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Ministry of Social Affairs
and Employment

Health-based recommended occupational exposure limit for piperazine

Dutch expert committee for occupational standards
(Met Nederlandstalige samenvatting)

RA 7/91

Labour Inspectorate

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This is a report of the Dutch Expert Committee for occupational standards (DEC). The draft-document has been prepared by J.Th.J. Stouten

January 1992

CIP-gegevens Koninklijke Bibliotheek, Den Haag

Health-based

Health-based recommended occupational exposure limit for piperazine / Dutch expert committee for occupational standards. - The Hague: Directorate-General of Labour. - Ill. - ([Report] / Dutch expert committee for occupational standards, ISSN 0921-9641; RA 7/91)
Met lit. opg. - Met samenvatting in het Nederlands.
ISBN 90-5307-243-8
Trefw.: chemische stoffen; bedrijfsgezondheidszorg.

INTRODUCTION

This review has been based on the consensus report on piperazine by the Swedish National Board of Occupational Safety and Health (Lundberg, 1985) and completed with other relevant data as indicated in the text.

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SAMENVATTING

1. FYSISCHE EN CHEMISCHE EIGENSCHAPPEN/GEBRUIK

CAS nummer	: 110-85-0
Molecuulformule	: $C_4H_{10}N_2$
Molecuulgewicht	: 86.14
Kookpunt (1 bar)	: 146°C
Smeltpunt (1 bar)	: 106°C
Dichtheid (20°C, 1 bar)	: 1,07 g/ml
Dampdruk (20°C)	: 0,23 mbar
Omrekeningsfaktoren (20°C, 1 bar)	: 1 mg/m^3 = 0,279 ppm 1 ppm = 3,58 mg/m^3

Piperazine (anhydraat) is een witte of transparante, kristallijne vaste stof. Het is zeer hygroscopisch en alkalisch ($k=6.4 \times 10^{-5}$) en oplosbaar in water en ethanol.
Piperazine komt ook voor in gehydrateerde vorm en in de vorm van zouten (adipaat, citraat).
Naast gebruik in de farmaceutische industrie (anthelminthica) vindt piperazine toepassing in o.a. de rubberindustrie, verfindustrie, als antioxidant en roestwerend middel.

2. MONITORING

Concentraties van piperazine in de omgevingslucht lager dan 1 ppm kunnen gemeten m.b.v. een gaschromatograaf uitgerust met een stikstof-specifieke detector.
Dergelijk lage concentraties in de urine worden, na derivatisering, gemeten m.b.v. een gaschromatograaf uitgerust met een stikstof-specifieke of massa-selectieve detector.

3. GRENSWAARDEN

In Zweden, waar piperazine wordt aangeduid als sensibiliserend, geldt een grenswaarde van 0,3 mg/m^3 (0,1 ppm), 8 u - tijdgewogen gemiddelde.

De ACGIH (VS) hanteert een grenswaarde van 5 mg/m^3, 8 u - tgg, voor piperazine dihydrochloride.

4. TOXICOKINETIEK

Beroepsmatige blootstelling zal vooral plaatsvinden via de ademhalingsorganen en de huid. Er zijn geen humane gegevens over de resorptie via deze routes. Na orale toediening wordt 30% van de geresorbeerde hoeveelheid onveranderd uitgescheiden in de urine. In de maag is omzetting in mononitrosopiperazine mogelijk. In tegenstelling tot bij proefdieren is geen omzetting tot het dinitroso-derivaat aantoonbaar gebleken.

5. EFFEKTEN

Piperazine anhydraat is een irriterende stof. Oplossingen en zouten zijn minder, respectievelijk niet irriterend. Beroepsmatige blootstelling kan allergisch eczeem veroorzaken.
Piperazine is weinig toxisch na eenmalige orale of inhalatoire toediening bij proefdieren. Therapeutische overdoses hebben tot ongecoördineerde spierbeweging, braken en anorexia geleid. Er zijn geen aanwijzingen dat piperazine mutageen of carcinogeen is.
Hoewel de proefdierstudies naar de carcinogeniteit van mononitrosopiperazine, dinitrosopiperazine en de combinatie piperazine-nitriet niet voldoen aan de OECD richtlijnen, moeten mononitrosopiperazine en dinitrosopiperazine als carcinogeen beschouwd worden en zijn er sterke aanwijzingen voor de carcinogeniteit van de combinatie piperazine-nitriet. De genotoxiciteit van mononitrosopiperazine kan niet beoordeeld worden. Dinitrosopiperazine moet worden beschouwd als genotoxisch.
Uit epidemiologisch onderzoek in een Zweedse fabriek blijkt, dat 30% van de hoogst blootgestelden asthma en 25% chronische bronchitis had. In een afdeling waar de expositieniveaus lager waren dan 0,3 mg/m^3, werden geen nieuwe gevallen van asthma gevonden. Er was geen verschil tussen de longfunktie van werknemers blootgesteld aan een gemiddeld niveau dat lager lag dan 0,1 mg/m^3 en die van niet blootgestelden. Een onderzoek naar bronchiale klachten d.m.v. een vragenlijst bracht evenmin verschillen aan het licht. Een verhoogd risico op kanker onder de werknemers van deze fabriek kon niet gerelateerd worden aan blootstelling aan een bepaalde stof.

6. ADVIESWAARDE

De advieswaarde is gebaseerd op de studies in de Zweedse fabriek. Geconcludeerd wordt, dat het meest kritische effekt t.g.v. beroepsmatige blootstelling het ontstaan van asthma is. Aangezien blootstelling aan niveaus lager dan 0,3 mg/m^3 niet tot nieuwe ziektegevallen leidde, wordt dit als no-adverse-effect-level beschouwd.

Echter, blootstelling aan 0.4 mg/m^3 veroorzaakte symptomen van asthma in gevoelige personen. Bovendien kan piperazine asthma induceren zowel via een type-I allergie als via een niet allergisch verkregen hyperreactiviteit, waarbij de binding van piperazine aan lichaamseigen eiwitten een voor individuele gevallen uiteenlopende rol speelt. Deze individuele gevoeligheid is niet voorspelbaar of beinvloedbaar, en daarom moet de overschrijding van een bepaalde drempel voorkomen worden, ook in verband met een wenselijke en mogelijke maximale arbeidsduur van 40 jaar. Omdat ook de gegevens tamelijk beperkt en afkomstig van één fabriek zijn, wordt een veiligheidsfaktor van 3 toegepast, resulterend in een advieswaarde van 0,1 mg/m^3 (0,03 ppm), gemiddeld over een werkdag van 8 uur.

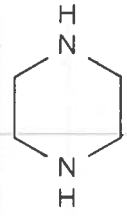
Omdat het ontstaan van asthma het gevolg kan zijn van kortdurende pieken, wordt een 15 min waarde van 0,3 mg/m^3 (0,1 ppm) voorgesteld.

Datum afronding advies: januari 1991

1. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, MONITORING

1.1 IDENTITY

- structure :



- name : piperazine (anhydrate)
- CAS registry number : 110-85-0
- systematic name : 1,4-diazocyclohexane
- synonyms : diethylenediamine
  hexahydropyrazine
  hexahydrodiazine
  piperazidine

In addition to the pure substance, the following hydrates and salts also occur (i.e. are manufactured in Sweden): piperazine hexahydrate, piperazine 65%, piperazine adipate, piperazine citrate, piperazine dihydrochloride, and piperazine hydrogen phosphate.
In the Netherlands, piperazine adipate and piperazine citrate may be of interest (VNCI, 1988).

1.2 PHYSICO-CHEMICAL CHARACTERISTICS

The physico-chemical characteristics of the aforementioned compounds are summarized in Table 1 (see page 2).

	piperazine						
	anhydrate	hexahydrate	65% (technical)	adipate	citrate	dihydrochloride	hydrogen phosphate
CAS no	110-85-0			142-88-1	144-29-6	142-64-3	1951-97-9
mol formula	$C_4H_{10}N_2$	$C_4H_{10}N_2.6H_2O$	$C_4H_{10}N_2$. ca 2.5 H_2O	$C_4H_{10}N_2$. $C_6H_{10}O_4$	$3C_4H_{10}N_2$. $2C_6H_{10}O_4$ x H_2O (x mostly ca 4)	$C_4H_{10}N_2$. $2HCl(H_2O)$	$C_4H_{10}N_2$. $H_3PO_4.H_2O$
mol weight	86.14	194.23	ca 131	232.28	642.68 (anhydrate)	159.06 177.07(monohydr)	20215
melting point (1 bar)	106°C	44°C	35-45°C	253°C (sublimates and desintegr)	182-187°C (desintegr)	83°C (both reported)	220-224°C 315-325°C
boiling point (1 bar)	146°C	125-130°C					
density	1.07 g/ml		1.015-1.020 g/ml(at 50°C)				
vapour pressure (20°C)	0.23 mbar						
Solubility: water	soluble	soluble		soluble	soluble	soluble(hydrate)	very slightly
ethanol	soluble	soluble		not soluble	not soluble		
ether	not soluble	not soluble			not soluble		
pH (eq. solutions)	10.8-11.8 (10%)			5.45 (<5%)	5.0-6.0 (10%)	3.2 (5%)	6.3 (1%)
physical state	white or transparant flat,rhom-boidal crystals	white or transparant flat crystals	white mass	white,pris-matic crystals		white, needle-shaped crystals	white crystals
conversion factors (20°C, 1 bar)	1 mg/m^3=0.279 ppm 1 ppm=3.58 mg/m^3						

Piperazine (anhydrate) is a strong base ($k=6.4x10^{-5}$). It absorbes water and CO_2 from the air.

* data from: Lundberg, 1985
Weast, 1988-1989
Windholz, 1983

1.3 ANALYTICAL METHODS

1.3.1 Environmental monitoring

Piperazine can be analysed routinely in samples obtained from working place air. Piperazine is collected in a dilute acidified aqueous adsorbing solution. After alkali treatment samples are injected directly and analysed by gas-liquid chromatography, using a nitrogen selective detector. Amounts at sub-ppm levels can be measured (Audunsson and Mathiasson, 1984).

1.3.2 Biological monitoring

Piperazine in urine can be determined by gas-liquid chromatography, using a flame ionization detector, after alkali treatment of the samples (Bellander et al, 1988b).
Skarping et al (1986) have published a method to determine piperazine at sub-ppm levels in urine. The method includes a two-phase derivatization procedure followed by a capillary gas chromatographic determination using nitrogen or mass selective detection.

2. SOURCES OF EXPOSURE

2.1 Natural occurrence

There are no data on the natural occurrence of piperazine.

2.2 Man-made sources

2.2.1 Production

Piperazine can be prepared from ethylenediamine, aminoethylethanolamine, diethylenetriamine, monoethanolamine, diethanolamine or triethanolamine. The principle route is by continuously passing ammonia and monoethanolamine over a Raney nickel catalyst under elevated pressure and temperature. Piperazine is separated from other reaction products by distillation (Axel et al, 1974; Mjos, 1978).
In Sweden piperazine is manufactured from ethylene oxide and ammonia in a closed, continuous proces (Lundberg, 1985).

2.2.2 Uses

Piperazine (as hexahydrate or salts like adipate, phospate, citrate) is mainly used as an anthelmintic drug for worm infections in animals and in some countries also in humans. Piperazine-ring containing compounds are also used as a basis for several other pharmaceuticals. Other uses are: as accelerators in the rubber industry, in antioxidants, corrosion inhibitors, surfactants, fibers, resins, insecticides, textile dyes and in analytical-chemical methods (Axel et al, 1974; Mjos, 1978).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 ENVIRONMENTAL LEVELS

No data were retrieved from literature on the occurrence of piperazine and its salts in ambient air, water or food.

With respect to the work place, the risk of exposure is in the handling of piperazine anhydrate and, to some extent, piperazine hexahydrate during the batchwise production of piperazine salts. Exposure to piperazine vapour occurs and in some extreme cases to its dust; exposure to salts is solely in the form of dust (Lundberg, 1985).

There is one report mentioning exposure levels in a Swedish factory. Most recent figures indicate that TWA levels (personal air sampling) for different work processes usually are below 0.1 mg/m^3 (0.03 ppm) (see Table 2) (Hagmar et al, 1987).

Table 2. Breathing zone levels of piperazine (PZ) in different work processes in a Swedish factory in 1980-1985. All levels expressed as 100% PZ (table from Hagmar et al, 1987).

Process (Exposure)	Year(s)	Pooled TWA[a]				Highest sample[b]	
		Sampling occasions	Total no. of samples	Sampling time (min)	Level (mg PZ/m^3)	Level (mg PZ/m^3)	Sampling time (min)
Flaking of anhydrous PZ (vapour)	1980	9	32	2255	1.2	100	0.5
	1981–1983	5	15	1239	0.73	6.4	93
	1984[c]	8	39	4800	0.63	9.2	2.3
Flaking of PZ hexahydrate (vapour)	1980	5	10	625	0.26	0.63	17
	1981–1983	4	10	980	0.42	2.0	113
	1984	3	11	1246	0.11	0.36	150
	1985	7	36	3829	0.05	0.40	2
Centrifuging of PZ salts (dust)	1980–1985	6	25	2960	0.06	0.80	67
Granulation of PZ salts (dust)	1982–1984	12	22	3128	0.09	0.42	70
	1985	6	30	2389	0.08	7.4	9

[a] Time-weighted average (TWA) over total sampling time, regardless of length and purpose of sampling
[b] Maximum concentration of a sample, regardless of length and purpose of sampling
[c] The work-process was closed down in 1984

3.2 HUMAN EXPOSURE

There are no reports presenting levels of piperazine in tissues, biological fluids etc. of the general or occupational population, except one study dealing with the in vivo metabolism: no piperazine was detected in blank urine samples of these subjects (Bellander et al, 1985).

4. GUIDELINES AND STANDARDS

The Netherlands have no occupational exposure limit for piperazine or one of its salts. The limits for some other countries are presented in Table 3.

Table 3. Occupational exposure limits in some countries

country	year	concentr. mg/m^3	concentr. ppm	time relation	reference
Sweden[1]	1987	0.3	0.1	TLV-TWA	National Board of Occupational Safety and Health, 1987
		1	0.3	STEL (15 min)	
UK[2]	1989	5		TLV-TWA	Health and Safety Executive, 1989
USA[2]	1989-1990	5		TLV-TWA	ACGIH, 1989
USSR[3]	1982	5		MAC-TWA	Izmerov et al, 1982

1. piperazine is indicated as a sensitizing substance
2. piperazine dihydrochloride
3. piperazine adipate.

5. TOXICOKINETICS

5.1 ABSORPTION

There are no data on the absorption of piperazine by the main industrial exposure routes: the respiratory tract and the skin. Piperazine is readily absorbed from the gastrointestinal tract (Webster, 1985).

Due to its extremely hygroscopic properties, it is assumed that piperazine is readily deposited in the respiratory tract after inhalation of the dust, dependent on the particle size distribution. From there it can reach the stomach either by mucociliary clearance followed by swallowing or by uptake from the lungs into the blood and excretion from the blood into the stomach.

The latter process is plausible due to the difference by a factor 10^9 in relative amount of unprotonated (i.e. membrane permeating) amine at pH 7.4 (blood) and 2.0 (stomach) (Bellander et al, 1988b).

5.2 DISTRIBUTION

There are no data available on the distribution of piperazine in the human or animal body.

5.3 BIOTRANSFORMATION

5.3.1 Piperazine

Little is known about the biotransformation of piperazine.

In man, piperazine can be converted in the stomach to N-mononitrosopiperazine. N-mononitrosopiperazine was detected in the gastric juice (highest levels at half an hour after intake and varying from 140 to 230 μg/l; total amount estimated to be 30-66 μg) and in the urine of all volunteers (n=4) after ingestion of 480 mg of piperazine (i.e. the therapeutic dose recommended to be taken for 2 to 7 days). About 10% of the amount estimated to be formed in the stomach, is excreted unchanged in the urine; the fate of the remainder is unknown. No N,N'-dinitrosopiperazine was found (minimum detectable concentration in urine: 0.5 μg/l) (Bellander et al, 1985).

In addition, N-mononitrosopiperazine was found in the urine of workers occupationally exposed for 12 h to TWA concentrations of piperazine ranging from 0.06 to 1.7 mg/m^3 (0.02-0.47 ppm).

In 5 out of 11 cases amounts varied from 0.3 to 4.7 μg/24h (during and after workshift). In the other cases it was detected occasionally (n=4) or not al all (n=2). The individual excretion was correlated with piperazine exposure (Spearman's rank correlation 0.78, P=0.01) (Bellander et al, 1988b).

5.3.2 Piperazine and nitrate/nitrite

Volunteers (n=4) were exposed to 0.3 mg/m^3 (0.08 ppm) of piperazine for 8 h. During this exposure, they received three different diets at three different days: nitrate- and ascorbate-poor; nitrate-rich and ascorbate-poor and nitrate- and ascorbate-rich. Ingestion of the first diet resulted in the excretion of N-mononitrosopiperazine in one volunteer (0.4 μg). In combination with the second diet, up to 1.7 μg of the mononitroso derivative was found in the urine of three volunteers, while addition of ascorbate resulted in a decline in N-mononitrosopiperazine excretion (highest excreted amount 0.6 μg). No N,N'-dinitrosopiperazine was detected (Bellander et al, 1988a).

As to animals, N,N'-dinitrosopiperazine was found in the urine of dogs given piperazine and nitrite-containing food and an infusion of a glucose solution and a diuretic to enhance urine production (see Table 4, page 10) (Sander et al, 1973).

Although the conversion of piperazine into N,N'-dinitropiperazine was demonstrated to occur in dogs under these experimental conditions, this conversion could not be quantified due to limited experimental design.

From rats (n=3), Hecht et al (1984) calculated that the administration of 19 μmol (1.65 mg) of piperazine followed by 191 μmol (13.2 mg) of nitrite resulted in the formation of 7.3 μmol (1.1 mg) of N,N'-dinitrosopiperazine (i.e. a 38% yield from piperazine). This calculation was based on the measurement of N-nitroso(2-hydroxyethyl)glycine, a metabolite of N,N'-dinitrosopiperazine in rats (see section 5.3.3).

5.3.3 N,N'-dinitrosopiperazine

The administration of N,N'-dinitrosopiperazine to rats resulted in the identification of 1-nitrosopiperazine-3-one and 3-hydroxy-N-nitroso-pyrrolidine in one and of N-nitroso-(2-hydroxyethyl)glycine (22% of the dose), N-nitrosodiethanolamine (3%), 3-hydroxy-N-nitroso-pyrrolidine (3%) and unchanged N,N'-dinitrosopiperazine (18%) in another study (Hecht et al, 1984).

Table 4. Excretion of N,N'-dinitrosopiperazine in the urine of dogs given nitrite-containing food and piperazine (table from Sander et al, 1973)

	Infusion[2] (ml)	Amount of urine excreted (ml)	Amount of dinitroso-piperazine excreted (μg)
200 g Chappi[1] + 3 g piperazine hexahydrate	2700	800	6
200 g Chappi + 0,5 g piperazine hexahydrate	2000	850	5
400 g Chappi + 400 mg $NaNO_2$ + 3 g piperazine hexahydrate	3725	950	104
700 g ham (10,8 mg $NaNO_2$/100 g) + 3 g piperazine hexahydrate	4500	3700	548
650 g ham (15,6 mg $NaNO_2$/100 g) + 3 g piperazine hexahydrate	2900	1700	65
400 g ham (10,8 mg $NaNO_2$/100 g) + 1 g piperazine hexahydrate	3000	500	26
100 g ham (10,8 mg $NaNO_2$/100 g) + 0,5 g piperazine hexahydrate + 100 g white bread	3000	500	3
100 g ham (3,6 mg $NaNO_2$/100 g)[3] + 0,5 g piperazine hexahydrate	2500	450	0,2
200 g ham (3,6 mg $NaNO_2$/100 g)[3] + 200 g Chappi + 1 g piperazine	2500	950	0,1

[1] commercially available dog food
[2] 5% glucose solution
[3] not abstemious

5.4 ELIMINATION

After oral intake of piperazine or its salts, piperazine is excreted in the urine. About 30% of the dose is excreted within 24 hours (half of this within the first 5 hours), although individual variations are considerable (Lundberg, 1985).

Bellander et al (1985) administered 480 mg to 4 volunteers. During the first 16 hours after challenge, 19 to 35% of the ingested dose was excreted as piperazine in the urine. During an additional period of about 24 hours another 2 to 3% were excreted. The elimination was not completed at that time. The elimination was concluded to occur in two phases; the half-life of the fastest and dominating phase is in the order of a couple of hours.

Two subjects were followed with respect to N-mononitrosopiperazine excretion: 24 hours after challenge no mononitrosopiperazine could be detected anymore; the total amounts were 1.0 and 2.2 μg, half of which had been excreted within 2 to 3 hours.

As can be seen from section 5.3, several metabolites of piperazine and the nitroso derivatives have been found in the urine of animals.

5.5 BIOLOGICAL MONITORING

From the previous sections it can be seen that in humans about 30% of the ingested dose is excreted unchanged and only minor amounts as the mononitroso derivatives. Therefore, measurement of piperazine in urine offers a possibility of biological monitoring of workers exposed to piperazine and its salts. Bellander et al (1988b) found a strong correlation between the level of piperazine in air and of piperazine in the urine of exposed workers (Spearman's rank correlation: $r=0.93$; $P<0.001$). However, the number of subjects was very limited ($n=8$) and the authors stated that the use of piperazine in urine as a biological index of exposure, even at levels below 0.3 mg/m^3 (0.08 ppm; i.e. the Swedish TLV-TWA), should require a more sensitive method as well as studies of kinetics at repeated exposure.

5.6 SUMMARY

There are no data on the absorption of piperazine by the main industrial exposure routes: the respiratory tract and the skin. It is readily absorbed by the gastrointestinal tract. There are no data on the distribution.

After oral administration 30% is excreted unchanged in the urine within 24 h. Elimination occurs in two phases: $t_{½}$ of the fastest phase is in the order of a couple of hours. Therefore,

measurement of piperazine in the urine of workers may offer a possibility for biological monitoring of workers exposed to piperazine. Furthermore, small amounts of N-mononitroso-piperazine were also found, but not the dinitroso derivative.

A nitrate-rich diet enhanced the formation of mononitrosopiperazine in the stomach. This could be partly inhibited by addition of ascorbate.

In animals, administration of piperazine and nitrite resulted in the excretion of dinitrosopiperazine and its metabolites.

6. EFFECTS

6.1 ANIMAL EXPERIMENTS

6.1.1 Irritation and sensitization

Piperazine scored an injury grade of 9 on a scale from 1 to 10, employed for rating the relative damage produced by chemicals in the eyes of rabbits (Carpenter and Smyth, 1946). Piperazine citrate was not a skin sensitizer as was shown from a classic anaphylaxis test in guinea pig (Ratner and Flynn, 1955).

6.1.2 Acute toxicity

In rats, the oral LD50 of anhydrous or hydrated piperazine ranges from 1900-3800 mg/kg and in mice from 600-4300 mg/kg. The LC50 for mice is 5400 mg/m^3/2h (1510 ppm). The subcutaneous and intravenous LD50-values are about 1100 mg/kg for mice and, respectively, 3700 mg/kg and 1340 mg/kg for rats. The piperazine salts are less toxic: oral LD50 for rats range from 7900 mg/kg (adipate) to 11200 mg/kg (citrate) and for mice from 8500 (citrate) to 20000 mg/kg (phosphate) (Lewis and Sweet, 1985; Reinhardt and Brittelli, 1981).
The therapeutic doses (administered as salts) range from 30 mg/kg for poultry to 500 mg/kg for sheep (Hapke, 1983). In veterinary literature cases have been reported concerning effects due to overdosing piperazine salts to cats and dogs. Instead of the therapeutic dose of 100 mg/kg, the animals received 5 to 8 times as much resulting in extreme weakness, muscular in-coordination, vomiting, diminished food intake (Bownass, 1987; Goddard and Johnston, 1986; Howroyd, 1984; Swift, 1984).

6.1.3 Short-term toxicity

Guinea pigs were exposed to a calculated concentration of 358 mg/m^3 (100 ppm), 3h/d, 7 times in 11 days. No gross adverse effects were detected (Reinhardt and Brittelli, 1981).
Oral administration of 150 mg/kg of piperazine hexahydrate (=70 mg of piperazine/kg) to rats, daily for 30 days, resulted in reductions in serum lipid levels. Oral administration of 300 mg/kg piperazine adipate (=110 mg of piperazine/kg) to rats, daily for 8 weeks, had no effect on weight gain and did not cause any histologically verifiable organ changes (Lundberg, 1985).

6.1.4 Long-term toxicity/carcinogenicity

Long-term toxicity

With respect to long-term toxicity studies, there is only one study available (carried out by Dow Chemical Company; cited by Reinhardt and Brittelli, 1981): rats were given 0.1% (i.e. 75 mg/kg/d) of piperazine (probably the hexahydrate) in the diet for 90 days and did not show changes as to appearance, behavior, growth, mortality, food consumption, final body or organ weights, and gross and microscopic examination of the tissues. Doses of 1 and 3% (i.e. 750 and 2250 mg/kg/d) caused (not further indicated) moderate liver and kidney pathological effects. Although the significance of this study cannot be evaluated because of complete lack of details, 75 mg/kg/d may be a no-adverse effect level.

Carcinogenicity

Carcinogenicity studies were done by administering piperazine alone or in combination with nitrite to rats and mice.

Piperazine. Rats were given 0.025% of piperazine in the drinking water, 5 d/w, 75 w (i.e. about 20-25 mg/kg/d; total dose 1.8 g). The animals were kept until death and subjected to complete pathological examination. No increase in the number of tumour-bearing animals was found (see Table 5). In addition, the incidence of tumours commonly found in control rats (pituitary adenomas, tumours of breast, uterus, testis) did not increase (Garcia and Lijinski, 1973).

Table 5. Number of tumour-bearing rats treated with piperazine with piperazine and nitrite (data from Garcia and Lijinski, 1973).

treatment	total dose (g)	number of animals		number of tumour-bearing animals	
		♂	♀	♂	♀
piperazine (0.025%)	1.8	15	15	5	8
piperazine (0.025%) + nitrite (0.05%)	1.8 3.7	15	15	8	13
untreated		15	15	5	4
nitrite (0.2%)	15	15	15	9	10

Swiss mice were given 6.25 g of piperazine/kg of food (i.e. 750 mg/kg/d), 5 d/w, 28 w. The animals were killed at w 40. There was neither an increase in the number of lung adenoma-bearing animals (15% vs 14% in controls) nor in the total number of lung adenomas (14 vs 26) (Greenblatt et al, 1971).

Treatment of male strain A mice with the same dose, but for only 20 w and followed for another 10 w, did not significantly increase the number of lung adenoma-bearing animals (28% vs 13% in controls). However, the number of adenomas (17 vs 5) and the number of adenomas/mouse (0.4 vs 0.1) were both significantly increased. In a separate experiment, 18,75 g of piperazine (i.e. 2250 mg/kg/d) was administered per kg of food, 5 d/w, for 25 w. The mice were killed at w 38. Despite the higher dose, the longer treatment and living, no significant differences were found with respect to the number of tumour-bearing animals, number of tumours and number of tumours/mouse, suggesting that the response in the 750 mg/kg/d dose group was without significance (Greenblatt and Mirvish, 1973).

Piperazine and nitrite. Rats received concomitantly daily doses of 0.025% of piperazine and 0.05% of nitrite in the drinking water, 5 d/w. The treatment lasted 75 weeks and total doses of 1.8 g of piperazine and 3.7 g of nitrite were administered.

After treatment, animals were kept until death. Data are presented in Table 5 (page 14). The authors concluded that under these conditions formation of nitrosamine derivatives in vivo from the free piperazine and nitrite was insufficient to induce tumours (Garcia and Lijinski, 1973).

Swiss mice were given 6.25 g of piperazine/kg of food (i.e. 750 mg/kg/d) together with 1.0 g of nitrite/l of drinking water, 5 d/w for 28 w and they were killed at w 40. The number of lung adenoma-bearing mice was significantly increased when compared to controls (64% vs 14%; only nitrite-receiving controls: 19%). The total number of lung adenomas increased from 26 in controls to 137 in treated animals (nitrite-receiving controls: 16) (Greenblatt et al, 1971).

Male strain A mice were treated with various amounts of piperazine in the food (0.69-18.75 g/kg of food; i.e. about 80-2250 mg/kg/d) together with a constant amount of nitrite in the drinking water (1.0 g/l), 5 d/w, for 25 w (killed after another 13 w) and, in a separate experiment, with a constant amount of piperazine (6.25 g/kg of food; i.e. 750 mg/kg/d) and various amounts of nitrite in the drinking water (0.05-2.0 g/l), 5 d/w, for 20 w (killed at w 30). Data are summarized in Table 6 (see page 17).

All administration regimens increased significantly the number of lung adenoma-bearing mice as well as the number of adenomas/mouse except the combination of 6.25 g of piperazine/kg of food with 0.05 g of nitrite/l of drinking water. As to the increase in the number of lung adenomas/mouse a dose-respons relationship was noted for piperazine as well as for nitrite. The number of adenomas/mouse was approximately proportional to the concentration of piperazine in the food and the square of the concentration of nitrite in the drinking water (Greenblatt and Mirvish, 1975).

The induction of lung adenomas in strain A mice by concomitant administration of piperazine (6.25 g/kg of food) and nitrite (1 g/l of drinking water) was reduced by sodium ascorbate in a

dose dependent way (Mirvish et al, 1975).

N-mononitrosopiperazine. The carcinogenic effect of the mononitroso derivative of piperazine was studied by oral or subcutaneous administration to rats and mice.
In rats, daily administration of 400 and 800 mg/l (40, 80 mg/kg) of N-mononitrosopiperazine in drinking water, 5 d/w, 75 w, resulted in an incidence of tumour bearing animals of 17/29 (59%) and 21/27 (78%) (cf controls: 38/69 (55%)). After subtracking the background levels of primarily endocrine and genital tumours experienced by the controls, a significantly elevated risk of cancer was noted for both groups, the principal target organ being the nasal cavity with some excess risk in the liver (no such tumours were found in controls) (Love et al, 1977). Previously, rats were given 50 and 200 mg/l (5 and 20 mg/kg), daily, in drinking water, 5 d/w, 60 w. An increase in the number of animals with tumours was found (12/20 and 10/20 vs 16/70 in controls), but tumours were scattered among several organs (Garcia et al, 1970).
In mice, daily doses of 34,5 mg/l of N-mononitrosopiperazine in drinking water (i.e. 7 mg/kg) for 25 weeks significantly increased the number of lung adenoma-bearing animals from 12/37 (32%) in controls to 30/39 (77%) in exposed animals and the total number of lung adenomas from 17 to 148. Animals were killed at w 40 (Greenblatt and Mirvish, 1973). In another study, a dose-related increase in the number of lung adenomas per mouse was reported, but only a figure was presented (Mirvish et al, 1975).

N,N'-dinitrosopoperazine. In a pilot study, 4 BD rats were given daily doses of 16 mg/kg/d (i.e. 0.1 LD_{50}) in the drinking water, 7 d/w. Two rats had papillomas and the two other rats had carcinomas of the oesophagus. Mean induction time: 274 d; mean carcinogenic total dose: 3.3 g/kg. A second group of fifteen rats received 8 mg/kg/d; after 5 months, doses were reduced to 4 mg/kg/d (7 d/w). Four animals died without tumours; nine had malignant tumours of the oesophagus. Mean carcinogenic total dose and mean induction time were estimated to be 2.2 g/kg and 364 d, respectively. A third group of 12 rats received 4 mg/kg/d, 7 d/w. In 4 rats papillomas and in 3 rats carcinomas of the oesophagus were observed, in two cases concomitantly with tumours of the liver. Another two tumours of the nasal cavity were seen. Mean induction time: 466 d; mean carcinogenic total dose: 1.2 g/kg.
With respect to controls, no data were presented, but it was stated that the tumours observed in the exposed groups did not occur spontaneously in the controls (Druckrey et al, 1967).
Garcia and Lijinsky (1972) administered 8 mg/kg/d, 5 d/w for 50 w to MRC rats and followed them to death. Tumours of the nasal cavity were found in 25 out of 29 animals. In addition, only a few other tumours were found.
When Sprague-Dawley rats were exposed according to the same regimen, 28 out of 29 animals were bearing tumours; 26 had tumours of the nasal cavity, 10 of the liver and 5 of the upper digestive tract (Lijinsky and Taylor, 1975).

Table 6. Lung adenoma induction in male strain A mice, treated with piperazine and nitrite (data from Greer blatt and Mirvish, 1973).

Treatment	Concentration[1] (g/kg food or g/liter water)	Effective No. of mice	Adenoma-bearing mice Number	Adenoma-bearing mice Percent	Total No. of adenomas	Adenomas/mice Mean	Adenomas/mice SD
Experiment							
None	-	37	12	32	17	0.5	0.8
$NaNO_2$	1.0	37	11	30	13	0.4	0.6
Piperazine + $NaNO_2$	18.75 + 1.0	40	35	88[2]	768	19.2[2]	10.9
Piperazine + $NaNO_2$	6.25 + 1.0	40	35	88[2]	536	13.4[2]	10.0
Piperazine + $NaNO_2$	2.08 + 1.0	40	33	83[2]	246	6.2[2]	5.5
Piperazine + $NaNO_2$	0.69 + 1.0	40	32	78[2]	115	2.8[2]	2.5
Experiment							
None	-	39	5	13	5	0.1	0.3
$NaNO_2$	2.0	39	7	18	8	0.2	0.5
Piperazine + $NaNO_2$	6.25 + 2.0	40	39	98[2]	1232	30.8[2]	12.3
Piperazine + $NaNO_2$	6.25 + 1.0	37	37	95[2]	318	8.2[2]	5.6
Piperazine + $NaNO_2$	6.25 + 0.5	39	30	77[2]	88	2.3[2]	2.0
Piperazine + $NaNO_2$	6.25 + 0.25	40	20	50[2]	27	0.7[2]	0.8
Piperazine + $NaNO_2$	6.25 + 0.05	39	10	26	12	0.3	0.6

[1] Piperazine was given in the food; $NaNO_2$ in the drinking water.
[2] Significantly different from controls ($p < 0.001$).

Greenblatt et al (1971) administered daily doses of 40 mg/l (i.e. 8 mg/kg/d), 5 d/w for 28 w, in the drinking water, to Swiss mice and observed them for another 12 w. Forty-nine out of 75 animals surviving more than 16 w from the beginning of experiment were bearing lung adenomas (vs 20/84 in controls). The total number of lung adenomas was 319 (vs 26) and the number of adenomas/mouse 4.9 (vs 0.18).

Börszönyi et al (1980) gave daily doses of 100 mg/l (i.e. 20 mg/kg) for 52 w resulting in a significant increase in the incidence of lung adenomas when compared to controls (14/36 vs 10/79). Administration of the same dose to pregnant mice either during pregnancy days 15 to 21 or during lactation (postpartum d 1-20) caused a significant increase in the incidence of lymphomas and leukemias in the transplacentally exposed (16/25 vs 26/79) and hepatocellular carcinomas (16/30 vs 6/79) in the translactationally exposed offspring.

Finally, N,N'-dinitrosopiperazine was injected subcutaneously in rats and mice.

Sixteen BD rats received 15 mg/kg (sc LD_{50}: 160 mg/kg), 2 times a week. Eight rats had carcinomas of the oesophagus and 6 rats tumours of the nasal cavity; 4 had no tumours. Mean induction time: 370 d; mean carcinogenic total dose: 1.6 g/kg. A second group of 12 rats received 10 mg/kg, 2 times a week. Seven tumours of the oesophagus and 10 of the nasal cavity were observed. Mean induction time: 522 d; mean carcinogenic total dose 1.1 g/kg. According to the authors, these types of tumours did not occur spontaneously in unexposed rats (Duckrey et al, 1967).

Schmähl and Thomas (1965) injected 20 mice subcutaneously with 10 mg/kg/d, 2 times a week, until death. Three mice died within 6 months. Fourteen out of the remaining 17 mice receiving a total dose of about 1.1 g/kg had tumours of the lung and 4 of the liver. No tumours of the nasal cavity were seen. No data on controls were presented.

Conclusion. The studies concerning the carcinogenicity of piperazine, piperazine and nitrite, N-mononitrosopiperazine and N,N'-dinitrosopiperazine do not generally meet the OECD guidelines with respect to dose range, duration and number of animals (as published by ECETOC, 1985).

Therefore, the significance of the findings as to piperazine are limited and can indicate at most, that piperazine is not likely to be carcinogenic.

With respect to piperazine and nitrite, indications are rather strong, that this combination can produce malignant tumours in experimental animals.

The mononitroso as well as the dinitroso derivative are carcinogenic in rats and mice.

6.1.5 Mutagenicity

Piperazine. Piperazine is neither mutagenic to S. typhimurium in vitro (Alba et al, 1988), nor in host-mediated assays (Lundberg, 1985).

Piperazine citrate and piperazine adipate were analyzed in a diploid mitotic recombination and

gene conversion assay and in a haploid yeast reversion assay: both were not genotoxic in either S. cerevisiae strain (Hennig et al, 1987).
Lundberg (1985) refers to a study not reported in detail in which the addition of piperazine together with microsomal liver fraction was stated to increase the TK mutant frequency in mouse lymphoma cells.

Piperazine and nitrite. Piperazine did increase the frequency of mutation in S. typhimurium TA 1950 in a host-mediated assay test when nitrite was simultaneously added at an equimolar amount. When nitrite was added before the piperazine, an 80% reduction of mutations was found (Lundberg, 1985).
Products of in vitro nitrosation of piperazine were mutagenic in S. typhimurium, both with and without metabolic activation by induced rat liver S9 mix. Metabolic activation increased the frequency of mutations (Alba et al, 1988).

N-mononitrosopiperazine. N-mononitrosopiperazine is mutagenic to S. typhimurium strains in host-mediated assay tests. It was weakly mutagenic in S. typhimurium TA 1535, both with and without rat liver S9 mix, but in another study results were negative. When tested in S. cerevisiae and E. coli Wv 3610, either with or without activation, it was not mutagenic. Finally, there is a study (not reported in detail) in which N-mononitrosopiperazine did not induce SCE in human leucocytes in vitro (Lundberg, 1985).

N,N'-dinitrosopiperazine. N,N'-dinitrosopiperazine was positive in the Drosophila wing mosaic assay system after feeding it to larvae for 2-4 days (Surjan et al, 1985).
I.p. injections induced chromosomal translocations and x-bound recessive lethal mutations in Drosophila melanogaster. N,N'-dinitrosopiperazine is mutagenic in the host-mediated assay with S. typhimurium and in the presence of rat liver S9 mix in S. typhimurium, E. coli and S. cerevisiae, as well (Lundberg,1985).

6.1.6 Reproduction toxicity

There are no data available with respect to reproduction toxicity of piperazine.

6.2 OBSERVATIONS IN MAN

6.2.1 Irritation and sensitization

Piperazine anhydrate is strongly irritating to skin, mucous membranes, etc, because of its

alkaline and hygroscopic properties. The hexahydrate is not hygroscopic, it irritates by its alkalinity. Piperazine solutions are less irritating, this being dependent on the concentration (pH).
Application of a solution of 250 g/l of piperazine hexahydrate (=115 g/l piperazine) resulted in skin irritation in 10 out of 12 volunteers; concentrations < 50 g/l (=23 g/l of piperazine) did not cause primary skin irritation (Lundberg, 1985).
The piperazine salts are not alkaline, but almost neutral and will be not irritating.
Several cases have been reported, in which dermal effects due to oral, dermal or rectal application of piperazine containing drugs have been described. Piperazine was positive in patch tests. Furthermore the reaction to piperazine has been ascribed to cross-sensitization as a result of contact to ethylenediamine (Menezes Brandao and Foussereau, 1982; Fernandez de Corres et al, 1986; Lundberg, 1985; Price and Hall-Smith, 1984; Wright and Harman, 1983).
Occupational handling of piperazine can lead to allergic eczema (Menezes Brandao and Foussereau, 1982; Lundberg, 1985).

6.2.2 Acute toxicity/case reports

All cases reported on piperazine intoxication are related to its use as a drug.
The therapeutic dose of piperazine for adults as well as for children is about 30 mg/kg and there is a wide range between this dose and the overtly toxic doses (Webster, 1985). However, after oral treatment at doses of piperazine below these levels, transient neurological disorders have been observed: fatigue, disorientation, confusion, hallucinations, tremor, ataxia (coordination problems), clonic spasms, weakness, nausea, vomiting. These disorders were accompanied by consistent, but reversible, electroencephalographic changes. Although these effects can appear in previously healthy adults and children, patients with renal insufficiency or a history of CNS disease (epilepsy) run a higher risk (Lundberg, 1985; Neau et al, 1984).
In addition, one case of non-infectious hepatitis in a young woman related to the intake of 10 g of piperazine phosphate (=4.3 g of piperazine) has been referred to, as well as to one case of hemolytic anemia in a child with glucose-6-phosphate dehydrogenase deficiency (Lundberg, 1985).

6.2.3 Short-/long-term exposure

Webster (1985) states that laboratory studies on patients receiving treatment with piperazine for several days have not showed abnormality and that piperazine has been used during pregnancy without adverse effect. However, no detailed information on, for instance, doses has been presented, nor has been retrieved from other literature.

6.2.4 Epidemiological studies

Occupational exposure to piperazine (anhydrate, hexahydrate, salts) has caused late asthma reactions. The latency time before the onset of symptoms varied from a few months to several years and symptoms often reappeared immediately on renewed exposure (Lundberg,1985).

Hagmar et al (1984) have studied the asthmatic reactions among a cohort of 610 factory workers by means of a questionnaire. They handled, or had handled, amines and other chemicals in the period 1942-1979. They were divided into 4 groups according to a piperazine time index, related to a score for the period of time during which specified piperazine working operations were done. A strong exposure-response relationship as to frequency of work-related asthma in terms of attacks of dyspnea was found. In the most exposed group, 39% of the workers had developed asthma (unexposed group: 5%). Length of employment and the used time index were strongly correlated with the asthmatic symptoms. An intensity of exposure index as such did not add any further explanation. This finding did apply in a comparable way to the association found between piperazine exposure and chronic bronchitis: in the most exposed group 25% had chronic bronchitis (unexposed: 5%). TWA exposure levels ranged from 0.3 mg/m^3 (0.08 ppm) - highest level 0.63 mg/m^3 (0.19 ppm) - at one workplace to 1.2 mg/m^3 (0.33 ppm) with excursions to 100 mg/m^3 (28 ppm) in another. It was not clear whether brief exposure to high concentrations or the total dose from long-term exposure was relevant for induction of asthma (Lundberg, 1985). The data on exposure levels (frequency, duration, level of peaks) are too limited to evaluate the role of peak levels concerning the occurrence of the effects. However, induction of astma was not associated with particular working operations at an average concentration 0.3 mg/m^3 (0.08 ppm), although attacks of dyspnea in sensitized subjects could be provoked at a TWA level of 0.4 mg/m^3 (0.11 ppm) and the most recent case of asthma was associated with an exposure level of 0.7 mg/m^3 (0.20 ppm) (Hagmar, 1986).

Several other studies were carried out in this factory. In 5 out of 72 workers (7%) exposed to piperazine, specific IgE antibodies against a conjugate between human serum albumine and piperazine were demonstrated with the radioallergent sorbent test (RAST) and RAST inhibition techniques. No such antibodies were found in 64 nonexposed workers at the same plant and in 60 healthy reference subjects. Four out of the 5 RAST-positives had unequivocal histories of piperazine-induced asthma, with an induction time of 6-168 months. In addition, 4 out of 67 RAST-negatives had a piperazine-induced asthma, however with an induction time of less than a month. These data suggest, that piperazine may cause asthma in different ways: either type-I allergy, pseudo-allergic reactivity, or nonspecific bronchial irritation (Hagmar and Welinder, 1986).

In order to detect early respiratory effects due to piperazine exposure the small airway function of 22 male workers was studied and compared with this function in 22 male referent subjects (from a food processing factory at the same town) with similar smoking habits and age. The selection criteria were not mentioned. The workers were interviewed about symptoms of bronchial obstruction, chronic bronchitis and atopic symptoms (atopic eczema, allergic rhinitis,

urticaria, asthma). No statistically significant association was found between stated airway symptoms and any of the lung function parameters examined; neither was there a significant difference in the prevalence of symptoms between piperazine-exposed and referent subjects. No difference was found in the volume of trapped gas before and after bronchial provocation, indicating that a TWA exposure of about 0.1 mg/m^3 (i.e. the level below which these workers were exposed for at least half the work-time during the year preceding the investigation) does not cause small airways disease in non-asthmatic subjects (Hagmar et al, 1987).

Finally a retrospective cohort study was done on a group of 664 male workers employed for at least one month during the period 1942-1979. Besides piperazine, urethane, ethylene oxide, formaldehyde and organic solvents were handled. With respect to mortality, a significantly increased overall mortality rate for the period 1961-1984 was found. The increase was mainly due to violent death (no occupational accidents) and cardiovascular diseases (not specified). There was no significant increase for death from asthma, bronchitis or emphysema. As to cancer morbidity, a significant increase was observed for malignant lymphoma/myelomatosis, when the observation time was at least 10 years (since all cases of lymphoma were of the non-Hodgkin's type, i.e. B-lymphocyte tumours, lymphoma and myelomatosis were treated as one entity). An increase in bronchial cancer was noted, but it was statistically significant only when the observation time was at least 15 years. With respect to smoking as a possible confounding factor for bronchial cancer, some of the increased morbidity was attributed to the higher proportion of smokers in the cohort when compared with the referent group. A case-referent study within the cohort could not reveal an association between piperazine exposure and cancer morbidity, due to the few cases of cancer and due to the complexity of the exposure (Hagmar et al, 1986).

In order to find out whether exposure to mutagens and mutagenic effects still occurred in this plant, and if so, whether specific exposures were responsible, structural chromosomal aberrations and micronuclei in peripheral lymphocytes were determined in combination with differential white blood cell counts and lymphocyte subset determinations (Hagmar et al, 1988a). Eighty-five male workers (mean age: 40 y; range: 19-65 y) were compared with 48 male clerks (mean age: 36 y; range: 25-58 y). Both groups were interviewed with respect to smoking and alcohol habits, tea and coffee consumption, X-ray examination, influenza and other virus infections during the preceding year (except ordinary colds), current drug intake.

The only significant difference that was related to exposure to one of the chemicals handled in the plant, was found when venous blood, sampled to determine lymphocyte subsets and immunoglobulins, obtained from 20 workers with the highest exposure to piperazine was compared with blood from 20 unexposed controls.

The number of B-lymphocytes (as was demonstrated by the number of IgM^+ cells) was significantly increased, as well as the number of T-suppressor/ cytotoxic ($T8^+$) cells. These findings are of interest, since an increased incidence of B-lymphocyte malignancies (possible relationship unknown) and of occupational asthma was observed.

The monitoring program also included unscheduled DNA synthesis, the activities of adenosine diphosphate ribosyl transferase, epoxide hydroxylases and glutathione transferase in resting

mononuclear leukocytes (Pero et al, 1988), urinary mutagenicity (bacterial fluctuation test) and thioether excretion (Hagmar et al, 1988b). No significant associations with exposure to piperazine or other specific compounds or groups of compounds could be established.

6.3 SUMMARY

Piperazine (anhydrate/hexahydrate) is irritating to the skin, mucous membranes, etc. Piperazine solutions and salts are less, respectively not irritating. Occupational handling can lead to allergic eczema.

After inhalation or oral administration the acute toxicity is low. In therapeutic cases, overdosing resulted in extreme weakness, muscular incoordination, vomiting and anorexia.

Data on the toxicity due to short-term or long-term exposure are very limited.

Piperazine is not likely to induce tumours in rats and mice after oral administration, but simultaneous administration of nitrite did increase the incidence of tumours. Both the mononitroso and the dinitroso derivatives caused tumours at several sites after oral or subcutaneous administration to rats or mice. In some studies a dose-response relationship was noted. Oral administration of the dinitroso derivative to pregnant or lactating mice increased the incidence of tumours in the offspring.

Piperazine and two of its salts were not mutagenic in mutation assays in bacteria and yeast cultures. The positive result in an in vitro mutation assay in a mammalian cell system could not be interpreted. Results on the mutagenicity of N-mononitrosopiperazine in mutation assays in S. typhimurium were conflicting, but it was positive in E. coli and S. cerevisiae and also in S. typhimurium in a host-mediated assay. The negative result of testing chromosomal effects in a human cell system could not be evaluated, due to inadequate documentation. N,N'-dinitrosopiperazine was positive in mutation assays in bacteria and yeast and to bacteria in a host-mediated assay. It was mutagenic when tested in insects. It is considered as a genotoxic agent.

In man, transient neurological disorders have been noted after oral therapeutical treatment, primarily in patients with renal insufficiency or a history of CNS disease. Several epidemiological studies in one Swedish factory revealed that of the most exposed group about 35% had developed asthma and 25% had chronic bronchitis. No new cases of asthma were noted when exposure levels were ≤ 0.3 mg/m^3 (0.08 ppm). Some workers had specific IgE antibodies against a conjugate of human serum albumine and piperazine. At 0.1 mg/m^3 (0.03 ppm) no difference in small airway function of exposed workers and in that of unexposed was found. Asthma may be caused by either type-I allergy or by nonspecific bronchial irritation. Finally, an increased mortality rate was observed, mainly due to violent death and cardiovascular diseases - not to airway system diseases. The increased cancer morbidity could not be associated to any specific chemical exposure.

7. PREVIOUS EVALUATION BY (INTER)NATIONAL BODIES

ACGIH considers, that, since piperazine dihydrochloride definitely has some biological activity, a TWA-TLV of 5 mg/m^3, below the nuisance dust level, seems indicated and that this level should prevent systemic toxicity. Mentioned are the low systemic toxicity, the slight irritation of eyes and skin, the mild to moderate skin burns and sensitization and the mild to moderate asthma (ACGIH, 1986).

The Criteria Group for Occupational Standards of the Swedish National Board of Occupational Safety and Health concluded in their consensus report for piperazine, that the critical effect of occupational exposure seems to be the induction of asthma and that no new cases are noted in a workplace where the average concentration is 0.3 mg/m^3 (Lundberg, 1985). The Swedish TWA-TLV is 0.3 mg/m^3 or 0.1 ppm and piperazine is indicated as a sensitizing substance (National Board of Occupational Safety and Health, 1987).

IARC did not mention piperazine or its nitroso derivatives in one of its monographs on the evaluation of carcinogenic risks of chemicals to humans.

8. EVALUATION OF HUMAN HEALTH RISKS

8.1 GROUPS AT EXTRA RISK

Since piperazine exerts effects on the respiratory tract, people with airway system problems form a group at extra risk, in particular those who may develop or have developed (atopic) hypersensitivity against inhalable foreign substances. Workers who have been sensitized to ethylenediamine, may have an increased risk of being sensitized to piperazine.
Finally, persons with renal insufficiency or a history of CNS diseases may run a higher risk, although the reported, transient neurological disorders in these patients are due to oral, therapeutic doses which are above occupational levels.

8.2 ASSESSMENT OF HUMAN HEALTH RISKS

Although most studies concerning the carcinogenicity of piperazine and its nitroso derivatives do not meet generally accepted criteria, they indicate that piperazine is not likely to be carcinogenic. It is not mutagenic either. In animals, it can be transformed to mononitroso and dinitroso piperazine. Simultaneous administration of piperazine and nitrite increases the incidence of tumours in mice. N-mononitrosopiperazine is carcinogenic to animals. Its mutagenicity can not be evaluated. N,N'-dinitrosopiperazine can be classified as a genotoxic carcinogen.
There are no data on the reproductive effects.
In man, some minor (at the μg level) amounts of mononitrosopiperazine were detected in the gastric juice and the urine of volunteers and in the urine of workers exposed to piperazine, but no dinitrosopiperazine (detection limit 0.5 μg/l). However, too little is known about the toxicokinetics to draw conclusions with respect to occupational exposure and endogenous formation of the N-nitroso derivatives of piperazine.
Human data on occupational exposure are exclusively coming from studies done at one Swedish factory. A retrospective cohort study revealed an increased risk for malignant lymphomas and myelomas, but no association between this risk, and any specific chemical exposure could be found by an internal case-control study. With respect to airway system diseases, an investigation among workers divided into categories related to exposure duration, demonstrated an increased incidence in asthma and chronic bronchitis in the most exposed group. At a workplace with TWA levels of 0.3 mg/m^3 (0.08 ppm) no new cases of asthma were noted, but exposure to 0.4 mg/m^3 (0.11 ppm) provoked attacks of dyspnea in sensitized subjects and exposure to 0.7 mg/m^3 (0.20 ppm) resulted in a case of astma. The level of 0.3 mg/m^3 (0.08 ppm) included peak levels

of 0.6 mg/m^3 (0.16 ppm) (sampling time 17 min) (see Table 2, page 5). Examination in a small group of workers exposed to average levels below 0.1 mg/m^3 (0.03 ppm) showed no difference in small airway function when compared with unexposed controls.

Therefore, the most critical effect of occupational exposure to piperazine seems to be the induction of asthma and 0.3 mg/m^3 (0.08 ppm) can be regarded as a no-adverse-effect level. The exposure data are too limited to allow an evaluation of the etiologic role of high peak levels. In addition, duration of exposure may be of greater importance than the intensity. In order to arrive to a health-based occupational exposure limit, a safety factor of 3 should be applied for a number of reasons. Exposure to a level of 0.4 mg/m^3 (0.11 ppm), leaving only a small margin with respect to the NAEL of 0.3 mg/m^3 (0.08 ppm), provoked attacks of dyspnea in sensitized subjects. Furthermore, piperazine may induce asthma as well by type-I allergy as by idiosyncrasy. The binding of a small molecule like piperazine to endogenous proteins may play a varying role in individual cases. This individual hypersensitiveness can neither be predicted nor be influenced. Therefore, crossing a certain - unkown - threshold should be avoided, especially with respect to a 40-y working life. In addition, the amount of data is limited, originating from studies in one plant. Finally, care should be used with respect to the possible formation of nitroso derivatives.

Therefore, an occupational standard of 0.1 mg/m^3 (0.03 ppm) is proposed. As the induction of asthma may be due to short-term peak levels, the establishment of a short-term exposure limit is advisable. Therefore, a 15 min value of 0.3 mg/m^3 (0.1 ppm) is proposed. These limits are supposed to be sufficiently low to protect workers against airway system diseases.

Note. Although only minor amounts of N-nitrosopiperazine are likely to be formed in the stomach of piperazine-exposed humans (less than 0.5%) and, hence, the risk of N-nitrosopiperazine-induced tumours is likely to be very small, a risk of cancer for the occupational population can be calculated from the animal experiments by Greenblatt and Mirvish (1973), in which simultaneous administration of piperazine and nitrite increased the incidence of lung adenomas in mice.

At exposure to the proposed exposure limit of 0.1 mg/m^3 (0.03 ppm) for 40 y, this risk will be 2.10^{-3} (see Appendix). Table 7 presents some risks of cancer and corresponding exposure levels.

Table 7. Risk of cancer and corresponding levels of exposure to piperazine

risk	exposure level in mg/m^3
4.10^{-3}	0.16
1.10^{-5}	4.10^{-4}
1.10^{-6}	4.10^{-5}

8.3 RECOMMENDED OCCUPATIONAL EXPOSURE LIMIT

The Dutch Expert Committee for occupational standards recommends a health-based occupational exposure limit for piperazine of 0.1 mg/m^3 (0.03 ppm) as an 8-h TWA concentration.

For short-term exposure a 15-min value of 0.3 mg/m^3 (0.1 ppm) is recommended.

9. RECOMMENDATIONS FOR RESEARCH

The following research is suggested to fill up certain gaps of knowledge:

- elucidation of the toxicokinetics of piperazine to evaluate the risk of the formation of the carcinogenic nitroso derivatives in relation to occupational exposure

- supplementary information on the mutagenicity of piperazine and nitrosopiperazine

10. REFERENCES

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APPENDIX

Calculation of the risk of cancer for the occupational population at exposure to 0.1 mg/m^3 (0.03 ppm) of piperazine for 40 y.

experiment: Greenblatt/Mirvish, 1973 (see section 6.1.4).
Mice are given 0.69 g of piperazine/kg of food together with 1 g of nitrite/l of drinking water. The total daily dose is 2.1 mg and equals the dose taken up by inhalation of 190 mg/m^3 for 8 h (from: dose = concentration x respiratory volume x retention, assuming respiratory volume = 23 ml/min and retention = 1).

number of exposed animals:	40
number of tumour-bearing animals:	32
number of controls:	37
number of tumour-bearing controls:	12
observation time:	38 w
life span exposed animals:	38 w
exposure duration:	25 w.

The risk can be calculated by*:

$$I = I_e \frac{C}{C_e} \times \frac{L_e}{O} \times \frac{L_e}{E_e} \times \frac{E_w}{L_w}$$

I = expected incidence

I_e = tumour incidence in experimental animals = $\frac{32-12}{40} = 0.5$

C = concentration for which incidence is calculated = 0.1 mg/m^3

L_e = life span experimental animals = 130 w

O = observation period = 38 w

E_e = duration of experimental exposure = 25 w

E_w = duration of exposure of worker = 40 y

L_w = life expectancy of worker = 75 y

$$I = 0.5 \times \frac{0.1}{190} \times \frac{130}{38} \times \frac{130}{25} \times \frac{40}{75} = 2.10^{-3}$$

* It is assumed that there are no differences between mouse and human with respect to toxicokinetics, mechanism of tumour induction, target susceptibility, etc.

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Code		*Prijs*
RA 2/79	Koolmonoxyde	f. 23,=
RA 1/80	Fosfine	f. 12,=
RA 2/80	Anorganisch Lood	f. 18,=
RA 3/80	Carcinogene stoffen	f. 16,=
RA 4/80	Tolueen Diisocyanaat	f. 7,=
RA 5/80	Cadmium	f. 16,=
RA 6/80	Chloor	f. 13,=
RA 1/81	n-Heptaan	f. 11,=
RA 2/81	Pentaan	f. 9,=
RA 3/81	1,1,1-Trichloorethaan	f. 18,=
RA 4/81	Formaldehyde	f. 17,=
RA 5/81	Metallisch Kwik	f. 13,=
RA 1/82	Mangaan	f. 17,=
RA 2/82	Monochloorethaan	f. 11,=
RA 3/82	Anorganische Kwikzouten	f. 15,=
RA 4/82	Organische Kwikverbindingen *(Uitsluitend phenylkwik en alkoxyalkylverb.)*	f. 13,=
RA 5/82	Kwikalkylverbindingen - Korte keten *(Uitsluitend methylkwik en ethylkwik)*	f. 18,=
RA 1/83	Methyleenchloride	f. 17,=
RA 2/83	Triethylamine	f. 16,=
RA 3/83	Trichloorethyleen	f. 18,=
RA 1/84	Asbest	f. 28,=
RA 2/84	Anorganische Arseenverbindingen *(Exclusief Arseenwaterstof)*	f. 20,=
RA 4/84	Caprolactam	f. 17,=
RA 1/85	2-Nitropropaan	f. 12,=
RA 2/85	Lachgas	f. 21,=

Code		*Prijs*
RA 3/85	Nikkel en nikkelverbindingen	*f.* 21,=
RA 4/85	Zwaveldioxide	*f.* 17,=
RA 5/85	Stikstofdioxide	*f.* 15,=
RA 6/85	Chroom en chroomverbindingen	*f.* 20,=
RA 1/86	Epichloorhydrine	*f.* 19,=
RA 1/87	1,4-Dioxaan	*f.* 13,=
RA 2/87	Hydrazine, dimethylhydrazine, hydroxyethyl-hydrazine en fenylhydrazine	*f.* 21,=
RA 3/87	Formaldehyde *(Engelse uitgave)*	*f.* 22,=
RA 4/87	4,6-Dinitro-ortho-cresol	*f.* 13,=
RA 5/87	Dibroomethaan	*f.* 13,=
RA 6/87	Aflatoxine B1, B2, G1 en G2	*f.* 16,=
RA 7/87	Chloroform	*f.* 18,=
RA 8/87	1,1-Dichloorethaan	*f.* 9,=
RA 9/87	Trimethylamine	*f.* 13,=
RA 10/87	Vanadium metaal en anorganische verbindingen	*f.* 16,=
RA 11/87	n-Hexaan	*f.* 21,=
RA 12/87	2-Propoxyethanol, 2-Propoxyethylacetate, 2-Isopropoxyethanol *(Engelse uitgave)*	*f.* 9,=
RA 13/87	Acrilaten	*f.* 13,=
RA 14/87	Trichlorofluoromethane *(Engelse uitgave)*	*f.* 16,=
RA 15/87	Fluorcarbons(except FC11) *(Engelse uitgave)*	*f.* 21,=
RA 1/88	Para-Dichloorbenzeen	*f.* 15,=
***		
RA 2/88	Hexachlorobenzene	*f.* 24,=
RA 3/88	Carbonylfluoride and PTFE Pyrolysis products	*f.* 11,=
RA 4/88	Beryllium and Beryllium compounds	*f.* 22,=
RA 1/89	Fluorine, Hydrogenfluorine and Inorganic fluorine compounds	*f.* 22,=
RA 2/89	Aniline	*f.* 17,=

Code		*Prijs*
RA 3/89	Phtalic anhydride	*f.* 12,=
RA 4/89	Ethyl Methanesulphonate (EMS) Methyl Methanesulphonate (MMS)	*f.* 22,=
RA 5/89	Benzeen *	*f.* 10,=
RA 6/89	Ethyleenoxide *	*f.* 13,=
RA 7/89	Selenium en verbindingen *	*f.* 18,=
RA 8/89	Styreen *	*f.* 17,=
RA 9/89	Evaluatie van risico op kanker bij beroepshalve blootstelling aan asbest (aanvullend op RA 1/84) *	*f.* 12,=
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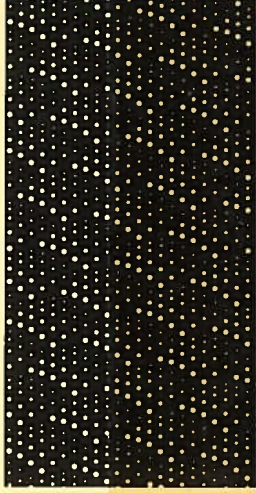

Uitgave van het Directoraat-Generaal van de Arbeid van het
Ministerie van Sociale Zaken en Werkgelegenheid,
Postbus 90804, 2509 LV Den Haag

ISBN 90-5307-243-8
ISSN 0921-9641/2.09.791/9202