Cyclohexylamine

(CAS reg no: 108-91-8)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

No. 2000/15OSH/021, The Hague, 13 November 2001

Preferred citation:
Health Council of the Netherlands: Committee on Updating of Occupational Exposure Limits. Cyclohexylamine; Health-based Reassessment of Administrative Occupational Exposure Limits. The Hague: Health Council of the Netherlands, 2001; 2000/15OSH/021.

1 Introduction

The present document contains the assessment of the health hazard of cyclohexylamine by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by KJ van den Berg, Ph.D., A Spooren, Ph.D. and H Stouten, M.Sc. (TNO Nutrition and Food Research, Zeist, the Netherlands).

The evaluation of the toxicity of cyclohexylamine has been based on the review by the American Conference of Governmental Industrial Hygienists (ACG99). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, literature was retrieved from the online data bases Medline, Toxline, and Chemical Abstracts covering the period 1966 to 26 April 1999 (19990426/UP), 1965 to 29 January 1999 (19990129/ED), and 1967 to 24 April 1999 (19990424/ED; vol 130, iss 18)*. HSDB and RTECS, data bases available from CD-ROM, were consulted as well (NIO99, NLM99). The final literature search has been carried out in April 1999.

In April 2001, the President of the Health Council released a draft of the document for public review. Comments were received from the following individuals and organisations: L Whitford (Health & Safety Executive, London, United Kingdom). These comments were taken into account in deciding on the final version of the document.

* Medline was searched with the following search profiles: 1) (cyclohexylamine OR aminocyclohexane OR cyclohexanamine OR 108-91-8 OR aminohexahydrobenzene OR hexahydroaniline OR monocyclohexylamine) AND cyclohexylamines/CT(L)(AE,PO,TO)/CT, 2) (cyclohexylamine OR aminocyclohexane OR CYCLOHEXANAMINE OR 108-91-8 OR aminohexahydrobenzene OR hexahydroaniline OR monocyclohexylamine) AND cyclohexylamines/CT (NOT 1), and 3) (cyclohexylamine OR aminocyclohexane OR cyclohexanamine OR 108-91-8 OR aminohexahydrobenzene OR hexahydroaniline OR monocyclohexylamine) NOT (1 OR 2). Toxline was searched with the profile: 108-91-8 NOT results Medline, and CA with: (108-91-8 OR 4998-76-9 OR 19834-02-7)/ADV NOT results Medline, Toxline (4998-76-9: CAS reg no of cyclohexylamine hydrochloride; 19834-02-7: CAS reg no of cyclohexylamine sulphate).

2 Identity

name	:	cyclohexylamine
synonyms	:	hexahydroaniline, aminocyclohexane, cyclohexanamine, aminohexahydrobenzene
molecular formula	:	$C_6H_{12}S$
structural formula	:	
CAS reg no	:	108-91-8

3 Physical and chemical properties

molecular weight	:	99.17°
boiling point	:	135°C
melting point	:	-18°C
flash point	:	31°C
vapour pressure	:	at 20°C: 1.4 kPa
solubility in water	:	miscible
Log $P_{octanol/water}$	:	1.49 (experimental); 1.63 (estimated)
conversion factors (20°C, 101.3 kPa)	:	1 ppm = 4.1 mg/m^3 1 mg/m^3 = 0.24 ppm

Data from ACG99, Ben94, NLM99, http://esc.syres.com.

Cyclohexylamine is a liquid with a strong, fishy amine odour.

4 Uses

Cyclohexylamine is used as a corrosion inhibitor in boiler feed water and has important applications as a chemical intermediate in organic synthesis and in the manufacture of insecticides, plasticisers, dry-cleaning soaps, rubber chemicals, dye stuffs, and gas absorbents (ACG99, Ric93).

5 Biotransformation and kinetics

There were no data on the kinetics of cyclohexylamine following exposure by inhalation.

Orally administered cyclohexylamine is rapidly and almost completely absorbed in both man and animals (dog, rat, guinea pig, rabbit). After administration of oral doses of 2.5, 5, and 10 mg/kg bw to volunteers, peak blood or plasma levels occurred between 1 and 2 hours and the half-life ranged from 3 to 5 hours. In rats and dogs, the peak cyclohexylamine levels in blood or plasma were achieved within the first hour, and the half-lifes were about 1 to 2 hours and 3 hours, respectively (Bop86).

In rats, cyclohexylamine readily penetrated into the body tissues, with the highest concentrations occurring in the lungs, spleen, liver, adrenals, heart, gastrointestinal tract, and kidneys. The levels in most tissues were higher than those in plasma. The apparent volume of distribution of 2.1 to 2.9 L/kg reported for man, agreed well with the values of 2.7 L/kg calculated for rats. In rats, only 8% of the cyclohexylamine was bound to the plasma proteins while the binding to human serum albumin averaged 33% at 5 µg/mL (Bop86).

Cyclohexylamine has been observed to freely diffuse across the placenta and to enter the fetus in studies with pregnant rhesus monkeys (Bop86).

Following oral administration of ^{14}C-cyclohexylamine, 1 to 2%, less than 10%, and about 30% of the dose administered were found to be metabolised in man, in female rats and guinea pigs, and in rabbits, respectively. In man, only the deaminated products, cyclohexanol, and *trans*-cyclohexane-1,2-diol were detected. In rats, the principal metabolic pathway involved ring hydroxylation, leading to isomers of 3- or 4-aminocyclohexanol, while both deamination and ring hydroxylation occurred in guinea pigs and rabbits. The deaminated products, cyclohexanone and cyclohexanol, have been identified in dogs, but no definitive information is available about the existence of the ring-hydroxylated metabolites in that species. N-hydroxycyclohexylamine was identified in rabbit urine but was not found in the urine of rats, guinea pigs, or man. It has been demonstrated that cyclohexylamine was deaminated by rabbit liver microsomes to form the ketones, which in turn were reduced to the alcohols. The deamination reaction required molecular oxygen and NADPH, and was inhibited by carbon monoxide, SKF-525A, metapyrone, and potassium cyanide. Cyclohexylamine formed type II spectral changes with the hepatic microsomes. Deamination reactions appeared more prevalent in rabbits than rats and ring hydroxylation was favoured in rats.

Metabolism of cyclohexylamine by microflora in the gastrointestinal tract cannot be excluded (Bop86).

A scheme summarising the metabolic pathways for cyclohexylamine is given in Figure 1, Annex I.

The main elimination route is via the urine. Both in man and animal species, about 90% or more of ingested doses of cyclohexylamine was excreted in the urine. In man, renal clearance values exceeded the creatinine clearance, indicating that cyclohexylamine was probably removed by tubular secretion as well as glomerular filtration. The renal clearance of cyclohexylamine decreased as the dose increased (range: 2.5 to 10 mg/kg bw), suggesting that the secretion process might be easily saturated (Bop86).

The pharmacokinetic differences of cyclohexylamine in rats and mice were investigated in greater detail in a follow-up study. Cyclohexylamine was found to be absorbed and eliminated more rapidly by mice. The steady-state plasma clearance in rats was approximately one-half that in mice.The renal tubular secretion of cyclohexylamine by rats *in vivo* was saturated at levels of cyclohexylamine used in the oral dosing studies. The concentration of cyclohexylamine in testes of rats, but not of mice, showed a non-linear relationship to dietary intake. The kinetics of cyclohexylamine appears to be important in the difference in sensitivity to testicular atrophy in rats and mice (Rob89b).

Cyclohexylamine is a biotransformation product of the artificial sweetener cyclamate (Lun91).

6 Effects and mechanism of action

Human data

In 48-hour patch tests with a 25% cyclohexylamine solution, no, slight, and severe irritation were found in 45, 52, and 3% of the volunteers, respectively. From a challenge 14 days later resulting in a positive reaction in 13% of the volunteers, cyclohexylamine was concluded to induce a slight sensitising effect (Mal52).

Cyclohexylamine was found negative in a patch test performed on an agricultural worker having occupational allergic contact dermatitis caused by rubber gloves and other rubber parts (Ber88).

Data on effects following occupational exposure to cyclohexylamine were limited to 3 cases, originating from the physician's department of one plant, of

transitory systemic effects from acute accidental exposures to unknown concentrations. The symptoms reported were lightheadedness, drowsiness, anxiety and apprehension, and nausea, and, in one of these cases, slurred speech, vomiting, and pupillary dilatation. Measurements following one of these events showed levels of 16 to 41 mg/m^3 (4-10 ppm), at which operators were stated to be free of symptoms. No details (on *e.g.*, sampling time, representativeness) were given (Wat50).

Cyclohexylamine was found to produce cardiovascular effects. One hour following administration of single doses of 5 and 10 mg/kg bw to male healthy volunteers, maximum increases (dose dependent, statitically significant) in mean systolic and diastolic blood pressure were observed; no significant changes occurred after a dose of 2.5 mg/kg bw. A slight decrease in the heart rate accompanied the vasopressor effects of the two high doses. The cyclohexylamine levels in plasma were closely correlated with the increases in the mean arterial blood pressure; it was estimated that the lowest plasma level of cyclohexylamine to cause a significant hypertensive effect was about 0.7 to 0.8 µg/mL. Furthermore, the blood glucose and serum potassium levels of adult males were not affected, but the free fatty acid concentrations were slightly elevated at the highest dose of 10 mg/kg bw (Eic74).

Animal data

Acute toxicity

When instilled into the eyes of rabbits, cyclohexylamine scored an injury grade of 10 (*i.e.*, a severe burn from 0.5 mL of a 1% solution) on a scale from 1-10 (Smy69). In a separate study, cyclohexylamine was concluded to be corrosive following instillation of 0.1 mL into the conjunctival sac of New Zealand albino rabbits (observation period: 7 days) (Ran90).

When applied to the clipped skin of rabbits, cyclohexylamine scored an injury grade of 7 (*i.e.*, necrosis) on a scale from 1-10 (Smy69). After application of 0.5 mL of cyclohexylamine under semi-occlusive dressings to the clipped, intact and abraded skin of New Zealand albino rabbits for 24 hours, cyclohexylamine was concluded to be corrosive (observation time: 7 days) (Ran90). Undiluted material was reported to be severely irritating to the skin of a not specified species (Mal52).

Referring to unpublished information, it was reported that a 1% solution of cyclohexylamine did not cause sensitisation in guinea pigs (Bea81,Ben94).

Undiluted cyclohexylamine was concluded to be moderately sensitising in not specified experimental animals (no more details presented) (Mal52).

Sensory irritation of the upper respiratory tract was evaluated in mice (male Swiss OF_1) during a 15-minute oronasal exposure to increasing concentrations of cyclohexylamine. The airborne concentration resulting in a 50% decrease in the respiratory rate (RD_{50}) was 210 mg/m^3 (51 ppm). Cyclohexylamine was also tested for pulmonary toxicity in mice and for the effects of a 120-minute exposure on the respiratory rates of non-anaesthetised, tracheally cannulated mice ($RD_{50}TC$). The $RD_{50}TC$ value for cyclohexylamine was found to be 750 mg/m^3 (184 ppm). From these results, it was concluded that cyclohexylamine is essentially an upper respiratory tract-irritating compound (Gag89). In another study with male Ssc:CF-1 mice, the RD_{50} and $RD_{50}TC$ were determined to be 110 and 320 mg/m^3 (27, 78 ppm), respectively (Nie89).

A single 7-hour inhalation exposure to 4900 mg/m^3 (1200 ppm) cyclohexylamine was reported to be lethal to all rabbits and guinea pigs, and to all but one rat. The major effects observed were irritation of the respiratory tract and eye irritation with the development of corneal opacity (ACG99, Gre98). Further, LC_{50}s of 7500 and 10,700 mg/m^3 (1800, 2570 ppm; exposure duration and observation time not indicated) were calculated for rats and mice, respectively. In this study, concentrations of 1800, 4300, and 11,500 mg/m^3 (430, 1030, 2760 ppm) were stated to be maximum tolerable and minimum and absolutely lethal, respectively, in rats. In mice, these figures were 50, 100, and 4300 mg/m^3 (12, 24, 1032 ppm), respectively, (duration of exposure not indicated). In these experiments, effects on the mucous membranes, the central nervous system (increased excitability: unrest, clonic spasms of trunk and paw muscles) and blood parameters, decreased body weight, decreased body temperature, and slowed respiration were seen. Upon necropsy, relative organ weights were affected and there were histological lesions in several organs (Lom65).

The acute dermal toxicity of cyclohexylamine was studied by applying single doses of 398, 631, 1000, and 1580 mg/kg bw to rabbits (n=1/dose) under semi-occlusive conditions (observation period: 14 d). Mortality occurred at doses of 1000 and 1580 mg/kg bw. Clinical signs included reduced appetite and activity (5 to 7 days in survivors), increasing weakness, collapse, and death. At autopsy of decedents, lung and liver hyperaemia, slightly enlarged gall bladder, and darkened spleen and kidneys were seen. Viscera of surviving animals appeared normal at sacrifice (Ran90).

Following oral administration to rats, neat or as a 5.0% solution in water, LD_{50}s were 11.0 and 590 mg/kg bw, respectively (95% confidence limits: 9.5-13.0 mg/kg bw and 500-700 mg/kg bw, resp) (observation period: 14 or 21 d). The minimal lethal doses were 10.0 and 398 mg/kg bw, respectively. Clinical signs included reduced appetite and activity, increasing weakness, collapse and death. At autopsy of decedents, liver and/or lung hyperaemia, discolouration of the liver (neat preparation only), and acute gastrointestinal inflammation were seen. In survivors of the neat preparation sacrificed after 21 days, there were areas of liver discolouration (Ran90). Summarising other studies, oral LD_{50}s in rats ranged from *ca*. 150 to 615 mg/kg bw (Bop86).

Following intraperitoneal injection, doses of 100 mg/kg bw caused little changes in rats, except for a decrease in spontaneous motility. Doses of 200 to 300 mg/kg bw induced various symptoms such as abnormal gait, scratching, salivation, lacrymation (tinged tear), epistaxis, oedema at eyelids, piloerection, mydriasis, exophthalmus, and, in some cases, sexual excitation as defined by ejaculation and frequent occurrence of mating behaviour. In addition, laboured respiration, which slowly developed and lasted for many hours, occurred and this was followed by respiratory arrest. Remarkable haemorrhage in the lungs was observed in all of 12 autopsied cases. The LD_{50} was presumed to be about 350 mg/kg bw (Miy69). In mice, the LD_{50} was 770 mg/kg bw when tested in animals housed singly at 20°C; under other conditions (aggregated, 28°C) values ranged from 300 to 520 mg/kg bw (Lee72). In another study in mice, an LD_{50} of 619 mg/kg bw was found. Doses of 20-50 mg/kg bw caused sedation as defined by a marked decrease in spontaneous motility and by depression of responses to external stimuli. At higher doses of 100-200 mg/kg bw, sedation became more remarkable, while continuous scratching and muscular rigidity followed by slight paralysis in hind legs were observed. Following injection of 500-800 mg/kg bw, severe tremors, tail rising, and clonic convulsions occurred, and the animals died of respiratory failure (Miy69). In a not specified species, an LD_{50} of 200 mg/kg bw was reported. Signs of severe shock and degenerative changes in the brain, liver, and the kidneys were seen (Mal52).

Intravenous injections of 5 to 50 mg/kg bw of cyclohexylamine produced sympathomimetic symptoms such as restlessness, piloerection, mydriasis and tachycardia, and muscular rigidity followed by paralysis of hind legs in dogs. Furthermore, slight swelling of the face and continuous scratching, which might be caused by liberation of histamine, were observed. At doses of 100 to 200 mg/kg bw, tonic and clonic convulsions were additionally seen. Doses higher than 200 mg/kg bw induced respiratory arrest during convulsive seizures. The

LD_{50} was presumed to be about 200 mg/kg bw (Miy69). In rabbits, iv LD_{50}s of 150-175 mg/kg bw were reported (Bop86).

Effects of cyclohexylamine observed on the central nervous system of rats were a prolonged hexobarbital sleeping time at intraperitoneal doses exceeding 200 mg/kg bw , a definite analgesic action (ED_{50} of *ca*. 20 mg/kg bw), suppression of the conditioned avoidance response at 100 to 200 mg/kg bw, and inhibition of aggressive behaviour at 100 to 150 mg/kg bw, and suppression of fighting behaviour in mice (ED_{50} of 51 mg/kg bw) (Miy69). Other studies have reported on central stimulant effects, such as hyperactivity, hyperexcitability, increased responsivity to external stimuli, and aggressive behaviour, resembling the signs following amphetamine treatment. In addition, the body temperature was found to be increased (Lee72).

The cardiovascular effects of cyclohexylamine have been well documented. In anaesthetised cats or dogs, intravenous administration of 0.4 to 5 mg/kg bw caused hypertension, positive chronotropic and ionotropic effects, and peripheral vasoconstriction. Cyclohexylamine was considerably less potent when given orally. The minimal effective doses in cats were estimated to be 0.05 mg/kg bw intravenously and 10 to 15 mg/kg bw orally. Orally administered cyclohexylamine increased blood pressure in unanaesthetised animals, but instead of the positive chronotropic effects, a reflex bradycardia was observed. Cyclohexylamine given in a subcutaneous dose of 50 mg/kg bw to rats, twice daily, for 2 days, did not induce visible lesions in the heart (Clas70). In contrast to the acute effects of orally administered cyclohexylamine, most chronic studies in animals have failed to demonstrate any significant cardiovascular effects. It appears that cyclohexylamine is primarily an indirectly acting sympathomimetic agent, similar to tyramine, but 100 to 1000 times less potent. Recent data indicate that the hypertensive effect of cyclohexylamine occurs primarily during rapid increases in plasma concentrations (Bop86, Bus92, Clas68, Miy 69, Ros68, Yam65).

Cyclohexylamine can also exert other sympathomimetic effects, including contraction of the nictitating membrane in anaesthetised cats at 3-3.7 mg/kg bw iv (Clas68), and contraction of the *vas deferens* in rats (Bop86). In vitro, cyclohexylamine caused contractions of isolated *rectus abdominis* muscles of frogs at concentrations higher than 10^{-3} g/mL and an increase in contractile force in isolated perfused frog hearts at applications of 10^{-6} to 10^{-5} g/mL. An histamine-releasing action was indicated by studies on rat lung preparations. Isolated guinea pig ileum showed contractions with increased peristalsis

following treatment with cyclohexylamine concentrations higer than 10^{-4} g/mL (Miy69).

Subacute/subchronic studies

Rabbits, guinea pigs, and rats (n=unknown) were exposed to cyclohexylamine vapours at concentrations of 620 mg/m^3 (150 ppm), 3300 mg/m^3 (800 ppm), or 4900 mg/m^3 (1200 ppm), 7 hours/day, 5 days/week. At 4900 mg/m^3 (1200 ppm), all animals but one rat died after a single exposure. All animals of this exposure group showed extreme irritation of the mucosal membranes and haemorrhage of the lungs. In the mid-concentration group, 1 rabbit and 2 guinea pigs died after the second exposure period, while 5 rats survived 24 hours of exposure. At 620 mg/m^3 (150 ppm), 4/5 rats and 2 guinea pigs survived 10 days of exposure, but 1 rabbit died after 7 hours. The main effects observed were irritation of the respiratory tract and eye irritation with the development of corneal opacities at the higher concentrations. No convulsions were observed (Wat50).

When albino rats were exposed to 0 and 700 mg/m^3 (170 ppm), 2 hours/day, for 2 months or to 0 and 100 mg/m^3 (25 ppm), 4 hours/day, for 5 months, mortality occurred in 3/6 (towards the end of the experimental period) and 1/10 animals (in month 4), respectively. In the animals exposed to 700 mg/m^3 (170 ppm), weight loss, gradually decreased body temperature and respiratory rate, soft stools (after day 70), reduced haemoglobin, reduced number of erythrocytes, and increased number of reticulocytes were seen. At necropsy, there were increases in the relative heart and kidney weights. At microsopic examination, deposition of haemosiderin in liver, spleen, and lungs and elongated follicles with squamous epithelium in the thyroid were found. Other lesions reported included vascular changes, fatty and granular degeneration of the myocardium and kidneys, inflammatory changes in the trachea and lungs, elimination of fat from the adrenal cortex but it was not clear in which of the experimental group(s) these lesions were seen. In the animals exposed to 100 mg/m^3 (25 ppm), there was an increase in body weight from month 3 onwards. Similar effects on body temperature, respiratory rate, and stools as found in the high-concentration group were observed. The incidence of albuminuria was 8/20 and 16/20 after 70 days and by the fourth month, respectively. Furtermore, there was a significant reduction in oxygen consumption. Exposure to 100 mg/m^3 (25 ppm) caused an increase in the number of neutrophils and leucocytosis while there were no changes in the red blood cell parameters. At necropsy, there was an increase in relative kidney weights. At microscopic examination, there were no indications

for a haemolytic effect (no haemosiderosis). In the thyroid, changes (reduced dimensions of the nuclei in the follicular epithelial cells) were observed. Other histological changes may have been present as well in this group (see above) (Lom65).

In a 90-day oral study, rats (CFE; n=15/sex/group) were given diets containing cyclohexylamine hydrochloride at concentrations of 600, 2000, or 6000 mg/kg diet, stated to result in average daily doses of cyclohexylamine of 30, 104, or 342 mg/kg bw, based on food intake and body weight data. In the high-dose group, body weight gain, food and water intake were reduced, and basal metabolic rate was increased. In addition, alterations were observed in haematological parameters (haemoglobin, packed cell volume, red blood cells, and total leucocytes), renal function, absolute organ weights (brain, heart, liver, spleen, kidneys, stomach, small intestine, caecum, adrenals, pituitary, thyroid) and relative organ weights (brain, stomach, small intestine, caecum, adrenals, thyroid). In males, absolute and relative testis weights were reduced and relative weights of female gonads were increased. The only histological findings were reduced spermatogenesis and tubular atrophy in the testes. In the mid-dose group, body weight gain, food and water intake were reduced. In addition, alterations were observed in haematological parameters of males (packed cell volume, total leucocytes), absolute organ weights (brain, heart, liver, spleen, kidneys, small intestine, caecum, adrenals, pituitary, thyroid) and relative organ weights (brain, and adrenals). The relative weights of female gonads were increased. Histological examination of the testes revealed reduced spermatogenesis and tubular atrophy. In the low-dose group, a decreased food consumption being statistically significant in females was the only finding (Bop86, Gau74). The decrease in food consumption in the low-dose group is considered a marginal probably due to the unpalatability of diet. Obviously, this did not result in adverse or unwanted effects. Therefore, the committee consideres this effect toxicologically not relevant, and places the NOAEL at 600 mg/kg diet, equivalent to 30 mg/kg bw/day.

In another 90-day study, male Wistar and Sprague Dawley rats (n=25/strain/group) were given diets containing cyclohexylamine hydrochloride at concentrations of 600, 2000, or 6000 mg/kg diet, calculated by Bopp *et al*. to result in a daily intake of cyclohexylamine of 46, 149, or 416 and 44, 140, or 406 mg/kg bw for Wistar and Sprague-Dawley rats, respectively, based on food intake and body weight data. In the high-dose group, body weight, body weight gain, and food intake were reduced. In addition, alterations were reported in absolute organ weights (heart, liver, kidneys, adrenals, pituitary, thyroid,

prostate), but not in relative organ weights. Haematological, clinical chemistry, and urinalysis parameters were not determined. Absolute testis weights were found to be reduced. Sperm analysis showed that the count and motility of spermatozoa differed significantly. The testicular lesions of the epididymides observed were an almost complete absence of motile sperm and increased numbers of decapitated spermatozoa. Upon histological examination, a reduction or a complete absence of spermatogenesis was seen in over 80% of the tubules. In the mid-dose group, body weight, body weight gain, and food intake were reduced. No effects on organ weights were reported, and no testicular lesions were observed in this dose group. In the low-dose group, no treatment-related effects were observed (Mas77). For this study primarily aimed at the investigation of potential effects on the testes, the NOAEL is set at 600 mg/kg diet, equivalent to *ca*. 45 mg/kg bw/day.

In addition, data from an unpublished 90-day study were cited, in which male rats (Wistar; n=15-16/group) received diets containing cyclohexylamine hydrochloride at concentrations of 100, 500, 1000, 2000, 5000, or 10,000 mg/kg diet, calculated by Bopp *et al*. to result in daily cyclohexylamine doses of 3.5, 17.6, 36, 69, 174, or 352 mg/kg bw, based on food intake and body weight data. A dose group receiving 25,000 mg/kg diet was included as well, but all animals of this group died within 5 days, showing intestinal haemorrhages at necropsy. No deaths occurred at the lower doses. Body weight gain and food intake were decreased at dietary levels of 1000 mg/kg and higher. The haematology, clinical chemistry, and urinalysis parameters were reported to be unaffected. Many organ weights were lower in the rats given doses of 2000 mg/kg and higher. These changes were considered to be related to the decreased body weight of the animals. The only histological changes involved the testes. The absolute testicular weights were found to be decreased at 5000 and 10,000 mg/kg diet, while the relative weights were increased at 5000 mg/kg diet and decreased at 10,000 mg/kg diet. Histological changes reported were degeneration of tubular epithelium of rats dosed at 10,000 mg/kg diet, and hydropic degeneration in the 5000 mg/kg diet group (Bop86). No effects were reported for a dietary dose of 500 mg/kg diet (*ca*. 18 mg/kg bw/day) or lower. On the basis of this study, a NOAEL of 18 mg/kg bw/day is established.

In a(n unpublished) study specifically designed to investigate the effects on the testes (see Section *Reproduction toxicity*), male rats (Sprague-Dawley; n=100/group) were fed daily cyclohexylamine doses of 50, 100, 200, and 300 mg/kg bw, for 3 months. *Ad libitum* and pair-fed control groups were included as well. Treatment induced decreased body weights accompanied by decreased

food consumption. These decreases were statistically significant in all dose groups when compared to *ad libitum* controls but only in the two highest dose groups when compared to pair-fed controls (Bop86).

In other studies on testicular effects, significantly decreased food intake and body weight gain were found in male rats (Wistar, n=45; DA, n=120) but not in male mice (MF1; n=45), given cyclohexylamine hydrochloride in the diet at a constant cyclohexylamine intake of 400 mg/kg bw/day for 13 weeks (Rob89a). When given orally (gavage) to 15 male rats (CD; 200 mg/kg bw/day) and 4 male dogs (Beagle; 250 mg/kg bw/day), for 9 weeks, with a subsequent exposure-free recovery period of 13 weeks, body weight gain, and food consumption were decreased in both species. Clinical signs were vomiting, unusually quiescence, a tendency to pass loose faeces in dogs, and reduced motor and grooming activity in rats (Jam81).

A diabetogenic effect was found in rats (Wistar; n=11) given a high dose of 1% cyclohexylamine in the diet (*ca*. 500 mg/kg/d) for 1 to 3 months; after an intravenous challenge of glucose (1 g/kg bw), blood glucose levels returned more slowly to normal than in a group of control animals (n=34). The insulin levels were not found to be affected in the rats fed with cyclohexylamine (Gon71).

Chronic/carcinogenicity studies

In a chronic study, rats (Wistar; n=48/sex/group) were given diets containing cyclohexylamine hydrochloride at concentrations of 600, 2000, or 6000 mg/kg diet for 2 years, resulting in an average daily cyclohexylamine intake of 18, 60, or 219 and 26, 88, and 321 mg/kg bw, for male and female animals, respectively, based on food intake and body weight data. There was no treatment-related increase in the incidence of any tumour (including the bladder) in any of the dose groups. Mortality was lower in the treated groups (especially in the two higher dose groups) than in the control group. In all dose groups, body weight gain as well as food and water intake were reduced. In the high-dose group, altered haematological parameters (haemoglobin, packed cell volume, reticulocyte counts, total leukocytes, neutrophils, lymphocytes), altered clinical chemistry parameters (serum urea concentrations, serum albumin levels, LDH), altered urinalysis parameters, altered absolute organ weights (heart, liver, spleen, kidneys, stomach, small intestine, caecum, adrenals, pituitary, and thyroid), and relative organ weights (brain, liver, spleen, kidneys, stomach, small intestine, caecum, and thyroid) were observed. In males, absolute testis weights were

decreased, while relative female gonadal weights were increased. Histological changes were observed in the lungs (alveoli with foamy macrophages), the kidneys of males (a mild glomerulonephrosis), and the testes (bilateral atrophy, tubules with reduced activity, deposits in tubules). In the mid-dose group, the main findings were altered haematological parameters (haemoglobin, total leukocytes), altered clinical chemistry parameters (serum urea concentrations, serum albumin levels), altered absolute organ weights (heart, liver, spleen, kidneys, stomach, small intestine, caecum, adrenals) and relative organ weights (brain, stomach, caecum, and thyroid). In males, absolute testical weights were decreased, while relative female gonadal weights were increased. Histological changes observed were lesions in the testis, consisting of tubules with few or no spermatids. In the low-dose group, haematological parameters (total leukocytes), clinical chemistry parameters (serum urea concentrations), absolute organ weights (heart, liver, kidneys), and relative organ weights (brain) were affected (Gau76). Hence, the committee could not establish an NOAEL from this study; a LOAEL of 600 mg/kg diet, equivalent to 18 mg/kg bw/day, was found.

In a multigeneration study, weanling rats (FDRL; n=30/sex/group) were given diets containing cyclohexylamine hydrochloride resulting in cyclohexylamine doses of 15, 50, 100, or 150 mg/kg bw/day, for 2 years. Treatment did not affect mortality rates. There was a dose-dependent decrease in mean terminal body weights. Body weight gains were statistically significantly depressed at doses of 50 mg/kg bw and higher in female rats and of 100 mg/kg bw and higher in male rats. Data on absolute and relative organ weights and on histological findings were summarised but no statistical analysis was presented. With a few exceptions, all absolute organ weights were decreased in all exposure groups while the relative weights were either higher or similar when compared to those in controls (no statistical evaluation presented). There was no clear dose-response relationship. Upon microscopic examination, no clear dose-response relationships were found either with respect to findings in specific organs. There were increased incidences in calcification of the kidneys (5/24, 11/35, 10/27, and 8/41 *vs* 2/33 in controls), mucosal thickening of the bladder ((9/58, 13/56, 9/56, and 13/56 *vs* 8/57), testical atrophy (6/15, 9/13, 3/10, and 12/20 *vs* 5/19), and abnormal germinal epithelium (0/15, 1/13, 1/10, and 3/20 *vs* 0/19). No effects were seen in haematological, clinical chemistry, and urinalysis parameters. No differences in tumour incidence were found between treated and control groups (Ose76). From this study, the committee establishes an NOAEL of 15 mg/kg bw/day.

In a limitedly reported carcinogenicity study with rats (Charles River), groups of 25 males and 25 females animals were given daily doses of cyclohexylamine sulphate of 0.15, 1.5, or 15 mg/kg bw, for 2 years. At the end of 2 years, a bladder tumour (diagnosed as an invasive transitional cell carcinoma) was found in one out of eight male survivors in the high-dose group while no such tumours were found in any of the other experimental groups. There were no treatment-related changes in any of the other organs examined (not further specified). It was reported that, apart from a slight decrease in body weight gain in the male animals of the high-dose group, no significant differences between treated and control animals were found as to mortality, food consumption, and blood chemistry and haematological parameters (Pri70).

Daily cyclohexylamine doses of 200 mg/kg bw fed to groups of 52 male and 52 female rats (Sprague-Dawley), for 30 months, did not induce an increased incidence of bladder or any other tumours (Sch73).

In a chronic study with mice (ASH-CS1; 48 males and 50 females/group), the animals received dietary doses of cyclohexylamine hydrochloride of 300, 1000, or 3000 mg/kg diet, equivalent to cyclohexylamine doses of 38, 128, or 376* mg/kg bw/day, for 80 weeks. Treatment did not cause statistically significant differences in tumour incidences (including those of the bladder). Mortality rates were not affected. There were no effects on relative organ weights and on female body weight (gain). In the male animals of the mid- and high-dose groups, body weight gain differed statistically significantly from controls at most of the examination time points; in the low-dose group, differences were observed only occasionally (at wk 1, 12, and 26). Other effects found were limited to the high-dose group and included changes in haematological parameters (neutrophils, lymphocytes) in male animals and histological lesions in the liver (cell vacuolation or polyploidy) and lungs (leucocyte infiltration or deposits) in female animals. No abnormalities were found in the testis (Har76). Based on effects on body weight gain, the committee places the NOAEL in this study at 38 mg/kg bw/day.

In a six-generation study, mice (Swiss SPF derived outbred; n=50/sex) were given diets containing 0.5% cyclohexylamine sulphate (*ca*. 50 mg/kg bw/day), for 21 months. No differences in tumour incidences between treated and control groups were found. Growth retardation was observed, particularly in the females, but survival was increased in the cyclohexylamine group. Haematology

---

* Assuming a mean body weight of 40 g and a mean food intake of 7 g/dag (see Pau98), and correcting for the fact that cyclohexylamine hydrochloride was administered.

examinations did not reveal any changes attributable to treatment, and the histological findings were similar in the control and experimental group (Kro77).

An (unpublished) study is reported in which dogs (Beagle; n=3/sex/group) were given daily oral (capsules) doses of cyclohexylamine sulphate of 0.15, 1.5, and 15 mg /kg bw. No effects of cyclohexylamine were observed in growth, behaviour, haematology, serum chemistry, urinalysis, or hepatic and renal function tests. One male and one female from each group were sacrificed after 1 year of treatment, and the organ weight data and histological examinations of the tissues did not reveal any abnormalities attributable to cyclohexylamine. After about 4 years, the doses were increased to 50, 100, and 150 mg/kg bw/day. The animals lost weight after the dosage increase (dose groups not reported), but subsequently slowly regained weight. Clinical pathology tests were not affected by the higher doses, and no histological changes attributable to cyclohexylamine were seen in the animals that died during the study or those sacrificed at the end of the 9.5 year period (Bop86, Gre98).

Mutagenicity and genotoxicity

The mutagenicity and genotoxic effects of cyclohexylamine have been extensively investigated in a number of systems, including bacterial, mammalian cell, and Drosophila mutagenicity tests, *in vitro* cytogenetic studies, *in vivo* cytogenetic studies with mammalian somatic cells, mammalian germ cell studies, dominant lethal tests, and various other types of studies. The data from these mutagenicity and genotoxicity assays have been summarised (Bop86, Cat76) and are presented in tables (see Annex III).

Generally, negative results were found in bacterial, mammalian cell, and *Drosophila* gene mutation test systems and were confirmed by additional tests in bacteria (Mor86) and *Drosophila* (Bru89). In some *in vitro* cytogenetic tests, small increases in frequency of chromosome gaps and/or breaks were observed. Because no exchange figures, translocations, or other chromosome abberations were found while forward HGPRT and UDS tests (Bru89) were negative, it is concluded that cyclohexylamine is not clastogenic *in vitro*. No consistent pattern of chromosome abnormalites was observed in somatic cells of animals in *in vivo* studies. Most of the studies with mammalian germ cells, except one study, failed to demonstrate genetic damage by cyclohexylamine. After a re-evaluation of these data (Adl89), it was concluded that cyclohexylamine cannot be regarded as aspecific germ-cell mutagen. No general effect of cyclohexylamine on the induction of dominant lethal mutations was observed.

The few positive findings could not be confirmed by other investigators. In the various other mutagenicity tests, no conclusive positive results for cyclohexylamine were found. From these data, the committee does not consider cyclohexylamine to be a mutagenic/ genotoxic compound.

Reproduction toxicity

As can be seen from the subchronic studies, administration of cyclohexylamine to male rats induced effects on the testes at mean daily dietary cyclohexylamine levels of 60 mg/kg bw. However, the test compound was given at fixed dietary concentrations resulting in relatively high doses, expressed as mg/kg bw, in the first weeks of the study and a progressive decrease in the remaining part. Therefore, a(n unplubished) study was performed in which male rats (Sprague-Dawley; n=100/group) were fed daily doses of cyclohexylamine of 50, 100, 200, and 300 mg/kg bw, for 3 months. *Ad libitum* and pair-fed control groups were included as well. Besides effects on body weights - statistically significantly decreased in all dose groups when compared to *ad libitum* controls and in the two highest dose groups when compared to pair-fed controls -, testes weights were decreased in the animals of the 200- and 300-mg/kg bw-dose groups. These decreases were statistically significant for both dose groups when compared to *ad libitum* controls and for the 300-mg/kg bw group when compared with pair-fed controls. Upon microscopic evaluation, testicular scores (for tubular alterations on a 0 to 4 scale) differed significantly in the two highest dose groups when compared to both control groups. The most severe lesions consisted of degenerative changes in the tubules, giant cell formation, and complete testicular atrophy, and, in some cases, only Sertoli cells remained within the affected tubuli (Bop86). From this study, the committee establishes an NOAEL for testicular effects of 100 mg/kg bw.

Other studies confirmed the occurrence of these effects in rats using high doses of cyclohexylamine. Given 200 and 250 mg/kg bw by oral gavage to male rats (CD; n=15) and dogs (Beagle; n=4), respectively, for 9 weeks, serum follicle stimulating hormone levels increased and testosterone levels decreased in rats. In dogs, no such effects were found, but reversible effects on sperm morphology were induced. There were no statistically significant effects on the weight of the pituitaries, testes, or secondary sex organs of either species. The only lesion detectable by conventional histological examination was focal atrophy of seminiferous tubules in one rat examined 13 weeks after cessation of cyclohexylamine treatment. Quantitative assessment of testicular

spermatogenesis showed that cyclohexylamine administration reduced the counts of pachytene spermatocytes and of early and late spermatids in both species. These effects were reversible in dogs but not in rats (Jam81).

Given dietary cyclohexylamine doses of 400 mg/kg bw/day to rats (Wistar; n=10-15/group), for 1, 3, 7, 9, or 13 weeks, body weights and food intake were decreased throughout the study. Absolute testis weights were significantly decreased from 7 weeks onwards; relative weights were significantly increased at weeks 1, 3, and 7. Histological examination of the testes showed changes after 3 weeks of cyclohexylamine administration. The lesion observed consisted of a focal, basal vacuolation of the Sertoli cell cytoplasm associated with the local loss of spermatocytes and spermatogonia. After 7 weeks, the Sertoli cell vacuolation was extensive while the germ cell population showed mild to moderate degeneration and depletion. With longer periods of treatment, the lesion was more severe and affected a greater number of tubules leading to general disruption of the germinal epithelium. *In vitro*, cyclohexylamine at 1-10 mM caused morphological changes in cultures of Sertoli and germ gells comparable with those seen *in vivo*. Sertoli cell vacuolation was the earliest change with progressive germ cell degeneration and exfoliation from the Sertoli cell monolayer (Cre90).

In a comparative study, male Wistar rats (n=45), DA rats (n=120), and MF1 mice (n=45) were given cyclohexylamine hydrochloride in the diet at a constant cyclohexylamine intake of 400 mg /kg bw/day, for 13 weeks. Significantly decreased food intake and body weight gain were found in both strains of rats but not in mice. After 7 and 13 weeks, testicular atrophy was observed by a decrease in organ weight and histological changes in both rat strains but not in mice.Cyclohexylamine concentrations in the plasma and testes were lower in mice than in rats, suggesting that kinetics play a role in the difference in sensitivity of these species (Rob89a).

In a multigeneration study in which rats were fed daily doses of cyclohexylamine (given as the hydrochloride) of 15, 50, 100, and 150 mg/kg bw, for 2 years, a slight decrease in fertility index was found at the 150 mg/kg-dose level in the 4^{th} and 5^{th} matings (*i.e.*, 79 and 77, resp, *vs* a 'normal' range of 87-93; no statistical analysis presented) (Ose76). Although at the end of the study testicular effects were found in the animals of this group, the committee finds it difficult to assess an effect on fertility in this group since similar fertility index values (*i.e.*, 79 and 80) were found in an earlier mating (3rd) at lower doses (15, 50 mg/kg bw).

As reported in an abstract, no consistently significant effects on fertility, reproduction, embryogenesis, and perinatal and postnatal development were observed when rats and rabbits were given daily oral doses of cyclohexylamine sulphate of 1.5 or 15 mg/kg bw/day 1) before and during mating, gestation, and lactation (rats only), or 2) during organogenesis (rats and rabbits), or 3) from gestational day 15 through weaning (rats only) [*The committee notices that the conclusion on fertility cannot be verified since the duration of the pre-mating period is not presented*] (Ken69).

When male and female rats (n=5-15/group) received cyclohexylamine at oral (gavage) doses of 22, 44, 89, or 178 mg/ kg/day, the fertility of the females was not impaired, but male fertility appeared to be decreased in the first of three mating trials. No adverse effects were seen on embryo viability, litter size, litter weight, postnatal viability, or the weight gain of the pups (Khe71).

In another experiment, daily oral (drinking water) doses of cyclohexylamine sulphate of 142 mg/kg bw were administered to both male and female rats (n=10-15/group). After the first three mating trials, only the males were given daily doses of 220 mg/kg bw for another 4 mating trials. Thereafter, males were untreated for 3 trials, but recieved 220 mg/kg bw for the final 2 trials. Male fertility, expressed as the number of females impregnated relative to the number exposed, and the total number of implantation sites were slightly decreased. The numbers of resorption sites, nonviable embryos, and malformed fetuses were similar in the control and test groups (Khe70).

No effect on fertility (mean number of pups, sex ratio, total litter weight, mean pup weight) was seen when males rats (n=5) given 0.6% cyclohexylamine (*ca*. 340 mg/kg bw/day) in the diet for 10 months were caged with 3 young untreated females for 10 days (Bop86, Gau74).

When male (n=30) and female (n=90) rats were mated following a 10-week exposure to daily oral (diet) doses of cyclohexylamine (given as the sulphate) of 136 mg/kg bw, no differences in fertility, pre-implantative and post-implantative losses were found between treated and control groups. Treatment did not induce effects on appearance, behaviour, and weight gain (Lor75).

Preimplantation losses were reported in female rats (random-bred Holtzman) mated with males that had been given two intraperitoneal doses amounting to 100 (50 + 50) or 300 (200 + 100 or 150 + 150) mg/kg bw of cyclohexylamine. About 35% of the ova taken from the females did not show any evidence of cleavage, suggesting that fertilization had not occurred (Gre72).

In a 2-year multigeneration study rats (FDRL; n=30/sex/group) were fed 0, 15, 50, 100, or 150 mg cyclohexylamine/kg bw (given as the hydrochloride). The

parental generation (F_0) was mated to produce five litters, and rats from the first litter of each generation from F_0 through F_4, were mated to produce the next generation. Rats from the second litter of the F_1 through F_4 generations were also mated, with about half of the dams being used for teratology studies and the other half raising their young to maturity. The body weights of the dams, the size of the litters, and the weight of the pups at weaning were all slightly reduced, especially at the two higher doses. Comparison of the results in the control and highest dose group (150 mg/kg) indicated that the reductions in the number of pups cast alive primarily occurred in the first litters of the different generations and that covariance analysis, with the dam weight at mating as the covariant, decreased or eliminated the statistical significance. The data for the pup weights on day 28 showed a more persistent effect through successive litters, but covariance analysis reduced or eliminated the significance of the differences. This suggests that the effects on the number of pups born alive and the pup weight on day 28 may have been related to the decreased maternal weight, which in turn probably resulted from decreased consumption of the unpalatable diets. Other females from this study were sacrificed prior to parturition, and the fetuses were examined *in utero*. The number of implantation sites, the number of live fetuses, and the incidence of malformations were not affected by cyclohexylamine treatment. Fetal weight appeared to be reduced with the highest dose in the F_1, generation, but was less affected in subsequent generations (Bop86, Ose76).

A six-generation reproduction study was performed in mice (Swiss; n=50/sex) given 0.5% cyclohexylamine in the diet (*ca*. 750 mg/kg bw/day) (administered as the sulphate). Growth retardation was seen in the mice receiving cyclohexylamine and was more pronounced in the females. Cyclohexylamine significantly decreased the number of live born fetuses, increased the postnatal mortality, and decreased mean pup body weights. In the litters examined *in utero,* the number of implantation sites was reduced by cyclohexylamine, but no treatment-related malformations were seen (Kro77).

In a multigeneration study in which TR mice were fed doses of 0.5 or 1% cyclohexylamine (*ca*. 750, or 1500 mg/kg bw/day) and NMRI mice doses of 1.0% during gestation and lactation, an increased postnatal mortality rate has been reported. No such effect was seen in TR mice dosed with 0.1% (*ca*. 150 mg/kg bw) (NMRI mice were given 1% only) (Gon72).

A few studies have been performed in which cyclohexylamine was administered to the females during the period of organogenesis and in neither of these studies treatment-related fetotoxic or teratogenic effects were seen.

No consistently significant effects on perinatal and postnatal development were observed when rats and rabbits were given daily oral doses of cyclohexylamine sulphate of 1.5 or 15 mg/kg bw/day during organogenesis (Ken69).

Pregnant rats (Wistar; n=15/group) received cyclohexylamine in the drinking water at doses of 1.8, 3.6, 18, or 36 mg/kg bw between gestational day 8 and 14. Toxic symptoms, disappearing within a few hours after each administration, such as lacrimation, nasal discharge, and slight ataxia were seen in the maternal animals, as well as a decreased body weight gain in the animals given 36 mg/kg bw/day. No effect was observed on fetal growth and survival. Except for three fetuses with abnormalities found in one dam dosed with 18 mg/kg bw/day, no other deformity, including skeletal anomaly, was found in any of the other animals. It was concluded that cyclohexylamine did not produce any fetal malformations (Omo70).

When cyclohexylamine was given at oral doses of 10, 30, or 100 mg/kg bw/day to rats (Long Evans; n=25/group) and mice (NMRI; 25/group) on gestational days 6 to 15 (the hydrochloride was administered), treatment with up to 30 mg/kg bw/day in rats and 100 mg/kg bw/day in mice had no adverse effect on the number of implantations, the resorption rate, sex ratio of the fetuses, fetal weight, placental weight, the incidence of malformations, and skeletal development. In the rats given 100 mg/kg bw/day, reductions in the weights of the placenta and fetuses were observed but these changes were accompanied by a decreased maternal body weight gain. Neither effects on maternal mouse body weights nor maternal impairment of external appearance or behaviour were seen in any of the exposure groups (Lor83).

In Rhesus monkeys receiving 4 daily oral doses of cyclohexylamine (25, 50, or 75mg/kg bw) between gestational days 20 and 45, no increase in intrauterine deaths or malformations was reported. There was some tendency toward lower fetal weights in the females treated earlier in the gestational period, but the small number of animals precluded any statistical analysis of the data (Bop86).

In a study with mice (Swiss-Webster), primigravid animals were dosed intraperitoneously with 61, 77, or 122 mg/kg bw on gestational day 11. All doses significantly depressed fetal body weights. Increased *in utero* deaths were observed at the highest two doses.The incidence of anomalies was not found to be different (Bec70).

The effect of cyclohexylamine was studied in *in vitro* rat embryo cultures. Concentrations of 0.1 and 0.3 mM (10 to 30 µg/mL) had few adverse effects on the growth and differentiation of the ratembryo, but growth retardation and

abnormal morphogenesis were seen at 1 mM or 100 µg/mL. According to the authors this would correspond to a dose of 100 mg/kg bw (Bop86).

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for cyclohexylamine in the Netherlands is 20 mg/m^3 (5 ppm), 8-hour TWA, with a skin notation.

Existing occupational exposure limits for cyclohexylamine in some European countries and in the USA are summarised in annex II.

8 Assessment of health hazard

The committee did not find data on the kinetics following inhalation. After oral administration, cyclohexylamine is rapidly and well absorbed from the intestinal tract in both men and animals (ca. 86-95%). Peak levels in human blood occurred 1 to 2 hours following single oral doses up to 10 mg/kg bw. The plasma half-life in humans ranged from 3 to 5 hours. Cyclohexylamine readily penetrates body tissues, with the highest concentrations occurring in the lungs, spleen, liver, adrenals, heart, gastro-intestinal tract, and kidneys. Cyclohexylamine may freely diffuse across the placenta and enter the fetus. Cyclohexylamine bound moderately to blood serum proteins. The metabolism of cyclohexylamine was rather low in man (1 to 2%), somewhat higher in rats and guinea pigs (less than 10%), and about 30% in rabbits. In man, only the deaminated products cyclohexanone and *trans*-cyclohexane-1,2-diol were found. In rats, the principal metabolic pathway involves ring hydroxylation, leading to isomers of 3- or 4-aminocyclohexanol. Cyclohexylamine was readily excreted, and the renal elimination of the unchanged compound accounts for 80 to 90% of the dose in most species.

Only very limited information on effects following occupational exposure were available. In 3 cases concerning accidental exposure to unknown high concentrations symptoms of central nervous system toxicity were reported. Measurements following one of these events showed levels of 4 to 10 ppm (16-41 mg/m^3) at which operators were stated to be free of symptoms of any kind.

From experimental human and animal studies, the committee concludes that cyclohexylamine is a severely eye and skin irritating and corrosive compound and that it may have skin sensitising properties as well.

No valid LC_{50} studies were available. A single 7-hour exposure to 4900 mg/m^3 (1000 ppm) was lethal to all rabbits and guinea pigs, and to all but one rats. There were no valid dermal LD_{50} studies. Dermally applied doses of 631 mg/kg bw did not induce mortality in rabbits while doses of 1000 or 1580 mg/kg bw did. Following single oral administration to rats, an LD_{50} of 11 mg/kg bw was estimated for undiluted cyclohexylamine while LD_{50}-values ranging from approximately 150 to 615 mg/kg bw were reported in other studies. Effects of acute toxicity observed in rats included cardiovascular effects, nervous system effects (from altered behaviour to paralysis and convulsions), irritation of eyes and the respiratory tract, and brain, lung, liver, and kidney lesions.

After repeated exposure of rats, rabbits, and guinea pigs to 800 ppm (3300 mg/m^3) or 150 ppm (620 mg/m^3), mortality occasionally occurred. The main effects observed were irritation of the respiratory tract, and eye irritation with the development of corneal opacities.

From the results of 2-year oral studies in rats and mice, the committee concludes that there is no evidence that cyclohexylamine is a carcinogenic compound. Data from 90-day, 2-year, and multigeneration studies showed that repeated exposure generally resulted in effects on body weight, organ weights, and the testes. Overall, the committee concludes that for chronic oral exposure, 15 and 18 mg/kg bw are the NOAEL and LOAEL, respectively, for these effects.

The mutagenicity of cyclohexylamine has been extensively studied in a wide variety of mutagenicity test systems, including bacterial, mammalian cell, and *Drosophila* mutagenicity tests, *in vivo* and *in vitro* cytogenetic studies with mammalian cells, mammalian germ cell tests, dominant lethal tests, and various other types of studies. Based on the mostly negative results of these studies, the committee does not consider cyclohexylamine to be a mutagenic/genotoxic compound.

Reproduction toxicity studies in mice and rats indicated that the administration of high doses of cyclohexylamine appeared to be associated with slight decreases in the number of pups born alive, placental and fetal weights, pup survival, and pup growth, as well as effects on the testes. The former pup effects were generally accompanied by, and might have been secondary to, the nutritional status of the females. The effects on the testes were examined in a study especially designed to control dose levels in terms of mg/kg bw, and an NOAEL of 100 mg/kg bw was established. None of the *in vivo* studies in mice, rats, or monkeys has given any indication of a teratogenic effect associated with the administration of cyclohexylamine.

The committee takes a chronic, multigeneration study in rats (Bap86, Ose76) as a basis for deriving a health-based recommended occupational exposure limit (HBROEL) for systemic effects. In this study, the NOAEL was 15 mg/kg bw. Decreased body weight gain and food intake, decreased absolute liver and kidney weights, and histological changes in testes, kidneys, and bladder were found at the higher doses. For the extrapolation to a HBROEL, a factor of 4 for the allometric scaling from rat to man, based on basal metabolic rate, and an overall factor of 9 for intra-and interspecies variation are applied, resulting in an NAEL for humans of 0.42 mg/kg bw/day. Assuming a 70-kg worker inhales 10 m^3 of air during an 8-hour working day and a retention of 100%, and applying the preferred value approach, a health-based occupational exposure limit of 5 mg/m^3 (1.2 ppm) is recommended for cyclohexylamine. Cyclohexylamine is an irritating compound but adequate data on irritation following inhalation exposure are lacking. Therefore, it is not clear whether the proposed limit will protect workers against irritation.

The committee recommends a health-based occupational exposure limit for cyclohexylamine of 5 mg/m^3 (1.2 ppm), as an 8-hour time-weighted average (TWA).

Because of lack of adequate data, the committee is not able to indicate whether or not a skin notation should be added.

References

ACG99 American Conference of Governmental Industrial Hygienists (ACGIH). Cyclohexylamine. In: TLVs® and other occupational exposure values -1999. [CD-ROM]. Cincinnati OH, USA: ACGIH®, Inc, 1999.

ACG00 American Conference of Governmental Industrial Hygienists (ACGIH). Guide to occupational exposure values - 2000. Cincinnati OH, USA: ACGIH®, Inc, 2000: 33.

ACG01 American Conference of Governmental Industrial Hygienists (ACGIH). 2001 TLVs® and BEIs®. Threshold Limit Values for chemical substances and fysical agents. Biological Exposure Indices. Cincinnati OH, USA: ACGIH®, Inc, 2001: 25.

Adl89 Adler ID, Ashby J. The present lack of evidence for unique rodent germ-cell mutagens. Mutat Res 1989; 212: 55-66.

Arb00a Arbejdstilsynet. Grænseværdier for stoffer og materialer. Copenhagen, Denmark: Arbejdstilsynet, 2000; At-vejledning C.0.1..

Arb00b Arbetarskyddstyrelsen. Hygieniska gränsvärden och åtgärder mot luftföroreningar. Solna, Sweden: National Board of Occupational Safety and Health, 2000: 22 (Ordinance AFS 2000/3).

Bea81 Beard RR, Noe JT. Aliphatic and alicyclic amines. In: Clayton GD, Clayton FE, eds. Toxicology. 3rd ed. New York: John Wiley & Sons, 1981:3135-73 (Patty's industrial hygiene and toxicology; Vol 2B).

Bec70 Becker BA, Gibson JE. Teratogenicity of cyclohexylamine in mammals. Toxicol Appl Pharmacol 1970; 17: 551-2.

Ben94 Benya TJ, Harbison RD. Aliphatic and alicyclic amines. In: Clayton GD, Clayton FE, eds. Toxicology. 4th ed. New York: John Wiley & Sons, 1994:1087-1175 (Patty's industrial hygiene and toxicology; Vol II, Pt B).

Ber88 Beretta E, Zerboni R, Nava C. Dermatite allergica da contatto con gomma. Evidenza di una nuova sensibilizzazione di gruppo a derivati della p-fenilendiamina. Med Lav 1988; 79: 482-8.

Bop86 Bopp BA, Sonders RC, Kesterson JW. Toxicological aspects of cyclamate and cyclohexylamine. Crit Rev Toxicol 1986; 16: 213-306.

Bru89 Brusick D, Cifone M, Young R, *et al.* Assessment of the genotoxicity of calcium cyclamate and cyclohexylamine. Environ Mol Mutagen 1989; 14: 188-99.

Bus92 Buss NE, Renwick AG. Blood pressure changes and sympathetic function in rats given cyclohexylamine by intravenous infusion. Toxicol Appl Pharmacol 1992; 115: 211-5.

Cat76 Cattanach BM. The mutagenicity of cyclamates and their metabolites. Mutat Res 1976; 39: 1-23.

CEC00 Commission of the European Communities (CEC). Commission Directive 2000/39/EC of 8 June 2000 establishing a first list of indicative occupational exposure limit values in implementation of Council Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work. Official Journal of the European Communities 2000; L142 (16/06/2000): 47-50.

Clas68 Classen G, Marquardt P, Späth M. Sympathicomimetische Wirkungen von Cyclohexylamin. Arzneimittelforsch 1968; 18:590-4.

Clas70 Classen HG, Gardell C, Kovacs K, *et al.* Electrocardiographic and histological changes in the adrenergic cardiopathy aggravated by cyclohexylamine. Arzneimittelforsch 1970; 20: 27-9.

Cre90 Creasy DM, Ford GR, Gray TJB. The morphogenesis of cyclohexylamine-induced testicular atrophy in the rat: *in vivo* ands *in vitro* studies. Exp Mol Pathol 1990; 52: 155-69.

DFG01 Deutsche Forschungsgemeinschaft (DFG): Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. List of MAK and BAT values 2001. Maximum concentrations and biological tolerance values at the workplace. Weinheim, FRG: Wiley-VCH, 2001: 43 (rep no 37).

Eic74 Eichelbaum M, Hengstman JH, Rost H, *et al.* Pharmacokinectics, cardiovascular and metabolic actions of cyclohexylamine in man. Arch Toxikol 1974; 31: 243-63.

Gag89 Gagnaire F, Azim S, Bonnet P, *et al.* Nasal irritation and pulmonary toxicity of aliphatic amines in mice. J Appl Toxicol 1989; 9: 301-4.

Gau74 Gaunt IF, Sharratt M, Grasso P, *et al.* Short-term toxicity of cyclohexylamine hydrochloride in the rat. Food Cosmet Toxicol 1974; 12: 609-24.

Gau76 Gaunt IF, Hardy J, Grasso P, *et al.* Long-term toxicity of cyclohexylamine hydrochloride in the rat. Food Cosmet Toxicol 1976; 14: 255- 67.

Gon71 Gondry E. Action diabétogène de la cyclohexylamine. Ann Endocrinol 1971; 32: 711-5.

Gon72 Gondry E. Recherches sur la toxicité de la cyclohexylamine, cyclohexanone et du cyclohexanol, métabolites du cyclamate. Eur J Toxicol 1972; 5: 227-38.

Gre72 Green S, Palmer KA, Legator MS. Effects of cyclohexylamine on the fertility of male rats. Food Cosmet Toxicol 1972; 10: 29-34.

Gre98 Greim H (ed). Cyclohexylamin. In: Gesundheitsschädliche Arbeitsstoffe. Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten. 1st-27th ed. Weinheim, FRG: Wiley-VCH, 1998.

Har76 Hardy J, Gaunt I F, Hooson J, *et al.* Long-term toxicity of cyclohexylamine hydrochloride in mice. Food Cosmet Toxicol 1976; 14: 269-76.

HSE01 Health and Safety Executive (HSE). EH40/2001. Occupational Exposure Limits 2001. Sudbury (Suffolk), England: HSE Books, 2001: 15.

Jam81 James RW, Heywood R, Crook D. Testicular responses of rats and dogs to cyclohexylamine overdosage. Food Cosmet Toxicol 1981; 19: 291-6.

Khe70 Khera KS, Stoltz DR. Effects of cyclohexylamine on rat fertility. Experientia 1970; 26: 761-2.

Khe71 Khera KS, Stoltz DR, Gunner SW, *et al.* Reproduction study in rats orally dosed with cyclohexylamine sulfate. Toxicol Appl Pharmacol 1971; 18: 263-8.

Kro77 Kroes R, Peters PWJ, Berkvens JM, *et al.* Long term toxicity and reproduction study (including a teratogenicity study) with cyclamate, saccharin and cyclohexylamine. Toxicology 1977; 8: 285-300.

Lee72 Lee IP, Dixon RL.Various factors affecting the lethality of cyclohexylamine. Toxicol Appl Pharmacol 1972; 22: 465-73.

Lom65 Lomonova GV. Toxicity of cyclohexylamine and dicyclohexylamine. Fed Proc 1965; 24: T96-8 (translation from Gig Trud Prof Zabol 1963; 7 (11): 51-6).

Lor75 Lorke D, Machemer L. Einfluss einer mehrwöchigen Behandlung männlicher und weiblicher Mäuse mit Saccharin, Cyclamat oder Cyclohexylaminsulfat auf Fertilität und Dominant-Lethal-Effekte. Humangenetik 1975; 26: 199-205.

Lor83 Lorke D, Machemer L. The effect of cyclohexylamine on the embryo following oral administration to mice and rats. Toxicol Lett 1983; 17: 137-43.

Lun91 Lundberg P, ed. Consensus report for cyclohexylamine. In: Scientific basis for Swedish ocupational standards. Arbete och Hälsa 1991; 1991/8: 85-8.

Mal52 Mallette FS, Von Haam E. Studies on the toxicity and skin effects of compounds used in the rubber and plastic industries. AMA Arch Ind Hyg Occup Med 1952; 5: 311-7.

Mas77 Mason PL, Thompson GR. Testicular effects of cyclohexylamine hydrochloride in the rat. Toxicology 1977; 8: 143-56.

Miy69 Miyata T, Kasé Y, Kamikawa Y, *et al.* Pharmacological characteristics of cyclohexylamine, one of metabolites of cyclamate. Life Sci 1969; 8: 843-53.

Mor86 Mortelmans K, Haworth S, Lawlor, *et al. Salmonella* mutagencitiy tests: Results from the testing of 270 chemicals. Environ Mutagen 1986; 8:1-59.

Nie89 Nielsen G D, Yamagiwa M. Structure-activity relationships of airway irritating aliphatic amines. Receptor activation mechanisms and predicted industrial exposure limits. Chem Biol Interact 1989; 71: 223-44.

NIO99 National Institute of Occupational Safety and Health (NIOSH). Cyclohexylamine. In: Registry of Toxic Effects of Chemical Substances (RTECS) [CD-ROM], issue July 1999. SilverPlatter International, 1999 (last update cyclohexylamine file: July 1999).

NLM99 US National Library of Medicine (NLM). Cyclohexylamine. In: Hazardous Substances Data Bank (HSDB) [CD-ROM], issue July 1999. SilverPlatter International, 1999 (last update cyclohexylamine file: March 1999).

Omo70 Omori Y, Kuwamura T, Tanaka S, *et al.* Experimental studies on cyclohexylamine in relation to congenital anomalies. EMS Newsl 1970; 3: 30.

Ose76 Oser BL, Carson S, Cox GE, *et al.* Long-term and multigeneration toxicity studies with cyclohexylamine hydrochloride. Toxicology 1976; 6: 47-65.

Pau98 Paulussen JJC, Mahieu CM, Bos PMJ. Default values in occupational risk assessment. Zeist, the Netherlands: TNO Nutrition and Food Research Institute, 1998; TNO rep V98.390.

Pri70 Price JM, Biava CG, Oser BL, *et al.* Bladder tumors in rats fed cyclohexylamine or high doses of a mixture of cyclamate and saccharin. Science 1970; 167: 1131-2.

Ran90 Randall DJ, Bannister RM. Acute toxicologic evaluation of cyclohexylamine. Acute Toxic Data 1990; 1: 65-6

Ric93 Richardson ML, Gangolli S, eds. C498 Cyclohexylamine. In: The dictionary of substances and their effects. Cambridge, UK: Royal Society of Chemistry 1993: 766-8 (Vol 2).

Rob89a Roberts A, Renwick AG, Ford GM, *et al.* The metabolism and testicular toxicity of cyclohexylamine in rats and mice during chronic dietary administration Toxicol Appl Pharmacol 1989; 98: 216-29.

Rob89b Roberts A, Renwick AG. The pharmacokinetics and tissue concentrations of cyclohexylamine in rats and mice. Toxicol Appl Pharmacol 1989; 98: 230-42.

Ros68 Rosenblum I, Rosenblum G. Cardiovascular responses to cyclohexylamine. Toxicol Appl Pharmacol 1968; 12: 260-4.

Sch73 Schmähl D. Fehlen einer kanzerogenen Wirkung von Cyclamat, Cyclohexylamin und Saccharin bei Ratten. Arzneimittelforsch 1973; 23: 1466-70.

SZW01 Ministerie van Sociale Zaken en Werkgelegenheid (SZW). Nationale MAC-lijst 2001. The Hague, The Netherlands: Sdu, Servicecentrum Uitgevers, 2001: 22.

TRG00 TRGS 900. Grenzwerte in der Luft am Arbeitsplatz; Technische Regeln für Gefahrstoffe. BArbBl 2000; 2.

Wat50 Watrous RM, Schulz HN. Cyclohexylamine, p-chlornitrobenzene, 2-aminopyridine: toxic effects in industrial use. Ind Med Surg 1950; 19:317-20.

Yam68 Yamamura HI, Lee IP, Dixon RL. Study of the sympathomimetic action of cyclohexylamine, a ossible metabolite of cyclamate. J Pharm Sci 1968; 57: 1132-4.

Annex I

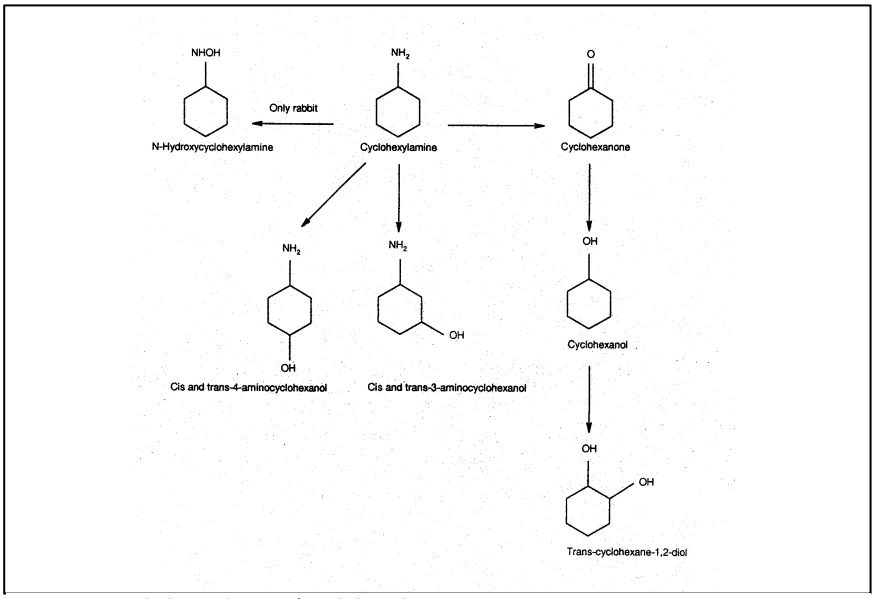

Figure 1 Metabolic pathway of cyclohexylamine.

Annex II

Occupational exposure limits for cyclohexylamine in various countries

country -organization	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	lit ref[b]
	ppm	mg/m^3				
the Netherlands						
-Ministry	5	20	8 h	administrative	S	SZW01
Germany						
-AGS	10	40	15 min		S	TRG00
-DFG MAK-Kom.	10	41	8 h	MAK		DFG01
	20	82	10 min[c]		[d]	
Great-Britain						
-HSE	10	41	8 h	OES		HSE01
Sweden	5	20	8 h			Arb00b
	10	40	15 min			
Denmark	10	40	8 h		S	Arb00a
USA						
-ACGIH	10	-	8 h	TLV	A4[e]	ACG01
-OSHA	-	-				ACG00
-NIOSH	10	40	10 h	REL		ACG00
European Union						
-SCOEL	-	-				CEC00

[a] S = skin notation; which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation

[b] Reference to the most recent official publication of occupational exposure limits

[c] Maximum 4 times per shift; momentary value, *i.e.*, a level which the concentration should never exceed. It presents a limit to be observed in work area technical planning; the analytical testing can then be carried out by use of sampling procedures designed for recording average values

[d] Classified in pregnancy risk group D, *i.e.*, classification in one of the groups A-D is not yet possible because although the data available may indicate a trend they are not sufficient for a final evaluation.

[e] Classified in carcinogenicity category A4, *i.e.*, not classifiable as a human carcinogen: agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of a lack of data. *In vitro* or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.

Annex III

Summary of mutagenicity/genotoxicity data (tables taken from Bop86)

Table 1 Gene mutation studies with cyclohexylamine in microbial and mammalian test systems .

test	organism	concentration	result
Ames test	*S. typhimurium* (TA1535,TA1537, TA98, TA100)	10-1000 µg/plate	-
Ames test	*S. typhimurium* (TA1535, TA1538,TA98, TA100)	4-2500 µg/plate	-
Ames test	*S. typhimurium* (TA1535, TA1537, TA98, TA100)	2500 µg/plate	-
Ames test	*S. typhimurium* (TA1535)	15 µg	-
Ames test	*S. typhimurium*	not specified	-
fluctuation test (streptomycin resistance)	*K. pneumoniae*	not specified	-
	C. freundii		-
	E. aerogenes		-
	S. typhimurium		-
	E. coli		+
pol A^+/pol A^-	*E. coli*	50 µg/well	-
phage Induction	*E. coli*	not specified	-
host-mediated in mouse	*S. typhimurium*	not specified	-
	S. cerevisiae	0.05-0.3 M	+
host-mediated in mouse	*K. pneumoniae*	not specified	-
	C. freundii		-
	E. aerogenes		-
	S. typhimurium		-
	E. coli		-
host-mediated in mouse	*S. typhimurium*	100 mg/kg bw, sc	-
	S. marcescens		-
8-azaguanine resistance	Chinese hamster	1000 µg/mL	-

Table 2 Drosophila studies with cyclohexylamine.

parameters	concentration	route	results
sex-linked lethals	0.1-5 mg/mL	adult injection	-
II-III translocations and mosaic lethals	0.l-0.2%	larval feeding	-
sex-linked lethals and II-III translocations	0.01-1.0%	adult injection	-
sex-linked lethals	1 mg/mL	adult feeding	-
X-loss and non-disjunction	0.08-0.86 mg/mL	adult and larval feeding	na[a]

[a] na: not available

Table 3 *In vitro* cytogenetic studies.

cell system	concentration	duration of exposure	results
human leukocytes	1-100	5-25 h	+
human leukocytes	1-500	24 h	+
human leukocytes	20-500	15 h	-
kangaroo rat kidney	1-500	24 h	+
Chinese hamster fibroblasts	l0-1000	3-124 days	+

Table 4 *In vivo* cytogenetic studies with mammalian somatic cells.

cell system	species	dose	route	duration	results
bone marrow	rat	1-50 mg/kg bw	ip	5 days	+
bone marrow	rat	50 mg/kg bw	po, ip	5 days	-
bone marrow	rat	50-150 mg/kg bw	food	up to 18 months	-
bone marrow	rat	15-60 mg/kg bw	po	>4 months	-
fetal kidney					-
bone marrow	Chinese hamster	50-450 mg/kg bw	ip	3 days	-
host-mediated with human leukocytes					-
leukocytes	Chinese hamster	200 mg/kg bw	po	3 days	+
leukocytes	rat	20-50 mg/kg bw	ip	5 days/ week for 7 weeks	-
leukocytes	fetal lamb	50-250 mg/kg bw	iv	single	+

Table 5 Germ cell studies.

cell system	species	dose	route	duration	results
spermatogonia	rat	1-50 mg/kg bw	ip	5 days	+
spermatogonia	rat	50 mg/kg bw	ip	5 days	-
testes	rat	50-150 mg/kg bw	food	up to 18 months	-
spermatogonia	Chinese hamster	100 mg/kg bw	po	5 days	-
spermatocytes for changes induced in spermatogonia	mouse	50-100 mg/kg bw	ip	5 days	-
spermatogonia and spermatocytes	mouse	40-80 mg/kg bw	ip	single	-

Table 6 Dominant lethal studies.

species	treated sex	mating schedule	dose	route	duration	results
mouse	m	6 weekly matings	100 mg/kg bw	ip	5 days	+
mouse	m	3 weekly matings	100 mg/kg bw	ip	5 days	+
mouse	m	8 weekly matings	102 mg/kg bw	po	5 days	-
mouse	m + f	single mating	0.11% or 136 mg/kg bw	food	10 weeks	-
mouse	f	single mating	102 mg/kg bw	po	single	-
mouse	m	8 weekly matings	5-25 mg/kg bw	ip	single	-
mouse	m	8 weekly matings	13.7-27.3 mg/kg bw	po	5 days	-
mouse	m	3 weekly meetings	50-100 mg/kg bw	ip	5 days	-
mouse	m	3 weekly matings	50 mg/kg bw	ip	single	-
rat	m	2 weekly matings	100--300 mg/kg bw	ip	1 day	preimplantation loss
rat	m		150 mg/kg bw	po	65 days	decreased fertility and implantations

Table 7 Miscellaneous tests with cyclohexylamine.

test system	concentration	results
cell transformation (human lung and Syrian hamster kidney cells)	0.08-250 µg/mL	-
mouse sebaceous gland suppression	2.4 mg/mouse	+
degranulation of endoplasmic reticulum from rat liver	12 µg/mL	+
tetrazolium-reduction by mouse skin	not specified	-
subcutaneous implant in mouse	0.02 mmol or 2 mg	-
in vitro sister chromatid exchange Chinese hamster ovary cells and human lymphocytes	10-100 µg/mL	+
in vivo sister chromatid exchange Chinese hamster bone marrow	not specified	-
mouse spot test	100-200 mg/kg bw, ip	+ (variable)

021-35 Cyclohexylamine