Cyanamide and calcium cyanamide

(CAS No: 420-04-2, 156-62-7)

Health-based Reassessment of Administrative Occupational Exposure Limits

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

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1 Introduction

The present document contains the assessment of the health hazard of cyanamide and calcium cyanamide 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 MA Maclaine Pont, MSc (Wageningen University and Research Centre, Wageningen, the Netherlands).

In August 2000, literature was searched in the databases Toxline, Medline, and Chemical Abstracts, starting from 1981, 1966, and 1992, respectively, and using the following key words: cyanamide, carbimide, carbodiimide, cyanoamine, cyanogen amide, cyanogen nitride, hydrogen cyanamide, N-cyanoamine, calcium cyanamide, 156-62-7, 420-04-2, and 6860-10-2. Data of unpublished studies were generally not taken into account. Exceptions were made for studies that were summarised and evaluated by the German MAK committee (Gre02). The final literature search was carried out in September 2003.

In October 2003, the President of the Health Council released a draft of the document for public review. Comments were received from the following individuals and organisations: A Aalto (Ministery of Social Affairs and Health, Tampere, Finland). These comments were taken into account in deciding on the final version of the document.

2 Identity

Name	:	cyanamide	cyanamide, calcium salt (1:1)
Synonyms	:	amidocyanogen; carbimide; carbodiimide; hydrogen cyanamide; *N*-cyanoamine; cyanogenamide; alzogur	calcium cyanamid; calcium carbimide; Alzodef, Dormex, Temposil
molecular formula	:	CH_2N_2	$CaCN_2$
structural formula	:	H_2N-CN	CaN-CN
CAS number	:	420-04-2	156-62-7

Data from: ACG99a, ACG99b, How92.

3 Physical and chemical properties

		cyanamide	calcium cyanamide
molecular weight	:	42.04	80.10
boiling point	:	at 0.07 kPa: 83°C	sublimes at 1150-1200°C
melting point	:	45-46°C	ca. 1340°C (decomposes)
flash point	:	140.6°C (open cup)	not available
vapour pressure	:	at 20°C: 0.5 Pa	not applicable
solubility in water	:	at 15°C: soluble (77.5 g/100 mL)	insoluble (decomposes)
log $P_{octanol/water}$	:	- 0.82 (experimental); -0.81 (estimated)	-0.20 (estimated)
conversion factors	:	at 20°C, 101.3 kPa: 1 ppm = 1.75 mg/m^3 1 mg/m^3 = 0.57 ppm	at 20°C, 101.3 kPa: 1 ppm = 3.3 mg/m^3 1 mg/m^3 = 0.30 ppm

Data from: Bud96, Lid99, NLM02, http://www.syrres.com/esc/est_kowdemo.htm.

Cyanamide, as the undiluted material, is a colourless deliquescent, almost odourless crystalline solid. It is commonly used as a 25% liquid solution. Pure calcium cyanamide is a non-volatile, non-combustible, white crystalline solid. Commercial calcium cyanamide is a crystalline grey material or a powder, containing small amounts of calcium carbide and other contaminants, such as carbon, calcium hydroxide, calcium oxide, calcium carbonate, and sulphides, oxides, and nitrides of silicon, iron, and aluminium (ACG99a, ACG99b).

Cyanamide is weakly acidic and hygroscopic. It is a highly reactive chemical, which can polymerise explosively if the liquid solution evaporates to dryness. Cyanamide can be stabilised in acidic solutions such as phosphoric acid, sulphuric acid, boric acid, or acetic acid, and by storage at low temperature. The compound decomposes upon heating or burning, forming nitrogen dioxide, ammonia and hydrogen cyanide. It reacts vigorously with strong acids, strong bases, and strong oxidising agents (ACG99a, Gre02, NLM02).

4 Uses

Cyanamide is used as a chemical intermediate for dicyandiamide in melamine manufacture. It is also used in fumigants, metal cleaners, refining of ores, production of synthetic rubber, and in chemical synthesis (ACG99a).

Calcium cyanamide is commercially used as raw material for the manufacture of calcium cyanide and dicyandiamide. It is also used in the desulphurisation of some types of speciality steels. The product is no longer used as a defoliant, fertiliser, or herbicide (ACG99b). According to the database of the

Dutch Pesticide Authorisation Board (CTB)*, neither cyanamide nor calcium cyanamide are permitted in the Netherlands for use as an active ingredient in pesticides.

Both compounds were introduced in Canada, Europe, and Japan in 1956 as new pharmacological adjuncts with few side effects in the treatment of alcoholism. Its use, however, is restricted in some countries, including the USA (Kaw97). It is sold either as a aqueous solution (stabilised with citric acid) or as a slow-release tablet, with various trade-names, such as Abstem, Colme, Dormex and Temposil (Bri80, Bri83, God94, Kaw97).

5 Biotransformation and kinetics

Human data

Cyanamide

To study dermal penetration, the inner side of the 2 forearms (16 cm^2/arm) of 6 male human volunteers were treated with 10 mg cyanamide for 6 hours, under occlusion (total dose: 20 mg per person). On average, 17.7 mg cyanamide was still present on the patches, indicating that maximally 2.3 mg of cyanamide (11.5% of the dose) was available for dermal absorption. Of this amount, 7.7% was excreted in the urine as the metabolite *N*-acetylcyanamide. A maximum dermal absorption rate of 12 μg/cm^2/ hour was calculated (Mer91a).

The kinetics of cyanamide were studied in male human volunteers (n=4/ group), given single oral doses of 0.3, 1.0, or 1.5 mg cyanamide/kg bw. Mean peak cyanamide concentrations in plasma (197-1706 g/L) were achieved at 10.5 to 15.5 minutes after administration. Mean cyanamide elimination half-lives from the plasma were 40, 77, and 62 minutes, respectively. Absorption was not complete, the mean oral bioavailability being 53 and 70% at doses of 0.3 and 1.0 mg/kg bw, respectively. In another experiment, male human volunteers (n=4/ group) received single intravenous infusions of 0.1, 0.3, 0.6, or 1 mg cyanamide/ kg bw for 20 minutes. The mean elimination half-lives from the plasma were 42, 44, 52, and 52 minutes, respectively (Oba91).

After oral administration of 0.25 mg cyanamide/kg bw to male human volunteers, approximately 35 and 40% were excreted in the urine as *N*-acetylcyanamide within 12 and 48 hours after administration, respectively. No significant changes were found in the cyanide concentrations in the blood or in

* at: http://www.ctb-wageningen.nl.

the thiocyanate concentrations in the urine, indicating that cyanamide is not metabolised to cyanide (Mer91a).

Calcium cyanamide

At pH 5, the approximate pH of moist on human skin, 92-98% of calcium cyanamide was converted into cyanamide within 2.5 minutes. At 22ºC, 95-100% was converted within 10 minutes (Mer91b). Under simulated human gastric conditions (at pH 1.15 and 37ºC), calcium cyanamide was hydrolysed to cyanamide to the extent of 92% within 1 hour (Loo81).

Animal data

Cyanamide

In an unpublished dermal penetration study, ^{14}C-labelled cyanamide was applied to the unoccluded skin of rats (12.5 cm^2) at doses of 0.1, 1.0, or 10 mg/animal for 0.5, 1, 2, 4, 10, or 24 hours. The peak concentrations of cyanamide in blood were achieved at 24 hours after application of 0.1 mg and at 10 hours after application of 1.0 or 10 mg. The 24-hour urinary excretion was 0.93, 1.7 or 7.7% of the administered doses at 0.1, 1.0, or 10 mg, respectively; the 24-hour excretion in faeces was less than 0.2%. Twenty-four hours after application, the amount of radioactivity retained in the skin and in the carcass was 20 and 0.1- 4% of the administered doses, respectively. From these data, it was calculated that the 24-hour dermal absorption of radioactivity was 1.8%, 2.8%, or 11.1% at doses 0.1, 1.0, or 10 mg, respectively (SKW89a).

Following administration of a single oral (gavage) dose of aqueous cyanamide of 2 mg/kg bw to Sprague-Dawley rats, the peak concentration of cyanamide in plasma (mean concentration: 0.39 mg/L) was achieved within 5 minutes; the mean plasma elimination half-life was 27 minutes. The mean bioavailablity was 69%. Following oral administration of 4 mg/kg bw to beagle dogs, the peak plasma concentration (mean level: 2.3 mg/L) was reached within 33 minutes; the mean elimination half-life was 62 minutes, the mean bioavailability 65% (Oba89). Single intravenous injections of 2 or 35 mg cyanamide/kg bw into rats resulted in mean elimination half-lives of 33 and 57 minutes, respectively; in beagle dogs given intravenous injections of 1, 2, or 4 mg cyanamide/kg bw, they were 39, 47, and 61 minutes, respectively (Oba86, Oba89).

In rats, the kinetics of cyanamide were studied in fasting and non-fasting animals, given an oral dose of 35 mg/kg bw. No significant differences were found between the bioavailability in fasted animals (mean: 93.3%) compared

with non-fasting animals (mean: 85.5%). However, the peak cyanamide plasma concentrations were reached much faster in fasted animals (mean: 6.3 minutes) compared to non-fasting animals (mean: 14.7 minutes), indicating retardation of drug absorption (Oba86). When ^{14}C-labelled cyanamide was administered intraperitoneally to rats (10 µC/kg bw), 93.9% of the radioactivity was excreted in the urine and 1.4% as expired CO_2 within 6 hours (Dei76).

In an unpublished biokinetic and metabolism study, 4 groups of rats (n=5/sex/group) were given ^{14}C-labelled cyanamide. Group 1 received a single intravenous dose of 1 mg/kg bw, groups 2 and 3 received single oral doses of 1 or 20 mg/kg bw, respectively, and group 4 received oral doses of 1 mg non-radiolabelled cyanamide/kg bw for 14 days, followed by a single oral dose of 1 mg/kg bw of ^{14}C-labelled cyanamide. In all groups, the amount of radioactivity recovered within 7 days was at least ca. 99% of the administered dose, most of it within 12-24 hours after administration. The radioactivity excreted in urine, faeces, and expired air (as $^{14}CO_2$) amounted to 67-98, 2.8-15, and 1.5-10.5% of the administered dose, respectively. Rats in groups 3 and 4 excreted less CO_2 compared to rats in groups 1 and 2, probably due to saturation of metabolism of cyanamide into CO_2. At post-administration day 7, rats in groups 1 and 2 retained traces of radioactivity in the blood (ca. 0.5-0.9%) and the liver (ca. 0.3-1.2%), but not in the gonads (<0.02%) while the carcass contained 2.9- 4.4%. Smaller amounts of radioactivity were found in the tissues after rats given 20 mg/kg bw. The bioavailability was 100% or 95% for rats given an oral dose of 1 or 20 mg/kg bw, respectively. Most of the urinary radioactivity (32 to 61% of the dose) was present as the metabolite *N*-acetylcyanamide. Three other metabolites, of unknown identity, were excreted in amounts of 1.6-14, 6.5-8.9%, and 3.2-4.8%, respectively (SKW93).

Beagle dogs, given a single dose of ^{14}C-labelled cyanamide of 1.7 mg/kg bw, excreted 67-83 and 80-90% of the radioactivity in the urine within 27 and 120 hours after administration, respectively. When the same dose was injected intravenously, 62 and 75% of the radioactivity was excreted in the urine within 27 and 120 hours, respectively. About 87% of the urinary radioactivity excreted between 0 and 27 hours was present as the metabolite *N*-acetylcyanamide, and 11% as unchanged cyanamide. *N*-acetylcyanamide was also found to a large extent (no quantitative data given) in the urine of rats and rabbits, given single intravenous doses of 42 and 75 mg/kg bw, respectively. The acetylation reaction is catalysed by an acetyl-*S*-CoA-dependent hepatic *N*-acetyltransferase, which capacity is variable among different species, with dogs, and possibly also slow acetylator-phenotype rabbits, exhibiting the lowest activity (Shi84).

In rats given single oral doses of unlabelled cyanamide of 10 mg/kg bw, an average of 42.7 and 45.6% of the dose was excreted in the urine as *N*-acetylcyanamide within 22 and 48 hours, respectively (Mer91a).

The ability of a cyanamide metabolite to inhibit the activity of hepatic aldehyde dehydrogenase (ALDH), an enzyme involved in the biotransformation of ethanol, is thought to be an important step in the mechanism of the alcohol-deterrent effect of cyanamide (see Section 6). *N*-acetylcyanamide did not inhibit purified preparations of ALDH, indicating that another, yet unidentified metabolite of cyanamide, is the ALDH blocking agent (Shi84).

Calcium cyanamide

After a single oral dose of 7 mg calcium cyanamide/kg bw (equals 3.68 mg cyanamide/kg) to rats, the peak plasma cyanamide concentration was achieved at 60 minutes after administration. No cyanamide residues were detected in the liver (Bri83).

In vitro data

In an unpublished *in vitro* dermal penetration study, 6 µL of cyanamide solutions of 27 g/L or 544 g/L were applied on 0.64 cm^2 human skin for 6 hours. The amounts absorbed were 34 and 80% at the low and high concentration, respectively. The rate of absorption was faster at the high than at the low concentration (SKW00).

In an unpublished *in vitro* dermal penetration study, 6 µL of cyanamide solutions of 27 g/L or 544 g/L were applied on 0.64 cm^2 of rat skin for 6 hours. The amounts absorbed were 26 and 75% at the low and the high concentration, respectively. The rate of absorption was faster at the high than at the low concentration (SKW00).

6 Effects and mechanism of action

Human data

Cyanamide

A few case studies of skin sensitisation have been reported, most of them related to the use of cyanamide as a therapeutical medicine. A 61-year-old man, treated with cyanamide for alcoholism during 2 years, started to develop erythematous scaly skin lesions. He reacted positively to patch tests with 0.1-5% aqueous solutions of cyanamide (Aba99). Of 7 patients who were treated for alcoholism with cyanamide daily during their stay in a clinic, 6 developed exfoliate dermatitis, and one a lichen-planus-like skin eruption. All subjects had positive patch tests with cyanamide at concentrations ranging between 0.001% and 1% (Kaw97). Three persons, who worked in hospitals and had handled many drugs, among which cyanamide (Colme), for treatment of alcoholic patients, developed erythema and oedema on the hands and also facial dermatitis. All reacted positively to patches containing 0.1 to 5% aqueous solutions of cyanamide (God94). In an earlier case report, a 35-year-old woman, who had given a cyanamide (Colme) solution to her husband as treatment for alcoholism for 4 months, developed redness and swelling on the back of her hand, marked oedema of the tip of her nose, and small papules on her plate and lips. She reacted positively to a patch with 1 and 10% Colme solution (0.6 and 6 g cyanamide/L, respectively) (Fer82). A 32-year-old man, who had handled numerous medicines, developed erythemato-squamous dermatitis. He reacted positively to patches containing 0.1 to 5% aqueous solution of cyanamide (Con81). A case of eczematous dermatitis with diffuse symmetrical involvement of the face, including the eyelids, the region under the chin, and the retroauricular folds, was reported in a worker 20 days after starting as a supervisor of the synthesis of phosphorylcreatine. Symptoms disappeared when away from the workplace, with the aid of medical treatment, but developed again when returning to work on the same job. Patch testing showed positive responses to phosphorylcreatine as well as to dibenzyl phosphite and cyanamide, both used as reagents in phosphorylcreatine synthesis (Fot03). In 2 older case reports, dermatitis and positive patch tests were described in a worker following occupational exposure to lead cyanamide (Bla75), and in a chemist who worked with several chemicals, among which cyanamide (Cal70). No allergic skin reactions were observed in 29 workers engaged in cyanamide and calcium

cyanamide manufacture upon patch testing with a 0.5% aqueous solution of cyanamide (Mer91b).

A 29-year-old Hispanic worker experienced hypotension, vertigo, nausea, puffiness of the face, and hypokalaemia, without exposure to alcohol, while spraying kiwi trees hydrogen cyanamide stabilised with phosphoric acid (Les98).

Calcium cyanamide

In the older German literature, 11 cases were reported of farmers who allegedly died after application of 'Kalkstickstoff', used as an agricultural product. The main ingredients were calcium cyanamide (60%) and calcium oxide (15%). In 5 of the cases, the victims probably had consumed alcohol on the day of application. Within the first days after application, symptoms were irritation of the mucous membranes of the throat, trachea, or bronchi, with a rapid deterioration of the health of the patient. Death followed generally with an unspecific cause, diagnosed, e.g., as a complication of an infection, a disease of the gastrointestinal tract, or a disease of the respiratory or circulatory system (Hau53). The effects of 'Kalkstickstoff' on the mucous membranes were likely caused by the calcium oxide in the product (Sch81). In another case, a causal connection between exposure to calcium cyanamide and the death of a farmer, 8 days after application of calcium cyanamide was doubtful, according to the author (Olk58). A more recent case is described of a 34-year-old housewife with alcohol dependence, who started vomiting, lost consciousness, and died after she took more than 20 mL of a 1% calcium cyanamide solution, together with an alcoholic beverage containing about 129 g of ethanol (Koj97).

The health effects of exposure to calcium cyanamide were investigated in a cross-sectional study on 65 workers who had been employed for 5 to 41 years in a 'Kalkstickstoff'-producing plant. At the time of the study, the airborne concentrations of calcium cyanamide in different units of the plant, measured with static air monitoring, varied between 0.23 and 8.4 mg/m^3. The workers were divided into 3 groups: group 1 comprised 18 workers employed in units of the plant where exposures were higher than 2.5 mg/m^3, group 2 24 workers employed in units with exposures between 1 and 2.5 mg/m^3, and group 3 23 workers employed in units where exposures were below 1 mg/m^3. There were no differences in age, duration of employment, and alcohol use between the 3 groups. The medical examination did not reveal diseases or health impairments related to exposure to calcium cyanamide. Special attention was given to the skin and mucous membranes, the respiratory system, the gastro-intestinal tract, the urogenital tract, the central and peripheral nervous system, the coronary and circulatory system, the thyroid gland, as well as to complications of infections.

The main effects of calcium cyanamide exposure were vasomotoric disturbances after drinking alcohol, leading to flushing, i.e., redness of the head, the neck, and the upper part of the body, often combined with tachycardia and dyspnoea. The percentages of workers who had experienced this effect during their employment were 83, 79, and 74% in group 1, 2, and 3, respectively, and the frequency of occurrence of the effect per year (median) 8, 9, and 15, respectively. According to the authors, the possible cause of the effect is exposure to high levels of calcium cyanamide dust during cleaning operations, combined with heavy drinking during the weekends (Sch81). The committee concluded that a connection between this so-called antabuse effect of workers and potential exposure to calcium cyanamide air levels could not be demonstrated in this study.

Some 10 years later, another occupational health study was conducted in the same factory on 2 groups of workers, engaged in the manufacture of cyanamide and calcium cyanamide. Group 1 comprised 31 males and 1 female, who had been employed for 1.5-35 years. Dermal exposure to (calcium) cyanamide, assessed by quantification of the amount of cyanamide on both hands of the workers, varied from 0.2 to 140 mg (mean: 17 mg; median: 5 mg). Patch tests on the workers did not reveal any case of allergy to cyanamide. Examination of the health status of these persons, including haematological tests, thyroid function tests (serum triiodothyronine, T_3; serum thyroxine, T_4; serum thyroid-stimulating hormone, TSH), or urine analyses did not reveal abnormalities. However, most of the workers had experienced the antabuse effect after drinking alcohol. The incidence of these effects varied largely, and no correlation was found with the amount of alcohol consumption. The effect was also experienced after consumption of cola, coffee, spices, and analgesics. In the 30 workers of group 2 (duration of employment not given), total absorption of (calcium) cyanamide via all routes of exposure was measured by determination of the concentration *N*-acetylcyanamide in urine samples, collected at the end of the 8-hour shift. Concentrations, expressed as cyanamide equivalents, varied from, <0.01 to 4.1 mg/L (mean: 1.1 mg/L; median: 0.2 mg/L). The estimated cyanamide uptake during the workshift, calculated from these urinary levels, ranged from <0.01 to 15 mg/day (mean: 4.0 mg/day; median: 0.7 mg/day). Airborne cyanamide levels were not reported (Mer91b). In a subsequent study, 21 male workers from the calcium cyanamide-production unit of the same factory were examined specifically for effects on thyroid and gonadal function. A control group comprised 9 males, not occupationally exposed to calcium cyanamide. Thyroid function was examined by determination of serum concentrations T_3, T_4, TBG (thyroxine-binding globulin), and TSH. Gonadal function was examined by

determination of serum concentrations FSH (follicle-stimulating hormone), LH (luteinising hormone), and testosterone. Internal exposure to calcium cyanamide was assessed by determination of the concentration *N*-acetylcyanamide in urine samples, collected at the end of the 8-hour shift. Concentrations, expressed as cyanamide equivalents, ranged from <0.01 to 11.54 mg/L (mean: 2.1 mg/L; median 1.2 mg/L), demonstrating appreciable absorption of calcium cyanamide. All control persons had urinary levels below the limit of detection (<0.01 mg/L). The calculated cyanamide uptake varied from <0.04 to 43 mg/day (mean: 8.0 mg/day; median: 4.6 mg/day). No statistically significant abnormalities were found in any of the thyroid or gonadal laboratory tests in exposed workers compared to the control group. No other work-related health disturbances were found in any of the workers in the calcium cyanamide production plant. Airborne cyanamide levels were not reported (Mer93). The committee concludes that mean daily absorption of approximately 0.1 mg cyanamide/kg bw, or 0.2 mg calcium cyanamide/kg bw, via all routes of exposure, in workers engaged in calcium cyanamide processes, does not induce effects on the thyroid or the gonadal function of the workers.

In several, mainly Spanish studies, specific hepatocyte lesions have been reported in patients with chronic alcoholism, who were taking cyanamide as a therapeutic medicine at daily doses of 20 to 180 mg, for 1 to 13 months. These lesions consist of distinctive cytoplasmatic 'inclusion bodies', similar to those observed in Lafora's disease, and to the ground-glass hepatocytes observed in type B hepatitis infections. 'Inclusion bodies' consist of glycogen, secondary lysosomes, and degenerated organelles. In addition, portal and periportal inflammation, as well as portal fibrotic lesions have been observed in patients with 'inclusion bodies' (Bru86, Mor84, Vaz80, Vaz83, Tho81). Apparently, these cytoplasmatic liver changes develop only by simultaneous intake of cyanamide and alcohol, but not of cyanamide alone (Yok95). Further, this effect is probably specific for plain cyanamide, because only scarce cases of this hepatic lesion have been reported in countries, where the medicine is used in its calcium form (Val89).

The antabuse effect of calcium cyanamide, reported in the above studies, is due to interference of cyanamide with the hepatic biotransformation of ethanol, by inhibiting the hepatic enzyme ALDH (See Section 5). This results in an increased blood acetaldehyde level, which implicates unpleasant reactions, including peripheral cutaneous vasodilatation - manifested as flushing -, tachycardia, hypotension, headache, nausea, and vomiting (Sch81, Bri83); also, faintness and sweating are reported (Kaw97).

In several studies, the relationship between alcohol consumption and clinical responses in human subjects exposed to or receiving calcium cyanamide, has been investigated.

As part of the above study on workers in a calcium cyanamide-producing plant (Sch81), 22 workers were asked to drink about 15 grams of alcohol, 1 to 7 hours after the working shift. Modest, weak, and no flushing reactions were observed in 6, 7, and 9 workers, respectively. No correlation was found between flushing reaction and calcium cyanamide exposure. The same author conducted a study with human volunteers who were given 100 mg calcium cyanamide followed by 20 grams of alcohol. Flushing and tachycardia were observed 5 hours after the alcohol consumption. The increase in acetaldehyde levels in blood was about 3 times the increase of a control group who did not ingest calcium cyanamide. Based on these studies, the authors calculated that the antabuse effect started with a daily intake of more than 25 mg calcium cyanamide (Sch81). The interindividual variability in the calcium cyanamide-alcohol interaction was studied in 4 volunteers who consumed 400 mg ethanol/kg bw, 10 hours after oral administration of 0.7 mg calcium cyanamide/kg bw (equals 0.37 mg cyanamide/kg bw). The peak acetaldehyde concentration in blood was achieved at about 30 minutes after administration of alcohol, and ranged from 3.92 to 11.94 µg/mL (mean: 8.87 µg/mL). There was a statistically significant correlation between blood acetaldehyde concentrations and heart rate. The intraindividual variability was studied in 1 individual who drank 400 mg ethanol/kg bw, 12 hours after oral administration of 0.7 mg calcium cyanamide/kg bw, with one-week interval between the sessions. The peak acetaldehyde concentration in blood ranged from 5.87 to 16 µg/mL. The coefficient of variation was 49.9% (Bri80). In another study, 4 male and 3 female volunteers were given an oral dose of 50 mg calcium cyanamide per person (equals 26.3 mg cyanamide per person), followed by an intravenous dose of 200 mg ethanol/kg bw 4 hours later. The peak acetaldehyde blood concentrations ranged from 12.2 to 185 µg/mL, which demonstrates that individual blood levels may vary a factor 15. The antabuse effect of calcium cyanamide started to occur at blood acetaldehyde levels between 30 and 50 µg/mL. Flushing reaction and pulse rate appeared to correlate best with blood acetaldehyde levels (Joh92).

Animal data

Irritation and sensitisation

Cyanamide

Cyanamide is severely irritating to the skin of rabbits, producing erythema, oedema and necrosis, when applied as a 50% aqueous solution under an occlusive patch for 4 hours (SKW82a). In another experiment, a 4-hour treatment of rabbit skin with a 50% aqueous solution under a semi-occlusive cover, caused erythema but only weak oedema (SKW89b). Cyanamide was a skin sensitiser in the standard guinea pig maximisation test of Magnusson-Kligman (SKW82b). However, no skin sensitisation was found when the Buehler test was used (SKW88a).

Cyanamide was severely irritating, when 0.1 mL of 50% aqueous solution was instilled into the rabbit eye (SKW74, SKW91a).

Calcium cyanamide

Calcium cyanamide caused severe skin irritation, several days after application on rabbit skin for a 24-hours period under a polyethylene cuff, at doses of 5 or10 mg/kg (ACG99b).

It was irritating when 100 mg was instilled directly into the conjunctival sac of the eye of a rabbit (ACG99b).

Acute toxicity

Results of acute lethal toxicity tests with cyanamide and calcium cyanamide are summarised in Table 1.

Table 1 Summary of acute toxicity studies in experimental animals.

	exposure route (duration)		species (sex)	LD_{50}/LC_{50}	reference
cyanamide	inhalation	(4 h)	rat (male, female)	>1000	SKW73a
	dermal		rabbit	590 mg/kg bw	Lew00
			rabbit (male, female)	2100-3200 mg/kg bw	SKW73b
			rabbit (male)	901 mg/kg bw	SKW88a
			rabbit (female)	742 mg/kg bw	SKW88b
			rat	84 mg/kg bw	Lew00
	oral		rat	125 mg/kg bw	Lew00
			rat (male)	100-125 mg/kg bw	SKW73c
			rat (female)	>175 mg/kg bw	SKW73a
			rat (male, female)	223 mg/kg bw	SKW94
	intraperitoneal		mouse	200 mg/kg bw	Lew00
calcium cyanamide	inhalation	(4 h)	rat	LCLo: 86 (4 hours)	Lew00
	dermal		rabbit	590 mg/kg bw	Lew00
	oral		rat	158 mg/kg bw	ACG99b
			rabbit	1400 mg/kg bw	ACG99b
			mouse	334 mg/kg bw	ACG99b
			cat	100 mg/kg bw	ACG99b
	intravenous		rat	125 mg/kg bw	Lew00
			mouse	282 mg/kg bw	Lew00
	intraperitoneal		mouse	100 mg/kg bw	Lew00

Cyanamide

In an acute inhalation study, rats (n=5/sex) were exposed to 1 mg/m^3 of a cyanamide aerosol for 4 hours. Particle sizes of the aerosol were 1.5 μm and below. No mortality was observed. Signs of toxicity were irritation of mucous membranes of the respiratory tract. Pathological examination did not show abnormalities (SKW73a).

Following the acute oral and dermal studies, signs of toxicity were lethargy, lachrymation, miosis, tremor, unsteady gait, piloerection (SKW73b, SKW73c, SKW88a, SKW94). In addition, dermal application caused erythema, oedema, and necrosis of the skin (SKW73b).

The effect of cyanamide on the levels of circulating ketone bodies was studied in fed and 18-hour fasted rats, that received a single intraperitoneal injection of 20 mg cyanamide/kg bw and were killed 4-hours later. Blood acetone levels were elevated 10-fold over controls, with a commensurate 5- to 7-fold increase in blood acetoacetate levels and a 2.5-fold increase in blood β-hydroxybutyrate concentrations (fed rat only). The threshold value required to elevate blood levels of ketone bodies was approximately 10 mg/kg bw. The

authors suggested that cyanamide caused a change in the rate of ketone body formation, as well as an inhibition of acetone metabolism. The latter effect is likely due to cyanamide-inhibition of the activity of ALDH (DeM88).

Calcium cyanamide

Rats given a single oral dose of 7 mg calcium cyanamide/kg bw (equals 3.7 mg cyanamide/kg), 30 minutes prior to an intraperitoneal dose of saline at 0, 48, and 96 hours, showed an increased activity of liver enzymes aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and glutamate dehydrogenase (GLDH) in blood, at 48 hours after the last dose of saline was given. No effect was found on the urinary concentrations of glucose, GLDH, *N*-acetyl-β-glucosaminidase (NAG), or coproporhyrin III, or on the urinary concentrations of any of the aldehydes investigated. However, calcium cyanamide caused a potentiating effect on the urinary excretion of several aldehydes in rats treated with diquat (Zwa99).

Subacute and subchronic toxicity

Cyanamide

In an unpublished 2-week 'nose-only' inhalation study, Wistar male and female rats (n=5-8/sex/group) were exposed to cyanamide aerosols at actual concentrations of 0, 148 (85-192), 263 (149-327), or 799 (408-1118) mg/m^3, 6 hours/day, 5 days/week. At the end of the treatment, animals in the high-concentration and control groups were observed for another 2 weeks, before macroscopic and microscopic examination was carried out. Particle sizes of the aerosol were smaller than 5 μm. No mortality or signs of intoxication were observed. In all treatment groups, male and female body weights were significantly reduced, and male and female relative heart and kidney weights significantly increased. Males showed increased absolute and relative gonad weights and increased relative brain weights and females increased relative liver and adrenal weights. In the high-concentration group, macroscopic and microscopic changes were found in the brain, liver, lungs, and heart. The LOAEL was 148 mg/m^3, based on reduced body weight and changes in absolute and relative organ weights (SKW96a). The committee has doubts on the validity of this study, as apparently the actual aerosol concentration was only 5 to 8% of the nominal concentration. In addition, particle size and distribution were not given, and macroscopic and microscopic examination did not include the upper respiratory tract (Gre02).

In an unpublished dermal study, New Zealand white rabbits (n=3/sex/group) received aqueous cyanamide (50% active ingredient) on occluded skin at doses of 12.5, 25, or 37.5 mg active ingredient (a.i.)/kg bw/day, 6 hours/day, 5 days/week, for 3 weeks. No mortality was observed. At all dose levels, some animals showed weakness and lethargy. Skin reactions (erythema) were observed at the mid and high doses. At 12.5 mg/kg bw and above, absolute liver weight was significantly increased in males. Non-dose-related significant changes were noted for absolute or relative kidney, adrenal, or gonad weights, in particular at the high and low dose. At the high dose, microscopic examination revealed congestion in the kidneys, haemorrhages in the lungs, and microcavities in the brain. The LOAEL for systemic changes was 12.5 mg/kg bw/day (SKW96b).

In an unpublished study, Sprague–Dawley rats (n=5/sex/group) were given doses of aqueous cyanamide (50% a.i.) of 5, 10, 20, or 40 mg a.i./kg bw/day by gavage, for 28 days. Reduced body weights were observed in male rats at 20 mg/kg bw/day and higher and in female rats at 40 mg/kg bw/day. Body weight gain was reduced in both sexes at 20 mg/kg bw and higher. At the high dose, a significant decrease in haemoglobin and haematocrit was observed in both males and females and in erythrocyte counts and mean corpuscular haemoglobin concentration in males. Monocyte counts were significantly increased in males. At 40 mg/kg bw/day, males showed significantly increased plasma bilirubin and uric acid levels, and at 10 mg/kg bw/day and above, significantly decreased total protein levels. At 40 mg/kg bw, relative liver and kidney weights were significantly increased in males and females and relative brain, thyroid, and parathyroid weights in males. At 20 mg/kg bw, relative liver and kidney weights were significantly increased in males only. Significant decreases in absolute testicular and epididymis weights were found at 20 mg/kg bw and above. Microscopic examination revealed a significantly increased incidence of small, colloid-poor thyroid follicles in males at 10 mg/kg bw and above, and in females at the high dose. High-dose females also showed follicular cellular hyperplasia. At the high dose, these changes were accompanied by increased serum thyreotropin and decreased T_3 and T_4 levels (not significant). At 10 mg/kg bw and above, a dose-relatedly increased incidence of bile duct hyperplasia was observed in male rats. The NOAEL was 5 mg/kg bw/day (SKW88c). In another unpublished study, Wistar rats (n=10/sex/group) were given cyanamide at dietary doses equivalent to 0, 0.5, 1.5, or 4.5 mg a.i./kg bw/day, for 90 days. No mortality or signs of toxicity were observed at any dose level. At the high dose, erythrocyte counts and relative liver weights were significantly increased in males and relative thymus weights decreased in females. Microscopic examination revealed an increased incidence of small thyroid follicles without

colloid and of proliferating epithelium and interfollicular cells at 1.5 mg/kg bw and above. The NOAEL was 0.5 mg/kg bw/day (SKW75).

Groups of Sprague-Dawley rats (n=20/sex/group) received an aqueous cyanamide solution at doses of 0, 2, 7, or 25 mg a.i./kg bw daily by gavage, for 6 months. At the end of the study, body weights were significantly decreased at 7 mg/kg and above, compared with the control group. Of liver function tests, plasma alkaline phosphatase and bilirubin were significantly increased in males and ALAT activity in females at the high-dose. Plasma bilirubin levels in both sexes were significantly increased at 25 and 7 mg/kg bw/day. Microscopic examination, focussed on the presence of hepatocyte inclusion bodies, did not show treatment-related abnormalities in the liver, compared with control animals. The NOAEL was 2 mg/kg bw/day, based on changes in body weights and in liver function tests (Oba85).

In another study on cyanamide-induced changes in the liver, 20 male Wistar rats were given an aqueous solution of cyanamide in Tween 80, at a dose level of 16 mg a.i./kg bw/day by gavage, for 25 weeks. Control animals (n=5) received daily doses of 2 mL of Tween 80. During cyanamide treatment, 3 animals died. Treatment-related effects were significantly reduced body weights, increased plasma ALAT and ASAT levels, and inclusion bodies in the liver cells of all animals. No inclusion bodies were observed in the control animals or in groups of rats treated with calcium cyanamide or disulfiram (Val89).

In an unpublished study, male and female beagle dogs (n=4/sex/group) received orally (gavage) aqueous cyanamide solutions at doses of 0, 0.6, 2, or 6 mg a.i./kg bw/day, continuously for 90 days. No mortality was observed during the treatment. At 6 mg/kg bw, a decreased body weight gain was observed in the high-dose animals. Clinical chemical tests showed decreased serum T_3 and T_4 levels at 2 mg/kg bw and above. However, these changes were statistically significantly for T_4 levels in males at 6 mg/kg bw/day only. At 6 and 2 mg/kg bw, male dogs had significantly reduced serum ASAT activities and significantly increased serum ALAT activities. At 6 mg/kg bw/day, males had significant decreases in haemoglobin, haematocrit, and erythrocyte and reticulocyte counts, and at 2 mg/kg bw, platelet counts were significantly decreased. Monocyte counts were significantly increased in female dogs at 6 and 2 mg/kg bw/day and in males at 2 mg/kg bw/day. Gross and microscopic examination revealed decreased (not statistically significant) absolute and relative testicular weights at 6 mg/kg bw/day. Atrophy of testicular tubuli, reduced spermatogenesis, or reduced spermatocyte counts in the epididymis were found in dogs at all dose levels, in a dose-related fashion. The LOAEL was 0.6 mg/kg bw/day, based on testicular effects (SKW82c).

In another unpublished study, focussed on testicular effects, beagle dogs (n=4/group) received orally (gavage) aqueous cyanamide solution at doses of 0, 0.6, and 6 mg a.i./kg bw/day, for 13 weeks. At the high dose, absolute and relative testicular weights were significantly decreased in 2 out of 4 animals. Macroscopic examination revealed enlarged cervical lymph nodes in 3 out of 4 animals at 6 mg/kg bw/day, and 1 out of 4 animals at 0.6 mg/kg bw/day. Microscopic examination revealed atrophy and necrosis of germ epithelium cells and a reduced spermatocyte count in the epididymis. According to the authors, the NOAEL was 0.6 mg/kg bw/day (SKW86).

In a next unpublished study, beagle dogs (n=4 /sex/dose group) received 50% aqueous cyanamide solutions by gavage at dose levels of 0, 0.1, 0.5, or 2.5 mg a.i./kg bw/day and of 0, 0.2, 1.0, and 5.0 mg a.i./kg bw/day, continuously for the first 2 weeks and the subsequent 50 weeks, respectively. No mortality was observed during the treatment. Effects in high-dose animals were tremor, salivation, and reduced body weight gain, compared with the controls. At week 52, high-dose females showed decreased haemoglobin levels (not significant), and high-dose males significantly decreased leukocyte counts and significantly increased monocyte counts. However, leukocyte counts were significantly depressed in males at 1 mg/kg bw/day. Mean corpuscular haemoglobin concentration (MCHC) was significantly decreased at 5 and 1 mg/kg bw/day in both males and females. At the high dose, serum T_4 was significantly decreased in males, serum creatinine, uric acid, and calcium in females and serum albumin in both males and females. A statistically significant increase in relative, but not in absolute thyroid and parathyroid weights was found in females. Microscopic examination revealed extramedullary haemopoiesis in the spleen, thymus atrophy, chronic inflammation of the testes, hypo- and aspermatogenesis, hypospermia, and immature sperm in the epididymis in males, enlarged and pale spleens in females, and pigmentation of liver (Kupffer) cells and (little) stones in the gall bladder in both sexes at 5 mg/kg bw/day. At 1 mg/kg bw, there was only immature sperm observed in 1 out of 4 dogs. The NOAEL was 0.2 mg/kg bw/day, based on effects on the male reproductive system (immature sperm) at 1.0 mg/kg bw (SKW89c).

Groups of 40 male Wistar rats and 40 male Sprague-Dawley rats were given intraperitoneal injections of aqueous cyanamide solutions, at doses of 0, 8, or 16 mg a.i./kg bw/day, for 1 year. A statistically significant dose-related decrease in body weight gain was observed in both strains. Also in both strains, increased plasma bilirubin levels and decreased plasma albumin levels and albumin/globulin ratios were found in rats treated with 16 mg/kg bw/day. No hepatocyte inclusion bodies were detected in any of the animals (Oba85).

Calcium cyanamide

In 2 separate range-finding experiments for a carcinogenicity study (see 'Chronic toxicity and carcinogenicity'), F344 rats (n=5/sex/dose level) were fed commercial calcium cyanamide (63% a.i.) at doses equivalent to 0, 13, 19, 25, 28, 31, 38, 47, 94, 126, 252, 315, 504, and 945 mg a.i./kg bw/day, for 7 weeks. All rats given doses of 252 mg/kg bw and above died. No mortality was observed at lower doses. A treatment-related decrease in body weight was observed. At 13 mg/kg bw/day, males and females showed mean body weight decreases of 11 and 3% of mean body weight of controls, respectively. Microscopic examination revealed bile duct hyperplasia in the livers of male and female rats at doses of 47 mg/kg bw/day and above. Thyroid hyperplasia was found in all treated groups, except at 13 mg/kg bw/day. The NOAEL for female rats was 13 mg/kg bw/day. For male rats, a LOAEL could not be established (NCI79).

Male albino Sherman rats (n=24) received calcium cyanamide via the diet at doses equivalent to approximately 52 mg a.i./kg bw/day, for 4 weeks. Then, levels were increased to approximately 60 mg a.i./kg bw/day for 6 weeks, followed by an increase to approximately 102 mg/kg bw/day during the last 4 weeks. A group of 30 control animals was also included. Groups of treated and control animals were killed immediately and 2, 4, and 16 weeks after the last treatment. One treated animal died during the last week of the study. Effects at the end of treatment were significant reductions of food intake and of body weight compared with the control group, which continued until 16 weeks of recovery. Haematology changes included a decreased red blood cell volume, accompanied with an increase of reticulocytes, and increased white blood cell counts. Macroscopic examination showed a marked increase in absolute and relative thyroid weights and increased relative weights of pituitary and adrenal glands, testis, liver, and spleen. Absolute and relative weights of seminal vesicles and absolute ventral prostate weights were significantly reduced compared with control animals. Major microscopic changes were decreased spermatogenesis in 3 out of 5 animals and testicular atrophy. After 16 weeks of recovery, there was still a moderate general oedema, slight general anaemia, extreme epithelial proliferation in the thyroid gland, numerous thyroidectomy cells, marked dystrophy in the anterior pituitary gland, moderate atrophy of the adrenal cortex, moderate fibrosis in the liver, moderate siderosis in the spleen, slight osteoporosis in the femur, and moderate reduction of spermatogenesis in the testis (Ben65).

In a study on calcium cyanamide-induced changes in the liver, 20 male Wistar rats were given a solution of calcium cyanamide in Tween 80, at a dose level of 30 mg a.i./kg bw/day by gavage, for 25 weeks. During treatment, 9

animals died. Control animals (n=5) received daily doses of 2 mL of Tween 80. Treatment-related effects were significantly reduced body weights and significantly increased plasma ALAT and ASAT levels. No inclusion bodies were observed in liver cells of the treated or control animals (Val89).

In a range-finding experiment for a carcinogenicity study (see 'Chronic toxicity and carcinogenicity'), B6C3F_1 mice (n=5/sex/group) were given calcium cyanamide (63% a.i.) via the diet at doses equivalent to 0, 100, 200, 270, 540, 675, 1080, and 2025 mg a.i./kg bw/day, for 7 weeks. In the high-dose group, all mice died. No mortality occurred in the other groups. A treatment-related decrease in body weight was observed; at 100 mg/kg bw/day, males and females showed mean body weight decreases of 8% and 13%, respectively, compared with mean body weight of controls. At 1080 mg/kg bw, males and females showed trace amounts of bile duct hyperplasia. Periportal hepatocytes with pale-staining vacuolated cytoplasm were seen in the males. Focal hepatic necrosis occurred in 4 females. At 675 mg/kg bw/day, the livers of all animals were essentially normal. The LOAEL was 100 mg/kg bw/day, based on reduced body weights (NCI79).

A summary of short-term toxicity studies with cyanamide or calcium cyanamide is given in Table 2.

Table 2 Summary of short-term toxicity studies with cyanamide or calcium cyanamide in experimental animals.

substance; exposure route	species (strain, number, sex)	dose level; exposure duration	critical effect	NOAEL[a]	reference
cyanamide					
inhalation	rat (Wistar; n=5-8/sex/group)	0, 148, 263, 799 mg/m^3; 6 h/d, 5 d/wk, 2 wks	body weight decrease; changes in absolute and relative organ weights	LOAEL: 148 mg/m^3	SKW96a
dermal	rabbit (New Zealand; n=3/sex/group)	0, 12.5, 25, 37.5 mg/kg bw;3 wks	organ weight changes	LOAEL: 12.5 mg/kg bw	SKW96b
oral	rat (Sprague-Dawley; n=5/sex/group)	0, 5, 10, 20, 40 mg/kg bw; 4 wks	thyroid changes; bile duct hyperplasia	5 mg/kg bw	SKW88c
	rat (Wistar; n=10/sex/group)	0, 0.5, 1.5, 4.5 mg/kg bw; 13 wks	thyroid changes	0.5 mg/kg bw	SKW75
	rat (Sprague-Dawley; n=20/sex/group)	0, 2, 7, 25 mg/kg bw; 25 wks	body weight decrease; liver function changes	2 mg/kg bw	Oba85
	rat (Wistar; n=20 males; controls: n=5)	0, 16 mg/kg bw; 25 wks	body weight decrease; liver function changes, incl. inclusion bodies	LOAEL: 16 mg/kg bw	Val89
	dog (beagle; n=4/sex/group)	0, 0.6, 2, 6 mg/kg bw; 13 wks	male reproduction system	LOAEL: 0.6 mg/kg bw	SKW82c
	dog (beagle; n=4 males/group)	0, 0.6, 6 mg/kg bw; 13 wks	male reproduction system	0.6 mg/kg bw	SKW86a

	dog (beagle; n=4/sex/ group)	0, 0.1, 0.5, 2.5 mg/kg bw, 2 wks, then 0, 0.2, 1.0, 5.0 mg/kg bw, 52 wks	male reproduction system	0.2 mg/kg bw	SKW89c
intraperitoneal	rat (Wistar, Sprague-Dawley; n=40 males/strain/ group)	0, 8, 16 mg/kg bw; 52 wks	body weight decrease	LOAEL: 8 mg/kg bw	Oba85
calcium cyanamide					
oral	rat (F344; n=5/sex/dose)	0, 13-945 mg/kg bw; 7 wks	body weight decreases; thyroid changes	LOAEL: 13 mg/kg bw	NCI79
	rat (Sherman; n=24 males; controls: n=30)	0, 52 mg/kg bw, 4 wks, then 60 mg/kg bw, 6 wks, then 102, 4 wks	body weight decrease; anaemia; testicular, thyroid, liver changes	LOAEL: 52-102 mg/kg bw	Ben65
	rat (Wistar; n=20 males; controls: n=5)	0, 30; 25 wks	mortality; body weight decrease; liver injury	LOAEL: 30 mg/kg bw	Val89
	mouse ($B6C3F_1$; n=5/sex/ group)	0, 100-2025 mg/kg bw; 7 wks	body weight decrease	LOAEL: 100 mg/kg bw	NCI79

Chronic toxicity and carcinogenicity

Cyanamide

Male and female Sprague-Dawley Crl:CD BR rats (n=20/sex/group) received aqueous cyanamide solutions by gavage at dose levels of 0, 2.5, 7.5, or 30 mg/kg bw/day for 16 weeks, followed by doses of 0, 1.0, 2.5, or 7.5 mg/kg bw/day for the subsequent 75 weeks. During the first 16 weeks, signs of toxicity (weakness, tremor) were observed in the high-dose animals and decreased body weights and body weight gains in the mid-dose group. In addition, changes in haematological parameters (decrease in erythrocyte counts, haemoglobin and haematocrit values; decrease in platelet counts; increase in leukocyte counts), as well as in clinical chemistry parameters (decrease in serum albumin, total protein, glucose, ALAT, and T_3 levels) were found. From week 17 onwards, signs of toxicity disappeared. However, at 7.5 mg/kg bw, body weights and feed intake were significantly decreased in both males and females. Leukocyte and lymphocyte counts were significantly increased at 1 and 7.5 mg/kg bw in female rats, but not at 2.5 mg/kg bw/day. These changes were, therefore, considered to be unrelated to exposure. Serum T_3 values were decreased in males, dose relatedly, at 1 mg/kg bw/day and above, and in females at 7.5 mg/kg bw/day. This decrease was statistically significant at 7.5 mg/kg bw/day for both males and females. Serum T_4 levels were significantly decreased in high-dose males only. Microscopic examination revealed a dose-related, statistically significantly increased incidence of small, colloid-poor thyroid follicles at 2.5 mg/kg bw/day for males and at 7.5 mg/kg bw/day for females. High-dose females showed an increased

incidence of adrenal haematocysts, breast fibroadenomas, and ovarian cysts. In females, uterus dilatation was found at 1 mg/kg bw/day and above, and polyps of the endometrium at 2.5 mg/kg bw/day. No data were reported on the incidences of neoplastic lesions in organs and tissues. The NOAEL was set at 1.0 mg cyanamide/kg bw/day (SKW91b).

Crl:CD-1 mice (n=60/sex/group) were given cyanamide via the drinking water at doses equivalent to on average 0, 11, 30, or 76 mg/kg bw/day for males and on average 0, 14, 35, or 101 mg/kg bw/day for females. Terminal sacrifices were scheduled at 100 and 104 weeks for males and females, respectively. Mortality was increased in high-dose females, compared to the control group. Treatment-related decreases in body weight gain were found at all dose levels for males and at the mid- and high-dose level for females. Relative brain weight was significantly increased at the top dose in males only. Microscopic examination revealed a dose-related increase of chronic cystitis, characterised by urothelial hyperplasia, fibrosis, and leukocyte infiltration in males and females at the mid and high dose. In the kidneys, focal nephropathy, vacuolar degeneration, and atrophic tubuli were found in high-dose females and in mid- and high-dose males. Other, non-neoplastic changes were treatment-related lung oedema in high-dose males and in females treated with 14 mg/kg bw and above. Hypertrophy and ventricular dilatation of the heart were observed in males at all dose levels and in females at the low dose only. A statistically significant proliferation of biliar liver cells was observed in the high-dose males. In high-dose females, a statistically significantly increased incidence of ovarian granulosa-theca cell tumours was found (controls: 2/60, low-dose: 1/60, mid-dose: 6/60, high-dose: 8/58). The diagnosis of granulosa-theca cell tumour in a third control animal was equivocal because of the occurrence of necrotic lesions. If included, the incidence of this tumour in the control group would have been 3/60. Comparison of this incidence with that of the high-dose group (8/58) did not give a statistically significant difference. In none of the other organs or tissues, an increased incidence of tumours was found at any dose level, compared to the controls. The LOAEL was 11 mg/kg bw/day for males, and 14 mg/kg bw/day for females (SKW90a).

Calcium cyanamide

In a carcinogenicity study, F344 rats (n=50/sex/group) were given a commercial formulation of calcium cyanamide (63% a.i.) via the diet at doses equivalent to 3.2 or 6.4 mg a.i./kg bw/day for males and to 3.2 or 12.6 mg a.i./kg bw for females, for 107 weeks. The control group comprised 20 animals of each sex. Survival was 70% or greater in all dosed and control groups of each sex at the

end of the study, and sufficient numbers of animals were at risk in all groups for the potential development of late-appearing tumours. Mean body weights of the dosed rats were presented in a figure only reporting quantitative data and statistical analyses. Those of the high-dose males and females were stated to be slightly lower when compared to the corresponding controls and those of the low-dose animals slightly and inconsistently lower. No statistically significant differences in neoplasms in any organ or tissue occurred between the treated rats of either sex compared with the controls. No treatment-related non-neoplastic lesions were observed in any organ or tissue although there was a dose-related, not statistically significant increase in the incidence of pheochromocytomas in females when compared with control animals. The authors concluded that under the conditions of the study, the test formulation of calcium cyanamide was not carcinogenic to F344 rats of either sex (NCI79).

B6C3F_1 mice (n=50/sex/dose level) were given the same commercial formulation of calcium cyanamide (63% a.i.) as in the above rat study, via the diet at doses equivalent to 31.5 or 126 mg a.i./kg bw/day for 107 weeks. The control group comprised 20 animals per sex. A dose-related increased mortality occurred in male mice, but survival was 76% or greater in the dosed and control groups, and sufficient numbers of animals were at risk in all groups for the potential development of late-appearing tumours. Mean body weights of the treated animals, which as for the rats (see above) were presented in a figure only, were stated to be slightly decreased compared with the controls, except for the low-dose female mice, whose mean body weights were unaffected by the test chemical. Except for haemangiosarcomas in males and malignant lymphomas or leukaemias in females, microscopic examination did not reveal differences in tumour incidences between treated and control groups. Analyses of the incidences of haemangiosarcomas (controls 1/20 (5%); low dose 2/50 (4%); high dose 10/50 (20%)) showed a dose-related linear trend (p=0.006; Cochran-Armitage test) and no statistically significant difference (Fisher exact test) when comparing incidences in the individual dose groups with those in the control group. The incidence of these tumours in historical control male B6C3F_1 mice was 4%, and the highest incidence observed was 10%. Similar analyses of the incidences of malignant lymphomas or leukaemias (controls: 1/20 (5%), low dose: 11/46 (24%); high dose: 18/50 (36%)) showed a dose-related linear trend (p=0.009; Cochran-Armitage test) and a statistically significantly increased incidence in the high-dose group (p=0.006; Fisher exact test) when compared to the control group. However, the incidence of the lymphomas or leukaemias in historical control female B6C3F_1 mice was 21%, suggesting that the incidence of these tumours in the matched control group of the present study may have been

abnormally low, according to the authors. Thus, neither the incidences of haemangiosarcomas in the male mice nor of lymphomas or leukaemias in the female mice can clearly be related to administration of the test chemical. The authors concluded that under the conditions of the study, the test formulation of calcium cyanamide was not carcinogenic for $B6C3F_1$ mice of either sex. No treatment-related non-neoplastic lesions were observed (NCI79). The committee concludes that under the conditions of these NCI studies, calcium cyanamide is not carcinogenic to rats and mice and does not induce non-neoplastic lesions at the highest doses tested, i.e., 6.4 and 12.6 mg/kg bw/day in male and female rats, respectively, and 126 mg/kg bw in male and female mice. Therefore, the animals might have been able to tolerate higher doses for the duration of the study (107 weeks), and a higher top-dose level should have been used in this study. Since quantitative data and statistical analyses with respect to body weights were lacking and haematology and clinical chemistry end points were not investigated, the committee cannot establish NOAELs.

Mutagenicity and genotoxicity

Cyanamide

In vitro tests:

- Gene mutation assays. Cyanamide was negative when tested with and without rat liver metabolic activation in several strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) at doses of 10-5000 µg/mL (Cad84) or of 0.02-2.54 mg/plate (SKW87a). Tests in *E. coli* strains GY4015 and GY5027, with and without induced-rat liver metabolic activation at doses up to 50-5000 µg/ml, were negative as well (Cad84).
- Other genotoxicity assays. Cyanamide did not induce DNA single strand breaks in rat hepatocytes at doses of 1.3, 13, or 126 mg/L (Sin83). In a DNA repair assay with primary rat hepatocytes, cyanamide did not induce unscheduled DNA synthesis (UDS) at doses between 5.95 and 143 mg/L (SKW87b).

In vivo tests:

- Cytogenicity assays. Swiss mice (n=6/sex/group) received oral doses of cyanamide of 0, 10, 49, or 247 mg/kg bw/day, for 2 consecutive days. At 24 hours after the last dose, no increase was observed in the frequency of micronuclei in normochromic and polychromic bone marrow cells, compared with control animals (Men84). In ICR mice (n=5/sex/group), oral (gavage) of single doses of cyanamide of 0, 32, 157, or 330 mg/kg bw did not result in

changes in the incidence of micronuclei in polychromatic erythrocytes of bone marrow cells at any dose level, compared with control animals. At the high dose, mortality was observed in 2 out of 5 male animals (SKW87c).

Calcium cyanamide

In vitro tests:

- Gene mutation assays. Tests for reverse mutations in several strains of *S. typhimurium* (TA98, TA100, TA1537) were negative both with and without rat and hamster liver metabolic activation. In strain TA1535, it was weakly positive with but negative without metabolic activation systems (Haw83, Ten86).
  The chemical did not induce sex-linked recessive lethality in *D. melanogaster* following feeding or injection of doses of 2000 or 1000 ppm, respectively (Yoo85).
- Other genotoxicity assays. The compound did not induce gene conversion, chromosomal non-disjunction, or chromosomal crossing-over in *A. nidulans* with mouse liver metabolic activation (Val83).

In vivo tests:

Calcium cyanamide did not induce hepatic DNA damage, by an alkaline elution assay, in female Sprague-Dawley rats, when 2 oral doses of 96 mg/kg bw were given 21 and 4 hours before sacrifice (Kit93).

The committee concluded that cyanamide or calcium cyanamide has no mutagenic or genotoxic potential.

Reproduction toxicity

Cyanamide

In a 2-generation study, 8-week-old male (n=20/group) and female (n=40/group) Sprague-Dawley rats received oral (gavage) doses of aqueous cyanamide of 0, 2, 7, or 25 mg a.i./kg bw/day, for 70 days and 15 days, respectively, until mating. In females, treatment continued throughout pregnancy and lactation. Twenty dams per group were sacrificed on day 13 of gestation. F1 male and female offspring from dams that delivered normally (day 21 of gestation) were selected at random to be mated, and treated with 0, 2, 7, or 25 mg cyanamide/kg bw/day, from their weaning to the weaning of the F2 generation. In F0 animals, a significant decrease in fertility index was observed at the high dose, compared with the controls. No effects were shown on mating percentages in any of the treated

groups. In a separate experiment, a significant decreased fertility index was observed when untreated females were mated with males dosed with 25 mg/kg bw cyanamide. However, no statistical difference in fertility index was found when females were treated with 25 mg/kg bw cyanamide and mated with untreated males. The mating percentages were not affected. This result suggests that the decrease in fertility index in F0 animals is related to cyanamide treatment of males. In rats sacrificed on day 13 of gestation, significantly decreased body weight gains, number of corpora lutea, and number of implantations were obtained at 25 mg/kg bw/day, compared with the controls. No abnormalities were noted in number of resorptions, living or dead embryos, or mean embryo weight in any of the groups. In F0 animals that delivered normally, the number of live pups born and litter weights were significantly increased at 2 mg/kg bw/day, as well as the number of implantations, and live born pups at 7 mg/kg bw/day group. However, at 25 mg/kg bw/day, the number of implantations and live born pups, as well as maternal body weight gain were significantly reduced. Mean individual birth weights were significantly increased. In the F1 generation, no treatment-related effects were observed with respect to mean pup weights during the lactation period and in the development of pups. At delivery of the F2 generation, no differences were observed between treated and control groups in mean body weight gain, number of implantations, resorptions, live born or dead pups, mean living birth weight, or mean litter weight. Mean pup weights of the F2 generation during lactation were not different in treated groups, compared with controls. The authors suggest that the F1 generation could have become adapted to cyanamide. Gross examination of organs and tissues revealed that no abnormalities occurred in female rats of both F0 and F1 generations. In F0 males, treated with 25 mg/kg bw/day, 'relative'* epididymis and prostate weights, and at 7 mg/kg bw, 'relative' prostate weights were significantly reduced, compared with controls. 'Relative' testes weights remained unaffected. No abnormalities in reproductive organ weights were seen in F1 animals. Microscopic examination showed testicular atrophy in 4 out of 20 F0 males in the high-dose group. This effect was observed at a low incidence in the F1 generation: 1 out of 6 males in the high-dose group and 1 out of 18 in the control group. The parental NOAEL was 7 mg/kg bw/day, based on reduced body weights at 25 mg/kg bw/day. The reproductive NOAEL was also 7 mg/kg bw/day, based on effects on the reproductive system, reduced fertility index, and reduced number of implantations and live born pups in F0 animals at 25 mg/kg bw/day (Val87).

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* According to an accompanying table, organ weights were relative to brain weights and not to body weights, and are therefore in fact absolute weights (see Fer73).

In another 2-generation study, 7-week-old Crl:CD rats (n=26/sex/group) received aqueous cyanamide by gavage, initially at dose levels of 0, 2.5, 7.5, or 30 mg a.i./kg bw/day. Because of decreases in body weight at 30 mg/kg bw/day and in food consumption and body weight gain at 7.5 mg/kg bw/day and above, doses were reduced after 12 weeks, shortly before mating, to levels 0, 1.25, 3.75, or 15 mg/kg bw/day. F1 animals were also treated with these doses. The fertility indices were low at all dose levels (including controls). In F0 animals treated with 0, 1.25, 3.75, or 15 mg/kg bw day, indices were 77, 91, 83, or 65%, respectively, and in F1 animals 96, 88, 88, or 83%, respectively. The gestation index in the high-dose F1 rats was decreased compared to the low- and mid-dose groups, or the controls. The survival rates of F1 pups after 4 days were 92 (controls), 83, 88, or 84, respectively, and in F2 pups, 93 (controls), 87, 82, or 81, respectively. Based on data from a historical control group (10% mortality) and on the lack of any developmental effects in pups, the authors did not consider the reduced survival rates in F2 pups as treatment-related effects. However, based on control data from other sources (5% mortality), the committee considers the reduced survival rates in F1 and F2 pups as dose-related effects. Mean litter sizes in the low- and mid-dose rats were increased compared to the controls, both for F1 and F2 progenies. Body weights of F1 progeny were decreased at 3.75 mg/kg bw/day and above for both sexes compared to the control group. No treatment-related changes were observed in body weights of F2 pups. According to the authors, the NOAEL for maternal and reproductive toxicity was 1.25 mg/kg bw/day (SKW90b). However, according to the committee, methodological flaws do not allow the setting a NOAEL/LOAEL for maternal or reproductive toxicity in this study.

In a developmental toxicity study, pregnant rats (n=25/group) were given oral (gavage) doses of aqueous cyanamide of 0, 5, 15, or 45 mg a.i./kg bw/day, during days 6 to 15 of gestation. Animals were sacrificed on gestation day 20. Effects on dams were a decrease in body weight gain at 5 mg/kg bw/day and in feed consumption and body weight at the high and mid dose, compared to controls. A decrease in uterus weight of pregnant rats was observed at the top dose. Fetuses of dams treated with 45 mg/kg bw/day showed a decreased body weight and an increased incidence of hernia of the diaphragm. Gross and microscopic examination showed visceral abnormalities in fetuses at the high dose. No further details were provided. The LOAEL for maternal toxicity was 5 mg/kg bw/day. For developmental toxicity, the NOAEL was 15 mg/kg bw/day (SKW89d).

In another developmental toxicity study, pregnant New Zealand White rabbits received aqueous cyanamide by gavage at doses 0, 2, 5.9, or 17.6 mg a.i./kg bw/day, during days 6 to 19 of gestation. Animals in the high-dose

group showed slightly decreased body weights, not statistically significant increases in early resorptions, dead fetuses, or post-implantation loss, and a statistically significant decrease in live fetuses. Fetuses of dams treated with 17.6 mg cyanamide/kg bw/day had not significantly decreased body weights. Microscopic examination revealed a significantly increased incidence of anomalies of the eyes in fetuses at the mid and high dose. Focal disintegration of liver structure was demonstrated in fetuses at the high dose. The NOAELs for maternal and developmental toxicity were 5.9 and 2 mg/kg bw/day, respectively (SKW89e).

7 Existing guidelines

The current administrative occupational exposure limits in the Netherlands (MAC) for cyanamide and calcium cyanamide are 2 mg/m^3 and 0.5 mg/m^3, 8-hour TWA, respectively.

Existing occupational exposure limits in some European countries and in the USA are summarised in Annex I (cyanamide) and Annex II (calcium cyanamide).

8 Assessment of health hazard

The health hazard assessment of (calcium) cyanamide is based to a large extent on unpublished studies presented and discussed in a toxicology review by the German MAK committee (Gre02).

Occupational exposure to cyanamide or calcium cyanamide may occur by inhalation of vapour or dust or by skin contact during the manufacture and use of the substances. Following skin contact or oral ingestion, calcium cyanamide is readily converted into cyanamide. No quantitative data is available of the percentage of pulmonary absorption of the compounds or on the conversion of inhaled calcium cyanamide into cyanamide. In a human volunteer study, the dermal absorption was approximately 10%, when 20 mg cyanamide, as an aqueous solution, was administered on the occluded skin for 6 hours. In rats, the dermal absorption varied from 2 to 11%, when cyanamide was applied on the skin for 24 hours at doses of 0.1-10 mg/animal. Following administration of single oral doses of 0.3-1.5 mg cyanamide/kg bw, the extent of absorption in human volunteers varied from 53 to 70%, depending on the dose level. In rats, 99-105% of a single oral dose of cyanamide was absorbed and in dogs at least 67-83%. No accumulation of cyanamide residues was found in tissues of rats, 7 days after administration of single or repeated oral doses. Following absorption,

a large proportion of cyanamide is acetylated into *N*-acetylcyanamide (ca. 40% in humans, ca. 45% in rats, and ca. 87% in dogs), and most of the metabolite is excreted in the urine within 24 hours. The committee concludes that following exposure, the compound is readily absorbed, metabolised, and eliminated from the body.

Case studies in humans show that cyanamide induced skin sensitisation, mostly related to its medicinal use. Fatalities have been reported in humans consuming alcohol after exposure to calcium cyanamide. In a number of occupational health studies, conducted between 1981 and 1993, no health impairments related to calcium cyanamide exposure have been reported. Patch tests did not reveal cases of skin sensitisation. Exposures to calcium cyanamide, measured with static air monitoring, varied from 0.23 to 8.3 mg/m^3. In a special study in workers engaged in calcium cyanamide processes, a mean daily absorption of approximately 0.1 mg cyanamide/kg bw or 0.2 mg calcium cyanamide/kg bw, via all routes of exposure, did not induce effects on the thyroid or the gonadal function.

The main effect of calcium cyanamide exposure were vasomotoric disturbances after drinking alcohol, leading to flushing, i.e., redness of the head, the neck, and the upper part of the body, often combined with tachycardia and dyspnoea. A connection between this so-called antabuse effect of workers and potential exposure to calcium cyanamide air levels could not be demonstrated. Based on several human volunteer studies, it was suggested that the antabuse effect started with a daily intake of more than 25 mg calcium cyanamide (equivalent to 13.1 mg cyanamide). When cyanamide is given to alcoholics to induce the antabuse effect, the dose is generally 50-70 mg per day.

In experimental animals, both cyanamide and calcium cyanamide were irritating to the eyes and the skin. Cyanamide also was a skin sensitiser. Inhalation of the compound caused irritation of the mucous membranes. Based on the results of acute lethal toxicity studies, the committee considers the substances to be of low toxicity by inhalation, but toxic after dermal and oral exposure. The committee did not find data from valid inhalation studies. Subacute and subchronic oral toxicity studies, varying from 2 to 52 weeks, showed that the male reproductive system, the thyroid, and the liver are the most sensitive target organs following exposure to cyanamide. The NOAEL in the dog was 0.2 mg/kg bw/day, based on effects on the male reproductive system (52-week study), and in the rat, 0.5 mg/kg bw/day, based on thyroid changes (13-week study). In a 3-week dermal study in rabbits, the LOAEL was 12.5 mg/kg bw/day, based on relative and absolute organ weight changes. In a 7-week oral study with calcium cyanamide, a NOAEL for thyroid changes in male and

female rats of 13 mg/kg bw/day was observed (equivalent to 6.8 mg/kg bw/day cyanamide).

Unpublished chronic oral toxicity studies with cyanamide in rats and mice showed a NOAEL of 1.0 mg/kg bw/day for the rat, based on thyroid changes, a LOAEL of 14 mg/kg bw for female mice, based on treatment-related lung oedema, and a LOAEL of 11 mg/kg bw for male mice, based on effects on the heart and decreased body weights. Female mice showed a dose-related increased incidence of ovarian granulosa-theca cell tumours, which was statistically significant at the high dose. The committee considers the statistical significance at the high dose as equivocal, because of uncertainty on the incidence of this tumour in control animals (either 3/60 or 2/60). No carcinogenicity data were reported in the rat study. In NTP carcinogenicity studies with calcium cyanamide, no treatment-related neoplasms were observed in rats. In male mice, an increased incidence of haemangiosarcomas of the circulatory system was found at the high dose (126 mg/kg bw/day), which was not statistically significantly different from concurrent and the historical control groups. In female mice, at the high dose, the incidence of lymphomas and leukaemias was significantly increased compared with the concurrent control group, but not with the historical control group. The committee concluded that under the conditions of these studies, the test formulation of calcium cyanamide was not carcinogenic to rats or mice. However, since no non-neoplastic lesions were observed at the highest dose levels tested, the animals might have been able to tolerate higher doses for the duration of the study (107 weeks), and, therefore, a higher top-dose level should have been used in this study.

Neither cyanamide nor calcium cyanamide induced gene mutations or cytogenetic effects in *in vitro* and *in vivo* studies, which supports the non-carcinogenicity of these substances.

In a reproductive toxicity study, cyanamide induced a decreased fertility rate and testicular atrophy in rats at 25 mg/kg bw/day. No effects on fertility, embryotoxic, and reproductive parameters were observed at 7 mg/kg bw/day. Unpublished developmental toxicity studies reported a NOAEL for developmental abnormalities of 15 mg/kg bw/day for the rat and of 2 mg/kg bw/day for the rabbit.

The committee considers the effects on the reproductive system of the male dog as the critical effect of exposure to cyanamide. These effects described in 3 studies with dosing regimens ranging from 0.2 to 6.0 mg/kg bw/day for 13 weeks and from 0.2 to 5.0 mg/kg bw/day for 52 weeks included chronic inflammation of the testes, atrophy of testicular tubuli, atrophy and necrosis of germ epithelium

cells, hypo- and aspermatogenesis, and reduced spermatocyte counts and immature sperm in the epididymides.

The committee takes the (unpublished) 52-week dog study with cyanamide as a starting point in deriving a health-based recommended occupational exposure limit (HBROEL). In this study, 0.2 mg/kg bw was the NOAEL, based on immature sperm seen at the next higher dose level of 1.0 mg/kg bw. Since workers are exposed for 5 days a week, this NOAEL from a continuous study (i.e., 7 days a week) is adjusted by multiplying with a factor of 7/5 resulting in a no-adverse-effect level (NAEL) of 0.28 mg/kg bw. For the extrapolation to a HBROEL, a factor of 1.4 for allometric scaling from dogs to humans, based on caloric demand, and an overall factor of 9, covering inter-and intraspecies variation, are applied, resulting in a NAEL for humans of 0.022 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 HBROEL of 0.2 mg/m^3 is recommended for cyanamide.

The committee recommends a health-based occupational exposure limit for cyanamide and calcium cyanamide together of 0.2 mg/m^3, expressed as cyanamide, as the inhalable fraction, as an 8-hour time weighted average. Since calcium cyanamide is readily converted into cyanamide into the body, this occupational exposure limit applies for cyanamide and calcium cyanamide together.

The committee considers a skin notation warranted if the amount taken up via the skin of hands and forearms (i.e., 2000 cm^2) for 1 hour is more than 10% of the amount taken up by inhalation at a HBROEL based on systemic toxicity for 8 hours. Using a penetration rate of 12 $\mu g/cm^2/h$ determined in human volunteers, the amount calculated to be taken up by the worker via the skin under the aforementioned premises amounts to 24 mg, which is considerable compared with the amount of 2 mg that could be taken up following an 8-hour exposure to the HBROEL of 0.2 mg/m^3. Therefore, the committee recommends a skin notation.

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Annex I

Occupational exposure limits for cyanamide in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	-	2	8 h	administrative		SZW04
Germany						
- AGS	0.58	1[c]	8 h		S	TRG04
	2.32	4	15 min			
- DFG MAK-Kommission	0.58	2	8 h		S, sens,[e]	DFG04
	1.19		15 min[d]			
Great Britain						
- HSE	-	2	8 h	OES		HSE02
Sweden	-	2	8 h		S	Swe00
	-	4	15 min			
Denmark	-	2	8 h			Arb02
USA						
- ACGIH	-	2	8 h	TLV		ACG04b
- OSHA	-	-				AGG04a
- NIOSH	-	2	10 h	REL		ACG04a
European Union						
- SCOEL	-	2	8 h	ILV[f]		EC04

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

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

[c] Measured as inhalable fraction of the aerosol.

[d] Maximum number per shift: 4, with a minimum interval between peaks of 1 hour.

[e] Listed among substances for which there is no reason to fear a risk of damage to the embryo or fetus when MAK and BAT values are observed.

[f] Listed among compounds for which OELs are already included in Commission Directives.

Annex II

Occupational exposure limits for calcium cyanamide in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	-	0.5	8 h	administrative		SZW04
Germany						
- AGS	-	1[c]	8 h		S	TRG04
	-	4	15 min			
- DFG MAK-Kommission	-	1[c]	8 h		S	DFG04
	-	2	15 min[d]			
Great Britain						
- HSE	-	0.5	8 h	OES		HSE02
	-	1	15 min			
Sweden	-	-				Swe00
Denmark	-	0.5	8 h			Arb02
USA						
- ACGIH	-	0.5	8 h	TLV	A4[e]	ACG04b
- OSHA	-	-				ACG04a
- NIOSH	-	0.5	10 h	REL		ACG04a
European Union						
- SCOEL	-	-				EC04

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

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

[c] Measured as inhalable fraction of the aerosol.

[d] Maximum number per shift: 4, with a minimum interval between peaks of 1 hour.

[e] Classified in carcinogen 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 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.