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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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Dicyclo-hexylamine

No. 212

CAS No. 101-83-7

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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Dicyclohexylamine

1 Summary and assessment

Dicyclohexylamine is absorbed from the gastrointestinal tract as well as via the skin and the respiratory tract. Following oral administration to rats, 26 to 44% of a single dicyclohexylamine dose of 17.5 mg/animal is excreted unchanged in urine within 3 days.

Upon acute oral administration, dicyclohexylamine has proved harmful and on acute dermal application it has been shown to be toxic (LD_{50} rat oral approx. 200 to 373 mg/kg body weight; LD_{50} rabbit dermal > 200 and < 316 mg/kg body weight). Clinical signs of toxicity seen after acute oral and dermal treatment include depressed general condition, dyspnoea, sedation as well as convulsions, while hyperaemia, haemorrhages in the lungs, discoloration of the liver and gastrointestinal inflammation are reported as autopsy findings. Terminal necropsy of the animals surviving to the end of the study was without findings. In inhalation risk tests, 8- and 6-hour exposure of rats to dicyclohexylamine-saturated test atmospheres generated at 20 and 27 °C, respectively, (according to the investigators the concentration at 27 °C was approx. 1400 mg/m³) was not lethal and caused only mild mucous membrane irritation and lethargy during exposure. In mice, concentration levels as low as ≥ 150 mg/m³ are reported to have been lethal after 1 to 2 hours.

Serious inadequacies in study conduct and documentation render the available 30-day inhalation studies in the rat and mouse unsuitable for the assessment of the systemic effects of dicyclohexylamine following repeated exposure.

When applied to the skin of rabbits, dicyclohexylamine causes corrosion which is associated with erythema and oedema formation and is dependent on the duration of exposure. If exposure lasts 4 hours or longer, full-thickness necrosis of the skin occurs. A 1-hour application results in superficial necrosis. Following a 3-minute application, no necrotic changes are observed, but there is well-defined reddening of the skin. Dicyclohexylamine is corrosive to the rabbit eye.

From the Salmonella/microsome test in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, the umu test conducted in

Salmonella typhimurium strain TA 1535/pSK1002 and the DNA synthesis inhibition test in HeLa S3 cells, there are no indications that dicyclohexylamine has a genotoxic potential.

The available long-term carcinogenicity studies with approx. 12-month oral and subcutaneous administration to rats of unspecified strain and the D mouse, respectively, are unsuitable for the assessment of the carcinogenic potential of dicyclohexylamine due to serious inadequacies in study conduct and documentation.

In cell transformation tests using human lung fibroblasts (W1-38), human liver cells (Chang) as well as kidney cells of the Syrian hamster (BHK-21 C13), dicyclohexylamine does not cause transformation either in the absence or presence of metabolic activation.

Without giving further details, one report states that a 60-minute inhalation exposure to dicyclohexylamine at 388 mg/m³ caused severe toxic damage in man.

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 dicyclohexylamine to Section IIb of the List of MAK and BAT Values, the section covering "substances for which no MAK value can be established at present". Because of the danger of percutaneous absorption, the chemical has been designated with "H".

On behalf of the Japanese Ministry of Health and Welfare, dicyclohexylamine is currently being investigated in a subchronic toxicity study, a preliminary study to a reproductive toxicity study, a Salmonella/microsome test as well as a chromosome aberration test.

2 Name of substance

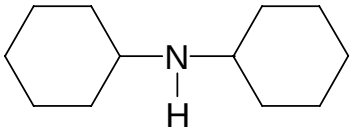
2.1	Usual name	Dicyclohexylamine
2.2	IUPAC name	N,N-Dicyclohexylamine
2.3	CAS No.	101-83-7
2.4	EINECS No.	202-980-7

3 Synonyms, common and trade names

Aminodicyclohexane
Cyclohexanamine, N-cyclohexyl-
N-Cyclohexylcyclohexanamine
N-Cyclohexylcyclohexylamine
DCHA
di-CHA
DICHA
Dicyclohexylamin
N,N-Dicyclohexylamine
Dodecahydrodiphenylamine
Inhibitor HO 735

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_{12}H_{23}N$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	181.32	
5.2	Melting point, °C	–0.1	(EC, 1996; Lide and Frederikse, 1996)
5.3	Boiling point, °C	133 (at 26 hPa)	(Heilen et al., 1985)
		130–134 (at 27 hPa)	(BASF, 1987)
		255.8 (at 1013 hPa; decomposition)	(EC, 1996)
		256 (decomposition)	(Lide and Frederikse, 1996)
5.4	Vapour pressure, hPa	0.1 (at 20 °C)	(BASF, 1987)
		1 (at 65 °C)	
		12 (at 114 °C)	(EC, 1996)

5.5	Density, g/cm³	0.91 (at 20 °C) (BASF, 1987) 0.911–0.913 (at 20 °C) (BASF, 1985 a) 0.9123 (at 20 °C) (Lide and Frederikse, 1996) 0.91 (at 25 °C) (EC, 1996)
5.6	Solubility in water	0.8 g/l (at 25 °C) (EC, 1996) 0.16% (at 28 °C) (Heilen et al., 1985)
5.7	Solubility in organic solvents	Miscible with nearly all usual solvents (BASF, 1985 a; Falbe and Regitz, 1990; Heilen et al., 1985) Soluble in ethanol, diethyl ether and acetone (Lide and Frederikse, 1996)
5.8	Solubility in fat	Partition coefficient n-octanol/water, log P_{ow}: 3.5 (calculated) (EC, 1996)
5.9	pH value	Ca. 11.5 (at 1 g/l and 20 °C) (Bayer, 1992)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 7.40 mg/m³ 1 mg/m³ ≙ 0.135 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

Intermediate for use in producing corrosion inhibitors, insecticides, paper and textile auxiliaries, emulsifiers, oil additives, vulcanisation accelerators, plasticisers and dyestuff precursors (BASF, 1985 a; Falbe and Regitz, 1990).

7 Experimental results

7.1 Toxicokinetics and metabolism

According to an older report (Filov, 1968), dicyclohexylamine can be absorbed via the skin, the respiratory tract and the gastrointestinal tract. The investigator demonstrated that in 2 rats which had inhaled dicyclohexylamine (no details of the duration or level of exposure) there was an increase in blood amine level. In one rabbit receiving an oral dicyclohexylamine dose of 85 mg/kg body weight as well as 2 other rabbits which had their ears im-

mersed in dicyclohexylamine for 3 hours, no increase in amine concentration was measured in the blood, but amine excretion in urine was observed to be increased. The orally treated animal died 160 minutes after dosing. Quantitative excretion data were reported for a study in 5 rats receiving single oral doses of 17.5 mg dicyclohexylamine per animal (equivalent to 62.5 to 106 mg/kg body weight). Urinary excretion of unchanged compound measured over a period of 3 days amounted to 26 to 44% of the administered dose, the predominant portion of which was excreted within the first 24 hours (no further details; Filov, 1968).

Absorption of dicyclohexylamine via the skin of rabbits was also reported without any further details by Carswell and Morrill (1937).

7.2 Acute and subacute toxicity

Acute toxicity

The results from studies investigating the acute toxicity of dicyclohexylamine following oral and invasive administration, dermal application and inhalation exposure are compiled in Table 1.

Beginning of Table 1

Table 1. Acute toxicity of Dicyclohexylamine

Species, strain, sex*	Route	Dose (mg/kg body weight or mg/m³)	Purity	Effects	Observation period	Reference
Rat, Wistar, male	oral	ca. 200 (0.22 ml)	n. d.	LD_{50}; tonic convulsions, sedation, depressed general condition, lethal within 2 days	14 days	Bayer, 1978
Rat	oral	ca. 237 (260 µl)	pure	LD_{50}; reeling, dyspnoea, tonic-clonic convulsions; no autopsy findings	7 days	BASF, 1965
Inhibitor HO 735 was used as the test substance.						
Rat, Sprague-Dawley, male, female	oral	240	n. d.	LD_{50}; reduced food consumption and activity, weakness, lethal within one day; necropsy of deceased animals: haemorrhages in the lungs, discoloration of the liver, gastrointestinal inflammation; terminal necropsy: no findings	14 days	Younger Laboratories, 1977
Rat, Wistar, male	oral	373	99.4%	LD_{50}; agitation, convulsions, lethal within 24 hours; necropsy: hyperaemia	14 days	Marhold et al., 1967
Rat, albino	oral	300–500	n. d.	300 mg: maximum non-lethal dose; 500 mg: minimum lethal dose; convulsions, laboured breathing in isolated cases	n. d.	Lomonova, 1963
Rabbit, New Zealand, male, female	dermal	> 200 < 316	n. d.	LD_{50}; mortality was 1/5 at 200 mg/kg body weight and 1/1 at 316 and 501 mg/kg body weight, death within 16 hours; reduced food consumption and activity, weakness; necropsy of deceased animals: haemorrhages in the lungs, discoloration of the liver, gastrointestinal inflammation, enlarged gall bladder; terminal necropsy: no findings	14 days	Younger Laboratories, 1977
Rat	inhalation	atmosphere generated at 20 °C, enriched or saturated (8 hours)	pure	mortality: 0/12; mild mucous membrane irritation; no autopsy findings	n. d.	BASF, 1965
Inhibitor HO 735 was used as the test substance.						
Rat, Sprague-Dawley, male	inhalation	saturated atmosphere (ca. 1.4 mg/l) generated at 27 °C (6 hours)	n. d.	mortality: 0/6; slight lethargy during exposure; no autopsy findings	14 days	Younger Laboratories, 1977
Mouse, albino	inhalation	110, 150 and 250 (no details as to duration of exposure)	n. d.	110 mg/m³: no findings; 150 mg/m³: minimum lethal concentration; 250 mg/m³: absolutely lethal concentration; convulsions, restlessness, increased motor activity, death after exposure for 1 to 2 hours	n. d.	Lomonova, 1963
Mouse, D, male, female	subcutaneous	135	n. d.	LD_{50}	n. d.	Pliss, 1958

Table 1. Acute toxicity of Dicyclohexylamine						
Species, strain, sex*	Route	Dose (mg/kg body weight or mg/m³)	Purity	Effects	Observation period	Reference
Mouse	intraperitoneal	ca. 82 (90 µl)	pure	LD_{50}; reeling, dyspnoea, tonic-clonic convulsions; no autopsy findings	7 days	BASF, 1965
Inhibitor HO 735 was used as the test substance.						
Rabbit	injection (no further details)	500–1000	n. d.	500 mg: not lethal, convulsions, temporary hindlimb paralysis; 1000 mg: convulsions ending in death	n. d.	Carswell and Morrill, 1937
* where specified n. d. no data						

End of Table 1

With LD_{50} values determined in the rat ranging from approx. 200 to 373 mg/kg body weight, dicyclohexylamine has proved harmful following acute oral administration (see Table 1; BASF, 1965; Bayer, 1978; Marhold et al., 1967; Younger Laboratories, 1977). Following acute dermal application, dicyclohexylamine was toxic as indicated by an inaccurately determined LD_{50} value of > 200 but < 316 mg/kg body weight in the rabbit (Younger Laboratories, 1977). Depressed general condition, dyspnoea, sedation and convulsions were reported *inter alia* as signs of intoxication following acute oral administration and dermal application. Necropsy of the animals which died revealed hyperaemia, haemorrhages in the lungs, discoloration of the liver and gastrointestinal inflammation or was without findings, as was terminal autopsy of the survivors (see Table 1). The findings after inhalation exposure do not present a consistent picture. On the one hand, 8- and 6-hour exposure of rats to dicyclohexylamine-saturated test atmospheres generated at 20 and 27 °C, respectively, (according to the investigators the concentration at 27 °C was approx. 1400 mg/m³) caused only mild mucous membrane irritation and lethargy during exposure; no deaths occurred among the 12 and 6 animals exposed, respectively, and there were no abnormal findings at autopsy (BASF, 1965; Younger Laboratories, 1977). On the other hand, concentration levels as low as ≥ 150 mg/m³ reportedly had lethal effects in mice after 1 to 2 hours (Lomonova, 1963). The LD_{50} values determined for subcutaneous and intraperitoneal administration of dicyclohexylamine to mice were 135 and appox. 82 mg/kg body weight, respectively (BASF, 1965; Pliss, 1958). In rabbits, injection (no further details) of 500 mg dicyclohexylamine/kg body weight caused convulsions, and a dose of 1000 mg/kg body weight was lethal (Carswell and Morrill, 1937).

Subacute toxicity

Rats and mice (albinos, no details of strain, number or sex) were subjected to daily 2-hour exposures to dicyclohexylamine at 110 mg/m³ for 30 days. In mice, the treatment did not result in any visible changes, and a functional test carried out at the end of the exposure period (forced swimming test) gave no abnormal results. The rats exhibited increased drowsiness in the first week of the study, and macroscopic and histopathological examination at study termination revealed dystrophic changes in the liver and kidney. No alterations in haematological parameters, prothrombin time or

organ weights were observed in the rats (Lomonova, 1963). Considerable inadequacies in the conduct and/or reporting of the study (e.g. no information is given on the number, strain or sex of the animals exposed and there are no details of the scope of examination, control groups or analytical verification of the concentration tested), render this study unsuitable for the assessment of the systemic effects of dicyclohexylamine following repeated exposure.

7.3 Skin and mucous membrane effects

The data on skin and mucous membrane effects of dicyclohexylamine are summarised in Tables 2 and 3.

When applied to the skin of rabbits, dicyclohexylamine caused corrosion which was associated with erythema and oedema formation and was dependent on the duration of exposure. Exposure lasting 4 hours or longer resulted in full-thickness necrosis of the skin. Superficial necrosis was observed following a 1-hour semi-occlusive application. A 3-minute application was not found to cause any necrotic changes, but well-defined reddening of the skin was observed (see Table 2; BASF, 1965, 1985 a, c; Bayer, 1979; Lomonova, 1963; Younger Laboratories, 1977).

Beginning of Table 2

Table 2. Skin irritancy of dicyclohexylamine

Species	Guideline and/or dose, duration, mode*	Findings	Reversibility	Assessment (by the authors)	Reference
Rabbit	short-term test, undiluted compound, dorsal skin, one minute	questionable reddening after 24 hours	mild scaling after 8 days, no further examination after day 8	mildly irritating	BASF, 1965, 1985 a
Inhibitor HO 735 (dicyclohexylamine pure) was used as the test substance.					
Rabbit	short-term test, 500 µl undiluted compound, dorsal skin, 3 minutes semi-occlusive	well-defined reddening, no oedema formation, irritation indices: 0.6 for erythema and 0.0 for oedema formation (mean values of 3 animals)	fully reversible after 72 hours in 2 animals, still very slight reddening in the third animal, no further examination after 72 hours	no information	BASF, 1985 c
Rabbit	short-term test, undiluted compound, dorsal skin, 5 minutes	slight reddening after 24 hours	mild scaling after 8 days, no further examination after day 8	mildly irritating	BASF, 1965, 1985 a
Inhibitor HO 735 (dicyclohexylamine pure) was used as the test substance.					
Rabbit	short-term test, undiluted compound, dorsal skin, 15 minutes	slight reddening after 24 hours	heavy scaling after 8 days, no further examination after day 8	mildly irritating	BASF, 1965, 1985 a
Inhibitor HO 735 (dicyclohexylamine pure) was used as the test substance.					
Rabbit	short-term test, 500 µl undiluted compound, dorsal skin, one hour semi-occlusive	well-defined to severe reddening and moderate to severe oedema extending beyond the area of exposure, irritation indices: 3.6 for erythema and 1.7 for oedema formation (mean values of 3 animals)	not reversible within 72 hours: superficial skin necrosis, well-defined to severe reddening as well as very slight to well-defined oedema extending beyond the area of exposure, haemorrhages in 1/3 animals, no further examination after 72 hours	no information	BASF, 1985 c
Rabbit	OECD guideline No. 404, 500 µl undiluted compound, dorsal skin, 4 hours semi-occlusive	moderate to severe reddening and oedema extending beyond the area of exposure, haemorrhages, irritation indices: 3.7 for erythema and 1.7 for oedema formation (mean values of 3 animals)	not reversible within 72 hours: full-thickness skin necrosis, moderate to severe erythema formation as well as slight oedema formation extending beyond the area of exposure, no further examination after 72 hours	no information	BASF, 1985 b
Rabbit	dorsal skin, undiluted substance, 20 hours	anaemia, severe necrosis and severe oedema after 24 hours	very severe necrosis after 8 days, no further examination after day 8	corrosive or severely irritating, depending on study	BASF, 1965, 1985 a
Inhibitor HO 735 (dicyclohexylamine pure) was used as the test substance.					

Table 2. Skin irritancy of dicyclohexylamine					
Species	Guideline and/or dose, duration, mode*	Findings	Reversibility	Assessment (by the authors)	Reference
Rabbit	skin of the ear, undiluted substance, 20 hours	anaemia, severe necrosis and mild oedema after 24 hours	very severe oozing necrosis after 8 days, no further examination after day 8	corrosive or severely corrosive, depending on study	BASF, 1965, 1985 a
Inhibitor HO 735 (dicyclohexylamine pure) was used as the test substance.					
Rabbit	500 µl undiluted substance, 24 hours semi-occlusive	No information	observation period 7 days, no details of findings	extremely irritating, corrosive	Bayer, 1979
Rabbit	500 µl undiluted substance, 24 hours	corrosion	loosening of scab after 17 to 21 days revealed injury in depth	corrosive (according to the Federal Hazardous Substances Act)	Younger Laboratories, 1977
Rabbit	500 mg undiluted substance, 24 hours	no information	no information	severely irritating	EC, 1996; Marhold, 1972
Rabbit	20 mg and more	immediate necrosis formation	necrosis, usually with inflammation at the edges, healed after 2 to 4 weeks	no information	Lomonova, 1963
* where indicated					

End of Table 2

Based on the findings in the rabbit eye, dicyclohexylamine was evaluated as being corrosive to the cornea and as causing mild to severe irritation. Following instillation of 100 and 50 µl dicyclohexylamine into the rabbit eye, moderate to severe reddening and oedema formation, copious discharge, slight cloudiness of the cornea and a sluggish reaction of the iris to light were observed. In one study, the findings were fully reversible after 14 days, and in a further study a tendency towards reversibility became apparent after 8 days in as much as there still was severe reddening, but oedema formation and corneal cloudiness were no longer seen (see Table 3; BASF, 1965, 1985 a; Bayer, 1979; Younger Laboratories, 1977).

Table 3. Mucous membrane effects of dicyclohexylamine					
Species	Guideline and/or dose, mode, duration*	Findings	Reversibility	Assessment (by the authors)	Reference
Rabbit	100 µl undiluted substance, 24 hours	up to 24 hours after instillation moderate to severe reddening, mild oedema, copious discharge and barely perceptible to slight corneal cloudiness, sluggish iris reaction to light	improvement from 48 hours after treatment, fully reversible after 14 days	moderately irritating (irritation index of 32.6 of a maximum of 110)	Younger Laboratories, 1977
Rabbit	100 µl undiluted substance	no information	observation period 7 days, no details of findings	corrosive to the cornea, mildly irritating	Bayer, 1979
Rabbit	50 µl undiluted substance	slight reddening, severe oedema formation and slight corneal cloudiness after one hour; severe reddening and oedema formation as well as slight cloudiness after 24 hours	severe reddening still present at day 8, no further examination after day 8	severely irritating	BASF, 1965, 1985 a
Rabbit	0.75 mg undiluted substance, 24 hours	no information	no information	severely irritating	EC, 1996; Marhold, 1972
* where specified					

7.4 Sensitisation

No information available.

7.5 Subchronic and chronic toxicity

No information available.

7.6 Genotoxicity

7.6.1 In vitro

Dicyclohexylamine (no indication of purity) was tested for mutagenic potential in the Salmonella/microsome assay using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1538 and concentration levels ranging from 4 to 2500 µg/plate (2 plates/concentration). The test, which was conducted as a standard-plate incorporation test with metabolic activation (S9 mix from Aroclor 1254-induced rat liver) only, gave no indication of a genotoxic potential of dicyclohexylamine. No bacteriotoxicity data were reported for the concentrations investigated (Purchase et al., 1978).

A Salmonella/microsome assay carried out as a preincubation test in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 with and without metabolic activation (S9 mix from Aroclor 1254-induced rat and hamster liver), also gave negative results for dicyclohexylamine. The test was performed with dicyclohexylamine of > 99% purity at concentration levels of 10 to 10000 µg/plate (3 plates/concentration with independent repetition). Concentrations ≥ 3333 µg/plate were toxic to the bacteria (Mortelmans et al., 1986).

The DNA-damaging effect of dicyclohexylamine was investigated in the umu test using *Salmonella typhimurium* strain TA 1535/pSK1002 and, in parallel, in the DNA synthesis inhibition test in HeLa S3 cells. Both tests, which were performed without metabolic activation in sets of 3 plates/concentration with 3 independent repeats and covered a concentration range of 0.6 to 20 mM dicyclohexylamine (approx. 109 to 3626 µg/ml, > 98% pure), gave no indication that dicyclohexylamine had a damaging effect on

DNA. No bacteriotoxicity or cytotoxicity data were reported for the concentrations investigated (Heil et al., 1996).

In an early cytogenetic study, the lymphocytes from human blood samples incubated with dicyclohexylamine sulfate for 5- and 24-hours showed a concentration-dependent increase in aberration rate from approx. 6% in the controls to approx. 16%. The test concentrations were 10^{-5}, 10^{-4} and 10^{-3} M (Stoltz et al., 1970). However, it is not possible on the basis of this study to arrive at any definite conclusion regarding the clastogenic potential of dicyclohexylamine, as in respect of study conduct and evaluation, there are considerable deviations from present standards (cf. OECD guideline No. 475; Madle et al., 1993). The investigators reported that the observed aberrations essentially consisted in gaps and breaks and, in deviation from current evaluation practice, they included the gaps in their calculation of aberration rates. It is not possible *post hoc* to estimate the aberration rates without including the gaps, as the frequencies of the various types of aberration were not reported. Although parallel cultures were tested, only 50 cells/culture were evaluated and pooled for calculation of the aberration rate. The investigators did not carry out any independent repeats of the experiment, nor did they include any positive or negative control substances in the tests. Moreover, no historical control data were given, and details of the purity of the test substance and the cytotoxic effect of the test concentrations are also lacking.

7.6.2 In vivo

No information available.

7.7 Carcinogenicity

Twenty-five male and 25 female rats of unspecified strain were given repeated subcutaneous dicyclohexylamine doses of 30 mg/animal (in oil, approx. 120 mg/kg body weight). As necrosis formation occurred at the injection sites, treatment was switched to oral administration after 2 weeks, and the test substance was then given in the feed at a dose level of approx. 25 mg/animal (0.5 ml of a 5-percent solution in oil, approx. 100 mg/kg body weight) on 6 days/week. The overall duration of exposure was 12 months. After 21 months – at which time 16 of the animals were still ali-

ve, while the majority had died of pneumonia – the liver of one animal exhibited a neoplastic change, and at 22.5 months, another animal was observed to have developed a sarcoma originating from the omentum. Without explaining the connection with dicyclohexylamine administration, the investigators defined, as a control, a group of 130 rats in total which received subcutaneous injections of octadecylamine and methylstearylamine for 10 months without developing tumours over a period of 20 months (Pliss, 1958). Considerable inadequacies in the conduct and reporting of the study (e.g. inadequate control group, no statistical evaluation, testing limited to one dose, undefined strain of experimental animal, no details of the number of subcutaneous doses, the observation period, the purity of the test substance or the scope of examination) render it unsuitable for the assessment of the carcinogenic potential of dicyclohexylamine (see also Greim, 2000; IARC, 1980).

Mice (strain D, 22 males and 35 females) received daily subcutaneous injections of dicyclohexylamine at approx. 1.3 mg/animal (0.05 ml of a 2.6-percent solution in oil, equivalent to approx. 65 mg/kg body weight) over a period of 11 to 12.5 months. After injection, the animals regularly had rapidly passing convulsions. In the first 6 months, 42 out of the 57 animals died. The cause of this high mortality rate was not discussed by the authors. In the 15 survivors, there were no more deaths until the first tumour occurred after 12 months. In total, 4 mice developed sarcomas at the site of injection after 12, 14, 15.5 and 16 months. Non-neoplastic changes reported by the investigators included oil granulomas at the site of injection and dystrophic changes in the liver and kidney (no data on incidence given). As controls, the investigators defined animals from their own laboratory which had not previously been included in experiments, but they did not specify the number or age of the animals, or the findings obtained (Pliss, 1958). Considerable inadequacies in the conduct and reporting of the study (e.g. inadequate control group, no statistical evaluation, testing limited to one dose, no details of treatment or study duration, purity of the test substance or scope of examination) render this study unsuitable for the assessment of the carcinogenic potential of dicyclohexylamine as well (see also Greim, 2000; IARC, 1980).

The short-term carcinogenicity tests carried out with dicyclohexylamine are presented in Table 4. The assessment of dicyclohexylamine in these short-term tests was part of a series of tests encompassing a total of 120 organic

compounds which was carried out to study the validity of the individual test systems (Purchase et al., 1976, 1978). In cell transformation tests conducted in human lung fibroblasts (W1-38), human liver cells (Chang) as well as kidney cells of the Syrian hamster (BHK-21 C13), dicyclohexylamine caused transformation neither in the absence nor in the presence of metabolic activation. According to the investigators' evaluation, the results of the transformation tests were in good agreement with the carcinogenic activity of the individual compounds, whereas the results of the other short-term tests (the Rabin test – also known as the degranulation test – in rat hepatocytes as well as the implantation test, sebaceous gland test and tetrazolium reduction test in the mouse) are of very limited predictive value as there was little concurrence between the individual compound's carcinogenic activity and its effect in those test systems. In the Rabin test, the implantation test and the sebaceous gland test, dicyclohexylamine gave negative results, while the tetrazolium reduction test was positive (Purchase et al., 1976, 1978).

Table 4. Data on the carcinogenicity of dicyclohexylamine – short-term test systems				
Test system	Concentration or dose	Toxicity	Results	Reference
Cell transformation, human lung fibroblasts (W1-38) and liver cells (Chang), ± rat liver S9 mix (no further details)	0.08–250 µg/ml	n. d.	+ S9 negative, – S9 negative	Purchase et al., 1976, 1978
Dicyclohexylamine was investigated in the context of a series of studies to evaluate the validity of the test system. According to the investigators' analysis, there was satisfactory concurrence between the carcinogenic activity of the individual compounds and the transforming activity in this test system.				
Cell transformation, Syrian hamster kidney fibroblasts (BHK-21C13) ± rat liver S9 mix (no further details)	0.08–250 µg/ml	n. d.	+ S9 negative, – S9 negative	Purchase et al., 1976, 1978
Dicyclohexylamine was investigated in the context of a series of studies to evaluate the validity of the test system. According to the investigators' analysis, there was satisfactory concurrence between the carcinogenic activity of the individual compounds and the transforming activity in this test system.				
Rabin test (degranulation test)	12 µg/ml	–	negative	Purchase et al., 1976, 1978
Determination of the number of ribosomes of the rough endoplasmic reticulum from rat hepatocytes after incubation with the test substance; decreases are said to indicate a positive result. Dicyclohexylamine was investigated in the context of a series of studies to evaluate the validity of the test system. According to the investigators' analysis, there was only limited concurrence between the carcinogenic activity of the individual compounds and the activity in this test system.				
Implant test, Alderley Park Swiss Mouse	subcutaneous implantation of filter discs overlain with dicyclohexylamine (0.02 mmol/disc, ca. 3.6 mg) for 90 days	n. d.	negative	Purchase et al., 1976, 1978
Only the dorsal skin tissue surrounding the filter disc was examined. Dicyclohexylamine was investigated in the context of a series of studies to evaluate the validity of the test system. According to the investigators' analysis, there was only limited concurrence between the carcinogenic activity of the individual compounds and the activity in this test system.				
Sebaceous gland test, male Alderley Park Swiss Mouse	0.4 mg/animal, 6 times in 3 days dermally, sacrifice at day 7 of the study	n. d.	negative	Purchase et al., 1976, 1978
Determination of the ratio of the number of sebaceous glands to the number of hair follicles; a reduction in the ratio was evaluated as a positive result. Dicyclohexylamine was investigated in the context of a series of studies to evaluate the validity of the test system. According to the investigators' analysis, there was only limited concurrence between the carcinogenic activity of the individual compounds and the activity in this test system.				
Tetrazolium reduction test, male Swiss mouse	ca. 2 mg/animal once dermally, sacrifice after 2 days	n. d.	positive	Purchase et al., 1976, 1978
Upon sacrifice, the treated skin was incubated with the leuco form of tetrazolium red; reduction of tetrazolium red (red coloration) was evaluated as a positive result. Dicyclohexylamine was investigated in the context of a series of studies to evaluate the validity of the test system. According to the investigators' analysis, there was only limited concurrence between the carcinogenic activity of the individual compounds and the activity in this test system.				
n.d. no data				

7.8 Reproductive toxicity

No information available.

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

No information available.

7.11 Other effects

Dicyclohexylamine exhibited hypoglycaemic activity upon administration of the hydrochloride to fasted rats and mice as well as hyperglycaemic, hepatectomised rats or rats rendered diabetic by streptozotocin, but it was inactive in pancreatectomised dogs, findings which the authors attributed to dicyclohexylamine-stimulated glucose transformation in the muscle tissue without enhancement of anaerobic glycolysis (Polacek and Breuer, 1978; Polacek and Ouart, 1978).

In experiments carried out in isolated rat vas deferens, dicyclohexylamine was observed to have sympathomimetic activity. At concentration levels of 0.1 to 1 mM (approx. 18 to 181 µg/ml), the compound potentiated the noradrenaline-induced contraction of the vas deferens and at higher levels induced vas deferens contraction even without the addition of noradrenaline (Mottram and Hickman, 1979; Kidman et al., 1979).

Numerous publications report inhibitory effects of dicyclohexylamine on spermidine synthase activity, e.g. in brain cells of the rat and mouse, pancreatic cells of the rat, various tumour cells, *Escherichia coli* and *Pseudomonas aeruginosa*, a compound referred to as dicyclohexylamine sulfate being used in most cases (Bitonti et al., 1982; Feuerstein et al., 1985; Hibasami and Ito, 1981; Hibasami et al., 1980; Ito et al., 1982; Mitchell et al., 1985; Pegg et al., 1983; Porta et al., 1984; Slotkin et al., 1984). Batchelor et al. (1986) were not able to confirm this inhibitory action of dicyclohexylamine on spermidine synthase activity in studies using human

skin fibroblasts. They demonstrated, however, that investigators who had reported inhibition of spermidine synthase activity by dicyclohexylamine sulfate obtained from a specific supplier of chemicals (e.g. Bitonti et al., 1982; Feuerstein et al., 1985; Hibasami and Ito, 1981; Hibasami et al., 1980; Ito et al., 1982; Pegg et al., 1983; Porta et al., 1984) had all been misled by the inappropriate chemical name on the supplier's label to test *bis*-cyclohexylammonium sulfate ($C_{12}H_{28}N_2SO_4$) and had therefore in fact tested cyclohexylamine (Batchelor et al., 1986).

8 Experience in humans

Without giving further details, one report states that a 60-minute inhalation exposure to dicyclohexylamine caused severe toxic damage at 388 mg/m³ (Goldblatt, 1955).

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 dicyclohexylamine to Section IIb of the List of MAK and BAT Values, the section covering "substances for which no MAK value can be established at present". Because of the danger of percutaneous absorption, the chemical has been designated with "H" (DFG, 2000).

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