinus after 2 treatment weeks as well. No deaths occurred during treatment with o-chloronitrobenzene. Test substance-related clinical signs of toxicity and damage to the eye were not observed. Body weight development was comparable to controls. A dose-dependent increase in blood methaemoglobin levels was observed after 2 weeks, and the increase in the mid and high concentration groups was statistically significant. At 4 weeks, methaemoglobin levels in the males and females of the high-concentration group were 5.7 and 4.8%, respectively. Haemoglobin levels and red blood cell count were decreased. Post-mortem of the exposed animals revealed no appreciable findings related to the test substance. Absolute and relative organ weights for liver and spleen were significantly increased in the mid and high concentration groups, whereas kidney weights showed a slight increase, which was none the less statistically significant. At the low concentration level, only the relative liver weights of the males showed a slight significant increase. Histopathological examination revealed changes in the spleen only. These included an increase in extramedullary haematopoiesis at the mid and high concentration levels and marked haemosiderosis even at the low exposure level. No other tissues had any remarkable findings. The investigators saw the most remarkable toxic effect of o-chloronitrobenzene in its action on the haematopoietic system, which was evident even at the low concentration of 10 mg/m³ (Nair et al., 1986).

In a preliminary study to a subchronic toxicity study, 6-week-old male and female rats (F344/N) underwent whole-body exposure to o-chloronitroben-

zene (> 99% pure). Groups of 5 animals/sex and concentration were exposed to o-chloronitrobenzene at levels of 0 (controls), 1.1, 2.3, 4.5, 9 and 18 ppm (equivalent to 0, 7, 14.7, 28.8, 57.6 and 115.2 mg/m³, respectively) for 6 hours/day on 5 days/week for 2 weeks. At the end of the treatment period, no effects on body weight development were noted. There were no deaths. Clinical signs of toxicity occurred at 18 ppm in the form of nasal discharge in the males and females, and the males additionally exhibited hypoactivity, ataxia and pallor. Gross pathology revealed no lesions. In the 18 ppm group, absolute and relative liver and spleen weights were increased, and there was a slight increase in relative kidney weight. Increases in absolute and relative liver weights were also seen at the lower exposure levels (no further details). Histopathological examination of the liver revealed minimal haemosiderin deposition in the portal areas and around central veins following 18 ppm treatment. The spleen also exhibited haemosiderin deposition of mild severity in this treatment group. There were no histopathological changes that could account for the observed increases in organ weights. No other investigations such as analyses of blood or urine were carried out (NTP, 1993). A *no effect level* could not be determined in this dose-finding study.

In parallel, 6-week-old male and female mice (B6C3F1) also underwent whole-body exposure to o-chloronitrobenzene (> 99% pure). Again, groups of 5 males and 5 females per concentration were exposed to o-chloronitrobenzene at levels of 0 (controls), 1.1, 2.3, 4.5, 9 and 18 ppm (equivalent to 0, 7, 14.7, 28.8, 57.6 and 115.2 mg/m³, respectively) for 6 hours/day on 5 days/week for 2 weeks. Treatment had no effect on body weight development. In the highest concentration group, there was one death on day 2 of treatment, examination revealing marked liver damage. Clinical signs of toxicity were only observed in the highest treatment group in the form of hypoactivity, abnormal posture and dyspnoea, especially in males. Marked concentration-dependent increases in absolute and relative liver weights were seen in all groups after o-chloronitrobenzene treatment. Absolute and relative kidney and spleen weights were increased only in the 18 ppm group. At necropsy, no gross lesions were noted except for discoloration and rough surface of the liver in some animals from the 18 ppm exposure group. There were histopathological changes of the liver and spleen. In the liver, centrilobular necrotic lesions occurred in a concentration-dependent manner and were accompanied by granulomatous inflammatory responses

in the animals of the 18 ppm group. The 9 and 18 ppm groups exhibited enlarged centrilobular hepatocytes. In the spleen, an increase in haemato-poietic activity was observed in the females of the 4.5, 9 and 18 ppm exposure groups, while in the males this effect was only seen in the 18 ppm group. In addition, 4 out of 5 males and all females of the 18 ppm group were found to have haemosiderin deposition of mild severity. No other investigations such as analyses of blood or urine were carried out (NTP, 1993). A *no effect level* could not be determined in this dose-finding study.

7.3 Skin and mucous membrane effects

The dermatological actions of o-chloronitrobenzene were studied in 6 New Zealand rabbits which received dermal applications of 0.5 ml slightly heated undiluted chemical for 24 hours. During the 168-hour observation period there were no signs of skin irritation, erythema or oedema (no further details; Younger Laboratories, 1973).

In a further study, 6 albino Himalayan rabbits (weighing 1.5 to 2.0 kg) had 0.5 ml of a 10-percent o-chloronitrobenzene solution in sesame oil applied to each of two 3 cm x 3 cm areas of skin, one intact and the other scarified, on the shorn flanks, upon which the sites were covered with gauze and an elastic bandage for 24 hours. Reactions were assessed after 24, 48 and 72 hours. Four of the rabbits showed mild signs of irritation (very mild, hardly perceptible erythema) of the intact and scarified flank skin immediately upon removal of the dressing, but the reactions were reversible after 48 hours. Under the experimental conditions of this study, o-chloronitrobenzene was not irritating to the skin (Hoechst, 1975 d).

Albino rabbits (weighing 3.5 to 4.0 kg) were used in order to assess the skin irritancy of o-chloronitrobenzene to the hairless inside of the pinna. Patches of tissue paper, sized 2 cm x 2 cm and pre-loaded with 500 mg o-chloronitrobenzene, were affixed to the skin of 2 rabbits with adhesive plaster for 24 hours. The treated skin areas showed no changes, either upon removal of the dressing or during the 7-day post-exposure observation period. Therefore, o-chloronitrobenzene had no irritating effect in this study, either (Bayer, 1976).

The effects of o-chloronitrobenzene on the eye were studied in 6 New Zealand rabbits which had 0.1 ml of slightly heated undiluted chemical instilled

into the conjunctival sac. Inspection at 24 hours revealed mild to moderate erythema of the conjunctivae, which cleared up after 24 hours and was reversible after 48 hours. The investigators thus evaluated o-chloronitrobenzene as not irritating to the eye (Younger Laboratories, 1973).

In another study on mucous membrane effects, groups of 6 albino rabbits had either 100 mg of undiluted o-chloronitrobenzene or 0.1 ml of a 10-percent solution of the chemical in sesame oil instilled into the conjunctival sac of the eye. With the undiluted sample, mild to moderate reddening of the conjunctivae was observed one hour after treatment, but the effect was completely reversible in 4 out of 6 animals after 7 hours, and in all animals after 24 hours. The 10-percent solution produced mild, reversible reddening of the conjunctivae in 3 of the 6 rabbits in the first hour. On the basis of these findings, the investigators evaluated o-chloronitrobenzene as hardly irritating to the mucous membranes (Hoechst, 1975 d).

The effects of o-chloronitrobenzene on the mucous membrane of the eye were studied in 2 rabbits (weighing 3.5 to 4.0 kg), which had 50 mg of the chemical instilled into the conjunctival sac of the right eye. The mucous membranes of the eye and the cornea showed no changes either on the day of treatment or during the 7-day observation period. o-Chloronitrobenzene thus did not exhibit any mucous membrane effects in this test (Bayer, 1976).

7.4 Sensitisation

In an insufficiently detailed study on the sensitising potential of o-chloronitrobenzene in long-term inhalation of small amounts, groups of 25 rats were exposed to a concentration level of 0.008 mg/m³ for 5 months (no further details), or left untreated (controls). Immediately subsequent to treatment, sensitisation of the rats was assessed by means of the microprecipitin test according to Vanier, by in-vitro determination of intracellular antibodies in erythrocytes, leukocytes and thrombocytes, by measuring passive sensitisation according to the Praustnitz-Küstner method and by injecting 0.2 ml of an o-chloronitrobenzene solution (105 mg/l) into the upper lip of the rats and evaluating the effects after 6 days (no further details). All test results were positive, thus indicating that o-chloronitrobenzene has a sensi-

tising potential. However, the reactions were weaker than with p-chloronitrobenzene, which was tested simultaneously (Rusakov et al., 1973).

The same authors also investigated the skin-sensitising potential of o-chloronitrobenzene in the guinea pig. Ten animals each had 3 drops of a 1-percent solution of the chemical in acetone applied to the shorn dorsal skin on 5 consecutive days. On the 7th day, the challenge was carried out by applying one drop of the same solution to a different area of shorn skin. None of the animals showed a reaction. The experiments were continued with the same animals and a 10-percent solution of o-chloronitrobenzene. On study day 22, the pads of the hind paws were injected with 0.2 ml Freund's adjuvant in combination with 0.5 mg o-chloronitrobenzene/kg body weight. On the 6th day after the injection, the challenge was carried out by applying one drop of a 10-percent solution of the chemical onto a shorn but otherwise untreated skin area. Fifty percent of the treated animals were described by the authors as showing a positive allergic reaction. In the case of p-chloronitrobenzene, which was tested simultaneously, response was 100% (Rusakov et al., 1973).

Final evaluation of the available studies on the sensitising potential of o-chloronitrobenzene is not possible due to incomplete documentation and methodology that is no longer in use today. None the less, there are indications that o-chloronitrobenzene may have sensitising properties both upon dermal application and longer-term inhalation.

7.5 Subchronic and chronic toxicity

In a comprehensive study on the subchronic toxicity of o-chloronitrobenzene, which was carried out as part of the US National Toxicology Program (NTP), groups of 10 male and 10 female rats (F344/N), aged 5 to 6 weeks, underwent whole-body inhalation treatment for 13 weeks. The inhalation concentration levels chosen were 0 (controls), 1.1, 2.3, 4.5, 9 and 18 ppm (equivalent to 0, 7, 14.7, 28.8, 57.6 and 115.2 mg/m³, respectively). Treatment was administered 6 hours/day on 5 days/week. The test substance (> 99% pure) was heated to well above melting point, and preheated nitrogen was passed through it. The resulting vapour was delivered into the closed, preheated exposure chamber and mixed with air that was preheated in order to avoid aerosol formation. Blood samples were taken by puncture

of the orbital sinus on days 1 (for determination of methaemoglobin levels only), 4 and 23 and at study termination. For the purpose of obtaining intermediate blood samples for haematology and clinical chemistry evaluations, an additional 10 males and 10 females were included in each group. At study termination, the animals were sacrificed, necropsies performed, and all relevant organs and tissues from the control group and the highest concentration group histopathologically examined. In addition, all organs and tissues that showed macroscopic changes were also examined by light microscopy in the other exposure groups. Organs and tissues that showed histopathological changes were also examined in the lower exposure groups. The liver, kidney, spleen and nasal cavity as the target organs were histopathologically examined at all levels of exposure. In addition, a comprehensive investigation of the reproductive organs was carried out (for a detailed account, see Section 7.8). None of the animals undergoing 13-week treatment died during this period. No differences in body weight development were observed. None of the animals showed clinical signs of intoxication while under treatment. Haematology evaluations showed that there was marked methaemoglobinaemia, which was identifiable at all exposure levels after 13 weeks' treatment and increased in a concentration-dependent manner. Methaemoglobin levels were elevated in most exposure groups after the first day of treatment, and after 23 days increased levels were seen in all males and the females exposed to 2.3 ppm and higher doses. At the end of treatment, the males and females of the 18 ppm group had 6-fold and 4-fold increases over control levels, respectively. Concentration-dependent moderate decreases in haematocrit, haemoglobin concentration and red blood cell count occurred in both sexes at and above 4.5 ppm. In the highest concentration range from 9 to 18 ppm, there were changes in all haematological parameters determined, with the exception of the females' leukocyte and lymphocyte counts. The animals were thus found to have concentration-dependent methaemoglobinaemia and normocytic, normochromic anaemia. The clinical chemistry parameters exhibited only minor deviations from the control values which, although more frequent at the higher exposure levels and, in part, statistically significant, were found to be independent of the duration of exposure and to exhibit variability similar to that seen between consecutive measurements in the controls. At terminal necropsy, no pathological alterations were observed other than dark discoloration of the spleen in 2 males and one female in the 18 ppm group. There was a significant, concentration-dependent increase in absolute and

relative liver weights in males and females at and above 2.3 ppm and at 4.5 ppm, respectively. A significant, concentration-dependent increase in absolute and relative spleen weights was seen in the females at and above the 4.5 ppm exposure level, while in the males it occurred only in the 18 ppm group. A slight increase in kidney weights was observed in both sexes in the 18 ppm group. Histopathologically, the liver showed minor cytoplasmic basophilia of the centrilobular hepatocytes in all male rats and the majority of the female rats of the 9 and 18 ppm exposure groups. Minimal to mild pigmentation of the proximal renal tubules, presumably a lipofuscin pigment, was occasionally found in males exposed to levels of 4.5 ppm and above and in all males and females exposed to 18 ppm. All females exposed to 9 ppm were also affected. More severe congestion of the spleen was observed in both sexes in the two highest exposure groups only. No increase in spleen haemosiderin deposition was apparent in exposed rats. All groups treated with o-chloronitrobenzene were observed to have hyperplasia/hypertrophy of the epithelium in the nasal cavity, with nearly all animals being affected. When the reproductive organs were investigated (for a detailed account, see Section 7.8), no substance-related alterations were apparent in the females. In the males of the highest concentration group, the cauda epididymal weights, spermatid heads per testis and spermatid count were decreased, indicating reduced spermatogenesis. As even the lowest, 1.1 ppm concentration level resulted in substance-related changes, a *no observed adverse effect level* (NOAEL) could not be determined (NTP, 1993; Travlos et al., 1996).

The same study design and dose regimen as in the rat study was employed in a study on the subchronic inhalation toxicity of o-chloronitrobenzene which the same investigators conducted in parallel in male and female mice (B6C3F1), aged 5 to 6 weeks. No blood samples were taken, however, and there were no haematology or clinical chemistry evaluations. The results were largely in agreement with those from the rat study, but there were differences in some respects. In this study, 2 males from the 18 ppm group died during treatment week 12. Differences in body weight development and clinical signs of toxicity did not occur during the treatment. Pale discoloration of the liver was seen in 6 male mice and one female in the 18 ppm group, while 3 females in the 9 ppm group and 4 females in the 18 ppm group had enlarged spleens. Absolute and relative kidney weights were increased in males and females exposed to concentration levels of and

above 2.3 ppm, but the increases were slight and not concentration-dependent. Liver weights were increased in female mice in all concentration groups and in males exposed to levels of and above 9 ppm. No increase in spleen weights was observed in mice. Histopathological examination of the liver revealed hepatocellular necrosis, mineralisation, chronic inflammation and hepatocytomegaly, primarily in the 18 ppm group. In the spleen, only a minimal increase in haematopoietic activity was observed in female mice in the 9 and 18 ppm groups, and there was no haemosiderosis. When the reproductive organs were investigated (for a detailed account, see Section 7.8), there were no findings in the females, while in the males reduced sperm motility was noted at the high exposure levels. Thus, the NOAEL for histopathological injury was 4.5 ppm (NTP, 1993; Travlos et al., 1996).

In an inadequately documented study, a total of 42 rats received oral treatment with o-chloronitrobenzene at dose levels of 0.0025, 0.005, 0.025 or 5.0 mg/kg body weight for 7 months. Treatment-related effects were only seen at 5.0 mg/kg body weight, the highest dose level. The most marked changes were those found in the blood picture. Methaemoglobin levels rose during the last months of treatment, while haemoglobin levels dropped. The reticulocyte count rose to 78% (controls 35%) and the percentage of Heinz bodies increased from 0 to 47%. Liver function tests showed elevated blood alkaline phosphatase activity, and there was a rise in the level of bilirubin in the urine. Very slight effects on the central nervous system (slowing down of the conditioned reflex reaction) were observed (no further details; Davydova, 1967).

7.6 Genotoxicity

7.6.1 In vitro

The available data on the in-vitro genotoxicity of o-chloronitrobenzene are summarised in Table 5.

Beginning of Table 5

Table 5. In-vitro genotoxicity tests with o-chloronitrobenzene					
Test system	Concentration range tested* (µg/plate)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
1. Tests in *Salmonella typhimurium* and *Escherichia coli*					
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1530, TA 1535, TA 1537, TA 1538, TA 1532, TA 1950, TA 1975, TA 1978, G46, standard-plate incorporation test	1–2000	S9 mix from Aroclor-induced rat liver	negative under aerobic and anaerobic conditions	negative under aerobic and anaerobic conditions	Gilbert et al., 1980
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1530, TA 1535, TA 1537, TA 1538, TA 1532, TA 1950, TA 1975, TA 1978, G46, fluctuation test	1–2000	S9 mix from Aroclor-induced rat liver	negative	negative	Gilbert et al., 1980
Salmonella/microsome test according to Ames (no further details)	no information	no information	positive in TA 100	no information	D'Addario and Jagannath, 1981
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, preincubation test	100–1000, bacteriotoxic at 1000 (99% pure)	S9 mix from Aroclor-induced rat and hamster liver	positive in TA 100	negative	Haworth et al., 1983; NTP, 1993
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1538, standard-plate incorporation test	no information (99.7% pure)	S9 mix from rat liver (no further details)	negative	negative	Graham et al., 1983
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538, preincubation test	25.6–3276.8, bacteriotoxic at the highest concentration (99% pure)	–	not investigated	positive in TA 98 and TA 1538	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	positive in TA 98 only on addition of norharman	negative	Suzuki et al., 1983
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, standard-plate incorporation test	833.3–2073.6, bacteriotoxic from 1000 (99.8% pure)	S9 mix from Aroclor-induced rat liver	positive in TA 100	negative	Bayer, 1984
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538, standard-plate incorporation test	4–2500, bacteriotoxic at 2500 (99% pure)	S9 mix from Aroclor-induced rat liver	positive in TA 98 and TA 100	positive in TA 1538	Hoechst, 1984
Standard-plate incorporation test, *Escherichia coli* WP2 uvrA	4–2500, bacteriotoxic at 2500 (99% pure)	S9 mix from Aroclor-induced rat liver	negative	negative	Hoechst, 1984

Table 5. In-vitro genotoxicity tests with o-chloronitrobenzene					
Test system	Concentration range tested* (µg/plate)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
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)	positive, no indication whether performed with or without metabolic activation or of the strains studied		Koch et al., 1985
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 98 NR, TA 98/1,8-DNP$_6$, preincubation test	5–20	S9 mix from PCB-induced rat liver	positive in all three strains	–	Suzuki et al., 1987
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, preincubation test	100–5000	S9 mix from Kanechlor-induced rat liver	negative	negative	Kawai et al., 1987
SOS chromotest, *Escherichia coli* PQ37	up to 1576	S9 mix from Aroclor-induced rat liver	negative	negative	von der Hude et al., 1988
umu Test, *Salmonella typhimurium* TA 1535/pSK 1002	100 µg/ml	S9 mix from phenobarbital- and 5,6-benzoflavone-induced rat liver	negative	negative	Ono et al., 1992
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, standard-plate incorporation test	10–1000, bacteriotoxic at 1000	S9 mix from Aroclor-induced rat and hamster liver	positive in TA 100 and TA 98 (TA 98 only with 30% S9 mix from hamster liver)	negative	NTP, 1993
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1535, preincubation test	10–1000, bacteriotoxic at 1000	S9 mix from phenobarbital- and 5,6-benzoflavone-induced rat liver	negative	negative	JETOC, 1996
Preincubation test, *Escherichia coli* WP2 uvrA	10–1000, bacteriotoxic at 1000	S9 mix from phenobarbital- and 5,6-benzoflavone-induced rat liver	negative	negative	JETOC, 1996
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 102, TA 104, TA 1535, TA 1537, preincubation test	39.1–625, bacteriotoxic from 500	S9 mix from phenobarbital- and 5,6-benzoflavone-induced rat and hamster liver	positive in TA 100 and TA 98	negative	JETOC, 1997
Preincubation test, *Escherichia coli* WP2 uvrA	39.1–625, bacteriotoxic from 500	S9 mix from phenobarbital- and 5,6-benzoflavone-induced rat and hamster liver	positive only in one test and with S9 mix from hamster liver	negative	JETOC, 1997

Table 5. In-vitro genotoxicity tests with o-chloronitrobenzene					
Test system	Concentration range tested* (µg/plate)	Metabolic activation system	Results		Reference
			with metabolic activation	without metabolic activation	
2. Tests in mammalian cells					
Chromosome aberration, Chinese hamster ovary cells (CHO), 100 metaphases examined per concentration	10–100 µg/ml (–S9; 99.8% pure), 25–250 µg/ml (+S9; 99.8% pure)	S9 mix from Aroclor-induced rat liver	negative	negative	Huntingdon, 1988
Gene mutation test, Chinese hamster lung cells (V79), HPRT test	100–900 µg/ml (–S9), 100–1200 µg/ml (+S9), cytotoxic at the respective highest concentration	S9 mix from Aroclor-induced rat liver	negative	negative	TNO, 1989
Chromosome aberration, Chinese hamster ovary cells (CHO), 200 metaphases examined per concentration	16–216 µg/ml (–S9), 50–500 µg/ml (+S9)	S9 mix from Aroclor-induced rat liver	weakly positive in two studies, negative in one	negative in one study, questionable in another	NTP, 1993
Sister-chromatid exchange test (SCE test), Chinese hamster ovary cells (CHO), 50 metaphases examined per concentration	5–75 µg/ml (–S9), 50–500 µg/ml (+S9)	S9 mix from Aroclor-induced rat liver	weakly positive in two studies, negative in one	weakly positive in one study, positive in another, negative in one study	NTP, 1993
UDS test, primary rat hepatocytes	up to 100 µg/ml, cytotoxic at 500 µg/ml	rat hepatocytes	negative	–	Monsanto, year not given
* unless stated otherwise, publications give no details of cytotoxic effects or of the purity of the o-chloronitrobenzene used and/or any impurities it may have contained					

End of Table 5

In numerous studies conducted in *Salmonella typhimurium* as standard-plate incorporation tests, preincubation tests or fluctuation tests, o-chloronitrobenzene showed no genotoxicity in strains TA 102, TA 104, TA 1530, TA 1532, TA 1535, TA 1537, TA 1950, TA 1975, TA 1978 and G46 either with or without metabolic activation with S9 mix. No gene mutations were induced in any of these strains. In the absence of metabolic activation, the additionally tested strains TA 98, TA 100 and TA 1538 gave only negative findings, with the exception of two positive results in strain TA 1538 and one positive result in strain TA 98 (Shimizu et al., 1983; Hoechst, 1984). The positive results are counterbalanced by two negative results in strain TA 1538 and by numerous negative results in strain TA 98 which were obtained under comparable experimental conditions (Gilbert et al., 1980;

Graham et al., 1983; and other authors, see Table 5). In the presence of metabolic activation, all studies conducted in strain TA 1538 were negative. Out of a total of 13 studies in strain TA 100 in which metabolic activation was achieved by adding S9 mix, the outcome was negative in 6 studies but positive in the other 7. The picture was very similar in the studies conducted with strain TA 98. Out of 12 tests carried out in the presence of metabolic activation, results were positive in 5 and negative in 7 studies. In this context it is interesting that in a preincubation test on strain TA 98, no genotoxic effects were seen up to the highest concentration of 100 µg o-chloronitrobenzene/plate under the given conditions in the presence of S9 mix, whereas when norharman (200 µg/plate) was added as well, this produced 9600 revertants (control 39; Suzuki et al., 1983). Another study, which was conducted in the nitroreductase-deficient TA 98 NR strain of *Salmonella typhimurium*, demonstrated that o-chloronitrobenzene requires reduction to o-chlorophenylhydroxylamine in order to develop its mutagenic activity and that in the normal TA 98 strain this reduction is catalysed by the bacteria's own nitroreductase. In strain TA 98 NR, o-chloronitrobenzene was clearly less mutagenic than in TA 98. Another strain of *Salmonella typhimurium*, TA 98/1,8-DNP_6 with a deficiency in an esterifying enzyme, exhibited markedly higher sensitivity towards the mutagenic effects of o-chloronitrobenzene than did the normal TA 98 strain, a finding which was interpreted to mean that esterification which takes place in the normal strain has an inhibitory effect on the mutagenic activity of o-chloronitrobenzene. The investigators' conclusion was that metabolic activation with S9 mix and additional norharman had no direct effect on the reduction of o-chloronitrobenzene or esterification which was of any significance with respect to the chemical's mutagenic activity. They in fact suggested that S9 mix and norharman stimulate ring-oxidation of o-chloronitrobenzene, which is also necessary in order to produce marked mutagenicity at low concentrations of o-chloronitrobenzene (Suzuki et al., 1987).

A further study was able to demonstrate that norharman forms adducts with aromatic amines in the presence of S9 mix and that the adducts were responsible for the mutagenicity of the respective amines. Aniline and norharman, in the presence of S9 mix, yielded aminophenylnorharman, which in turn was oxidised to hydroxyaminophenylnorharman and activated to form esters with, for instance, acetic acid. In this form, it reacted with the DNA bases to induce mutations in *Salmonella typhimurium* strains TA 98

and TA 100 (Totsuka et al., 1998). In the light of the data available, the positive results obtained with norharman in the above-mentioned bacterial strains must be considered doubtful as proof that o-chloronitrobenzene is mutagenic, since the chemical is metabolised to the amino and hydroxy-amino compounds which presumably react in the same way as the compound formed from aniline.

The overall conclusion from the results obtained with o-chloronitrobenzene in the Salmonella/microsome test is that the chemical causes no gene mutations in this test system in the absence of metabolic activation. With metabolic activation, only strains TA 98 and TA 100 gave both positive and negative results. The tests were negative for all other strains.

For *Escherichia coli* WP2 uvrA, two preincubation tests are reported to have given negative results (JETOC, 1996, 1997). Negative results were also obtained in the SOS chromotest with *Escherichia coli* PQ37 (von der Hude et al., 1988) and a umu test on *Salmonella typhimurium* strain TA 1535/pSK 1002 for identifying DNA-damaging activity (Ono et al., 1992).

Mammalian cells have also given contradictory results. A gene mutation test carried out in V79 lung cells of the Chinese hamster gave clearly negative results both with and without metabolic activation (TNO, 1989). When parallel SCE tests were performed in Chinese hamster ovary cells by two laboratories in order to assess o-chloronitrobenzene-induced DNA damage, the result from one laboratory was positive in the absence of metabolic activation and was reproducible in a second experiment. With metabolic activation, the result was negative. The other laboratory found no increase in SCEs (sister chromatid exchange rates) in the absence of metabolic activation, whilst in the presence of metabolic activation there was a weakly positive result which was reproducible in a second experiment (NTP, 1993). In a chromosome aberration test in Chinese hamster ovary cells, negative results were obtained with and without metabolic activation (Huntingdon, 1988). With a similarly conducted test, which was again carried out in parallel by two laboratories, one laboratory found a negative result in the presence of metabolic activation and a questionable result in the absence of metabolic activation. The other laboratory found a weakly positive result in the presence of metabolic activation, a finding which was reproducible in a second test. Without metabolic activation, the test result was negative (NTP, 1993). In a UDS test on primary rat hepatocytes,

o-chloronitrobenzene tested negative up to the highest noncytotoxic concentration.

7.6.2 In vivo

In an alkaline elution test, 8 male mice (Swiss CD1) were injected intraperitoneally with o-chloronitrobenzene at a dose level of 60 mg/kg body weight. The controls were given the same volume of solvent. After 4 hours, the animals were sacrificed, livers and kidneys removed and the cell nuclei isolated. The DNA obtained from them was fractionated, eluted with an alkaline buffer and analysed by a fluorometric method. DNA fragments of low molecular weight elute more rapidly than do the high-molecular weight DNA strands so that the method permits detection of single-strand DNA breaks. In this study, o-chloronitrobenzene caused a marked increase in elution rate of the rapid-elution DNA fraction both in kidney and in liver (no further details; Cesarone et al., 1982).

The mutagenic potential of o-chloronitrobenzene was also investigated in *Drosophila melanogaster* in a sex-linked recessive lethal test. Adult male flies (Canton-S wild type, no older than 24 hours) were kept in feeding vials and allowed to feed on a 5-percent sucrose solution containing 125 ppm o-chloronitrobenzene (99% pure), dissolved in 10-percent ethanol, for 72 hours. Subsequently, the males were mated with untreated females. No increase in mortality was detectable in the progeny. Similarly, when o-chloronitrobenzene was injected (no details of the amounts injected) at 10000 ppm into the abdomen of male flies fed on normal food for 1 to 3 days prior to mating with untreated females after another 24 hours, this did not result in increased mortality in the progeny. o-Chloronitrobenzene thus showed no mutagenic activity in this test. The two o-chloronitrobenzene concentrations used in this study were set at such levels that they would produce 30% mortality during treatment (Zimmering et al., 1985).

In a further sex-linked recessive lethal test in *Drosophila melanogaster*, the flies were treated with o-chloronitrobenzene at the larval stage. During their development from egg to adult, the flies were fed standard food containing 60 ppm o-chloronitrobenzene (99% pure, dissolved in 4-percent ethanol). The emergent males, about one day old, were mated with untreated females, and the mortality rate was studied in the progeny. The o-chloronitro-

benzene concentration was set at such a level that the treatment would cause about 30% mortality among the larvae. No increased mortality was observed among the progeny of the surviving males and untreated females, and thus o-chloronitrobenzene showed no mutagenic activity in this study, either (Zimmering et al., 1989).

7.7 Carcinogenicity

A long-term carcinogenicity study was conducted in male Sprague-Dawley rats (Charles River CD) and male and female HaM/ICR mice (Charles River CD-1), which were treated with o-chloronitrobenzene (97 to 99% pure) by daily dietary administration for 18 months. Dose groups each consisted of 25 animals aged 4 to 6 weeks. The controls were given ordinary feed. Two dose levels were tested, the high dose representing the maximally tolerated dose as determined in a preliminary 30-day study, and the low dose being half of the high dose (no further details). Because the high dose levels produced a decrease in body weight gain by ≥ 10% and increased mortality in the course of the study, the doses were lowered in rats and mice after 6 and 8 months, respectively. The rats were thus treated with 1000 or 2000 mg o-chloronitrobenzene/kg feed during the first 6 months, whereas for the following 12 months they received 500 or 1000 mg o-chloronitrobenzene/kg feed. The mice were given 3000 or 6000 mg o-chloronitrobenzene/kg feed during the first 8 months and 1500 or 3000 mg o-chloronitrobenzene/kg feed for the following 10 months. The respective doses were equivalent to 100 or 200 and 50 or 100 mg/kg body weight in the rats and to 300 or 600 and 150 or 300 mg/kg body weight in the mice (assuming that food consumption was $\frac{1}{10}$ of body weight). After 18 months' treatment, the rats and mice were fed a normal diet for another 6 and 3 months, respectively, before being sacrificed. All animals which died after 6 months of treatment and all animals killed at the end of the experimental period underwent gross necropsy and comprehensive examination. Data on clinical signs of toxicity, mortality and body weight development in the individual study groups are lacking. The data reported on the incidences and types of tumour observed in the study are summarised in Table 6.

Table 6. Carcinogenicity study of o-chloronitrobenzene in rats and mice, number of tumour-bearing animals/total number of survivors (Weisburger et al., 1978)					
Species and sex	Type of tumour found	Number of tumours found			
		Low dose	High dose	Simultaneous controls	Pooled controls*
Rat, male	various tumours**	7/22	1/19	1/22	14/111
Mouse, male	hepatomas	7/17	3/16	3/18	7/99
Mouse, female	hepatomas	5/22	5/19	0/20	1/102

* controls from parallel experiments

** thyroid adenocarcinoma, lymphosarcoma and cholangiosarcoma of the liver, stomach papilloma, pituitary adenoma and subcutaneous fibroma (no further details)

Table 6 shows that under the given experimental conditions, general tumour incidence in male rats was markedly increased relative to controls at the low dose level (initially 100 mg/kg body weight, later 50 mg/kg body weight), while the high dose level produced no such effect. In mice, only hepatomas were found, the males again showing an increased incidence only at the low dose level (initially 300 mg/kg body weight, later 150 mg/kg body weight). The high dose was not effective. In the female mice, the increase in hepatoma incidence was seen at both the low and the high dose level, but again it was not dose-dependent. No data on the time of tumour occurrence were given. The investigators arrived at the conclusion that o-chloronitrobenzene could not be considered a very active carcinogen due to the high dose levels and long latent periods required for tumour development (Weisburger et al., 1978). The study under discussion does not meet current requirements for such studies, because the numbers of animals used were too small and documentation of the results was inadequate. Insufficiently substantiated due to the small numbers of animals used, the increase in tumour incidence following administration of very high doses of o-chloronitrobenzene, which had to be reduced in the course of the study, can only be considered an indication that the chemical may have carcinogenic potential.

In order to investigate the potential hepatocarcinogenicity of o-chloronitrobenzene, a 4-week mouse study was conducted in which histochemical analysis was employed to identify preneoplastic foci in liver sections (see also Section 7.2). Groups of 12 male and 12 female mice (strain not specified) were treated by daily dietary administration of o-chloronitrobenzene at concentration levels of 0 (controls), 50, 500 and 5000 ppm in feed (equiva-

lent to 5, 50 and 500 mg/kg body weight, respectively, assuming that food consumption was 1⁄10 of body weight) for one week. Six animals from each group were then sacrificed and the remaining 6 were treated with the same concentrations for another 3 weeks prior to terminal sacrifice. The left hepatic lobe was removed from each animal, cut into 0.5 cm-thick tissue slices and shock-frozen at -140 °C. Upon thawing of the material, the following histochemical parameters were determined: stored glycogen, glucose 6-phosphatase as a measure of gluconeogenesis, glyceraldehyde 3-phosphate dehydrogenase as a measure of glycolysis and glucose 3-phosphate dehydrogenase as a measure of the pentose phosphate pathway activity. Enhancement of glycolysis and pentose phosphate pathway activity in conjunction with a decrease in gluconeogenesis and in the glycogen stored in liver tissue constitutes an integral part of the transformation of hepatocytes and, therefore, is an indication that the test substance has a hepatotoxic effect. Under the experimental conditions of this study, o-chloronitrobenzene did not induce the otherwise typical foci (clusters of cells exhibiting the metabolic changes mentioned above) in the liver tissue analysed. However, hepatocytes showed clearly dose-dependent alterations of carbohydrate metabolism, for which the investigators could not exclude the possibility that they covered up the foci induced by o-chloronitrobenzene. The findings are summarised in Table 7.

Table 7. Enzyme histochemically demonstrated alterations of carbohydrate metabolism in the liver of mice treated with o-chloronitrobenzene

Dose, sex	Glycolysis*	Pentose phosphate pathway*	Gluconeogenesis*	Glycogen level*
0, male	–	–	–	1 (+)
0, female	1 (+)	–	1 –, 2 (–)	1 –, 1 (–)
50 ppm, male	–	–	–	–
50 ppm, female	–	–	2 (–)	1 –, 3 (–)
500 ppm, male	1 (+)	1 +	1 – –, 6 –, 2 (–)	3 –, 2 (–)
500 ppm, female	2 (+)	–	7 – –, 4 –, 1 (–)	5 – –, 3 –, 4 (–)
5000 ppm, male	5 ++, 7 +	2 ++, 9 +, 1 (+)	12 – –	9 – –, 2 –, 1 (+)
5000 ppm, female	2 ++, 8 +	3 ++, 4 +, 3 (+)	11 – –	8 – –, 3 –

* number of animals with

marked increase	++	marked decrease	– –
increase	+	decrease	–
borderline increase	(+)	borderline decrease	(–)

The effects were generally observed after only one week of treatment and showed no further increase after 4 weeks, and so Table 7 summarises the data at the time points of sacrifice. Only the reduction in glycogen levels was slightly more pronounced after longer treatment. The investigators interpreted their results to mean that o-chloronitrobenzene induced a metabolic situation in the parenchymal cells of the liver which may possibly promote tumorigenesis (Bayer, 1991, 1993).

7.8 Reproductive toxicity

In order to assess the reproductive toxicity of o-chloronitrobenzene, a teratogenicity/embryotoxicity study was conducted in female rats (Sprague-Dawley Cr1:CD). Groups of 25 pregnant females (12 weeks old, weighing 198 to 298 g) were treated with o-chloronitrobenzene (commercial product) by oral gavage at daily doses of 0 (controls), 25, 75 and 150 mg/kg body weight in corn oil from days 6 to 15 of gestation. The control treatment consisted of pure corn oil. At the highest dose level, all animals died within a few days, and hence evaluation was not possible. A second study was therefore carried out with 100 mg/kg body weight, administered in the same manner as described above, and another concurrent control group was included. The results obtained with dose groups receiving 25, 75 and 100 mg/kg body weight and the control group were presented together. Body weight and food consumption was monitored in all dams during treatment and up to day 21 of pregnancy. On that day, all dams were sacrificed and given a post-mortem, the number of implantations was determined, and the foetuses were removed from the uterus. Body weight and sex distribution of the foetuses were determined and they were given a gross external examination. One-half of each litter was placed in Bouin's fixative for subsequent serial sectioning and visceral examination. The remaining foetuses were eviscerated and fixed in alcohol for subsequent staining with Alizerin Red S and skeletal examination. No evidence of maternal toxicity was exhibited at dose levels of 25 and 75 mg/kg body weight. At 75 mg/kg body weight, body weight development and food consumption were reduced only on gestation days 6 to 10 compared with controls, but returned to normal levels in the course of the study. There was one death per group at 75 and 100 mg/kg body weight, but no o-chloronitrobenzene-related toxicity could be detected. At 100 mg/kg body weight, there was body weight loss from

gestation days 6 to 10 and reduced food consumption from days 6 to 16. None of the groups treated with o-chloronitrobenzene exhibited any changes in fertility rate, mean numbers of live and dead foetuses, total implantations, late resorptions and corpora lutea or preimplantation losses compared with the control groups. An increase in early resorptions was seen in the group treated with 75 mg/kg body weight but this finding was not confirmed at 100 mg/kg body weight and was considered irrelevant in comparison with historical controls. There were no biologically meaningful differences from the controls with respect to the number of pups with visceral or skeletal malformations. Specific variations noted at dose levels of 25 and 75 mg/kg body weight were not observed at 100 mg/kg body weight. The investigators concluded from these findings that treatment of pregnant rats with daily, maternally toxic doses of 100 mg o-chloronitrobenzene/kg body weight did not result in teratogenicity, embryotoxicity or foetotoxicity (Monsanto, 1986).

In a study conducted in male rats (Fischer 344), which is available only as an abstract, the testicular toxicity of o-chloronitrobenzene was assessed upon single oral administration of 150 mg/kg body weight. The rats were killed 1 or 25 days after administration, and testicular weights, daily sperm production and histopathological changes in the testes were determined. Under the described conditions of exposure, o-chloronitrobenzene had no effect at all on the testis of the rat (no further details; Mohr and Working, 1988).

At the end of a subchronic toxicity study, in which rats and mice underwent 13-week whole-body inhalation exposure to o-chloronitrobenzene vapour, sperms were examined, and vaginal cytology was assessed in order to ascertain oestrus cycle (see also Section 7.5). Groups of 10 animals were exposed to o-chloronitrobenzene at concentration levels of 0 (controls), 4.5, 9 and 18 ppm (equivalent to 0, 28.8, 57.6 and 115.2 mg/m³, respectively) for 6 hours/day, 5 days/week. In the group of rats exposed to 18 ppm, a level with high systemic toxicity (marked methaemoglobinaemia and anaemia), the males were found to have reduced spermatogenesis in conjunction with decreased testicular weights. The cauda epididymal weight and epididymal weight, number of spermatid heads/testis and spermatid count were markedly lower than those of the controls. Sperm motility was not affected. In the females, no functional or morphological changes were seen in the reproductive parameters assessed, in comparison with controls. The male mice exposed to o-chloronitrobenzene at 18 ppm only exhibited markedly

reduced sperm motility. Again, the females showed no changes, compared with the controls (NTP, 1993).

Testicular weights and histopathological abnormalities of testicular tissue were also investigated in B6C3F1 mice after daily dietary administration of o-chloronitrobenzene for 4 weeks (see also Section 7.2). Only the highest dose with marked systemic toxicity, 5000 ppm (equivalent actually ingested amount: 1120 mg/kg body weight), was observed to result in a slight decrease in testicular weights. There were no cases of histopathological abnormalities of the testis (Bayer, 1993).

A comprehensive 2-generation study on the reproductive toxicity of o-chloronitrobenzene was conducted in mice (VAF Cr1:CD-1(ICR)BR strain) receiving daily oral administrations of the chemical by gavage ("Reproduction Assessment by Continuous Breeding"). The first-generation investigations were carried out with 40 pairs (separately housed pairs of one male and one female) serving as a control group and 3 groups of 20 pairs each which were treated with o-chloronitrobenzene at 40, 80 or 160 mg/kg body weight. The o-chloronitrobenzene used was more than 99% pure and dissolved in corn oil. The controls received the corn oil vehicle only. Pairs of approximately 11-week-old animals were initially housed in cages by sex for one week and treated with the aforementioned doses. Subsequently, the animals were housed as individual breeding pairs and treated with o-chloronitrobenzene for another 98 days. At the end of this treatment, 23 controls and 21 animals treated with 160 mg/kg body weight were held for collection of blood samples and spleen weights. In the control and 160 mg/kg body weight groups, the pups of the last litter born during the treatment period were reared by the dam until weaning, housed in pairs by sex and given the same treatment with o-chloronitrobenzene (160 mg/kg body weight) or corn oil (controls) as their parents. At sexual maturity, groups of 20 males and 20 females were cohabitated and then housed singly and treated for another 7 days. Upon delivery of the first litter, the animals were sacrificed. Overall, the following were endpoints evaluated: clinical signs of toxicity, parental body weight and water consumption, parental spleen weight and blood methaemoglobin levels in the control group and the highest dose group in the first generation (F_0), first-generation fertility (number of pairs producing a litter, number of litters/pair, number of live pups/litter), sex ratio and body weights of the pups immediately after birth. In the second generation (F_1), the same endpoints were investigated as in the first generation, excluding the num-

ber of litters/pair seeing that there was only one litter, but including determination of spleen weights and blood methaemoglobin levels. In addition, all F_1 males underwent a thorough examination of the testes and epididymides, while the F_1 females were evaluated with regard to vaginal cytology in order to ascertain oestrus cycle. The results are summarised in Table 8.

Table 8. Reproductive toxicity study of o-chloronitrobenzene in mice (NTP, 1992)

First generation (F_0)	Dose (mg/kg b. w.)		
	40	80	160
Body weights	no effect	no effect	slightly increased
Clinical signs	none	none	slight inactivity, cyanosis in a few females
Mortality (controls 4/40)	2/20	2/20	3/20
Water consumption	no effect	no effect	no effect
Number of pairs producing a litter	all	all	all
Number of litters/pair (controls 4.9)	mean value was 4.9 in all dose groups		
Number of live pups/pair (controls 11.2)	12.1	11.9	12.9
Sex ratio and body weight of the pups immediately after birth	no effect	no effect	no effect
Spleen weights	not determined	not determined	increased up to 1.5 to 2-fold
Methaemoglobin levels in blood	not determined	not determined	increased up to 4 to 6-fold
Second generation (F_1), litters from controls and animals treated with 160 mg/kg body weight			
Body weights	slightly increased in the group treated with o-chloronitrobenzene		
Clinical signs	none		
Mortality	none		
Water consumption	no difference between the controls and the group treated with o-chloronitrobenzene		
Number of pairs producing a litter	controls 19/20, treated group 19/20		
Number of live pups/litter	no difference between the controls and the treated group (11.6/11.4)		
Sex ratio and body weight of the pups immediately after birth	no difference between the controls and the treated group		
Spleen weights	increased 1.4 to 1.6-fold compared with the controls		
Methaemoglobin levels	increased 3-fold compared with the controls		
Oestrus cycle	not affected by treatment with o-chloronitrobenzene		
Sperm count, percent abnormal sperm and sperm motility in the epididymides and testes, and epididymal weights	not affected by treatment with o-chloronitrobenzene		

The study showed that the treatment with o-chloronitrobenzene did not alter reproductive function in mice of either generation. At the dose levels with which the first generation was treated, 40 and 80 mg/kg body weight, there were no indications that o-chloronitrobenzene had a toxic effect, whereas 160 mg/kg body weight led to marked increase in spleen weights and

blood methaemoglobin levels, and in some animals produced clinical signs of toxicity. In the second generation, o-chloronitrobenzene treatment affected spleen weights and methaemoglobin levels in the same manner. The investigators therefore concluded that although o-chloronitrobenzene did cause clinical signs of systemic toxicity in mice, it was not a reproductive toxicant (NTP, 1992).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

No information available.

7.11 Other effects

o-Chloronitrobenzene was investigated in rats with regard to its effect on lipid peroxidation, its antioxidant activity and its effect on the content of vitamin E in the liver and spleen. White male rats (weighing 200 to 300 g) were given o-chloronitrobenzene by oral gavage at daily doses of 51 mg/kg body weight in sunflower oil. Treatment was administered for 5 days in one group and for 30 days in another. Subsequently, the animals were killed, and the liver and spleen were removed and frozen in liquid nitrogen. After thawing, a 20-percent homogenate in phosphate buffer was prepared from the specimens. This was analysed for content of primary lipid peroxidation products (diene, triene, oxodiene and tetraene conjugates) and of malonic dialdehyde as a secondary lipid peroxidation product. Total antioxidant activity was measured using oleate thermoautoxidation, and the content of vitamin E was determined after alkaline hydrolysis of the homogenate. The 30-day treatment with o-chloronitrobenzene resulted in a decrease in the content of diene, triene and oxodiene conjugates and malonic dialdehyde in the liver. No such effects were observed after 5 days of treatment. In the spleen, there were no changes in the content of primary or secondary lipid peroxidation products after 5 and after 30 days of treatment. The vitamin E content in the liver was unaltered, relative to controls, after o-chloronitrobenzene treatment, whilst in the spleen it was markedly reduced after 5

days of treatment. Following 30-day treatment, the vitamin E content in the spleen was increased. Total antioxidant activity in the liver was 1.7-fold higher than in the controls after the 5-day administration, but after 30 days of treatment the control level was attained again. In the spleen, this parameter was not affected. The investigators arrived at the – very speculative – conclusion that o-chloronitrobenzene affects lipid oxidation, and they suspected that this system plays a role in the detoxification of o-chloronitrobenzene, particularly in the liver (Paranich et al., 1993).

An in-vitro study was conducted to investigate the effect of o-chloronitrobenzene on δ-aminolevulinic acid synthetase and ferrochelatase activities in rat liver. A 5-percent suspension of rat liver homogenate in buffer was used, which was sonicated to ensure disruption of mitochondrial membranes. At a concentration level of 10^{-3} M (equivalent to 0.16 mg/ml), o-chloronitrobenzene had no effect on δ-aminolevulinic acid synthetase activity, but slightly stimulated ferrochelatase activity, though the statistical significance of this finding could not be established (Johnson et al., 1985).

8 Experience in humans

Although it has long since been known that o-chloronitrobenzene is dangerous to humans, the relevant literature contains practically 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”. o-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 o-chloronitrobenzene in water was reported as 0.02 mg/l. However, no distinction was made between the isomers of chloronitrobenzene (Davydova, 1967).

There are a number of reports on cases of poisoning in the workplace with considerable involvement of o-chloronitrobenzene. 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).

Whilst isolating o- and p-chloronitrobenzene, workers were severely exposed to vapour emanating from the heated mixture. Two of the 3 employees exhibited severe signs of intoxication, one after 3 days and the other after 3.5 days. The signs included collapse, vomiting, headache and severe cyanosis. Although exposure was reduced by modifying the work shifts, there were two further cases of poisoning with the same signs (Renshaw and Ashcroft, 1926).

Another case of intoxication with a mixture of o- and p-chloronitrobenzene, to the vapours of which a worker was exposed in a hot room for a prolonged period of time, resulted in attacks of unconsciousness, severe headache, vomiting and convulsions after a latent period of one hour. Upon hospitalisation in a state of unconsciousness, cyanosis and methaemoglobinaemia were diagnosed. On the second day in hospital, complete recovery was reached except for a slight disturbance of renal function (Schwanke, 1930).

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).

Exposure to a mixture of o- and p-chloronitrobenzene resulted in severe intoxication when, during technical problems and subsequent repair work at

a plant for isolating the isomers, a worker inhaled large amounts of vapour. Collapse, signs of cyanosis and a drop in haemoglobin levels to 65% of the normal value were observed. The patient recovered only slowly, complained of dyspnoea and vertigo, and even after 7 weeks haemoglobin levels were still only 80% of the normal value (Gerbis, 1932).

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).

Although o-chloronitrobenzene has been in use for decades, there are no known indications that the chemical has an allergising effect in humans (BUA, 1985; Bayer, 1999).

9 Classifications and threshold limit values

In the Federal Republic of Germany, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") of the Deutsche Forschungsgemeinschaft has assigned o-chloronitrobenzene to category 3 of carcinogenic substances in the List of MAK and BAT Values (i.e. "substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data") and the chemical has been designated with "H" because of the danger of cutaneous absorption (DFG, 2000; Greim, 2000).

Furthermore, o-chloronitrobenzene has been legally classified in the TRGS 905 and placed into carcinogenicity category C3 of "substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment" and in category R_F3 of substances toxic to reproduction, the category comprising "substances which cause concern for human fertility", in accordance with the EU classification criteria (TRGS 905, 2000).

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