BG Chemie

Toxicological Evaluations

5 Potential Health Hazards of Existing Chemicals

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Preface

As part of its "Programme for the prevention of health hazards caused by industrial substances", the Berufsgenossenschaft der chemischen Industrie (BG Chemie, Employment Accident Insurance Fund of the Chemical Industry) began in 1977 to investigate the toxicity of those chemicals which are widely used, have many different applications and are suspected of being dangerous to health, in particular of having long-term effects. The investigations consist of a literature search and – depending on the results – commissions of experimental studies. It is hoped by means of this testing to close gaps in our knowledge and to increase the scientific validity of the required risk assessments. The results of the toxicological investigations carried out by BG Chemie, and the resulting substance assessments have been published in German since 1987 in the form of 113 „Toxikologische Bewertungen" ("Toxicological Evaluations") up to now.

In order to make this useful information internationally available, BG Chemie began in October 1990 to publish them as a book series in English, of which the fifth volume (containing 12 individual evaluations) is presented here. Therefore for 72 existing chemicals "Toxicological Evaluations" are available in English at the moment, a further 38 are in preparation and will be published soon.

Because of the short time between publishing volume 1–5, printing of "Introduction" (consisting of a general overview of the programme, lists with names of people involved as well as substances under investigation) was abandoned in volumes 2, 3 and 5. If more detailed information is required about the ongoing work, see volume 1 or 4 or contact BG Chemie at first hand.

BG Chemie hopes that, for many people working in the chemical industry, this information will be of practical help in assessing hazards to health at the workplace.

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Contents

1-Chloro-2,4-dinitrobenzene

A BUA Substance Report No. 42 (December 1989) is available for this chemical. Further work on the compound with regard to genotoxicity and toxicity to warm-blooded organisms has been conducted for BG Chemie. Any information which is supplementary to that in the BUA Substance Report is marked by an asterisk.

1. Summary and assessment

^{14}C-1-Chloro-2,4-dinitrobenzene is absorbed efficiently through the skin of monkeys and humans both in vitro (48.4 and 32.5%, respectively) and in vivo (approximately 53% in both species). In the guinea-pig given a single oral or intraperitoneal dose, 2,4-dinitrophenyl groups are detectable in the upper gastrointestinal tract, in the Peyer's patches, the mesenteric lymph nodes, the spleen and the peripheral blood lymphocytes. Intermediately the chemical binds to glutathione and is detectable as S-(2,4-dinitrophenyl)-glutathione. The activity of glutathione-S-transferase, involved in this reaction, varies between different species. Other metabolites include 1-methyl-mercapto-2,4-dinitrobenzene, S-(2,4-dinitrophenyl)-cysteine and m-phenylenediamine. After dermal application to monkeys and humans, ^{14}C-1-chloro-2,4-dinitrobenzene is eliminated in the urine within 5 days (52.5 and 53.14%, respectively). After a single intravenous injection of ^{14}C-1-chloro-2,4-dinitrobenzene in man, 64% of the administered radioactivity is found in the urine within 5 days, and after a single parenteral administration to monkeys, 85.8% is found in the urine after 5 days.

On acute administration, 1-chloro-2,4-dinitrobenzene is found to be slightly toxic or toxic (LD_{50} rat oral approximately 640 mg/kg and 1100 µl/kg; rabbit dermal 130 µl/kg). The chemical causes methaemoglobinaemia, Heinz body formation and secondary anaemia. Doses within the LD_{50} range cause excitation of the central nervous system and cramps.

1-Chloro-2,4-dinitrobenzene is a potent skin sensitizer, and is therefore frequently used as a positive control in sensitization tests

and in immunobiological tests. There are numerous publications on this, and most are well documented. The initial reaction is dose- and solvent-dependent, the best results being obtained with ethanolic solutions. Allergic reactions to 1-chloro-2,4-dinitrobenzene can be induced not only in the guinea-pig but also in other species, and in other target organs as well as the skin.

After subchronic and chronic oral administration of 1-chloro-2,4-dinitrobenzene, the maximum tolerated doses over a 12-month period are 2000 mg/kg of feed in the rat, 3000 mg/kg of feed in the female mouse and 6000 mg/kg of feed in the male mouse, respectively. However, the only criteria for this are body-weight gain and mortality. On the other hand, a concentration of 0.2 mg/m^3 inhaled for 4 months is still reported to be toxic in rats.

1-Chloro-2,4-dinitrobenzene induces dose-dependent point-mutations in the Salmonella/microsome test, the mutagenic activity being reduced by the addition of a metabolic activation system. The most sensitive strain appears to be TA 100. The lowest dose to cause mutations is 3.2 μg/plate, and according to Japanese data the chemical is one of the most active in this respect (revertant number 10,000/mg). Glutathione-deficient Salmonella strains are much less sensitive and the addition of glutathione to the S9-mix completely eliminates the mutagenic activity. 1-Chloro-2,4-dinitrobenzene causes increased DNA repair in rat hepatocytes, and chromosomal aberrations in Chinese hamster V79 cells. In vivo, 1-chloro-2,4-dinitrobenzene causes DNA single-strand breaks in the brain, kidneys and liver of mice and in the hepatocytes of rats, in a dose-dependent manner. Negative results have been obtained in the dominant lethal test after subchronic inhalation exposure of male rats to 1.1 mg/m^3. In Chinese hamster V79 cells, non-cytotoxic concentrations affect spindle function in up to 15% of the mitoses observed.

A positive dose-related effect is found in the cell transformation test on BHK21C kidney cells of the Chinese hamster.

No carcinogenicity tests have been conducted that conform with present-day protocol requirements, but an earlier study has found no evidence of carcinogenic activity in male rats or in male and female mice given 1-chloro-2,4-dinitrobenzene in the diet at the maximum tolerated dose for 18 months. Although the results with 1-chloro-2,4-dinitrobenzene are poorly documented, it may be concluded that if the chemical is a carcinogen, it is not likely to be a very potent one.

Subchronic inhalation exposure of male rats to 1-chloro-2,4-dinitrobenzene leads to certain testicular findings, but these have no effect on testicular histology or on fertility. In female rats exposed to 0.13 mg/m^3 throughout pregnancy, there is an increase in post-implantation losses, and during the first 7 days there is evidence of mild embryotoxicity.

The effect of 1-chloro-2,4-dinitrobenzene on the immune system must be considered mainly in conjunction with its sensitizing properties. The substance appears to have a tumour-promoting effect, based on a cellular immune reaction.

Experiences with 1-chloro-2,4-dinitrobenzene in man are based mainly on its skin-sensitizing action. The chemical is so potent that 95% of all normal people can be sensitized against it. Among experimental subjects the allergic reaction is dose-dependent. 1-Chloro-2,4-dinitrobenzene has frequently been used therapeutically as an immunobiological stimulant in certain diseases (alopecia, warts, malignant melanoma, AIDS), and here too, allergic reactions have been observed. A case of eye damage (retrobulbar neuritis) that was reported 100 years ago cannot be linked conclusively to 1-chloro-2,4-dinitrobenzene, due to concomitant exposure to other chemicals.

2. Name of substance

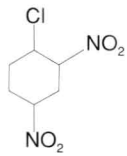
2.1	Usual name	1-Chloro-2,4-dinitrobenzene
2.2	IUPAC-name	1-Chloro-2,4-dinitrobenzene
2.3	CAS-No.	97-00-7

3. Synonyms, common and trade names

4-Chloro-1,3-dinitrobenzene
2,4-Dinitro-1-chlorobenzene
2,4-Dinitrochlorobenzene
DNCB
2,4-Dinitro-1-chlorbenzol
1-Chlor-2,4-dinitrobenzol

4. Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_6H_3O_4N_2Cl$

5. Physical and chemical properties

5.1	Molecular mass, g/mol	202.55
5.2	Melting point, °C	52–54 (Windholz, 1976)
5.3	Boiling point, °C	315 (Windholz, 1976)
5.4	Vapour pressure, hPa	10^{-4} (at 20° C) (Hoechst, 1988)
5.5	Density, g/cm^3	ca. 1.7 (at 20° C) (Windholz, 1976)
5.6	Solubility in water	0.36 g/l (at 20° C) (Hoechst, 1989)
5.7	Solubility in organic solvents	Soluble in ether, benzene, carbon disulphide (Windholz, 1976)
5.8	Solubility in fat	No information available
5.9	pH-value	–
5.10	Conversion factor	1 ppm $\triangleq$ 8.41 mg/m^3 1 mg/m^3 $\triangleq$ 0.12 ppm (at 25° C and 1013 hPa)

6. Uses

As an intermediate in the manufacture of dyes, crop protection agents, photochemicals, perfumes and explosives (Hoechst, 1989).

7. Experimental results

7.1 Toxicokinetics and metabolism

Dermal absorption studies with ^{14}C-labelled 1-chloro-2,4-dinitrobenzene in isolated skin sections from monkeys and humans have shown that the substance is rapidly absorbed through the skin. The maximum absorption rate is reached within the first 2 hours of application. This is followed by a rapid fall in the rate of absorption until the zero value is reached again after about 24 hours (Bronaugh and Maibach, 1985).

The excretion of a single parenterally-administered dose of ^{14}C-labelled 1-chloro-2,4-dinitrobenzene was studied in monkeys. Within 5 days, 85.8% of the administered radioactivity had been excreted in the urine (Bronaugh and Maibach, 1985).

In comparable studies, the excretion of ^{14}C-labelled 1-chloro-2,4-dinitrobenzene was studied after dermal application to humans and monkeys. No difference in elimination could be detected between species following a single application of 4 $\mu g/cm^2$ skin surface. 5 days after the application, 53.1% of the radioactivity was recovered in the urine in humans (Feldmann and Maibach, 1970) and 52.5% in the urine in monkeys (Bronaugh and Maibach, 1985).

In humans, 64% of an intravenous dose was excreted in the urine, with a half-life of 4 hours (Feldmann and Maibach, 1970).

1-Chloro-2,4-dinitrobenzene undergoes a glutathione-dependent biotransformation to S-(2,4-dinitrophenyl)glutathione in vivo and in vitro (Summer and Göggelmann, 1980; Kerklaan et al., 1987). Other metabolites that have been proposed include 1-methylmercapto-2,4-dinitrobenzene (Kerklaan et al., 1987), m-phenylenediamine (Weisburger et al., 1978) and S-(2,4-dinitrophenol)cysteine, which then enters the classical pathway for the formation of mercapturic acids (Kerklaan et al., 1987).

The distribution of dinitrophenyl groups was studied by means of immunofluorescence, following the oral administration of 125 mg 1-chloro-2,4-dinitrobenzene/kg body weight to guinea-pigs that had been fasted for one day. After 1 to 24 hours, dinitrophenyl groups were found in the epithelial cells of the upper digestive tract, the mesenteric lymph nodes, such as the Peyer's patches, the spleen and the peripheral blood. The nitrophenyl groups were localized in the cell membrane and the cytoplasm, but were not present in the

cell nucleus. No dinitrophenyl groups were found in the large intestine, the liver, the kidneys or the thymus gland (Takei et al., 1985).

Dermal application of a 0.5% solution of 1-chloro-2,4-dinitrobenzene had no effect on the cytochrome P450 system in rats. In addition to the cytochrome P450 content, the activities of cytochrome C-reductase, 7-ethoxycoumarin-dealkylase and aryl-hydrocarbon hydroxylase were measured. None of these enzyme activities differed significantly from control values (Kratka et al., 1979).

1-Chloro-2,4-dinitrobenzene has been found to bind specifically to glutathione (Summer et al., 1980*; Awasthi, 1983*; Jones et al., 1988*). The conjugate has been identified as S-(2,4-dinitrophenyl)glutathione. The enzyme concerned, glutathione S-transferase, is also found in human erythrocytes (Awasthi et al., 1983*) and human lymphocytes (Jones et al., 1988*).

The activity of glutathione S-transferase has been shown to vary between species, and decreases in the following order: Hamster (5.84) > rabbit (4.97) > guinea-pig (4.58) > mouse (3.06) > rat (1.0; Igarashi et al., 1986*).

7.2 Acute and subacute toxicity

The following table shows the results of acute toxicity studies on 1-chloro-2,4-dinitrobenzene:

Table 1. LD_{50} of 1-chloro-2,4-dinitrobenzene

Route of administration	Species	LD_{50} value (mg/kg body weight)	Reference
oral	rat	640	Sziza and Magos, 1959
oral	rat	939	Hoechst, 1977 a, b
oral	rat	1070	Smyth et al., 1962
intraperitoneal	rat	280	Sziza and Magos, 1959
dermal	rabbit	130	Smyth et al., 1962

Signs of pain were detected in rats after intraperitoneal injection of 1-chloro-2,4-dinitrobenzene (120 to 940 mg/kg body weight). In the surviving rats, urinary incontinence and severe weight loss

occurred. In rats that received an intraperitoneal dose corresponding to the LD_{50} value (280 mg/kg body weight), severe tonic-clonic spasms were seen within a few minutes. Death occurred within 30 minutes to 1 hour. Post mortems of the animals that died at the time of treatment and those killed after the 14-day observation period revealed severe inflammatory effects in the abdominal cavity, coalescence of the liver, spleen and peritoneum as well as a bloody exudate (Sziza and Magos, 1959).

On oral administration (120 to 4700 mg/kg body weight) the appearance of the above signs of toxicity was delayed. At the dose corresponding to the LD_{50} (640 mg/kg body weight), death occurred about 1 day after administration. At 4700 mg/kg, all of the animals died within 4 hours. In many cases, autopsy revealed rounding of the edges of the liver and hyperaemia of the intestines (Sziza and Magos, 1959).

Colitis was observed in guinea-pigs and rabbits after rectal instillation of 1-chloro-2,4-dinitrobenzene (10 ml of a 0.25% suspension of 1-chloro-2,4-dinitrobenzene in mineral oil in each case). However, this inflammation of the large intestine and accompanying diarrhoea occurred only in animals that had already been sensitized (Rabin and Rogers, 1978; Glick and Falchuk, 1981).

The ability of 1-chloro-2,4-dinitrobenzene to induce methaemoglobin formation was studied in rats after the administration of a dose corresponding to the appropriate LD_{50} either orally (640 mg/kg body weight) or by intraperitoneal injection (280 mg/kg body weight). After intraperitoneal administration, 25% methaemoglobin was found after 30 minutes, 39% after 1 hour and 63% after 2 hours, while the corresponding values after oral administration (20%, 17% and 12%) were clearly lower. According to the authors, there was not a close correlation between the size of the dose and the methaemoglobinaemia induced (Sziza and Magos, 1959). When compared with nitrobenzene, p-nitrochlorobenzene, p-nitroanisole and p-nitrophenetole, each administered at a dose corresponding to the LD_{50}, 1-chloro-2,4-dinitrobenzene caused the most marked methaemoglobin formation after intraperitoneal administration in rats, but caused a weaker methaemoglobinaemia than nitrobenzene and p-nitrochlorobenzene after oral administration (Sziza and Magos, 1959). In cats, methaemoglobin formation was one third less after subcutaneous administration of 1-chloro-2,4-dinitrobenzene than after nitrobenzene, while m-nitrochlorobenzene was 2 to 3 times more effective than nitrobenzene (Jung, 1947).

In another study, a slight increase in the methaemoglobin level (14.1%) was observed in rats, 5 hours after intraperitoneal administration of 20 mg 1-chloro-2,4-dinitrobenzene/kg body weight (Watanabe et al., 1976).

Rats were given either a single intraperitoneal injection of 280 mg 1-chloro-2,4-dinitrobenzene/kg body weight or a single oral dose of 640 mg/kg body weight. An increase in Heinz bodies in the blood (30 to 55%) was seen only after oral administration (Sziza and Magos, 1959).

7.3 Skin and mucous membrane effects

When 0.5 mg 1-chloro-2,4-dinitrobenzene was applied to the skin of rabbits, slight hyperaemia as well as slight oedema formation resulted after exposure for 1 hour; these were completely reversible within 24 hours. Pronounced intense reddening and strong oedema formation were observed after 24 hours exposure. Painting the skin with 0.02 and 0.05% 1-chloro-2,4-dinitrobenzene in acetone for 5 days produced no irritant effect in guinea-pigs. Reddening of the skin could be seen 24 hours after painting on a 0.1% solution. When a 1% solution was used extended necrosis soon appeared (within 24 hours; Sziza and Magos, 1959).

In a further study, severe symptoms of irritation and the beginnings of necrosis could be observed 24 hours after application of 500 mg 1-chloro-2,4-dinitrobenzene to the skin of rabbits (Hoechst, 1977 c).

In addition an orientating skin irritation study exists on white New Zealand rabbits using non-occlusive exposure for 24 hours. 0.01 ml of undiluted substance produced strong irritation with necrosis, degree 7 (Smyth et al., 1962*).

The irritant effect of 1-chloro-2,4-dinitrobenzene on the eye was studied in rabbits. Following application of 20 mg of finely powdered substance a strongly irritant effect was observed leading to complete blindness in the treated eye. 1 hour after application of 0.5 mg, strong reddening and pronounced chemosis (oedema of the conjunctiva) appeared, but were completely reversible within 96 hours (Sziza and Magos, 1959).

After applying a single dose of 100 mg 1-chloro-2,4-dinitrobenzene to rabbits eyes, the substance was again found to have a strongly irritant effect on the mucous membrane. The quoted irritation index after 24 hours (74 out of a maximum 90) corresponds to

severe eye damage. It was not possible to assess the cornea and iris because of the severe conjunctivitis (Hoechst, 1977 c).

In other studies, a level of burning of degree 10 on a 10-degree scale was observed after instilling 0.5 ml of a 1% solution (in propylene glycol) into the conjuctival sac of rabbits (Smyth et al., 1962*).

7.4 Sensitization

The potent sensitizing effects of 1-chloro-2,4-dinitrobenzene after dermal application have been described in detail in the literature (Sziza and Magos, 1959; Christensen et al., 1984; Oka et al., 1986; and others). Even after sensitization with 0.1% 1-chloro-2,4-dinitrobenzene in acetone and challenge with a 0.02% solution, severe, pronounced sensitization reactions have been obtained in guinea-pigs (Sziza and Magos, 1959).

Adult albino guinea-pigs (Rockefeller University strain) were injected intracutaneously with 5 µg ^{14}C-labelled 1-chloro-2,4-dinitrobenzene (4.86 mCi/mmol) in the right ear. About 90% of the injected dose was carried away from the injection site within 1 hour and was partly excreted in the urine. The rest remained at the application site for a considerable time. A challenge was carried out after 14 days by application of a 1% solution in olive oil to the clipped dorsal skin (evaluation after 24 and 48 hours). The effect of the primary injection site on immune reaction was then studied. When the ear was removed up to 6 hours after the local injection, no allergic reaction occurred. When the ear was removed 12 hours after the injection, only 1/28 guinea-pigs reacted positively. An increase in the interval between pre-treatment and removal of the ear to 24 or 28 hours caused an increase in the sensitization rate. If the injected ear was removed after 4 or more days, an optimum sensitization rate was obtained in the guinea-pigs. The authors concluded from these findings that sensitization was induced through the allergens that remained in the skin of the ear, and not through those that had been carried away from the injection site (Macher, 1969*).

According to the English summary of a Polish report, 1-chloro-2,4-dinitrobenzene was a more potent sensitizer than potassium dichromate or p-phenylenediamine in guinea-pigs (Kiss, 1983*).

Besides causing allergic skin reactions in guinea-pigs, 1-chloro-2,4-dinitrobenzene has also been reported to cause reactions in mice (Lubach, 1983*; Czerniecki et al., 1988*), rats (Berczi et al.,

1983*; Nagy et al., 1983*; Lukacs et al., 1986*), cats (Schultz and Maguire, 1982*) and chickens (Huynh and Chubb, 1987*). Other target organs that have been identified besides the skin include the lungs (Nakajima et al., 1983*), the kidneys (Nakajima, 1981*), the large intestine (Rabin and Rogers, 1978*; Glick and Falchuk, 1981*; Norris et al., 1982*) and the vagina (Newmann et al., 1983*).

7.5 Subchronic and chronic toxicity

No appropriate results from studies in warm-blooded species have been located. Some information can be gleaned from a carcinogenicity study (Weisburger et al., 1978, see section 7.7) from which the following maximum tolerated concentrations in the feed were derived:

Species	Sex	Concentration (mg/kg feed)
Rat	Male	2000
Mouse	Male	6000
Mouse	Female	3000

Further preliminary indications are provided by a study of the reproductive toxicology of 1-chloro-2,4-dinitrobenzene (Khipko et al., 1985, see section 7.8). After inhalation exposure of rats to 1-chloro-2,4-dinitrobenzene (1.1 mg/m^3, 4 hours/day, 5 days/week for 4 months), general signs of toxicity were seen after 2 to 3 weeks. The observed effects included restlessness, hyperaemia of the mucous membranes and breathing difficulties. In addition, 4 of the 23 rats died. Towards the end of the 4-month treatment period, specific changes in blood composition were found in the animals, including a decrease in the haemoglobin content and a reduction in the erythrocyte count. In addition a slight sulfhaemoglobinaemia was detected. Changes in blood protein level and increases in relative liver weight were still observed at a concentration of 0.2 mg/m^3. However the value of this study is limited because details of the protocol and methods are not given. It is therefore not possible to evaluate the findings.

7.6 Genotoxicity

There are numerous studies on the genotoxicity of 1-chloro-2,4-dinitrobenzene of which only a few can be considered in the form of examples in the following.

7.6.1 In vitro

1-Chloro-2,4-dinitrobenzene was studied in the Salmonella/microsome test in strains TA 98 and TA 100 with and without metabolic activation. Without metabolic activation, 10 µg/plate proved positive in TA 98 and TA 100 ($p=0.05$), while with metabolic activation only 100 µg/plate proved positive in TA 98 and 50 µg/plate in TA 100 (Kratka et al., 1979). Similar results were reported from Japan (Matsuda, 1981).

Other authors observed an increase in the number of revertants in the Salmonella/microsome test in TA 1538 to 71±6, 114±9, 206±6 and 231±30 (controls 9±1) following 1-chloro-2,4-dinitrobenzene doses of 0.05, 0.1, 0.25 and 0.4 mmol/plate without metabolic activation. With metabolic activation the mutagenic effect was again distinctly weaker. The same doses produced an increase in numbers of revertants of only 1.2-, 1.5-, 2.9- and 7.6-fold. Addition of up to 2.5 mmol GSH to the S9-mix cancelled the mutagenic effect of 1-chloro-2,4-dinitrobenzene completely (Summer et al., 1980).

The same authors also tested 1-chloro-2,4-dinitrobenzene in strains TA 1535, TA 100 and TA 98 without metabolic activation. Here too there was a strong, direct mutagenic effect at concentrations of 26 and 39 µg/plate, with 9-fold and 6-fold increases respectively in the number of revertants in the particularly sensitive TA 100 and TA 98 strains (Summer and Göggelmann, 1980).

In other studies, the mutagenic effect of 1-chloro-2,4-dinitrobenzene was compared in strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with and without metabolic activation. Doses were in the range of 6.3 to 200 µg/plate. Without metabolic activation the substance proved mutagenic in TA 98 from 10 µg/plate, in TA 100 from 50 µg/plate and in TA 1538 from 10 µg/plate. With metabolic activation the effective threshold doses for all three strains were between 50 and 100 µg/plate. It was thus possible in these studies to observe weakening of the mutagenic effect as a result of metabolic activation only in TA 98 and TA 1538. 1-Chloro-2,4-dinitrobenzene was not mutagenic in strains TA 1535 and TA 1537 (Strobel and Röhrborn, 1980).