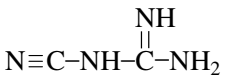
Dicyanodiamide

MAK value (2003)	**–**
Peak limitation	**–**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity	**–**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms:	cyanoguanidine dicyandiamid dicyandiamide
Chemical name (CAS):	guanidine, cyano-
CAS number:	461-58-5
Structural formula:	
Molecular formula:	$C_2H_4N_4$
Molecular weight:	84.08
Melting point:	209–211°C
Boiling point:	decomposition
log P_{OW}[1]:	–1.15

Dicyanodiamide is a colourless and odourless powder which is moderately soluble in water and polar organic solvents and of low or no solubility in apolar solvents. The substance is a starting material for the manufacture of guanamines, guanidine salts,

[1] *n*-octanol/water partition coefficient

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melamine, formaldehyde resins and is used in epoxy resin curing, in flame retardants and as an additive in fertilizers (Güthner *et al.* 2001).

1 Toxic Effects and Mode of Action

Dicyanodiamide has a very low acute toxicity in rodents. Dicyanodiamide may be slightly irritating to the skin and eyes. No sensitizing potential was derived from the findings obtained in humans and animals. There were no specific effects after repeated oral administration to rats. Non-specific effects such as reduced body weight gain and alterations of clinicochemical and haematological parameters were observed at high doses from about 2400 mg/kg body weight and day onwards. Furthermore, findings like intranuclear eosinophilic inclusion bodies in the proximal tubular epithelium of the kidney were sporadically obtained in some mid-term and long-term studies. There were no substance-induced pre-neoplastic or neoplastic changes after long-term treatment with dicyanodiamide.

Dicyanodiamide shows no genotoxicity in various test systems *in vitro*.

2 Mechanism of Action

Cyanamide and a number of other substances lead to an incompatibility reaction (antabuse syndrome), which is due to an accumulation of acetaldehyde in the blood, if the substance is ingested together with alcohol. Unlike in the case of cyanamide, no increase in the blood acetaldehyde level is caused in rabbits after a single oral administration of 1500 mg dicyanodiamide/kg body weight and 1500 mg ethanol/kg body weight in a 20% aqueous solution (Hald *et al.* 1952).

3 Toxicokinetics and Metabolism

There are no data available for the toxicokinetics and metabolism of dicyanodiamide.

4 Effects in Humans

There are no data available for the effects of single or repeated exposure of humans to dicyanodiamide nor for local effects of dicyanodiamide on skin or mucous membranes, reproductive toxicity, genotoxicity or carcinogenicity.

Allergenic effect

In a prophetic patch test (no other details), dry, pulverized material was investigated on 200 persons for skin irritation and sensitizing properties. Dicyanodiamide was not found to be either sensitizing or irritating (ACC 1959). 34 persons with acute eczema of the upper extremities, who came from different areas of the industry processing or producing epoxy resins and from research institutes, were investigated. The skin lesions occurred one week to two years after the first contact with epoxy resin, with the most frequent latency period being one to three months. The persons affected were investigated in the patch test with 2% dicyanodiamide in water. Dicyanodiamide did not lead to skin changes among any of the 34 persons affected (Jirasek and Kalensky 1962).

One worker from a factory for flame retardants developed ambilateral dyshidrosiform hand eczema after four years of working there. The epicutaneous testing with dicyanodiamide (pure substance and 10% preparation) revealed a highly positive reaction after 24 hours, which was marked even with the 1% preparation. The positive result was confirmed in another epicutaneous testing carried out one year after the worker had given up his job. It is not clear from the documentation whether and at what time a further reading was made. The patient showed no reactions to substances from a standard test series or to rubber chemicals, substances from the coating, plastic and adhesive series or to disinfectants. No reactions to 1% and 10% dicyanodiamide or to pure dicyanodiamide were obtained among 25 control persons (Senff *et al.* 1988).

Dermatoses were observed among 19 workers in the department of a chemical plant which produces melamine starting from calcium cyanamide via cyanamide and dicyanodiamide. Therefore, patch tests according to Jadassohn and Bloch were carried out with a 1% dicyanodiamide preparation in petrolatum or with petrolatum alone as a negative control on the persons affected and on 61 persons with healthy skin (a total of 57 men and 23 women between 18 and 65 years old). The 19 workers affected (24%) were additionally subjected to a patch test for a standard group of allergens. Reactions to dicyanodiamide assessed as allergic by the authors were observed on 7 workers affected and on 2 of the healthy workers (Szczeklik-Franek and Masalska 1977).

5 Animal Experiments and *in vitro* Studies

5.1 Acute toxicity

5.1.1 Inhalation

5 male and 5 female Wistar rats were exposed to dicyanodiamide dust (> 99.5% purity) with a maximally achievable dicyanodiamide concentration of 259 mg/m^3 in whole-body exposure chambers for 4 hours. The mean flow rate of the dicyanodiamide dust through the exposure chamber was 1 m^3/h. 99% of the particles had a size of 0.6 to 2.4 µm. The animals were somewhat restless during the first 15 minutes after the beginning of exposure and slept during the remaining exposure period. No deaths or signs of intoxication occurred during treatment or the 14-day post-exposure observation period. Accordingly, the LC_{50} of dicyanodiamide is above 259 mg/m^3 (SKW 1977b).

5.1.2 Ingestion

10 male and 10 female Wistar rats were given 30 ml/kg body weight of an aqueous 33% dicyanodiamide suspension (10000 mg dicyanodiamide/kg body weight) once by gavage. Necropsy of the animals carried out after a 14-day post-exposure observation period did not indicate any treatment-related gross-pathological alterations. A few hours following administration, the rats showed humpback behaviour, were sluggish and occasionally had slight diarrhoea, which was no longer observed 20 hours after administration. All animals survived the treatment. Accordingly, the LD_{50} is above 10000 mg dicyanodiamide/kg body weight (SKW 1977a).

In a further study, an LD_{50} of higher than 10000 mg dicyanodiamide/kg body weight was also determined for male rats (no other details; ACC 1959).

5.1.3 Dermal absorption

A dose of 2000 mg dicyanodiamide/kg body weight was applied occlusively to the abraded, intact dorsal skin of 5 male and 5 female white New Zealand rabbits. All animals survived the treatment and showed no clinical signs during the 14-day post-exposure observation period. Slight erythema (degree 1) was detected in one rabbit only on the first day after application (SKW 1985a). In a further study, a dose of 10000 mg dicyanodiamide/kg body weight (aqueous paste) was applied once for 24 hours to the closely clipped abdominal skin (no other details). There were no signs of intoxication or local reactions (no other details; ACC 1959).

5.1.4 Intraperitoneal injection

After intraperitoneal injection of dicyanodiamide, the LD_{50} was specified to be above 4000 mg/kg body weight for mice and above 3000 mg/kg body weight for rabbits (no other details; Hald *et al.* 1952).

5.2 Subacute, subchronic and chronic toxicity

Rat

A group of 5 rats received 1000 mg dicyanodiamide/kg body weight and day in edible oil by gavage on five consecutive days. No adverse effects were observed (no other details; Dupont 1947).

In a 28-day range-finding study, dicyanodiamide was administered to groups of 10 male and 10 female Wistar rats in doses of 0, 200, 2000 and 20000 mg/kg diet. Uptake of dicyanodiamide of about 0, 23, 240 and 2350 mg/kg body weight and day for males and of about 0, 24, 240 and 2400 mg/kg body weight for females was determined on the basis of the body weight and feed consumption in the middle of the study. The rats revealed no clinical signs. No substance-induced alterations were observed in the low and intermediate exposure groups. The increased body weight of the females of the intermediate exposure group (2000 mg/kg diet), which was interpreted by the authors as an incidental finding, was probably also responsible for the statistically significant increase of the absolute, but not of the relative kidney weight. The body weight of the males was slightly, but not significantly reduced in the high exposure group (20000 mg/kg diet). Water consumption was significantly elevated in the males compared with the control group, whereas feed consumption revealed no substantial variations among the groups. The analysis of the haematological parameters showed a statistically significant decrease of the leukocyte count in the males, which, according to the authors, was due mainly to an extremely low value in one of the treated animals of this exposure group. When this value was ignored in the calculation, the leukocyte count was still reduced, but not statistically significantly compared with the control. No alterations were detected either by gross pathology or in the histopathological examination of the liver and kidneys. The NOAEL (no observed adverse effect level) specified by the authors is 2000 mg dicyanodiamide/kg diet corresponding to about 240 mg dicyanodiamide/kg body weight and day (SKW 1983).

In a 13-week range-finding study, groups of 10 male and 10 female F344 rats received dicyanodiamide in the diet in concentrations of 0, 12500, 25000, 50000 and 100000 mg/kg diet corresponding to about 0, 570, 1250, 2600 and 5800 mg/kg body weight and day for males and 0, 700, 1500, 3000 and 6800 mg/kg body weight and day for females based on the body weight at the end of the study and the overall substance absorption throughout the study period. No animal died during treatment. Initial effects occurred in the male and female rats at 12500 mg/kg diet and above. The platelet count was increased in both sexes, the leukocyte count was increased only in the males, and the urea concentration and the aspartate aminotransferase activity were elevated only in

the female rats. In addition, the absolute liver weight was reduced in female rats. Increased feed consumption and a decrease in the serum concentrations of total protein, total cholesterol and calcium and in the alanine aminotransferase activity were recorded in male rats at 50000 mg/kg diet. Females revealed reduced body weight, which led to a reduced absolute thymus weight, and their relative brain and heart weights were increased. A lowering of the total serum protein concentration and an increase in alkaline phosphatase activity were also detected. At 100000 mg/kg diet, the organ weights of heart and kidneys were changed in the males in accordance with the reduced body weight. The absolute and relative brain weights and alkaline phosphatase and cholinesterase activities increased. Feed consumption was higher in the females, and the relative spleen and kidney weights were increased. The concentration of total cholesterol in the serum was reduced. Histopathologically, intranuclear inclusion bodies in the proximal tubular epithelium of the kidneys, which in 2 of 10 males occurred even at 50000 mg/kg diet, were diagnosed in both sexes (Matsushima *et al.* 1991).

In a 13-week study, dicyanodiamide (100% pure) was administered to 10 male and 10 female Sprague-Dawley rats with the diet. The doses were 0, 240, 2400, 8000 and 24000 mg dicyanodiamide/kg diet, corresponding to a mean dicyanodiamide uptake of about 0, 16, 150, 550 and 1600 mg/kg and day for males and of about 0, 18, 200, 650 and 1900 mg/kg body weight and day for females. A satellite group of 10 male and 10 female Sprague-Dawley rats which were given 24000 mg dicyanodiamide/kg diet was examined 4 weeks after the end of treatment. The survival rate, clinical parameters, body weight, organ weights of liver, kidneys, adrenals and testes including epididymides and the opthalmological findings yielded no deviations between the control and treated animals. Feed consumption was only statistically significantly increased in the female rats treated with 2400 and 24000 mg dicyanodiamide/kg diet. The authors did not regard this change or the increase in total protein and albumin concentrations in male rats (2400 mg dicyanodiamide/kg diet) as being induced by the test substance. The gross-pathological examination showed no changes in the treated groups compared with the control group. Findings such as uterine dilation, mononuclear infiltrations in the mucosa of the urinary bladder, hydronephrosis and chronic progressive nephropathy were detected by histopathology, but these were not statistically significant and were not interpreted by the authors as being related to the test substance. The NOAEL is thus above 24000 mg dicyanodiamide/kg diet, corresponding to about 1600 mg dicyanodiamide/kg body weight and day for male rats and 1900 mg dicyanodiamide/kg body weight and day for female rats (SKW 1985b).

A short communication reported that the 26-week administration of 10000 mg dicyanodiamide/kg diet to rats caused no toxic effects. Compared with the control group, no abnormalities were detected either for body weight, feed consumption and the results of haematological determinations or for the gross-pathological and histopathological examinations (ACC 1959).

The investigation of the chronic toxicity of dicyanodiamide (> 99.8% purity) in Sprague-Dawley rats was combined with a study for carcinogenicity. In the chronic toxicity study, 20 rats per sex were treated with 50000 mg dicyanodiamide/kg diet corresponding to 1980–5200 mg dicyanodiamide/kg body weight and day (male rats) and 2850–6350 mg dicyanodiamide/kg body weight and day (female rats) for 52 weeks.

The corresponding control group consisted of 10 animals per sex. In the carcinogenicity study, groups of 50 male and 50 female rats received 0, 5000, 15000 and 50000 mg dicyanodiamide/kg diet corresponding to about 0, 170–490, 540–1480 and 1740–5110 mg dicyanodiamide/kg body weight and day (males) and 0, 210–580, 690–1760 and 2420–6370 mg dicyanodiamide/kg body weight and day (females) for 104 weeks. There were no differences between the groups treated with dicyanodiamide and the control group for the survival rate, feed consumption, the opthalmological findings or the results of the thyroid function tests. According to the authors, the clinical signs observed are not related to the treatment with dicyanodiamide. Effects were recorded only in the animals of the high exposure groups (50000 mg dicyanodiamide/kg diet) both in the carcinogenicity and in the chronic toxicity study. The body weight gains were statistically significantly reduced. The organ weight changes were probably due to the altered body weight – except for a statistically significant decrease in the relative liver weight and an increase in the relative adrenal weight. No histopathological findings were obtained. Changes in the clinicochemical and haematological parameters were not consistent between the chronic toxicity study and the carcinogenicity study or were not dose-dependent or were not observed at all, but only at some times of examination. Therefore, the authors do not regard the effects observed as being induced by the substance. Only the blood urea value in the male rats of the chronic toxicity and carcinogenicity study (50000 mg/kg diet) was increased in animals of both groups after 26 and 52 weeks. The NOAEL is specified to be 15000 mg dicyanodiamide/kg diet corresponding to about 540–1480 mg/kg body weight and day for male rats and 690–1760 mg/kg body weight and day for female rats (SKW 1992a).

In another carcinogenicity study, groups of 50 male and 50 female F344 rats received 0, 25000 and 50000 mg dicyanodiamide/kg diet corresponding to about 0, 850 and 1870 mg dicyanodiamide/kg body weight and day (males) and about 0, 1200 and 2350 mg dicyanodiamide/kg body weight and day (females) for 104 weeks. The animals were observed up to the 113th week. The survival rate yielded no differences between the control group and the treated groups. The dose-dependent decrease in body weight gain was statistically significant in the rats treated with 50000 mg dicyanodiamide/kg body weight. Within a 4-week treatment-free period after substance administration had ended, the animals of both exposure groups showed a considerable body weight gain compared with the control group. Feed consumption did not differ in the control or the treated animals. In the highest exposure group (50000 mg/kg diet), a statistically significant increase of slight bile duct hyperplasia was detected in the females and intranuclear eosinophilic bodies in the proximal tubular epithelium of the kidney were observed in the males (Yasuhara *et al.* 1997). On account of the low number of parameters examined and insufficient documentation, the study can be assessed only with reservations.

Dog

Groups of 4 male and 4 female beagles initially received dicyanodiamide (99.5% purity) in concentrations of 0, 10000, 25000 and 50000 mg/kg diet corresponding to about 0, 330, 900 and 1750 mg dicyanodiamide/kg body weight and day for males and 0, 350, 900 and 1600 mg dicyanodiamide/kg body weight and day for females for 4 weeks. Since the dogs did not eat enough food apparently for reasons of palatability, half of the

animals were sacrificed after 4 weeks and the remaining 2 male and female animals were given dicyanodiamide in capsules in doses of 0, 250, 625 and 1250 mg/kg body weight and day for 22 days. None of the animals died during the study. Apart from emesis, no other clinical signs were detected. The body weight gain was lower by 33% and 64% compared with the control group in the male and female dogs of the highest dose group, respectively, between the 1st and 4th weeks. Thus, the feed consumption in this dose group was lower by 18% in the males and by 10% in the females. The analysis of clinicochemical and haematological parameters revealed sporadic, statistically signify-cant changes in the form of increased haemoglobin and haematocrit values in the pre-treatment period and reduced inorganic phosphorus, potassium and chloride concentrations after the 4-week treatment period, but the authors did not assess these effects as being due to treatment. The absolute and relative weights of the testes including the epididymides were slightly, but not statistically significantly reduced in 2 male dogs at 25000 mg dicyanodiamide/kg diet and above. No gross-pathological or histopathological findings were obtained. Since the body weight and a number of clinicochemical and haematological parameters varied considerably in the pre-treatment period so that substance-induced effects could not be identified and since the number of animals was limited in the further course of the study, this study cannot be used for the present assessment (SKW 1991).

A short communication reported that the 37-day administration of 10000 mg dicyanodiamide/kg diet to dogs caused no toxic effects. Compared with the control group, no abnormalities were detected for body weight or feed consumption, or in the haematological analysis or for the gross-pathological or histopathological examinations (ACC 1959).

Rabbit

A range-finding study for a pilot teratogenicity study was carried out in white New Zealand rabbits. For this purpose, 10 non-pregnant animals received 1000 mg dicyanodi-amide/kg body weight and day in an aqueous carboxymethyl cellulose suspension by gavage on 5 consecutive days. All rabbits survived. The clinical signs and the gross-pathological findings in the visceral thorax, abdomen and pelvis showed no abnormali-ties. Body weight was reduced between days 4 and 5 of exposure. Feed consumption was similar on all treatment days (SKW 1989).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

The effects on skin were investigated on 12 white New Zealand rabbits by applying 500 mg dicyanodiamide to the intact (6 animals) and abraded skin (6 animals) by means of an occlusive adhesive bandage. The duration of exposure was 24 hours. After this time, one animal with intact skin and two animals with abraded skin showed slight erythema, but no oedema. After a further 48 hours (72 hours after the beginning of

application), slight scaling was observed in 2 animals with abraded skin. The authors assess dicyanodiamide as virtually not irritating to the skin of rabbits (SKW 1977c).

Skin irritation resulted from the 24-hour occlusive application of 10000 mg dicyanodiamide/kg body weight (aqueous paste) to the abdominal skin of rabbits (no other details; ACC 1959).

5.3.2 Eyes

According to earlier data which were not specified in more detail, small amounts of dry dicyanodiamide were not irritating to the eyes of rabbits (ACC 1959).

Another eye irritation study was carried out on 6 white New Zealand rabbits; after instillation of 100 mg dicyanodiamide into the lower eyelid, both eyelids were kept closed for about 1 second to avoid a loss of substance. The eyes were not rinsed afterwards. Treatment led to slight iritis and corneal opacity in one animal within 24 hours and to moderate redness and swelling of the conjunctiva; in the remaining 5 animals, slight redness and swelling of the conjunctiva were observed. These reactions had subsided after one week except for slight redness of the conjunctivae in 2 rabbits. Dicyanodiamide is not classified as irritating to the eyes according to the FDA standard (SKW 1977d).

5.4 Allergenic effect

The Landsteiner/Draize test was carried out on 16 guinea pigs to investigate the sensitizing potential; for induction, 8 animals initially received 0.05 ml intradermally and then 0.1 ml of 2% dicyanodiamide in physiological saline on three days of the following three weeks. Two weeks after the last injection, the treated and the 8 control animals were given a further injection of 0.05 ml of 2% dicyanodiamide in physiological saline. Slight irritation during the treatment and challenge phases was seen in all animals treated. 24 hours after the challenge treatment, only one guinea pig of the control group, but no other animal showed a reaction. Dicyanodiamide was therefore classified as non-sensitizing (SKW 1977e), but no reading was carried out at later dates.

In a maximization test, the sensitizing effect of dicyanodiamide was investigated in 20 guinea pigs, with an aqueous 1.75% (w/w) preparation with Freund's complete adjuvant being used for intradermal induction and an aqueous 25% (w/w) solution for epicutaneous induction. The reactions to 0.5, 2.5 and 5% dicyanodiamide showed no significant differences between treated and control animals immediately after the 24-hour occlusive challenge treatment carried out on the 21st day or 24 and 48 hours later (Boman *et al.* 1985).

In a study which was not described in more detail, dicyanodiamide was tested for sensitization in the Freund's complete adjuvant test on 10 guinea pigs. No sensitizing potential was observed (Senff *et al.* 1988).

5.5 Reproductive toxicity

5.5.1 Fertility

In a 2-generation study with 26 male and 26 female CD®BR rats, dicyanodiamide (99.5% purity) was administered in doses of 0, 5000, 15000 and 50000 mg/kg diet. In the F_0 and F_1 parental generations, this corresponded to about 0, 310–370, 940–1170 and 3220–4130 mg dicyanodiamide/kg body weight and day (male) and about 0, 390–450, 1200–1360 and 4160–4950 mg/kg body weight and day (female) in the pre-mating period (14 weeks), to about 0, 330, 1000–1030 and 3650–3760 mg dicyanodiamide/kg body weight and day in the female rats in the gestation phase (21 days) and about 0, 490–570, 1670–1770 and 5600–5760 mg dicyanodiamide/kg body weight and day in the lactation phase. The clinical signs observed (pigmented lacrimation, ruffled fur, alopecia, ataxia, anorexia, tremor, inflammations, movable masses (no other details), swelling of the genitals and mammary gland, etc.) only sporadically occurred in the treated F_0 and F_1 parental generations and were interpreted by the authors as not being due to treatment. Body weight and body weight gain were reduced in the males of the F_0 generation from 15000 mg dicyanodiamide/kg diet. The body weights of the females of the F_0 generation and of the F_1 parental generation were significantly reduced only at 50000 mg dicyanodiamide/kg diet. The feed consumption of the treatment groups of the F_0 and F_1 parental generations showed significant deviations from the control group, but these occurred sporadically, were not dependent on the dose and were thus not regarded by the authors as being substance-induced. The gross-pathological and histopathological examinations revealed uterine dilation in the F_0 parental animals of the highest exposure group and dilation of the renal pelvis not related to the dose in the male parental animals of the F_1 generation. The fertility indices were statistically not significantly reduced in the highest exposure group. There was no impairment of the pregnancy indices in any of the treated F_0 and F_1 groups. The number of F_1 and F_2 live-born pups was reduced in the highest exposure group. The body weight of the pups of the F_1 generation was statistically significantly reduced at 15000 mg/kg diet on day 21 after birth and at 50000 mg/kg diet from day 0 to day 21 after birth, whereas a statistically significant body weight reduction was detected in the F_2 pups of the 50000 mg/kg group on the day of parturition and on days 14 to 21 after birth. Weakness and dehydration in the treated offspring of the F_1 and F_2 generations, and additionally swelling of the mammary gland in the F_2 offspring, were observed as clinical signs, but these were not related to the dose or statistically significant. Dilated renal pelvis found in the pups of the F_1 and F_2 generations was neither related to the dose nor statistically significant and was regarded by the authors as not being due to treatment. The NOAEL of this study is 5000 mg dicyanodiamide/kg diet (SKW 1992b).

5.5.2 Developmental toxicity

In a range-finding study on teratogenicity, groups of 6 female CD®BR rats were given dicyanodiamide (99.5% purity) from days 6 to 15 of gestation in doses of 0, 250, 500, 1000 and 2000 mg/kg body weight and day in 0.5% aqueous carboxymethyl cellulose by gavage. On day 20 of gestation, the dams were sacrificed, and the uterus and the ovaries were examined. The foetuses were weighed and examined for external changes. The dams showed no clinical abnormalities. The maternal body weight gain was slightly reduced (about 7–12%) in the highest dose group. The weight of the gravid uterus was not changed as compared with the control group. The gross-pathological examination of the dams revealed no changes. The number of *corpora lutea* increased with the dose. The preimplantation loss and thus the implantation efficiency of 4.2, 5.0, 22.0, 18.3 and 9.4% (corresponding to 0, 250, 500, 1000 and 2000 mg/kg body weight and day) was not increased in relation to the dose in the litters of the treated groups. A slight, but not significant increase of resorptions (postimplantation losses) was observed in the lowest and highest dose groups. The body weight of the foetuses was slightly reduced in the highest dose group (about 4%). There were no abnormal external changes in the foetuses (SKW 1990).

5.6 Genotoxicity

In vitro

In a *Salmonella* mutagenicity test, the *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 were incubated with up to 25 mg dicyanodiamide in dimethyl sulfoxide (DMSO)/plate in the presence and absence of metabolic activation. The S9 fraction from male Wistar rats treated with Aroclor 1254 was used as a metabolic activation system. No toxic effects were observed up to the solubility limit of 250 mg dicyanodiamide/ml. Dicyanodiamide was not mutagenic in the cultures investigated compared with the positive controls sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-aminoanthracene (SKW 1984).

In an assay carried out to test the mutagenic activity of antiphytoviral substances, dicyanodiamide was tested in the *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100. The concentrations were in a range between 0 and 10 mg dicyanodiamide/plate. Whereas the *Salmonella typhimurium* strains TA1535, TA1537 and TA1538 were investigated only in the absence of metabolic activation, the strains TA98 and TA100 were also tested with metabolic activation of the S9 fraction from mouse liver or of the S14 fraction from maize seedlings. Dicyanodiamide was mutagenic only in the strain TA98 in the presence of the S14 plant fraction. No mutagenic potential was detected in any of the other cultures treated with dicyanodiamide, as opposed to the different positive controls (El-Tarras *et al.* 1989).

A DNA repair test with primary hepatocytes of male Fisher-344 rats was used as a further *in vitro* test. The test concentrations of dicyanodiamide (in DMSO) were between

1 and 5000 µg/ml medium. There was no increased incorporation of ^{3}H-thymidine into the DNA of rat hepatocytes in the dicyanodiamide concentrations used or in the negative control (DMSO), as opposed to the positive control (0.05 µg 2-acetylaminofluorene/ml medium) (SKW 1985c).

In an HPRT (hypoxanthine guanine phosphoribosyl transerase) mutagenicity test, CHO cells (a cell line from Chinese hamster ovary) were incubated with concentrations of 1000 to 5000 µg dicyanodiamide/ml medium with and without metabolic activation (no other details). No increased mutation frequencies occurred in the cultures treated with dicyanodiamide or in the negative controls (DMSO or medium) either in the absence or in the presence of metabolic activation, whereas the positive control led to a significantly increased mutation rate (SKW 1985e).

CHO cells were investigated for chromosomal aberrations after treatment with concentrations of 20 to 2000 µg dicyanodiamide/ml medium in the absence and presence of metabolic activation. No cytotoxic effects were observed up to 2000 µg dicyanodiamide/ml medium. The cytogenetic analysis showed a significant increase of chromosomal aberrations only in the cultures treated with the positive controls, but not in the medium control or in the cultures treated with dicyanodiamide or DMSO (SKW 1985d).

There are no data available for the genotoxicity of dicyanodiamide *in vivo*.

5.7 Carcinogenicity

The carcinogenic potential was investigated in 50 CD®BR rats per sex, which received 0, 5000, 15000 and 50000 mg dicyanodiamide/kg diet for 104 weeks. This study and the results of a chronic toxicity study are presented in Section 5.2. No increased occurrence of tumours was observed (SKW 1992a).

In another carcinogenicity study, groups of 50 male and 50 female F344 rats received 0, 25000 and 50000 mg dicyanodiamide/kg diet corresponding to about 0, 850 and 1870 mg dicyanodiamide/kg body weight and day (males) and 0, 1200 and 2350 mg dicyanodiamide/kg body weight and day (females) for 104 weeks (see Section 5.2). The total tumour incidence of the treated groups was not different from that of the control group. The tumour rate of bronchioalveolar adenomas was statistically significantly increased in the male rats treated with 50000 mg/kg diet (4/49), but not in the females (0/49) compared with the control group. No bronchioalveolar adenocarcinomas were found. According to the authors, the increased incidence of adenomas was in the range of historical control data for this rat strain (no other details) and is therefore regarded as not being induced by the substance. The authors concluded that dicyanodiamide has no carcinogenic potential (Yasuhara *et al.* 1997).

6 Manifesto (MAK value/classification)

Results of studies of persons exposed to dicyanodiamide which would be suitable for the derivation of a MAK value are not available. In a series of mid-term and long-term studies in rats, no specific effects occurred even at high doses. There were only non-specific effects such as a reduction of body weight gain, changes in clinicochemical and haematological parameters and some sporadically occurring histopathological findings. No carcinogenic potential of dicyanodiamide was detected in long-term studies. A NOAEL of 240 mg dicyanodiamide/kg body weight and day was derived from a 4-week range-finding study in rats (SKW 1983). The NOAELs determined in a 13-week study and a carcinogenicity study are approximately between 500 and 2000 mg dicyanodiamide/kg body weight and day (see Section 5.2). At a dose level of 240 mg/kg body weight and day, an airborne concentration of 1610 mg dicyanodiamide/m^3 air is calculated in relation to a body weight of 70 kg and an amount of 10 m^3 air inhaled during 8 hours. If this dicyanodiamide concentration were inhaled, too much strain would be placed on the clearance function of the lungs resulting in dust overload and thus in lung changes. A health-based MAK value can not be derived for dicyanodiamide. Dicyanodiamide is therefore classified in Section IIb of the *List of MAK and BAT Values*. On the basis of the information mentioned above, neither toxic effects nor lung changes are expected to be induced by the slightly soluble dicyanodiamide if the general threshold limit value for dust (valid however for insoluble substances) is observed.

On account of the low systemic toxicity and the high dermal LD_{50}, which does not indicate good absorption through the skin, dicyanodiamide is not designated with “H”.

Only a few, incompletely documented findings in humans are available on sensitization to the skin. The results of the animal studies do not indicate any notable sensitization. Skin sensitization thus cannot be assessed conclusively. The substance is not designated with “Sh”. There are no data available for sensitizing effects on the respiratory tract. The substance is therefore not marked with “Sa” either.

No data are available which would justify a classification of dicyanodiamide in one of the categories for germ cell mutagens.

7 References

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