2,2'-Iminodiethanol

(CAS No: 111-42-2)

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/152 The Hague, October 27, 2005

Preferred citation:
Health Council of the Netherlands: Committee on Updating of Occupational Exposure Limits. 2,2'-Iminodiethanol; Health-based Reassessment of Administrative Occupational Exposure Limits. The Hague: Health Council of the Netherlands, 2005; 2000/15OSH/152.

1 Introduction

The present document contains the assessment of the health hazard of 2,2'-iminodiethanol 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 JJC Paulussen, 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 2,2'-iminodiethanol 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, in April 1999, literature was searched in the on-line databases Medline, Toxline, and Chemical Abstracts starting from 1966, 1965, and 1967, respectively, and using the following key words: diethanolamine, iminodiethanol, dihydroxydiethylamine, and 111-42-2.

In September 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: GM Wallace (Cefic, Brussels, Belgium). These comments were taken into account in deciding on a revised version of the document. Further, results of a literature search performed in Toxline and Medline in September 2004 were included.

In December 2004, the President of the Health Council released a revised draft of the document for public review. Comments were received from the following individuals and organisations: E Ball (Health and Safety Executive, London, UK). These comments were taken into account in deciding on the final version of the document.

2 Identity

name	: 2,2'-iminodiethanol
synonyms	: diethanolamine; *N,N*-diethanolamine; 2,2'-aminodiethanol; diethylolamine; 2,2'-dihydroxydiethylamine; diolamine; 2,2'-iminobisethanol
molecular formula	: $C_4H_{11}NO_2$
structural formula	: $HO\text{-}CH_2\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}OH$
CAS number	: 111-42-2

3 Physical and chemical properties

molecular weight	: 105.15
boiling point	: 269°C
melting point	: 28°C
flash point	: 134°C (closed cup); 152°C (open cup)
vapour pressure	: at 20°C: 0.04 Pa
solubility in water	: miscible
log $P_{octanol/water}$	: -1.43 (experimental); -1.71 (estimated)
conversion factors	: at 20°C, 101.3 kPa: 1 mg/m^3 = 0.23 ppm 1 ppm = 4.38 mg/m^3

Data from ACG99, BGC91, DFG03, NLM04, http://www.syrres.com/esc/est_kowdemo.htm.

2,2'-Iminodiethanol can be either a colourless solid or liquid at room temperature, but is usually offered as a viscous liquid. It is hygroscopic and a strong base with a mild ammonia-like odour. An odour threshold of 0.27 ppm (1.2 mg/m^3) has been reported in man (ACG99, BCG91).

The compound can react with nitrite or nitrogen oxides to form *N*-nitrosodiethanolamine (ACG99).

4 Uses

2,2'-Iminodiethanol is used as an intermediate in the manufacture of dyes, optical brighteners, rubber chemicals, wetting agents, and plasticizers, as an additive for agrochemicals, cosmetics, pharmaceuticals, cutting and drilling oils, cleaning and polishing agents, detergents, and textile chemicals, as a constituent of lubricants and surface-active preparations, and as a corrosion inhibitor in cooling lubricants for metal-cutting (BGC91).

5 Biotransformation and kinetics

There are several extensive reports available on the biotransformation and kinetics of 2,2'-iminodiethanol. However, there is no information concerning the inhalation route.

In a single-dose skin penetration study, radiolabelled 2,2'-iminodiethanol (vehicle: 95% ethanol) was applied to the clipped skin of rats and mice using a non-occlusive protective device. Forty-eight hours after application, 3-16% of doses of 2-27.5 mg/kg bw and 27-58% of doses of 8-81 mg/kg bw were absorbed (i.e., radioactivity found in tissues, urine, and faeces and at the skin dose site) in

rats (skin dose area: 2 cm^2) and mice (skin dose area: 1 cm^2), respectively (Mat97). Using 6-hour data from the aforementioned study, Knaak et al. calculated skin absorption rates in rats of 0.113, 1.48, and 5.8 $\mu g/cm^2/h$ for doses of 2, 7.6, and 27.5 mg/kg bw, respectively (Kna97). In an unpublished study, radiolabelled compound (1500 mg/kg bw; ca. 19.5 mg/cm^2; vehicle not reported) was applied under occlusion to the clipped back skin (dose area: 19.5 cm^2) of rats. After 6 hours, wrappings were removed and treated skin area of half of the animals and all wrappings were washed. From analysis of radioactivity in urine, faeces, and tissues (excluding skin) 48 hours after administration, absorption was estimated to be 1.4 and 0.64% in unwashed and washed animals, respectively. From the 6-hour data, Knaak et al. calculated skin absorption rates of 45 and 21 $\mu g/cm^2/h$ in unwashed and washed animals, respectively. In a repeated-dose skin penetration study, non-labelled compound (1500 mg/kg bw; skin dose area: 25 cm^2) was applied 6 hours/day, for 3 or 6 days, followed by application of the same dose as radiolabelled material which was left on the skin for 48 hours prior to removing wrappings and washing treated skin and wrappings. Absorption - estimated from analysis of radioactivity in excreta, carcass, internal organs and tissues excluding dosed skin site - was 21% and 41%, respectively. From the 48-hour data, Knaak et al. calculated skin absorption rates of ca. 70 and 137 $\mu g/cm^2/h$, in animals exposed for 3 and 6 days, respectively (Kna97). The skin penetration characteristics of undiluted and aqueous solutions of radiolabelled 2,2'-iminodiethanol (doses: 20 mg/cm^2) were examined *in vitro* in a dynamic, flow-through apparatus using full-thickness skin preparations from female rats (CD), mice (CD-1), rabbits (New-Zealand white), and humans (female mammoplasty patients). Under the experimental conditions of this study, time-course curves for appearing of undiluted material in effluent samples showed lag times required to reach steady-state penetration rate of 0.6, 0.9, 1.3, and 3.2 hours, respectively. Steady-state phases extended from 2.5-4.0 hours for rat skin preparations to 4.5-6.0 hours for human skin preparations. Steady-state penetration rates of 1.8, 46.3, 0.9, and 5.7 $\mu g/cm^2/h$ were calculated for rat, mouse, rabbit, and human skin, respectively. Using aqueous solutions, lag times were 0.8, 0.8, 1.5, and 2.4 hours for rat, mouse, rabbit, and human skin, respectively. Steady-state penetration rates were 23, 294, 132, and 12.7 $\mu g/cm^2/h$, respectively (Sun96).

Gastrointestinal absorption appeared to be nearly complete in rats given up to 200 mg/kg bw (Mat97).

The distribution of 2,2'-iminodiethanol after intravenous, oral, or dermal administration was comparable. 2,2'-Iminodiethanol was mainly found in liver, kidney, spleen, brain, and heart, with a particularly high affinity for liver and

kidney (Mat97). Next to the parent compound, metabolites like *N*-methyldiethanolamine and *N,N*-dimethyldiethanolamine were incorporated into phospholipid derivatives and taken up into several tissues (Mat95). Levels of radioactivity in plasma and red blood cells peaked at 5 minutes following intravenous administration of radiolabelled doses of 10 or 100 mg/kg bw to female rats. The concentrations of radiolabel in red blood cells were about 2-fold higher than those in plasma through 6 hours post-administration. After an initial rapid decline, the red blood cells gradually accumulated radioactivity starting at 6-12 hours post-administration. Analysis of radiolabel of tissues of the animals sacrificed 96 hours after injection showed that the majority of the radioactivity administered was in the carcass (35 and 28%, respectively), the liver (21 and 17%, respectively), and the kidneys (7 and 5%, respectively). The liver (15 and 137 µg equivalents 2,2'-iminodiethanol/kg tissue, respectively) and the kidneys 26 and 199 µg equivalents/kg, respectively) contained the highest concentrations. The skin had retained 5% of the radiolabel administered while the other organs and tissues each had less than 1% (Men01). After repeated oral administration of 2,2'-iminodiethanol for several weeks in rats, the distribution pattern appeared the same as after single administration. However, higher equivalents were found in the tissues, demonstrating a potential for 2,2'-iminodiethanol to bio-accumulate, resulting in a steady state for bioaccumulation in tissues after approximately 4 weeks. Dose proportionality for bioaccumulation occurred between 0.7 and 7 mg/kg bw/day, but saturation was evident at 200 mg/kg bw/day. However, there was still no steady state reached in the blood after 8 weeks (Mat97). It was shown that 2,2'-iminodiethanol (330 mg/kg bw/day) given repeatedly to male rats caused significant inhibition of the synthesis of hepatic, most notable, and renal phospholipid derivatives of choline and ethanolamine. These phospholipid derivatives showed a shorter half-life compared to 2,2'-iminodiethanol-phospholipid derivatives, which may favour the accumulation of 2,2'-iminodiethanol containing phospholipids during chronic exposure (Bar79a). Furthermore, a significant alteration in hepatic mitochondria was observed already 2 weeks after daily administration of 42 mg/kg bw of 2,2'-iminodiethanol, due to, at least in part by, elevation of Mg^{2+}-dependent ATPase activity (Bar79b). These effects were not seen in rats after single administration of 2,2'-iminodiethanol (Bar79a, Bar79b).

Within 96 hours following intravenous administration of radiolabelled doses of 10 or 100 mg/kg bw to female rats, 25 and 35% of the radioactivity administered was excreted in the urine, respectively, and only 1-2% via the faeces. The urinary excretion was more rapid at the high dose with ca. 23% of the radioactivity administered excreted in the first 12 hours and an excretion rate

over this period of ca 507 µg equivalents 2,2'-iminodiethanol/hour (low dose: 8.5% and 20 µg equivalents/hour, respectively). Elimination of radioactivity from plasma and red blood cells was biphasic with plasma half-lives of 0.2 and 270 and of 0.3 and 113 hours at the low and high dose, respectively, and red blood cell half-lives of 0.1 and 169 and of 0.6 and 154 hours, respectively (Men01). Within 48 hours after administration of a single oral or intravenous radiolabelled dose of 7 mg/kg to rats, only less than 30% of the administered radioactivity was excreted in urine (almost all parent compound as shown by additional HPLC analysis) and <3% in faeces. After repeated oral administration of 7 mg/kg bw/day, for 1, 2, 4, or 8 weeks to male rats, the mean fraction of the administered amount of radioactivity excreted in the urine in the last week was approximately 40, 70, 80, and 90%, respectively. Almost only parent compound was found, but following 8 weeks of exposure, there were also significant amounts of 2 metabolites, identified and tentatively identified as *N*-methyl iminodiethanol and *N,N*-dimethyl-2-oxomorpholinium, respectively. It was demonstrated that these compounds were incorporated into phospholipids, which appeared to be incorporated into tissues to account for the retention and bioaccumulation. Half-lives of approximately one week were calculated for liver, brain, and spleen. This is comparable to the half-live based on the rate of excretion. However, half-live of 2,2'-iminodiethanol in blood was much longer (ca. 54 days) (Mat97).

From experiments with human and rat liver slices, it might be assumed that the metabolism of 2,2'-iminodiethanol in man is similar to what has been reported for rodents (Mat95).

6 Effects and mechanism of action

Human data

The committee found only a limited amount of data on the toxicity of 2,2'-iminodiethanol in humans.

In its final report on the safety assessment of 2,2'-iminodiethanol, the Cosmetic Ingredient Review (CIR) Expert Panel presented only 2 reports on testing the irritating and sensitising potential of iminodiethanol-containing products in humans (data submitted by the Cosmetic, Toiletry and Fragrance Association (CTFA)). Repeat-insult patch testing of a shave gel containing 2.7% 2,2'-iminodiethanol and of a non-commercial dyeless base formulation containing 2% 2,2'-iminodiethanol in 100 and 165 subjects, respectively, did not produce irritation or sensitisation (Bra83). A 1% solution of 2,2'-iminodiethanol

in paraffin oil produced a positive reaction in 1 out of 32 patients who had been sensitised to ethylenediamine and were investigated for possible cross-sensitisation to, among others, 2,2'-iminodiethanol (Bal86).

In a case report on dermatitis following contact with cutting oil, patch testing with 0.1% 2,2'-iminodiethanol in water produced a positive result. Repeated testing 4 weeks later with serial dilutions showed positive results at concentrations of 1 and 2% in petrolatum; results of 0.05 and 0.1% in water were negative (Blu97). In an evaluation of 4 patch systems in which test materials, among which 1% NaOH and 20% sodium lauryl sulphate, were applied for 4 hours, undiluted 2,2'-iminodiethanol showed no irritation (positive in 1/15 volunteers in one of the tests only) while the results of other test compounds indicated that all patch systems were adequately sensitive (Yor95).

A 39-year-old male metal worker, without a history of atopy, began to experience respiratory complaints 1-2 years after introduction of a cutting fluid containing 0.32% triethanolamine and 0.15% 2,2'-iminodiethanol. In provocation tests employing heated (40°C) cutting fluid (undiluted and 1:1 diluted in water) as well as pure 2,2'-iminodiethanol (1.0 and 0.75 mg/m^3), an asthmatic airway obstruction with a slight dose-response relationship was observed. Specific IgE antibodies to 2,2'-iminodiethanol could not be found (Pii98).

Evaluation of data gathered between 1992 and 2000 by the German Information Network of Departments of Dermatology (IVDK) showed that there is an increased risk of sensitisation to 2,2'-iminodiethanol due to occupational exposure to ethanolamines, especially in workers involved in metal-removing operations. Out of 4701 patients, 77 showed a positive response (1.6%; 15 x 2+, 2 x 3+) while 61/1906 metalworkers (3.2%; 9 x 2+, 1 x 3+) had a positive response to 2,2'-iminodiethanol (2% in petrolatum). Thirty-six out of 325 patients being currently involved in metal-removing operations reacted positively (11.1%; 5 x 2+, 1 x 3+). Questionable or follicular reactions were seen in 20/325 (6.2%) metal-removing workers and in 97/4701 (2.1%) patients. Irritation was seen in 15/4701 patients, one of them being a metalworker (Gre01). Patch testing in one out of 5 dermatology departments, all being members of the German Contact Dermatitis Research Group and the IVDK, between April 2000 and July 2002 showed one positive reaction (i.e., positive when read at 72 hours) to 2,2'-iminodiethanol in 174 patients with potential current and former exposure to metalworking fluid allergens (0.6%) and one positive reaction in 86 metalworkers currently exposed to metalworking fluids with work-related dermatitis (1.6%). In addition, Geier et al. referred to another study, published in 2002 and exclusively focussed on metalworkers exposed to

water-based metalworking fluids with occupational dermatitis, in which 4.2% of the tested population reacted positively to 2,2'-iminodiethanol (Gei03).

Animal data

Irritation and sensitisation

Following a single 4-hour application of 2,2'-iminodiethanol (0.5 mL undiluted) to the clipped covered skin of rabbits, the mean erythema and oedema scores (Draize) were calculated to be 0.78 and 0.89, respectively (maximum score possible: 8). Signs of mild skin irritation were seen during the study (Bar85). In a study performed according to (not specified) French guidelines, pure 2,2'-iminodiethanol produced moderate skin irritation with a primary irritation index of 2.6 (maximum index possible: 8) (Dut82). When 0.1 mL was applied to rabbits (to the ear under uncovered conditions or to the shaved abdomen under 24-hour semi-occluded conditions), for 10 days over a 14-day period, 2,2'-iminodiethanol was concluded to be moderately irritating based on some denaturation on the ear and the abdomen found after 10 and 3 applications, respectively. No irritation was observed following similar applications of a 10% solution. In separate tests, irritation scores of 0.17 and 0.29 (maximum score: 8) were calculated after applying 50 and 30% solutions, respectively, under semi-occluded conditions to the intact and abraded shaved skin of rabbits (data submitted by CTFA, presented by the CIR Expert Panel) (Bra83).

2,2'-Iminodiethanol was concluded not to be a sensitiser when tested in the guinea pig maximisation test according to corresponding OECD and EU guidelines. Challenge application of 25% 'pure' 2,2'-iminodiethanol in physiological saline produced positive erythema reactions in 2/20 and 1/20 animals after 24 and 48 hours, respectively (BGC90).

2,2'-Iminodiethanol was reported not to be sensitising in guinea pigs (no more data presented) (ACG99, BGC91). The review of the CIR Expert Panel did not present data on testing of the sensitising potential of 2,2'-iminodiethanol or 2,2'-iminodiethanol-containing products in experimental animals (Bra83).

When tested for its eye-irritating potential in rabbits, 2,2'-iminodiethanol was graded 5 on a scale of 1 to 10, which was defined as producing a certain injury score, representative of 'severe injury', 18 to 24 hours after application of 5 µL of undiluted test compound (Car46). In a study performed according to (not specified) French guidelines, pure 2,2'-iminodiethanol produced severe eye irritation in rabbits with primary irritation indexes of 50, 56, 52, 45, and 41 after 1, 2, 3, 4, and 7 days, respectively (maximum index possible: 110) (Dut82).

Instillation of 0.2 mL of 50% solution in the conjunctival sac of the eye of rabbits (rinsing after 15 sec) caused moderate to severe irritation and corneal injury with slight reddening of the iris, which healed within 7 days. No irritation was seen upon testing of a 30% solution (data submitted by CTFA, presented by the CIR Expert Panel) (Bra83).

Acute toxicity

Without giving references, Knaak et al. stated that no mortality occurred in rats exposed to saturated vapour concentrations of 2,2'-iminodiethanol at room temperature or to a combination of saturated vapour and mist generated at 170°C (Kna97). In rats exposed to saturated vapours for 8 hours, no mortality occurred while no symptoms were reported following 8-hour exposures to 'air saturated with vaporous particles at 20°C' (Gre98). Exposure to approximately 6500 mg/m^3 resulted in lung oedema and mortality (ACG99, Gre98).

A dermal LD_{50} from a 24-hour occluded contact with rabbit skin was between 8100 and 12,200 mg/kg bw (Kna97). In mice, a subcutaneous LD_{50} of 3500 mg/kg bw has been reported (Mel92).

Acute oral toxicity studies gave LD_{50}s in the range of 770-3500 mg/kg bw in rats (Bra83, Gre98, Kna97). In mice, rabbits, and guinea pigs, LD_{50}s were found to be 3300-4570, 2200, and 2000 mg/kg bw, respectively (Kna97, NIO04, Sha82). Administration of single oral doses of 100-6400 mg/kg bw to rats caused dose-dependent increases in relative liver and kidney weights at 100 mg/kg bw and higher and in the activity of several serum enzymes at 800 mg/kg and higher. Histological changes were seen in the liver (minimal parenchymal cell damage, i.e., liver cells less acidophilic, some loss of basophilic stippling in cytoplasm) and kidney (degenerative changes with localised tubular necrosis) starting at doses of 200 and 400 mg/kg bw, respectively. These lesions became more widespread and severe with increasing doses (Kor73).

Following intraperitoneal injection, LD_{50}s in mice were 2300 and 400 mg/kg bw after observation periods of 24 hours and 7 days, respectively (Blu72, Gre98). Also lower values have been reported: 210 mg/kg for mice and 160 mg/kg for rats (Sha82). Toxicity signs in mice included sedation, ataxia, and loss of righting reflex. 2,2'-Iminodiethanol also produced hepatic steatosis and cellular degeneration (Blu72).

Repeated-dose toxicity

Reported in an abstract, short-term (not specified) inhalation of '200 ppm vapour or 1400 ppm aerosols' (874 and 6118 mg/m^3, respectively) resulted in respiratory difficulties and in some deaths in rats, while continuous exposure to 25 ppm (109 mg/m^3), for 216 hours, increased liver and kidney weights and serum aspartate aminotransferase and blood urea nitrogen levels. Exposure to 6 ppm vapour (26 mg/m^3), 8 hours/day, 5 days/week, for 13 weeks, induced decreased body weight gains, increased lung, liver, and kidney weights, and some mortality (Har70). The committee notices that in view of the very low vapour pressure of 2,2'-iminodiethanol probably aerosols were tested and not vapours.

Rats (Sprague-Dawley; n=10/group; sex not reported), dogs (beagle; n=3/group; sex not reported), and guinea pigs (Hartley; n=6/group; sex not reported) were exposed to 0 and 2.2 mg/m^3 (0, 0.5 ppm) 2,2'-iminodiethanol vapour, 6 hours/day, 5 days/week, for 9 weeks. The vapours were generated from pure 2,2'-iminodiethanol and from a formulation used in photography with approximately 80% water and less than 20% 2,2'-iminodiethanol as the major components. The actual measured 2,2'-iminodiethanol concentrations were slightly higher: 0.6 and 0.7 ppm (2.6 and 3.0 mg/m^3) for the pure compound and the formulation, respectively. Generally, no irritation or clinical effects, no haematological effects, and no macroscopic or microscopic lesions were observed in any of the species. Changes observed were limited to minor changes in relative liver weights in dogs (increases in the animals exposed to the pure compound, decreases in the animals exposed to the formulation), increased absolute and relative kidney weights in guinea-pigs exposed to the pure compound, and a slight growth rate retardation in rats exposed to 2,2'-iminodiethanol alone when compared to the animals exposed to the formulation (both were comparable to controls) (Ter67a)*.

In a separate study, weanling and adult rats (n=10/sex/group), dogs (n=2/sex/group), and guinea pigs (n=5/sex/group) were exposed to air saturated with vapours from pure 2,2'-iminodiethanol and the aforementioned formulation (mean analytical 2,2'-iminodiethanol concentrations: 0.25-0.26 ppm or 1.1 mg/m^3; the vapour generated from the formulation contained 0.05 ppm quinone), 24 hours/day, 7 days/week, for 90 days. In adult and weanling rats, some mortality was seen, but this was not considered to be compound related. Male and female adult rats showed a significantly decreased body weight compared to controls while in the weanling rats a decreased growth rate was seen in the male

* No tables and figures were available to verify the conclusions of Terhaar et al.

animals after the sixth week of exposure. During the first 4 weeks, occurrence of nasal discharge in the treated rats was noticeable. Haematological studies, urine analysis, and ophthalmological studies revealed no significant changes when compared to controls. Evaluation of the relative organ weight data did not demonstrate compound-related effects. Gross examination showed a slightly higher incidence of abnormalities of the lungs (congestion, 'blanched spots') and the livers (discoloured areas) of adult and weanling rats; no changes were seen in the brain, thyroid, parathyroid, heart, spleen, kidneys, trachea, intestines, adrenals, urinary and gall bladder, or gonads. Upon microscopic examination, no compound-related alterations were found in any of the organs examined (i.e., brain, lung, liver, spleen, kidneys, testes, bone marrow). In dogs, there was no mortality and there were no indications for effects on body weights. No signs indicative of irritation or affected health status were observed. No effects were found upon haematological studies and urine analysis. The corneas of the eyes of 2/4 dogs exposed to the formulation were affected. There were no effects on organ weights. Upon gross examination, treated dogs exhibited blackish elevated areas in the spleen, but microscopic evaluation did not show lesions in any of the organs examined. The effects on guinea pigs were more difficult to compare, because of the high mortality rate. After 6 weeks of exposure, a second group of guinea pigs was exposed for 13 weeks to approximately 0.32 ppm 2,2'-iminodiethanol (1.4 mg/m^3), because a high percentage of the original animals died of starvation due to the once-daily feeding schedule. From then, food and water were provided *ad libitum*. However, due to alteration of the air-conditioning later in the study, a lot of guinea pigs from both groups died of pneumonia. In the first group, loss of fur and rashes, and a greater incidence of abnormalities at necropsy (not further described) were seen. The surviving guinea pigs showed no effects on body weight, nasal discharge, ophthalmological examinations, urine analysis, haematology, and organ weights at the end of the exposure period (Leo67)*.

In a 14-day range-finding study, rats (Wistar; n=10/sex/group) were head-nose-only exposed to liquid 2,2'-iminodiethanol (purity: 99.5%) aerosols (mass median aerodynamic diameter - MMAD -: 0.4-1.0 µm) at (measured) concentrations of 0, 110, 210, and 400 mg/m^3, 6 hours/day, 5 days/week. Besides examinations as outlined in OECD guidelines for testing of chemicals, neurofunctional and neurohistological examinations were carried out as well. No treatment-related effects were found at 110 and 210 mg/m^3. Exposure to 400 mg/m^3 induced slightly decreased body weight and body weight gain in males,

* No tables and figures were available to verify the conclusions of Leong.

slightly decreased serum cholesterol in both sexes, and increased absolute and relative liver weights in female animals. There was no evidence of neurotoxicity (BGC93a)*.

In the subsequent subchronic study, rats (Wistar; n=13/sex/group) were head-nose-only exposed to liquid aerosols (MMAD: 0.6-1.9 µm) at mean (measured) concentrations of 15.2, 152.6, and 410.0 mg/m^3, 6 hours/day, 5 days/week, for about 90 days (65 exposures). The study was carried out according to corresponding OECD, EU, and EPA guidelines, and a neurotoxicity evaluation (following EPA test guidelines) was included as well. There was no mortality. Apart from a decrease found in the male animals of the high-concentration group, body weight (gain) was not affected. Clinical observations and ophthalmoscopy did not show treatment-related abnormalities in any of the test groups. From neurofunctional (functional observation battery, motor activity measurement) and neurohistological examinations, there was no evidence of effects on the nervous system. In the high-concentration group, a mild, non-regenerative, normochromic, microcytic anaemia was seen in both sexes. Some male animals of this group showed diffusive testicular atrophy accompanied by oligospermia in the epididymides and slight prostate atrophy.

Concentration-dependent changes in the stomach (erosion), liver (increased weights, increased serum alkaline phosphatase levels; not accompanied by histological changes), and kidneys (haematuria, increase in renal tubular cells and granular casts in the urine, increased kidney weights, tubular hyperplasia, intratubular lithiasis) were observed in both the mid- and high-concentration group. Generally, these changes were of low incidence often occurring in one of the sexes only and of minimal or slight degree, especially in the mid-concentration group. Finally, treatment induced local effects consisting of squamous metaplasia and hyperplasia of laryngeal epithelium and focal inflammation at the tracheal bifurcation at the 2 higher levels and squamous metaplasia of the laryngeal epithelium with some submucosal infiltration of inflammatory cells in a few animals at the lowest concentration of 15.2 mg/m^3 (BGC96)*. The committee concluded that in this study, the NOAELs for systemic and local effects are 15 mg/m^3 and <15 mg/m^3, respectively.

Because a NOAEL was not found in the study discussed above, an additional study was performed in which rats (Wistar, strain: CrlGlxBrlHan:Wl; n=10/sex/group) were head-nose-only exposed to liquid aerosols

* Only volume 1 - report section and summary tables – was available to the committee.

(MMAD: 0.6-0.7 µm; GSD: ca. 3) at target concentrations of 1.5, 3, and 8 mg/m^3*, 6 hours/day, 5 days/week, for 90 days. Furthermore, 2 additional groups consisting of 10 females each were exposed to 3 and 8 mg/m^3, respectively, and sacrificed after a 3-month exposure-free period. The study was carried out according to corresponding OECD, EU, and EPA guidelines and GLP principles. There were no clinical findings or consistent organ weight changes in any of the treated groups. Upon histological examination, only laryngeal lesions were observed especially with respect to the ventral laryngeal epithelium, at the base of the epiglottis. These included focal squamous metaplasia in 9/10 male and 9/10 female animals exposed to 8 mg/m^3. This was accompanied by inflammatory cell infiltration in 3/10 males (grade 2 or 3 on a 5-point scale) and 3/10 females (grade 1 or 2). In the animals exposed to 3 mg/m^3, only squamous metaplasia was seen in 3/10 males; there were no lesions in females. At 1.5 mg/m^3, 1/10 male animals showed inflammatory cell infiltration ('grade 1') while a similar lesion was seen in 2/10 male (grade 1 or 2) and 1/10 female (grade 1) control animals. No effects were observed in the recovery groups (Gam02). Gamer et al. considered the combined occurrence of laryngeal epithelial change and inflammation found at 8 mg/m^3 to be a borderline adverse effect, which was fully reversible within the 3-month recovery period. They considered the laryngeal epithelial change in the absence of inflammation seen at 3 mg/m^3 to be an adaptive change caused by the inhalation of the test compound rather than a true adverse effect. They concluded 3 mg/m^3 to be the NOAEL. However, although the epithelial changes found in 3 male animals exposed to 3 mg/m^3 most probably indeed are adaptive, the committee is of the opinion that they are indicative of cytotoxicity followed by regenerative hyperplasia and, finally, metaplasia, and, thus, are adverse and clearly unwanted effects. The committee considers 3 mg/m^3 to be a minimum observed effect level and places the NOAEL in this study at 1.5 mg/m^3.

Dermal application (vehicle: 95% ethanol) of doses of 125, 250, 500, 1000, or 2000 mg/kg bw/day, 5 days/week, for 2 weeks, to rats (F344/N; n=5/sex/dose) caused mortality in 5/5 females and 3/5 males of the 2000-mg/kg group and in 1/5 females of the 1000-mg/kg group and reduced body weight gains in these 2 dose groups. Treatment induced dose-dependent effects on haematological endpoints (indicating a poorly regenerative, microcytic, normocytic anaemia), the kidney, and the skin. Both for local and for systemic effects, the LOAEL was placed at 125 mg/kg bw, the lowest level tested (Mel92).

---

* Mean measured concentrations: 1.57, 3.43, and 8.18 mg/m^3, respectively.

Application (vehicle: 95% ethanol) of doses of 0, 32, 63, 125, 250, and 500 mg/kg bw/day, 5 days/week, for 13 weeks to rats (F344/N; n=10/sex/group) induced mortality in 1/10 males and 2/10 females of the 500 mg/kg group. Reduced body weight gain was seen in male animals at doses of 250 mg/kg bw and higher and in females at doses of 63 mg/kg bw and above. The primary clinical signs of toxicity were irritation and crusting of the skin at doses of 125 mg/kg bw and above. Treatment induced local effects being dose related in incidence and severity. They included ulceration, inflammation, acanthosis, and hyperkeratosis, the latter found in the female animals at all dose levels. Furthermore, as in the 2-week study, treatment induced anaemia. Red blood cell parameters were affected even at the lowest dose of 32 mg/kg bw; no histological changes were observed in femoral bone marrow. Clinical chemistry parameters were affected at doses as low as 125 mg/kg bw (increased alanine aminotransferase activity in males). There were dose-dependent effects on the kidney (increased relative and absolute weights, tubular necrosis and mineralisation; females more susceptible than males) and on the liver (increased relative and absolute weights with some biochemical changes in serum but without histological changes). At the highest dose level(s), demyelination in the medulla oblongata was found. Both for local and for systemic effects, the LOAEL was set at 32 mg/kg bw, the lowest level tested (Mel92, Mel94a).

Similar studies were performed with mice (B6C3F1; 2-week study: n=5/sex/group; 13-week study: n=10/sex/group) by applying doses of 160, 320, 630, 1250, or 2500 mg/kg bw/day and of 80, 160, 320, 630, or 1250 mg/kg, 5 days/week, for 2 and 13 weeks, respectively. In the 2-week study, mortality was found in the animals of the highest dose group (5/5 males, 3/5 females). No effects on body weight (gain) were found at doses up to 1250 mg/kg bw. Treatment induced effects at the site of application including ulceration and inflammation both in all male and female animals of the 2500 mg/kg group and in the male animals of the 1250 mg/kg group (incidences: 3/5 and 5/5, respectively) and acanthosis in all animals at all dose levels. Systemic effects included dose-dependent increases in absolute and relative liver weights with minimal cytological changes in hepatocytes at the highest dose level. Both for local and systemic effects, a LOAEL of 160 mg/kg bw, the lowest level tested, was established (Mel92).

In the 13-week study, there was mortality in 2/10 males and 4/10 females treated with 1250 mg/kg bw. Body weight (gain) decrease was seen only in the males of this group. Primary clinical signs of toxicity consisted of local effects: irritation, crust formation, thickening of the skin. Microscopically, skin lesions included acanthosis (found at all dose levels), hyperkeratosis, inflammation, and

ulceration. Other effects found were on the liver (increased absolute and relative weights associated with hepatocellular cytological changes - among which multinucleated giant cells resulting from fusion of several hepatocytes (syncytia) at the higher dose levels - and, in males only, necrosis), on the kidneys (increased absolute and relative weights with minimal to mild tubular necrosis in 4 males and 1 female of the highest dose group), and on the heart (increased absolute and relative weight and cardiac myocyte degeneration, at the highest dose only). Both for local and systemic effects, a LOAEL of 80 mg/kg bw, the lowest level tested, was established (Mel92, Mel94b).

Oral (gavage, drinking water, diet) studies are summarised in Table 1 (see Annex II). From these studies, it can be concluded that the blood, the kidneys, and the liver are the target 'organs'. From the 13-week drinking water study in rats, it is concluded that the NOAEL for this type of toxicity study will be less than 15 mg/kg bw, since administration of this dose, the lowest tested, caused changes in haematological endpoints and in the kidney.

Immunotoxicity

Studies to determine the effects of 2,2'-iminodiethanol on the immune system were performed in rats and mice.

Female F344 rats (n=48/group) were given oral (gavage) doses of 0, 50, 100, or 200 mg/kg bw, for 14 consecutive days. Thereafter, animals were sacrificed and groups of 8 animals/dose were evaluated for standard toxicology (body weights, organ weights, pathology, haematology, serum chemistry) (see Table 1, Annex II) and immunological end points including lymphocyte surface markers, spleen IgM antibody-forming cell response to sheep erythrocytes, spleen cell proliferative responses to mitogens and mixed leukocyte response to allogeneic spleen cells, natural killer cell activity, macrophage activity, and peritoneal cell differentials. All rats survived the treatment period and did not show overt signs of toxicity (gait, fur status, drainage from orifices). There were no changes in thymus or spleen weights in any of the exposed groups when compared to controls. 2,2'-Iminodiethanol treatment did not affect the number of B cells, T cells, or T cell subsets, the antibody-forming cell response to SRBC (sheep red blood cells), or the proliferative response to the mitogens concanavalin A or STM (*S. typhimurium* mitogen). There was a dose-dependent increase in the mixed leukocyte response, being statistically significant at 200 mg/kg bw. Expressing the data as a stimulation index, results from both the mid- and high-dose group differed statistically significant from control values. Immune

functional parameters including natural killer cell response and cytotoxicity of resident macrophages were slightly and dose dependently decreased, reaching statistical significance at doses of 200 and of 100 and 200 mg/kg bw, respectively, while the cytotoxicity of peptone-elicited macrophages in cultures without macrophage-activating factors was dose dependently increased being statistically significant in the 2 higher dose groups. Finally, the total number and percentage of lymphocytes were dose dependently increased (significant at 200 mg/kg bw); the total number and percentage of neutrophils were dose dependently decreased (significant at all doses and at 100 and 200 mg/kg bw, respectively). No NOAEL was found in this study. From a preceding range-finding study (data not available to the committee), Munson et al. and NTP concluded that 25 mg/kg bw was a no-effect level (Mun92, NTP03).

Female B6C3F_1 mice (numbers not available)* were given oral (gavage) doses of 0, 100, 300, and 600 mg/kg bw/day for 14 consecutive days and evaluated for standard toxicology (see Table 1, Annex II) and immunology end points. Thymus or spleen weights of the animals of the exposed groups did not differ from those of controls. Administration of 2,2'-iminodiethanol caused changes in the number of B-cells (increase) and of $CD4^+/CD8^-$ T-cell subsets (decrease) but not of total T-cells and other T-cell subsets. The antibody-forming cell response to SRBC was decreased. There was no effect on the proliferative response to mitogens and allogeneic cells (mixed leukocyte response). Apart from a decreased cytotoxic T lymphocyte activity when evaluated at the highest effector/target ratio (25:1), natural killer cell response was not affected. 2,2'-Iminodiethanol decreased the cytotoxicity of resident macrophages while no changes were observed in cytotoxicity of resident macrophages in cultures with macrophage-activating factors or of peptone-elicited macrophages with or without stimulation. Peritoneal cell differentials did not differ between exposed and control animals. Tested in 3 studies, host resistance was not affected in *L. monocytogenes* while there were decreases in resistance to *S. pneumoniae* and in the B16F10 melanoma tumour model. All changes observed were dose dependent. They almost all reached statistically significance at the high dose. However, the cytotoxic T lymphocyte activity and the tumour burden following challenge with the B16F10 melanoma tumour were statistically significantly changed at 100 mg/kg bw, the lowest dose tested; therefore, no no-effect level could be established in this mouse study (NTP98).

These studies show that 2,2'-iminodiethanol, tested at relatively high levels of 50-200 and 100-600 mg/kg bw in rats and mice, respectively, affects the

* Only abstract and summary tables available.

immune system. Although the biological relevance of changes in neutrophil (sub)populations in rats at 50 mg/kg bw is not clear, the committee is of the opinion that in mice, 100 mg/kg bw is a LOAEL based on the decreased resistance found in a bacterial and tumour model.

Carcinogenicity

The potential carcinogenicity of 2,2'-iminodiethanol has been examined in rats and mice by dermal application (vehicle: 95% ethanol). Male and female rats (F344/N, n=50/group) were treated with 0, 16, 32, or 64 and 0, 8, 16, or 32 mg/kg bw/day, respectively, for 2 years. Treatment did not affect survival rates. Decreased body weights were observed in the males and females of the high-dose group starting from week 8 and 97, respectively. The only clinical sign of toxicity observed was local skin irritation. No treatment-related increase in the incidence of any tumour was found. Compared to controls, non-neoplastic effects were limited to increased incidences of acanthosis (in high-dose males), hyperkeratosis (in mid-/high-dose males, in all females), and exudate of the skin (in high-dose males, in all females), and increased incidences (47/50, 48/50, 48/50 vs. 40/50) and severities (1.5, 1.9, 2.7 vs. 1.2) of nephropathy in females (nephropathy incidences in males: 48/50, 50/50, 48/50 vs. 48/50). When mice ($B6C3F_1$; n=50/sex/group) were treated with 0, 40, 80, or 160 mg/kg bw, for 2 years, survival of treated males was similar to that of controls while that of treated females was significantly lower. Decreases in mean body weights were observed in the animals of the mid- and high-dose groups starting from week 53 in high-dose females. 2,2'-Iminodiethanol caused increased incidences of liver neoplasms in male and female animals and of renal tubule neoplasms in male animals. Furthermore, cytoplasmic and syncytial alterations of the liver, thyroid gland follicular cell hyperplasia, haematopoietic cell proliferation of the spleen, and hyperkeratosis of the skin was observed (for summary of incidences of non-neoplastic and neoplastic effects: see Table 2, Annex III) (NTP99).

In evaluating the Tg.Ac transgenic mouse skin model which is being developed as a rapid alternative for the time-consuming cancer bioassays and thought to identify especially non-genotoxic and tumour-promoting compounds, 2,2'-iminodiethanol was one of the compounds tested. Female Tg.AC homozygous transgenic mice (n=20/dose) were treated topically with daily doses of 0, 5, 10, or 20 mg/mouse (vehicle: 200 µL acetone), 5 days/week, for 22 weeks. Negative (treated with acetone only) and positive (receiving 1.25 µg of 12-*O*-tetradecanoylphorbol, twice a week, for 20 weeks) control groups were included as well. Terminal sacrificed was scheduled 6 weeks after the final

applications. After 20 weeks (the end of treatment), survival was high in both treated and control groups, and there was no evidence of chronic irritation or ulceration at the application sites. While there were multiple papillomas in 18/20 animals of the positive controls, no increase in the incidence of skin tumours was found in the 2,2'-iminodiethanol-treated mice (Spa00).

Mutagenicity and genotoxicity

2,2'-Iminodiethanol was negative when tested with and without metabolic activation in bacteria in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 and *E. coli* strains WP2 and WP2 *uvrA* (Dea85, Haw83, Hed78, Mel92), or in yeast in *S. cerevisiae* JD1 (Dea85). In mammalian cell systems in vitro, it was not mutagenic in the L5178Y $TK^{+/-}$ mouse lymphoma cell assay with or without metabolic activation (Mel92). 2,2'-Iminodiethanol did not induce chromosome aberrations in Chinese hamster ovary cells (with and without metabolic activation) (Lov89, Mel92) or rat liver cells (RL1 and RL4) (Dea85) or SCEs in Chinese hamster ovary cells (with/without metabolic activation) (Lov89, Mel92, Sor88). It increased the frequency of non-disjunction on oocytes of orally treated D. melanogaster females (Muñ03).

In vivo, no increased frequency of micronuclei was found in peripheral blood erythrocytes of mice dermally exposed to daily doses of 80-1250 mg/kg bw for 13 weeks (Mel92).

In *in vitro* cell transformation assays, a negative result was reported in Chinese hamster embryo cells (concentration range tested: 25-500 µg/mL) (Ino82). Using Syrian hamster embryo cells, the morphological transformation frequency was significantly increased after exposure to concentrations of 2500-4500 µg/mL for 24 hours (Ker96) and to 250-2500 (Ker96) and 10-500 µg/mL (Leh00) for 7 days, while co-administration of excess choline prevented cell transformation (Leh00).

Mechanisms of action

It was stated that 2,2'-iminodiethanol could react with nitrite to *N*-nitrosodiethanolamine (Ash96). The latter compound produced hepatocellular carcinomas and renal adenomas in rats following oral administration and nasal cavity adenocarcinomas, tracheal papillomas, hepatocellular adenomas, and local fibrosarcomas in hamsters following subcutaneous injection (IARC78), and could, therefore, have contributed to the tumour formation in dermally treated mice. However, *N*-nitrosodiethanolamine was identified neither in urine and

blood from mice ($B6C3F_1$; n=5-6 males/group) treated with doses of 160 mg/kg bw via dermal application with (grooming) or without access to the application site or via oral intubation, 7 days/week, for 2 weeks, nor in blood or gastric contents of additional groups of mice similarly treated but with supplemental nitrite at doses of 40 mg/kg bw (Sto00).

Dietary deprivation of choline is known to induce hepatocellular carcinomas or to promote their formation following initiation by a chemical carcinogen. Mechanisms playing a role might include enhanced cell proliferation, altered methylation status, and altered signal reduction (Leh02). 2,2'-Iminodiethanol was found to alter chlorine homeostasis in a manner resembling choline deficiency. *In vitro*, using Syrian hamster embryo cells, 2,2'-iminodiethanol disrupted intracellular choline homeostasis by inhibiting choline uptake and altering phospholid synthesis. In addition, 2,2'-iminodiethanol increased the morphologic transformation frequency in these hamster cells. Excess choline blocked these biochemical effects as well as the cell transformation (Leh00). In male $B6C3F_1$ mice dermally and orally treated with doses of 160 mg/kg bw/day, 7 days/week, for 2 weeks (see also above), 2,2'-iminodiethanol decreased choline, phosphocholine, and glycerophosphocholine levels by as much as 64, 84, and 70%, respectively. The largest decreases were found in orally treated animals (Sto00). In a separate experiment in doses of 10-160 mg/kg bw were applied to the skin of male $B6C3F_1$ mice, 5 days/week, for 4 weeks, similar biochemical changes consistent with choline deficiency were observed. Phosphocholine was most sensitive to 2,2'-iminodiethanol treatment, decreasing at doses of 20 mg/kg bw and reaching a maximum decrease by 50% at 160 mg/kg bw. The no-adverse-effect level for changes in choline homeostasis was 10 mg/kg bw. In mice treated with 160 mg/kg bw and allowed to recover for 2 weeks prior to sacrifice, levels of choline, phosphocholine, glycerophosphocholine, phosphatidylcholine, *S*-adenosylmethionine, and *S*-adenosylhomocysteine had returned to control levels. Application of doses of 160 mg/kg bw/day to the back skin of C57BL/6 mice – a strain which was considered to be resistant to liver tumour development – caused decreases in phosphocholine concentrations, however, without affecting hepatic *S*-adenosylmethionine levels, suggesting that strain-specific differences in intracellular methyl group regulation may influence carcinogenic outcome with 2,2'-iminodiethanol treatment. Dermal application of 95% ethanol, which was used as a vehicle in the dermal 2,2'-iminodiethanol studies, reduced hepatic levels of betaine, which is an oxidation product of choline and which serves as the methyl donor in the conversion of homocysteine into methionine. Lehman et al. suggested that co-administration of ethanol might have exacerbated the

hepatic effects of 2,2'-iminodiethanol because of the substantial interplay between choline and methyl metabolism (Leh02).

Because of the lack of genotoxicity, enhanced cell proliferation and suppression of apoptosis, features of non-genotoxic liver tumour promoters, might have played a role in the dermal carcinogenicity studies. These characteristics were investigated by dermal application of doses of 2,2'-iminodiethanol (vehicle: ethanol) of 160 mg/kg bw/day to 8 male and 8 female B6C3F1 mice, for 1 week, followed by a 3-week exposure-free recovery period, of doses of 160 mg/kg bw/day to groups of 10 male mice for 1, 4, or 13 weeks, or of doses of 10, 20, 40, 80, 160, 630, or 1250 mg/kg bw/day to groups of 8 male mice, for 1 or 13 weeks. Animals were treated 5 days/week; during the period of BrdU-administration, the respective animals were treated 7 days/week. Control groups were included as well. Since the first study did not reveal major differences between males and females, the subsequent studies were performed with males only. No treatment-related mortality occurred. Severe skin lesions at the application sites were observed in animals treated with doses of 630 and 1250 mg/kg bw and the animals were sacrificed after 1 week. Single animals of the other groups, including controls, showed erythema and/or crust formation at application sites, but these were attributed to the administration procedure and/or ethanol (the vehicle, but not to 2,2'-iminodiethanol. Relative liver weights were increased by 8-15% at doses of 10-160 mg/kg bw, without being related to dose or duration, and by 26 and 32% at 630 and 1250 mg/kg bw/day, respectively. Liver weights of the males sacrificed 3 weeks after receiving 160 mg/kg bw/day were similar to those of controls. Microscopic examination did not reveal overt signs of liver toxicity at any of the doses. There were cytoplasmic eosinophilia in zone 1 (periportal zone) of the liver lobules at doses 40 mg/kg bw after 1 week and at doses of 80 and 160 mg/kg bw at 13 weeks and hepatocellular giant cells in zone 3 (central vein region) of the liver lobules at doses of 160 mg/kg bw after 13 weeks. Following 1-week treatment, cell proliferation was statistically significantly increased in zone 3 at doses of 80 and 160 mg/kg bw, but returned to levels lower than those of controls in the group receiving 160 mg/kg bw after a 3-week recovery period. Following 13-week treatment, cell proliferation, particularly in the central vein region, was increased reaching statistically significance in all treated groups except the 40 mg/kg bw group. Application of 2,2'-iminodiethanol did not affect apoptosis in any of the treated groups (Mel04).

Reproduction toxicity

In a 13-week inhalation study using rats (Wistar; n=13/group), effects on the male reproductive system consisting of diffusive testicular atrophy accompanied by oligospermia in the epididymides and slight prostate atrophy in some animals were found at concentrations of 410 mg/m^3, but not at 152 mg/m^3 (BGC96). In male rats (F344/N; n=5/group) given daily doses of 10,000 ppm (1016 mg/kg bw) of 2,2'-iminodiethanol in drinking water, for 2 weeks, mild to moderate seminiferous tubule degeneration characterised by a reduction in tubule size and in the number of spermatogenic cells and accompanied by the appearance of large numbers of degenerate cells in the lumen of epididymal tubules was observed while these effects were not seen at doses of 5000 ppm (622 mg/kg bw). In a subsequent 13-week study (n=10/group), testis and epididymis weights were decreased at doses of 1250 ppm (97 mg/kg bw) and higher. This was associated with degeneration of seminiferous epithelium and hypospermia. The lesions found were morphologically similar to those seen in the 2-week study. In the 2 highest dose groups given 2500 and 5000 ppm (202 and 436 mg/kg bw, respectively), there was testicular degeneration in 3/10 and 10/10 animals, respectively. In a 2-week dermal study, a daily dose of 2000 mg/kg bw induced similar mild to moderate seminiferous tubule degeneration in the testes of 4/5 animals while no such lesions were seen at 1000 mg/kg bw. No such effects were reported in a 13-week dermal study in rats (maximum dose: 500 mg/kg bw) or in 2- and 13-week drinking water and dermal studies in mice (maximum doses: drinking water: 1362 and 1674 mg/kg bw, respectively; dermal: 2500 and 1250 mg/kg bw, respectively) (Mel92).

No effects were seen on oestrous cycle length or oestrous stages of female rats (F344/N) following a 13-week oral (drinking water) or dermal administration/application of doses up to 242 or 500 mg/kg bw/day, respectively. Similar results were obtained in female mice ($B6C3F_1$) at maximum oral (drinking water) or dermal doses of 1128 or 630 mg/kg bw/day, respectively, given for 13 weeks (Mel92).

In assessing the potential developmental toxicity of 2,2'-iminodiethanol using the Chernoff-Kavlock screening assay, oral (gavage) dosing of mice with 450 mg/kg bw/day, during gestational days 6-15, did not affect maternal mortality, litter size, or pup birth weight. However, post-natal observations continued until post-natal day 3 showed treatment-related decreases in the number of viable litters and in the percent survival and the weight gain of the pups (Per87; see also Kna97 and Pri99). Using the results of this screening assay and a scoring method based on 5 indices for potential developmental toxicity, it

was concluded that 2,2'-iminodiethanol had high priority classification for potential developmental toxicity (Per87, Yor88).

Pregnant rats (Wistar; n=25/group) were head-nose-only exposed to liquid aerosols (MMAD: <1.2 µm) of 2,2'-iminodiethanol (purity: 98.7%) at mean measured concentrations of 0, 10.0, 50.2, and 202 mg/m^3, 6 hours/day, on post-coital days 6 through 15. Concentrations were selected from a range-finding study in which exposure to concentrations of 200 and 400 mg/m^3 resulted in dose-dependent increased liver weights and decreased serum cholesterol and triglyceride levels. In the main study, exposure to 10 or 50 mg/m^3 did not cause maternal toxicity or embryotoxic, fetotoxicity, or teratogenic effects. Exposure to 202 mg/m^3 produced maternal toxicity consisting of vaginal haemorrhages in 8 dams as well as a markedly increased number of fetuses with skeletal variations, especially rudimentary cervical ribs, which event was considered to be a manifestation of treatment-related stress on the dams. No other embryotoxic, fetotoxic, or teratogenic effects were seen. The NOAEL for maternal and fetal toxicity was concluded to be 50 mg/m^3, that for teratogenic effects 200 mg/m^3 (BGC93b)*.

Application of 0, 150, 500, 1500 mg 2,2'-iminodiethanol/kg bw to the covered clipped skin of rats (Charles River CD; n=25/group), 6 hours/day, on gestational days 6 through 15, did not result in external, visceral, or skeletal malformations or fetal body weight changes but slight developmental retardation, indicated by increased incidences of 6 skeletal variations in the skull, axial skeleton, and distal limb areas, was observed in the litters of the high-dose group. Effects indicative of maternal toxicity included slight microcytic anaemia with abnormal red blood cell morphology (at all dose levels with a dose-dependently increasing severity), a dose-dependent increase in absolute and relative kidney weights (statistically significant at the 2 higher dose levels), skin irritation (especially in the high-dose group with crusting in some animals of the 2 other groups), and decreased body weights in the high-dose group (Mar99, Nee92). A similar study was performed in rabbits (New Zealand White; n=15/group) dosed with 0, 35, 100, 350 mg/kg from gestational days 6 through 18. At the high-dose level, skin irritation, increased absolute and relative liver and kidney weights (by 10, 16, and 7%, respectively; not statistically significant), and colour changes in the kidneys occurred. Treatment did not induce significant changes in haematology parameters. There was no evidence of any kind of developmental toxicity in any of the treated groups (Mar99).

---

* Only volume 1 - report section and summary tables – was available to the committee.

Topical administration of 2 mL of a hair dye, containing 2% 2,2'-iminodiethanol (equivalent to 40 mg 2,2'-iminodiethanol/kg), to Charles River CD rats on days 1, 4, 7, 10, 13, 16, and 19 of pregnancy did not induce embryotoxic or teratogenic effects (Kna97, BGC91).

In a range-finding developmental toxicity study in which Sprague-Dawley rats (number not presented) were given daily oral (gavage) doses of 0, 50, 200, 500, 800, or 1200 mg/kg bw on gestational days 6 through 15, none of the developmental toxicity end points (total number of implants, resorptions, dead fetuses, live fetuses, and live fetal weight) examined was affected. Maternal toxicity reported included transient reduction in body weight on gestational day 8, corrected reduced body weight gain, and rough coat in 2 females at gestational day 9 in the animals given 200 mg/kg bw, and mortality or moribund sacrifice in all animals of the 3 higher dose groups (Kna97, Pri99).

In order to extend the post-natal findings in a developmental toxicity screening assay in mice (see above), a developmental toxicity study was designed in which time-mated female Sprague-Dawley-derived (CD®) rats (n=12/group) were orally (gavage) exposed to doses of 2,2'-iminodiethanol of 0, 50, 125, 200, 250, or 300 mg/kg bw/day on gestational days 6 through 19 and the maternal and developmental end points were monitored through the end of lactation at post-natal day 21 (termination). Because of excessive toxicity consisting of moribund sacrifice of 2 animals, decreased body weights by 9 and 26% on gestational day 9 and 12, respectively, tremors, lethargy, and piloerection, all animals of the 300-mg/kg bw group were sacrificed prior to scheduled necropsy and excluded from description of the study results. At 250 mg/kg bw, one animal was found dead at gestational day 15 and one sacrificed moribund on gestational day 21, while at 200 mg/kg bw, one animal was sacrificed moribund on gestational day 22. Other maternal effects included statistically significant decreases in feed intake (at doses 200 mg/kg bw), water intake (125 mg/kg bw), and body weight or weight gain (200 mg/kg bw) and increases in absolute kidney weights (125 mg/kg bw), while an increasing trend was seen for the relative kidney weights. Evaluation of developmental effects showed statistically significant increases in the incidences of post-implantation mortality (post-natal day 0) at doses 200 mg/kg bw and early post-natal mortality (post-natal day 0 to 4) at doses 125 mg/kg bw, but not of mortality for post-natal days 4 to 7, 7 to 14, or 14 to 21, and decreases in pup body weights at 200 mg/kg bw. For both maternal and developmental toxicity, the NOAEL was 50 mg/kg bw, based on decreased water intake and increased absolute kidney weights in maternal animals and early post-natal mortality in offspring at the next higher dose of 125 mg/kg bw (Pri99).

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for 2,2'-iminodiethanol in the Netherlands is 2 mg/m^3 (0.46 ppm), 8-hour TWA.

Existing occupational exposure limits for 2,2'-iminodiethanol in some European countries and in the USA are summarised in Annex I.

8 Assessment of health hazard

The committee did not find data on the (toxico)kinetics of 2,2'-iminodiethanol after exposure by inhalation. Dermal absorption varied, with increasing dose, between 3-16 and 27-58% in rats and mice, respectively. From *in vivo* studies in rats using different experimental conditions, skin absorption rates ranging from 0.113 to 137 µg/cm^2/h were calculated. *In vitro* penetration rates for undiluted material ranged from 1.8 to 46.3 µg/cm^2/h for rat and mouse skin, respectively (human skin: 5.7 µg/cm^2/h); figures for aqueous solutions were 23, 294, and 12.7 µg/cm^2/h for rat, mouse, and human skin, respectively. Oral absorption of 2,2'-iminodiethanol is nearly complete. After absorption, most of the 2,2'-iminodiethanol and its metabolites is taken up by the tissues, especially the liver and the kidneys, via incorporation into phospholipids, thereby competing with the 'normal' substrates (mono)ethanolamine and choline. Relatively small amounts (30% after 48 hours) are excreted via the main route, i.e., the urine. Accumulation may occur in tissues and red blood cells. In rats, half-life of 2,2'-iminodiethanol for liver, brain, and spleen are approximately one week after repeated administration, while blood half-life is much longer.

Although a respiratory response to triethanolamine present in the cutting fluid cannot be excluded, the committee concludes from a human case study that 2,2'-iminodiethanol is capable of inducing respiratory allergy. Both from human and animal data, the committee concludes that 2,2'-iminodiethanol can cause skin irritation. It causes eye irritation in animals, but does not seem to be sensitising.

From acute lethality data in rats (oral LD_{50}s: ca. 770-3500 mg/kg bw) and rabbits (dermal LD_{50} >8000 mg/kg bw), the committee concludes that 2,2'-iminodiethanol is harmful if swallowed while there are no reasons for concern following single dermal exposure. The committee did not find valid data on acute inhalation exposure.

Repeated inhalation, dermal, and oral exposure studies showed that the blood, kidneys, liver, and the male reproductive system are target 'organs' and

that local effects are induced as well. From two 90-day inhalation studies (BCG96, Gam02) carried out in (Wistar) rats, the committee concludes that 15 mg/m^3 is the NOAEL for systemic effects and 1.5 mg/m^3 the NOAEL for local effects with squamous metaplasia of the laryngeal epithelium seen at the next higher level of 3 mg/m^3. In 90-day oral and dermal studies in rats, effects were induced at 15 and 32 mg/kg bw, respectively, the lowest concentrations tested, and no NOAELs could be established. In 2-year dermal studies, no evidence of carcinogenic effects was found in male or female (Fischer) rats while in (B6C3F1) mice, incidences of liver neoplasms and renal tubule neoplasms (males only) were increased.

From the results of *in vitro* and *in vivo* tests, the committee concludes that 2,2'-iminodiethanol is not a genotoxic/mutagenic compound.

Oral studies in rats and mice at relatively high doses of 50-200 and 100-600 mg/kg bw showed that 2,2'-iminodiethanol affected the immune system. Although the biological relevance of changes in neutrophil (sub)populations in rats at 50 mg/kg bw is not clear, the committee is of the opinion that in mice, 100 mg/kg bw is a LOAEL based on the decreased resistance found in a bacterial and tumour model.

From the data available, the committee concludes that 2,2'-iminodiethanol does not have reproduction toxicity properties. Fetotoxic effects (increased skeletal variations) were seen in rats exposed by inhalation or via the skin on gestational days 6 through 15, but they were accompanied by maternal toxicity. The NOAELs for fetal toxicity were 50 mg/m^3 and 500 mg/kg bw, respectively, those for maternal toxicity 50 mg/m^3 and <150 mg/kg bw. In rats orally dosed on gestational days 6 through 19, the NOAEL for maternal and developmental toxicity was 50 mg/kg bw. The LOAEL was 125 mg/kg bw based on decreased water intake and increased absolute kidney weights in the dams and increased mortality during post-natal days 0 through 4 in the pups. In male rats, effects on the testes were found, but at levels higher than those inducing other effects.

Following dermal application, 2,2'-iminodiethanol induced increased incidences of liver adenomas and carcinomas in a mouse strain (i.e., $B6C3F_1$) which is known for its susceptibility to liver tumours. Furthermore, a small increase in the incidence of benign kidney tumours was found in male animals only while nephrotoxicity (tubular hyperplasia) was observed as well. The committee considers that these neoplastic events are very likely due to non-genotoxic/non-mutagenic mechanisms.

The committee is of the opinion that a threshold approach is warranted and takes the aforementioned 90-day inhalation studies in rats (Gam02) as a basis for deriving a health-based recommended occupational exposure limit (HBROEL).

The level of 1.5 mg/m^3, which is concluded to be the NOAEL for local effects (target organ: the larynx), was taken as a starting point for deriving an HBROEL. For the extrapolation to a HBROEL, an overall assessment factor of 4 is established. This factor covers the following aspects: inter- and intraspecies variation, differences between experimental conditions and the exposure pattern of the worker, and the type, low incidence, and slightness of the critical effect (reversible squamous metaplasia of the laryngeal epithelium in 3/10 males) at 3 mg/m^3. Thus, applying this factor of 4 and the preferred value approach, a health-based occupational exposure limit of 0.5 mg/m^3 is recommended for 2,2'-iminodiethanol.

Skin absorption

The systemic effects observed in rats and mice following 13-week dermal exposure to doses as low as 32 and 80 mg/kg bw/day, respectively, may indicate the need to assign a 'skin notation'. A skin notation is warranted if the amount taken up via the skin of hands and forearms (i.e., 2000 cm^2) during an exposure period of 1 hour is more than 10% of the amount taken up after inhalation exposure for 8 hours at an HBROEL based on systemic toxicity. The skin penetration data available vary widely, depending on the experimental conditions: in vivo rat data ranged from 0.1-137 µg/cm^2/h. The latter rate was determined in an experiment in which very large amounts of 1500 mg/kg were repeatedly applied which might have caused damage facilitating uptake. Taking a rate of 5.8 µg/cm^2/h which was calculated from a single application of 27.5 mg/kg bw, and assuming that rat skin is two to three times as permeable as human skin (as was found in a in vitro experiment), the amount calculated to be taken up by the worker via the skin under the aforementioned premises amounts to ca. 5 mg. Taking a NOAEL found in a 13-week rat inhalation study for systemic effects of 15 mg/m^3 and using an assessment factor of 10 would lead to a HBROEL for systemic effects of 1.5 mg/m^3. Assuming a worker inhales 10 m^3 in 8 hours, the amount that would be taken up after inhalation exposure for 8 hours is calculated to be 15 mg. These calculations support the need of a skin notation.

Therefore, the committee concludes that a 'skin notation' is warranted.

The committee recommends a health-based occupational exposure limit for 2,2'-iminodiethanol of 0.5 mg/m^3, as inhalable dust and vapour, as an 8-hour time-weighted average, and the addition of a skin notation.

Based on human data on metal workers, the committee notes that 2,2'-iminodiethanol may have skin-sensitising properties.

References

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

ACG04 American Conference of Governmental Industrial Hygienists (ACGIH). Guide to occupational exposure values - 2004. Cincinnati OH, USA: ACGIH®, 2004: 49.

ACG05 American Conference of Governmental Industrial Hygienists (ACGIH). 2005 TLVs® and BEIs® based on the documentation of the Threshold Limit Values for chemical substances and physical agents & Biological Exposure Indices. Cincinnati OH, USA: ACGIH®, 2005: 25.

Arb02 Arbejdstilsynet. Grænseværdier for stoffer og materialer. Copenhagen, Denmark: Arbejdstilsynet, 2002: 23 (At-vejledning C.0.1).

Ash96 Ashby J. Prediction of rodent carcinogenicity for 30 chemicals. Environ Health Perspect 1996; 104: 1101-4.

Bal86 Balato N, Cusano F, Lembo G, et al. Ethylenediamine dermatitis. Contact Dermatitis 1986; 15: 263-5.

Bar79a Barbee SJ, Hartung R. The effect of diethanolamine on hepatic and renal phospholipid metabolism in the rat. Toxicol Appl Pharmacol 1979; 47: 421-30.

Bar79b Barbee SJ, Hartung R. Diethanolamine-induced alteration of hepatic mitochondrial function and structure. Toxicol Appl Pharmacol 1979; 47: 431-40.

Bar85 Barber JE. Diethanolamine: skin irritation study. Macclesfield (Cheshire), UK: Imperial Chemical Industries PLC, Central Toxicology Laboratory, 1985; rep no CTL/T/2330 (available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0520483).

BGC90 Berufsgenossenschaft der chemischen Industrie (BG Chemie). Contact hypersensitivity to Diethanolamin, rein in albino guinea pigs maximization test. Heidelberg, FRG: BG Chemie, 1990; confidential study performed on behalf of BG Chemie within its 'Programme for the Prevention of Health Hazards caused by Industrial Substances'.

BGC91 Berufsgenossenschaft der chemischen Industrie (BG Chemie). Diethanolamine. In: Toxicological evaluations, potential health hazards of existing chemicals. Berlin, FRG: Springer-Verlag, 1991: 135-151 (Vol 2).

BGC93a Berufsgenossenschaft der chemischen Industrie (BG Chemie). Study on the inhalation toxicity including neurotoxicology examinations of Diethanolamin as a liquid aerosol in rats, 14-day test. Heidelberg, FRG: BG Chemie, 1993; confidential study performed on behalf of BG Chemie within its 'Programme for the Prevention of Health Hazards caused by Industrial Substances'.

BGC93b Berufsgenossenschaft der chemischen Industrie (BG Chemie). Study of the prenatal toxicity of Diethanolamin in rats after inhalation. Heidelberg, FRG: BG Chemie, 1993; confidential study performed on behalf of BG Chemie within its 'Programme for the Prevention of Health Hazards caused by Industrial Substances'.

BGC96 Berufsgenossenschaft der chemischen Industrie (BG Chemie). Diethanolamin - subchronic inhalation toxicity and neurotoxicity study in Wistar rats, 90-day liquid aerosol exposure. Heidelberg, FRG: BG Chemie, 1996; confidential study performed on behalf of BG Chemie within its 'Programme for the Prevention of Health Hazards caused by Industrial Substances'.

Blu72 Blum K, Huizenga CG, Ryback RS, et al. Toxicity of diethanolamine in mice. Toxicol Appl Pharmacol 1972; 22: 175-85.

Blu97 Blum A, Lischka G. Allergic contact dermatitis from mono-, di- and triethanolamine. Contact Dermatitis 1997; 36: 166.

Bra83 Brandt K. Final report on the safety assessment of triethanolamine, diethanolamine, and monoethanolamine. J Am Coll Toxicol 1983; 2: 183-235.

Car46 Carpenter CP, Smyth HF Jr. Chemicals burns of the rabbit cornea. Am J Ophthalmol 1946; 29: 1363-72.

Dea85 Dean BJ, Brooks TM, Hodson-Walker G, et al. Genetic toxicology testing of 41 industrial chemicals. Mutat Res 1985; 153: 57-77.

DFG05 Deutsche Forschungsgemeinschaft (DFG): Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. List of MAK and BAT Values 2005. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA, 2005: 53 (rep no 41).

Dut82 Dutertre-Catella H, Lich NP, Huyen VN, et al. Etude comparative de l'agressivité cutanée et oculaire des éthanolamines (mono, di, tri et poly). Arch Mal Prof 1982; 43: 455-60.

EC05 European Commission: Directorate General for Employment, Social Affairs and Equal Opportunities. Indicative occupational exposure limit values. http://europe.eu.int/comm/employment_social/health_safety/docs/oels_en.pdf.

Gam02 Gamer AO, Leibold E, Kaufmann W, et al. Diethanolamine - subchronic inhalation toxicity study in Wistar rats, liquid aerosol/vapor exposure. Study focus on irritation of upper respiratory tract. Ludwigshafen/Rhein, FRG: BASF Aktiengesellschaft, Experimental Toxicology and Ecology, 2002; proj no 51/0299/99125.

Gei03 Geier J, Lessmann H, Frosch PJ, et al. Patch testing with components of water-based metalworking fluids. Contact Dermatitis 2003; 49: 85-90.

Gre98 Greim H, ed. Diethanolamin. In: Gesundheidsschädliche Arbeitsstoffe. Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werte (Maximale Arbeitsplatz-Konzentrationen). 1st-27th ed. Weinheim, FRG: VCH-Wiley Verlag GmbH, 1998.

Gre01 Greim H, ed. Diethanolamin. In: Gesundheidsschädliche Arbeitsstoffe. Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werte (Maximale Arbeitsplatz-Konzentrationen). 31th ed. Weinheim, FRG: VCH-Wiley Verlag GmbH, 2001.

Har70 Hartung R, Rigas LK, Cornish HH. Acute and chronic toxicity of diethanolamine. Toxicol Appl Pharmacol 1970; 17: 308.

Haw83 Haworth S, Lawlor T, Mortelmans K, et al. Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen 1983; 5 (suppl 1): 3-142.

Hed78 Hedenstedt A. Mutagenicity screening of industrial chemicals: seven aliphatic amines and one amide tested in the Salmonella/microsomal assay. Mutat Res 1978; 53: 198-9.

HSE02 Health and Safety Executive (HSE). EH40/2002. Occupational Exposure Limits 2002. Sudbury (Suffolk), England: HSE Books, 2002: 20.

IARC78 International Agency for Research on Cancer (IARC). N-Nitrosodiethanolamine. In: Some N-nitroso compounds. Lyon, France: IARC, 1978: 77-82 (IARC monographs of the carcinogenic risk of chemicals to man; Vol 17).

Ino82 Inoue K, Sunakawa T, Okamoto K, et al. Mutagenicity tests in in vitro transformation assays on triethanolamine. Mutat Res 1982; 101: 305-13.

Ker96 Kerckaert GA, Brauninger R, Leboeuf RA, et al. Use of the Syrian hamster embryo cell transformation assay for carcinogenicity prediction of chemicals currently being tested by the national toxicology program in rodent bioassays. Environ Health Perspect 1996; 104: 1075-84.

Kna97 Knaak JB, Leung H, Stott WT, et al. Toxicology of mono-, di-, and triethanolamine. Rev Environ Contam Toxicol 1997; 149: 1-86.

Kor73 Korsud GO, Grice HG, Kuiper Goodman T, et al. Sensitivity of several serum enzymes for the detection of thioacetamide-, dimethylnitrosamine- and diethanolamine-induced liver damage in rats. Toxicol Appl Pharmacol 1973; 26: 299-313.

Leh00 Lehman-McKeeman LD, Gamsky EA. Choline supplementation inhibits diethanolamine-induced morphological transformation in Syrian hamster embryo cells: evidence for a carcinogenic mechanism. Toxicol Sci 2000; 55: 303-10.

Leh02 Lehman-McKeeman LD, Gamsky EA, Hicks SM, et al. Diethanolamine induces hepatic choline deficiency in mice. Toxicol Sci 2002; 67: 38-45.

Leo67 Leong KJ. Laboratory tests to determine effect of inhalation of two ethanolamines. Diethanolamine (DEA), methylaminoethanol (MAE), formulation 485K. Final report. Falls Church VA, USA: Hazleton Laboratories Inc, 1967 (report submitted to Eastman Kodak Company, Rochester NY, USA; available from National Technical Information Service, Springfield VA, USA; order no NTIS/ OTS0516742)..

Lov89 Loveday KS, Lugo MH, Resnick MA, et al. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro: II. Results with 20 chemicals. Environ Mol Mutagen 1989; 13: 60-94.

Mar99 Marty MS, Neeper-Bradley TL, Neptun DA, et al. Developmental toxicity of diethanolamine applied cutaneously to CD rats and New Zealand White rabbits. Regul Toxicol Pharmacol 1999; 30: 169-81.

Mat95 Mathews JM, Garner CE, Matthews HB. Metabolism, bioaccumulation, and incorporation of diethanolamine into phospholipids. Chem Res Toxicol 1995; 8: 625-33.

Mat97 Mathews JM, Garner CE, Black SL, et al. Diethanolamine absorption, metabolism and disposition in rat and mouse following oral, intravenous and dermal administration. Xenobiotica 1997; 27:733-46.

Mel92 Melnick RL. NTP technical report on toxicity studies of diethanolamine (CAS No. 111-42-2) administered topically and in drinking water to F344/N rats and B6C3F$_1$ mice. Research Triangle Park NC, USA: National Toxicology Program, 1992; Toxicity Rep Ser No 20.

Mel94a Melnick RL, Mahler J, Bucher JR, et al. Toxicity of diethanolamine. 1. Drinking water and topical application exposures in F344 rats. J Appl Toxicol 1994; 14: 1-9.

Mel94b Melnick RL, Mahler J, Bucher JR, et al. Toxicity of diethanolamine. 2. Drinking water and topical application exposures in B6C3F_1 mice. J Appl Toxicol 1994; 14: 11-9.

Mel04 Mellert W, Kaufmann W, Rossbacher R, et al. Investigations on cell proliferation in B6C3F_1 mouse liver by diethanolamine. Food Chem Toxicol 2004; 42: 127-34.

Men01 Mendrala AL, Waechter JM Jr, Bormett GA, et al. The pharmacokinetics of diethanolamine in sprague-Dawley rats following intravenous administration. Food Chem Toxicol 2001; 39: 931-9.

Mun92 Munson AE, White KL Jr, McCay JA. Immunotoxicity of diethanolamine in female Fischer 344 rats. Report to National Toxicology Program. Richmond VA, USA: Virginia Commonwealth University, Medical College of Virginia, Immunotoxicology Program, 1992.

Muñ03 Muñoz ER, Mazar Barnett B. Chromosome malsegration induced by the rodent carcinogens acetamide, pyridine and diethanolamine in Drosophila melanogaster females. Mutat Res 2003; 539: 137-44.

Nee92 Neeper-Bradley TL. Definitive developmental toxicity evaluation of diethanolamine (DEA) administered cutaneously to CD® (Sprague-Dawley) rats. Export PA, USA: Bushy Run Research Center, 1992; BRRC rep no 54-563 (report available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0543466).

NIO04 US National Institute of Occupational Safety and Health (NIOSH), ed. Ethanol, 2,2'-iminodi-. In: The Registry of Toxic Effects of Chemical Substances (RTECS) (last update 2,2'-iminodiethanol file: October 2002); http://www.cdc.gov/niosh.

NLM04 US National Library of Medicine (NLM), ed. Diethanolamine. In: The Hazardous Substances Data Bank (HSDB) (last revision date 2,2'-iminodiethanol file: March 2003; last review date: July 1991); http://toxnet.nlm.nih.gov.

NTP98 National Toxicology Pogram (NTP). The immunotoxicity of diethanolamine (CAS No. 111-42-2) in female B6C3F_1 mice. Research Triangle Park NC, USA: National Toxicology Program, 1998; NTP Rep No IMM98011.

NTP99 National Toxicology Pogram (NTP). NTP technical report on the toxicology and carcinogenesis studies of diethanolamine (CAS No. 111-42-2) in F344/N rats and B6C3F_1 mice (dermal studies). Research Triangle Park NC, USA: National Toxicology Program, 1999; NTP Techn Rep No TR-478 (report available from National Technical Information Service, Springfield VA, USA; rep no PB99-167553).

NTP03 National Toxicology Pogram (NTP). The immunotoxicity of diethanolamine (CAS No. 111-42-2) in female Fischer 344 rats. Research Triangle Park NC, USA: National Toxicology Program, 2003; NTP Rep No IMM20303.

Per87 Pereira M, Barnwell P. Screening of priority chemicals for reproduction hazards. Monoethanolamine (CAS No. 141-43-5), diethanolamine (CAS No. 111-42-2), triethanolamine (CAS No. 102-71-6). Cincinnati OH, USA: Environmental Health Research & Testing, Inc, 1987; EHRT proj no: ETOX-

85-1002 (report available from National Technical Information Service, Springfield VA, USA; rep no PB89139067).

Pii98 Piipari R, Tuppurainen M, Tuomi T, et al. Diethanolamine-induced occupational asthma, a case report. Clin Exp Allergy 1998; 28: 358-62.

Pri99 Price CJ. Developmental toxicity screen for diethanolamine (CAS No. 111-42-2) administered by gavage to Sprague-Dawley (CD®) rats on gestational days 6 through 19: evaluation of dams and pups through postnatal day 21. Research Triangle Park NC, USA: Research Triangle Institute, 1999; NTP Study No: TER-96-001 (available from National Technical Information Service, Springfield VA, USA; rep no PB2001-103718).

Sha82 Sharp RL. Toxicity and health hazard summary. Rochester NY, USA: Eastman Kodak Company, Health, Safety, and Human Factors Laboratory, 1982 (available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0516742).

Smy51 Smyth HF Jr, Carpenter CP, Weil CS. Range-finding toxicity data: list IV. Arch Ind Hyg Occup Med 1951; 4: 119-22.

Sor88 Sorsa M, Pyy L, Salomaa S, et al. Biological and environmental monitoring of occupational exposure to cyclophosphamide in industry and hospitals. Mutat Res 1988; 204: 465-79.

Spa00 Spalding JW, French JE, Stasiewicz S, et al. Responses of transgenic mouse lines $p53^{+/-}$ and Tg.Ac to agents tested in conventional carcinogenicity bioassays. Toxicol Sci 2000; 53: 213-23.

Sto00 Stott WT, Bartels MJ, Brzak KA, et al. Potential mechanisms of tumorigenic action of diethanolamine in mice. Toxicol Lett 2000; 114: 67-75.

Sun96 Sun JD, Beskitt JL, Tallant MJ, et al. In vitro skin penetration of monoethanolamine and diethanolamine using excised skin from rats, mice, rabbits, and humans. J Toxicol Cut Ocular Toxicol 1996; 15: 131-46.

Swe00 Swedish National Board of Occupational Safety and Health. Occupational exposure limit values and measures against air contaminants. Solna, Sweden: National Board of Occupational Safety and Health, 2000: 32 (Ordinance AFS 2000:3).

SZW05 Ministerie van Sociale Zaken en Werkgelegenheid (SZW). Nationale MAC-lijst 2005. The Hague, the Netherlands: Sdu Uitgevers, 2005: 30.

Ter67a Terhaar CJ, Wolf GL, Yanno FJ. Subacute inhalation toxicity of diethanolamine and bimat imbibant (485K). Rochester NY, USA: Eastman Kodak Company, Laboratory of Industrial Medicine, Toxicology Laboratory, 1967 (report available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0516742).

Ter67b Terhaar CJ, Krasavage WJ, Wolf GL, et al. Ethanolamine, diethanolamine and methylaminoethanol. A dietary feeding study. Rochester NY, USA: Eastman Kodak Company, Laboratory of Industrial Medicine, Toxicology Laboratory, 1967 (report available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0516742).

Ter68 Terhaar CJ, Krasavage WJ. Diethanolamine (66-227). A thirty day dietary feeding study. Rochester NY, USA: Eastman Kodak Company, Laboratory of Industrial Medicine, Toxicology Laboratory,

1968 (report available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0516742).

TRG04 TRGS 900. Technische Regeln für Gefahrstoffe. Grenzwerte in der Luft am Arbeitsplatz. BArbBl 2004; (7/8).

Yor88 York RG, Barnwell PL, Pierrera M, et al. Evaluation of twelve chemicals in a preliminary developmental toxicity test. Teratology 1988; 37: 503-4.

Yor95 York M, Basketter DA, Cuthbert JA, et al. Skin irritation testing in man for hazard assessment - evaluation of four patch systems. Hum Exp Toxicol 1995; 14: 729-34.

Annex I

Occupational exposure limits for 2,2'-iminodiethanol in various countries.

country - organisation	occupational exposure limit		time-weighed average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
The Netherlands						
- Ministry of Social Affairs and Employment	0.46	2	8 h	administrative	S	SZW05
Germany						
- AGS	-	15[c]			S, [d], [e]	TRG04
- DFG MAK-Kommission	-	-			S, sens, [d], [f]	DFG05
Great-Britain						
- HSE	3	13	8 h	OES		HSE02
Sweden	3	15	8 h		S	Swe00
	6	30	15 min			
Denmark	0.46	2	8 h		S	Arb02
USA						
- ACGIH	-	2	8 h	TLV	S	ACG05
- OSHA	-	-				ACG04
- NIOSH	3	15	10 h	REL		ACG04
European Union						
-SCOEL	-	-				EC05

[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] Inhalable fraction.

[d] Diethanolamine can be converted into carcinogenic nitroso compounds by nitrosation (e.g. reaction with NO).

[e] Limit value with inadequate database.

[f] Classified in carcinogenicity category 3A: i.e., among substances for which the criteria for classification in Category 4 or 5 are fulfilled but for which the database is insufficient for the establishment of a MAK value.

Annex II

Table 1 Repeated-dose toxicity studies with 2,2'-iminodiethanol in experimental animals; oral studies.

species	exposure	observations	reference
rat (female; F344; n=48/group)	0, 50, 100, 200 mg/kg bw/d, gavage, for 14 consecutive days	standard toxicology end points: body weights, organ weights, pathology, haematology, serum chemistry; investigated in 8 animals/dose as part of immunotoxicity study (results from macroscopic and microscopic evaluations were not reported). 200 mg/kg bw: decreased bw and bw change by 4 and 35%, resp., in first week and by 4 and 24%, resp., in 2nd week; increased absolute and relative liver and kidney weights (by 21-28%); increased BUN; decreased erythrocyte numbers and reticulocyte counts and Hb and haematocrit values. 100 mg/kg bw: decreased bw, change by 13% in 2nd w; increased absolute and relative liver (not stat. sign.) and kidney weights (by 17-20%); increased BUN (not stat. sign.); decreased reticulocyte counts and Hb and haematocrit values. 50 mg/kg bw: increased absolute and relative liver and kidney weights (both not stat. sign.); increased BUN; decreased reticulocyte counts and Hb and haematocrit values.	Mun92, NTP03
rat (male; n=10/ group)	0, 0.01, 0.1, 1.0% in the diet, for 30 days (75 and 330 mg/kg bw/d for the mid- and high-dose group, resp.; calculation by Ter67)	1.0%: weight loss, decreased food intake, mortality in 9/10; increased white blood cell count and decreased haemoglobin and haematocrit in one survivor. 0.1%: increased absolute and relative liver weight; decreased haemoglobin and haematocrit (stat. sign.; values within normal range). 0.01%: decreased bw and food intake between day 4 and 8, increased relative liver weight.	Ter67b[a]
rat (male; n=10/ group)	0, 0.01, 0.1, 1.0% in the diet, for 30 days	1.0%: hypersensitivity, weight loss, decreased food intake, mortality in 8/10; no microscopic changes observed. 0.01%, 0.1%: no mortality, no effect on bw or food intake, no consistent pattern of changes in organ weights, haematology, urinalysis, measurements of alkaline phosphatases and serum aspartate aminotransferase, histology. 0.1%: decreased relative liver weight, increased relative kidney weight, increased serum aspartate aminotransferase (stat. sign.; values within normal range). 0.01%: decreased relative liver weight, increased relative kidney weight.	Ter68[b]
rat (male; Wistar; n=10/group)	0, 5, 20, 90, 170, 350, 680 mg/kg bw/d, in the diet, for 90 days	680 mg/kg bw: mortality in 10/10. ≥170 mg/kg bw: some mortality; histological changes in liver (fatty degeneration) and kidney (cloudy swelling, degeneration of tubular epithelial cells). ≥90 mg/kg bw: increased liver and kidney weights. ≥20 mg/kg bw: no effects.	Smy51, ACG99, Bra83, Kna97

rat (male/female; F344; n=10/sex/ group)	0, 20, 50, 100, 200, 400 mg/kg bw/d, gavage, for 13 weeks	≥100 mg/kg bw: dose-dependent decreased bw gain; mortality. 50 mg/kg bw: decreased bw gain in males by 11%. microscopy: mineral deposition in kidneys of females of 200- and 400-mg/kg bw groups; hyperplasia and hyperkeratosis in stomach of males of the 400-mg/kg bw group	ACG99, BCG91[c]
rat (male/female; F344/N; n=5/sex/ group)	0, 630, 1250, 2500, 5000, 10,000 mg/L in drinking water, for 2 weeks (ca. 80-1040 mg/kg bw/d)	mortality in 2/5 males of highest dose group and in all females of the 2 highest dose groups; decreased water consumption in all treatment groups; reduced bw gain in males of the 2 highest dose groups and in the females of the 1250- and 2500-mg/L group; dose-dependent increased absolute and relative kidney weights accompanied by microscopic findings; dose-dependent changes in haematology endpoints indicative of anaemia; degeneration of seminiferous tubules of testes in high-dose males	Mel92
rat (male/female; F344/N; n=10/sex/ group)	males: 0, 320, 630, 1250, 2500, 5000 mg/L (25-436 mg/kg bw/d) females: 0, 160, 320, 630, 1250, 2500 mg/L (14-242 mg/kg bw/d), in drinking water, for 13 weeks	mortality in 2/10 males of highest dose group; dose-dependent decreased body weight gain, being statistically significant at doses ≥48 mg/kg bw in males and ≥32 mg/kg bw in females; decreased water consumption at ≥48 mg/kg bw in males and ≥242 mg/kg bw in females; dose-dependent changes in haematology (poorly regenerative, microcytic anaemia), kidney (increased weight, tubular necrosis, decreased renal function, tubular mineralization), and liver (increased relative weights accompanied by biochemical but not by microscopic changes); microscopic changes in brain and spinal cord at the 2 highest dose levels; testes lesions at ≥97 mg/kg bw.	Mel92, Mel94a
rat (male)	4000 mg/L in drinking water, for 7 weeks	many deaths (not specified); kidney and liver effects; pronounced normocytic anaemia without bone-marrow depletion and obvious increase in number of reticulocytes.	Har70[d]
mouse (female; $B6C3F_1$)	0, 100, 300, 600 mg/kg bw/d, gavage, for 14 consecutive	standard toxicology end points: bw, organ weights, pathology, haematology, serum chemistry; investigated as part of immunotoxicity study; only abstract and summary tables available. dose-dependently increased body and liver weights (stat. sign. at 600 mg/kg bw); dose-dependent decreases in erythrocyte number, reiticulocyte counts, and in Hb and haematocrit values (stat. sign. at 600 mg/kg bw).	NTP98
mouse (male/female; $B6C3F_1$; n=10/sex/ group)	0, 50, 100, 200, 400, 800 mg/kg bw/d, gavage, for 13 weeks	800 mg/kg bw: mortality in 2/10 males; microscopic lesions (increased and abnormal mitosis in cortex; casts and eosinophilic material in tubular lumina) in males. ≥200 mg/kg bw: decreased bw gain in males; alopecia in males. ≥100 mg/kg bw: alopecia in females.	ACG99, BCG91
mouse (male/female; $B6C3F_1$; n=5/sex/ group	0, 630, 1250, 2500, 5000, 10,000 mg/L in drinking water, for 2 weeks (males: 110-1362 mg/kg bw/d; females: 197-2169 mg/kg bw/d)	no mortality; bw reductions in males at 1362 mg/kg bw and in females at 1399 and 2169 mg/kg bw; decreased water consumption at the highest dose only; clinical signs observed only at the highest dose levels and included rough hair coat, emaciation, abnormal posture; dose-dependent increased relative and absolute liver weights accompanied by histological changes in both sexes.	Mel92

mouse (male/female; $B6C3F_1$; n=10/sex/ group)	0, 630, 1250, 2500, 5000, 10,000 mg/L in drinking water, for 13 weeks (males: 104-1674 mg/kg bw/d; females: 142-1128 mg/kg bw/d)	mortality in all animals of the 2 highest dose groups and in 3/10 females given 884 mg/kg bw; decreased body weight gain in males at doses of 422 mg/kg bw and in females at doses of 347 and 884 mg/kg bw; no effect on water consumption in these animals; toxic signs observed at the 3 highest dose levels included tremors, ruffled fur, emaciation, abnormal posture, hypoactivity; dose-dependent effects on liver (increased absolute and relative weights accompanied by biochemical and histological changes), kidneys (increased absolute and relative weights in especially males associated with nephropathy), and the heart (cardiac myocyte degeneration).	Mel92, Mel94b

[a] No haematology performed in low-dose group; unpublished report, no tables/figures present in the copy available to the committee.

[b] Repeat of Ter67 study because of findings in this study (decreased bw in low- but not in mid-dose group; lack of haematology data from low-dose group; lack of microscopic data in high-dose group); unpublished report, no tables or figures present in the copy available to the committee.

[c] Unpublished report, not available to the committee.

[d] Reported in abstract only.

Annex III

Table 2 Summary of non-neoplastic and neoplastic effects in male and female B6C3F$_1$ mice following 2-year dermal application of 2,2'-iminodiethanol (from NTP99).

	males				females			
	0	40[a]	80	160	0	40	80	160
skin:								
hyperkeratosis[b]	0/50	13/50	10/50	17/50	1/50	3/50	8/50	16/50
spleen:								
haematopoietic cell proliferation	13/50	22/50	29/50	32/50	24/50	31/50	41/50	43/50
thyroid:								
follicular cell hyperplasia	18/50	22/49	30/50	42/50	18/50	28/49	32/50	39/50
liver:								
cytoplasmic alteration	1/50	17/50	17/50	12/50				
syncytical alteration	0/50	28/50	38/50	23/50	0/50	2/50	17/50	18/50
hepatocellular adenoma	31/50	42/50	49/50	45/50	32/50	50/50	48/50	48/50
hepatocellular carcinoma	12/50	17/50	33/50	34/50	5/50	19/50	38/50	42/50
hepatoblastoma	0/50	2/50	8/50	5/50	0/50	2/50	1/50	1/50
adenoma or carcinoma					33/50	50/50	50/50	50/50
adenoma, carcinoma, or blastoma	39/50	47/50	50/50	49/50				
kidney:								
renal tubular hyperplasia[c]	3/50	7/50	7/50	10/50				
adenoma[d]	1/50	4/50	6/50	6/50				
adenoma[c]	1/50	6/50	8/50	7/50				
adenoma or carcinoma[d]	3/50	5/50	6/50	8/50				
adenoma or carcinoma[c]	3/50	7/50	8/50	9/50				

[a] Doses in mg/kg bw.
[b] Non-neoplastic lesions in italics.
[c] From standard and extended evaluation combined.
[d] From standard evaluation only.