Social Europe

Recommendation from the Scientific Committee on Occupational Exposure Limits for morpholine

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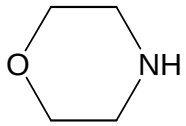
8 hour TWA	:	10 ppm (36 mg/m^3)
STEL (15 mins)	:	20 ppm (72 mg/m^3)
Additional classification	:	-

Substance

Morpholine



Synonyms : 1-oxa-4-azacyclohexane; tetra-2H-1,4-oxazine; diethylene oximide; diethyleneimide oxide

EINECS N° : 2038151

EEC N° : 613-028-00-9

Classification :

CAS N° : 110-91-8

MWt : 87.12

Conversion factor (20°C, 101.3 kPa) : 3.62 mg/m^3 = 1 ppm

1. Occurrence/use

Morpholine is a colourless, oily, hygroscopic, volatile liquid with a characteristic amine ("fishy") smell. The human olfactory threshold for morpholine is 0.036 mg/m^3. It is completely miscible with water, as well as with many organic solvents, but has limited solubility in alkaline aqueous solutions. Morpholine is a strong base, the 0.01% (w/w) mixtures having a pH of 9.4, and the 10% (w/w) mixtures having a pH of 11.2. It has a MPt of –3.1 °C (-3.1 to –5), a BPt of 128.9 °C (128-130) and a vapour pressure of 1.1 kPa at 20°C. The saturated vapour concentration is 38,000 mg/m^3 (20°C).

N-nitrosomorpholine (NMOR) can be formed by reaction of aqueous solutions of nitrite with morpholine or be reaction of gaseous nitrogen oxides in aqueous solutions of morpholine.

Morpholine is an extremely versatile chemical. It is most used as a chemical intermediate in the rubber industry, in corrosion control, and in the synthesis of a large number of drugs, crop protection agents, dyes and optical brighteners. It is also a solvent for a large variety of organic materials, including resins, dyes and waxes.

2. Health Significance

The irritating and corrosive properties of morpholine are due to its basicity. The mechanism of action of its systemic effects is not known.

Morpholine can undergo a variety of reactions. It behaves chemically as a secondary amine. Under environmental and physiological conditions, the proven animal carcinogen *N*-nitrosomorpholine (NMOR) is formed by reaction of solutions of nitrite or gaseous nitrogen oxides with diluted solutions of morpholine. Nitrogen oxide (NO) levels may be of importance in nitrosation. The conditions of nitrosation, in particular pH, play a significant role. Challis and Kyrtopoulos (1977) reported that nitrosamines could also be formed rapidly from nitrogen dioxide and amines in neutral, aqueous media. *In vitro* nitrosation of morpholine has been reported. NMOR was formed when morpholine was added to human saliva (Tannenbaum *et al*., 1978). Additionally, a new type of metabolite, *N*-cyanomorpholine, was identified when morpholine was incubated *in vitro* with whole human saliva (Wishnok and Tannenbaum, 1976).

Van Stee *et al*. (1995) exposed CD-1 mice to $^{15}NO_2$ (20 ppm) for 6 h/day for 4 days and for 2h on day 5, and to 1g morpholine/kg body weight by gavage daily for five consecutive days. *N*-nitrosomorpholine (NMOR) was found in intact mice, stomach, skin with hair, and remains. GC-MS analysis served to distinguish between the NMOR of $^{15}NO_2$ origin and that of other origin. 98.4% of NMOR in the whole mouse homogenate was identified as ^{15}NMOR, 1.6% was derived from $^{14}NO_2$ of other origin. In the stomach, 73% of NMOR was identified as ^{14}NMOR, and 17.5% as ^{15}NMOR. It was concluded that direct nitrosation of morpholine is less important than indirect nitrosation by the formation of cholesteryl nitrite. These findings point to endogenous nitrosamine formation as an ongoing mammalian process.

After oral and parenteral administration or after inhalation exposure, morpholine is well absorbed and is distributed in all tissues and body fluids. Morpholine is eliminated mainly in a non-metabolised form in the urine of the rat, mouse, hamster and rabbit (Griffiths, 1968; Tanaka *et al*., 1978; Van Stee *et al*., 1981; Sohn *et al*., 1982). However, Sohn *et al.* (1982) reported that morpholine is metabolised by *N*-methylation followed by *N*-oxidation in the guinea-pig. Elimination studies have been carried out by administering morpholine–HCl

orally and intravenously. In all cases, over 85% of the dose was excreted in urine within 24 h. A further portion, up to 5%, was excreted during the next three days. The urinary excretion rate was doubled when the pH of the urine was lowered by administration of ammonium chloride in drinking water prior to injection of [^{14}C]-morpholine (Van Stee *et al*., 1981). Elimination of ^{14}C from labelled morpholine (intraperitoneal injection) through expired air is minimal (Van Stee *et al*., 1981).

The acute toxicity of morpholine in rats is moderate (LD50: 1000 – 1900 mg/kg, oral administration; LC50: 7800 mg/m^3 (no details given); 43400 mg/m^3 (8h)). The causes of death or symptoms were diarrhoea, spasms, gastrointestinal haemorrhage, dyspnoea and haemorrhage of the nose, mouth, eyes and lung. Undiluted morpholine applied for 5-15 min to rabbit skin led to severe necrosis (BASF, 1967). Wang and Suskind (1988) observed no dermal irritation in a guinea-pig patch-test (solution of 10% morpholine in Vaseline, application of 0.1g, 1, 24 and 48 h). No data are available on long-term dermal exposure to morpholine.

Rats and guinea-pigs were fed morpholine at concentrations of 160 – 180 mg/kg and 90 – 450 mg/kg body weight, respectively, by gavage for 30 days (Shea, 1939). At concentrations of half of the LD50 (rats: 800 mg/kg body weight per day, guinea-pigs: 450 mg/kg body weight per day), nearly all the animals died within 30 days, the principal symptoms being severe damage to the secreting tubules of the kidney, fatty degeneration of the liver and necrosis of the stomach glandular epithelium. Fatty degeneration (lipidosis) of the liver in rats was noted after feeding morpholine (500 mg/kg) daily for 56 days (Sander and Bürkle, 1969). A 13-week toxicity study was carried out in B6C3F$_1$ mice by feeding morpholine as the fatty acid salt (morpholine oleic acid salt, MOAS), at dosage levels of 0%, 0.15%, 0.3%, 0.6%, 1.25% and 2.5% MOAS in the drinking-water (Shibita *et al*., 1987a). At the highest MOAS level, body weight gains were slightly reduced. Swelling of the proximal tubules was seen, no other alterations were observed in the organs of either sex. Urine analysis showed increases in both specific gravity and plasma urea nitrogen in some dosage groups, suggesting a possible malfunction of the kidney.

After repeated exposure to morpholine by inhalation at 65200 mg/m^3 (34h over 5 days), lung haemorrhage, severe damage to the secreting tubules of the kidney and fatty degeneration of the liver were observed in rats that died after 5 days (Shea, 1939). Rats inhaling morpholine at 3620 mg/m^3 and 18100 mg/m^3 for 9 days, 6h per day, died within the exposure period (Hazleton, 1981). At lower concentrations (1810 mg/m^3, 6h/9 days), weight loss and irritation to the nose and eyes, as well as two deaths were reported. No deaths were seen in rats after inhalation of 360 mg/m^3 morpholine, but red stains around nose and mouth, and weight loss in females were observed. The report concluded that the maximal tolerated dose for rats is about or just below 300 mg/m^3. An increased thyroid activity, shown as increased uptake of injected ^{131}I, was observed in male rats after exposure to 80 mg/m^3 morpholine, 4h/day for 4 days (Grodeckaja and Karamzina, 1973). An increase in lung weight, residual volume and total lung capacity was reported after exposure to morpholine (7200 mg/m^3, 4h/day for 4 days or 1630 mg/m^3 for 30 days) (Takezawa and Lam, 1979).

The induction of lysosomal enzymes (α-mannosidase and acid phosphatase) in lung alveolar macrophages was seen in rabbits exposed by inhalation of morpholine (905 mg/m^3, 6h/day, 5 days/week for a total of 33 days exposure) (Tombropoulos *et al*., 1983). The induction was also observed when macrophages were cultured in the presence of morpholine.

Conaway *et al*. (1984b) exposed groups of 40 rats to morpholine (0, 90, 360 and 900 mg/m^3) for 7 and 13 weeks. Slight, rapid breathing was occasionally noted in all groups except the controls. Lesions of the nasal septum, anterior cavities, nasoturbinates and maxilloturbinates were observed in the 360 and 900 mg/m^3 groups but not in the lowest exposure groups. Increased nervous system activity and increases in haemoglobin and peripheral red blood cell counts in rats and guinea-pigs exposed to morpholine (8 and 70 mg/m^3 for 4 months) were reported by Migukina (1973). An increase in chromosomal aberrations in bone marrow cells was also noted. This study was deficient with respect to the description of study methods.

Harbison *et al*. (1989) carried out an extensive long-term exposure inhalation study. Groups of 70 rats of each sex were exposed to morpholine (0, 36, 181, 543 mg/m^3, 6h/day, 5 days per week) for 104 weeks, with an interim sacrifice at week 53 (10 rats of each sex). Levels of nitrates and nitrites in the drinking water were reported to be <0.1 mg/l and 0.01 mg/l, respectively. Survival, body weight gain, organ weights, haematology and clinical chemistry data were normal in exposed groups, compared to the controls. In-life clinical examinations revealed increased incidences of irritation around the eyes and nose at the highest concentration (543 mg/m^3). Histomorphological changes are reported as inflammation of the cornea, inflammation and squamous metaplasia of the turbinate epithelium and necrosis of the turbinate bones in the nasal cavity of both male (6/60) and female rats (2/60) at 181 mg/m^3 morpholine. At a concentration of 36 mg/m^3 morpholine, there were no inflammatory signs reported.

Some mutagenicity data are available for morpholine. Negative results were reported in the Ames test up to 10,000 μg/plate and in the DNA-repair test (Texaco, 1979a; Haworth *et al*., 1983; Texaco, 1979b; Conaway *et al*., 1984a). Morpholine induced small increases in the frequency of SCEs in CHO cells (Litton Bionetics, 1980). In the BalbC/3T3 cell transformation assay, results were inconclusive (Texaco, 1979b; Litton Bionetics, 1979a,b; Litton Bionetics, 1982; Conaway *et al*., 1982). No chromosomal aberrations, micronucleus formation, or 8-azaguanine- or ouabain-resistant mutations were found in primary cells of hamsters exposed *in utero* (Inui *et al*., 1979). An increase in chromosomal aberrations in bone marrow of rats and guinea pigs was reported, but there were deficiencies in the study design (Migukina, 1973).

Greenblatt *et al*. (1971) observed no increase in lung tumour rates in mice orally treated with 900 mg/kg per day for 28 weeks. After a further 12 weeks of observation, the surviving animals were sacrificed. It should be noted that the duration of exposure in this study was shorter than normally used in a well-designed long-term carcinogenicity study.

Multi-generation oral studies were performed on Sprague-Dawley rats fed morpholine (5, 50 or 1000 mg/kg diet), together with various dietary concentrations of sodium nitrite (0, 5, 50 or 100 mg/kg diet) (Newberne and Shank, 1973; Shank and Newberne, 1976). From the day of conception, the pregnant animals were given 0 to 1000 mg morpholine/kg feed. The F_1 and F_2 generations were fed likewise for the length of the experiment. The estimated dosage for young animals was 10 mg/day and for mature animals, 20 mg/day. The average life-span was 117 weeks for the treated animals and 109 weeks for the controls F_1 and F_2 generations were studied, the survivors being sacrificed in the 125th week. Three liver cell carcinomas, two lung sarcomas and one other, and two malignant gliomas were found in the group of 104 rats (F_1 and F_2) treated with morpholine alone. No tumours were seen in the untreated control group.

In a similar study using Syrian golden hamsters, only the F_1 generation was studied, the survivors being sacrificed in the 110th week (Shank and Newberne, 1976). With morpholine

alone (1000mg per kg feed), no liver tumours were found but the number in the group (22) was small.

Morpholine oleic acid salt (MOAS), at dosage levels of 0%, 0.25% or 1.0%, was added to the drinking water of B6C3F_1 mice for 96 weeks, and this was followed by normal tap water for a further 8 weeks (Shibata *et al*. 1987b). Only the incidence of hyperplasia in forestomach epithelium in the males of the 1% MOAS group was statistically higher than in the controls; otherwise, no significant increases in the incidence of non-neoplastic and neoplastic lesions were found. Harbison *et al*. (1989), as noted above, reported no significant increase in the incidence of tumours in rats of either sex after a long-term inhalation study over 2 years.

N-nitrosomorpholine (NMOR) is mutagenic to many bacterial test systems and induces unscheduled DNA synthesis (UDS) in rat hepatocytes (BUA, 1991; Williams *et al*., 1989). NMOR has been shown to be carcinogenic in mice, rats, hamster and various fish. Benign and malignant tumours of the liver and lung in mice, of the liver, kidney and blood vessels in rats, and of the liver in hamsters, have been reported following oral administration of NMOR (IARC, 1978).

Sensitisation studies (modified Buehler) on guinea-pig skin using 2% morpholine in petrolatum gave negative results (Wang and Suskind, 1988).

The phenomenon known as blue vision, grey vision or haloes, "glaucopsia" is a well-documented effect on eyes of workers exposed to amines, including morpholine and its derivatives, particularly in the foam plastic industry (Mastromatteo, 1965; Jones and Kipling, 1972). The disturbances lasted for 4-6 h after leaving work. In a minority of the workers examined, mild conjunctival infection was observed; no corneal oedema or alteration in visual acuity was observed by inspection or by ophthalmoscopy. The atmospheric concentrations of morpholine and other similar compounds were not reported. Corneal oedema with "hazy vision" and halo phenomena around lights have also been described (Grant, 1986).

No epidemiological studies of morpholine have been reported and no data were available from human studies on the carcinogenicity of morpholine. The overall IARC evaluation was that morpholine was not classifiable for its carcinogenicity to humans (IARC, 1989).

No adequate studies on reproductive toxicity, embryotoxicity or teratogenicity have been reported.

Recommendation

The 2-year study of Harbison *et al*. (1989) and its 13-week dose-finding study (Conaway *et al*., 1984) were considered to be the best available bases for setting occupational exposure limits for morpholine. Overall assessment of these studies indicates a NOAEL of 36 mg/m^3 morpholine. The NOAEL is based on an extensive long-term exposure inhalation study in rats.. The SCOEL considers the use of an uncertainty factor of 1 justifiable because of the low incidence of histopathological nasal changes seen at 181 mg/m^3 in the long-term study and at 360 mg/m^3 in the 13-week study, and the absence of histopathological nasal changes and the very mild nature of the effects (occasional rapid breathing) at 90 mg/m^3 in the 13-week study. Although the negative results obtained in *in vivo* mutagenicity and carcinogenicity studies indicate that these endpoints are not of concern for exposure to morpholine, the possibility of nitrosation, forming *N*-nitrosomorpholine, cannot be excluded from the information available. The

recommended 8-hour TWA for morpholine is 36 mg/m^3 (10 ppm). A STEL (15 mins) of 72 mg/m^3 (20 ppm) was proposed to limit peaks in exposure which could result in irritation.

Observations on skin uptake are mostly due to the corrosive properties of morpholine, so no "skin" notation was considered necessary.

At the levels recommended, no measurement difficulties are foreseen.

Because of the potential for nitrosation of morpholine to form nitrosamines under some workplace conditions, monitoring of ambient nitrous oxides is highly recommended.

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