The MAK Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Morpholine

MAK Value Documentation, addendum – Translation of the German version from 2022

A. Hartwig[1,*] MAK Commission[2,*]

1 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

2 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords

morpholine; irritation; upper respiratory tract; focal necrosis turbinates; MAK value; maximum workplace concentration; momentary value; $BMDL_{05}$; developmental toxicity

Abstract

The German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) has re-evaluated the occupational exposure limit value (maximum concentration at the workplace, MAK value) and the pregnancy risk group of morpholine [110-91-8] considering also irritating and sensitizing effects. Relevant studies were identified from a literature search and also unpublished study reports were used. The critical effect of morpholine is the local effect on the upper respiratory tract. There are no human data to derive a MAK value. In a 2-year inhalation study with rats already reported in the evaluation 1996, the lowest concentration of 10 ml/m^3 is a NOAEC. As focal necrosis of turbinates at the next higher concentration of 50 ml/m^3 was not pronounced and had a low incidence, a benchmark dose (BMD) calculation based on focal necrosis of turbinates in male rats was performed, resulting in a $BMDL_{05}$ of 19.78 ml/m^3. Based on this $BMDL_{05}$ a MAK value of 5 ml/m^3 is established. Since a local effect is critical, Peak Limitation Category I is retained. As data on humans are not available and the calculated NAEC is close to the MAK value, an excursion factor of 1 and by analogy with other amines with a MAK value of 5 ml/m^3, a momentary value of 10 ml morpholine/m^3 is set. In 1996, no specific data on reproduction or developmental toxicity had been available. In a study conducted in the meantime, the NOAEL for developmental and maternal toxicity in rats was 52.9 mg/kg body weight and day, the LOAEL was 176 mg/kg body weight and day. In a one-generation toxicity study in rats, a NOAEL of 423 mg/kg body weight and day was obtained for perinatal toxicity whereas at this dose maternal toxicity occurred. In rabbits, the NOAEL for developmental and maternal toxicity is 49.7 mg/kg body weight and day, the LOAEL is 148 mg/kg body weight and day. The studies were conducted with the hydrochloride in order to be able to administer a maximum amount of morpholine by gavage to the animals. However, the oral hydrochloride dose does not correlate with real workplace exposure to morpholine vapours. The corresponding morpholine concentrations would lead to significant irritant effects, at least in the range of LOAECs. Therefore, such a high dose could not be tested via inhalation. After toxicokinetic scaling, the margins between the effect doses and the MAK value of 5 ml/m^3 are evaluated as sufficient and morpholine is assigned to Pregnancy Risk Group C. There are no indications of a sensitizing potential of morpholine in humans or animals. Studies investigating respiratory sensitization are not available. According to skin absorption models, percutaneous absorption is not expected to contribute significantly to systemic toxicity.

Citation Note:
Hartwig A, MAK Commission. Morpholine. MAK Value Documentation, addendum – Translation of the German version from 2022. MAK Collect Occup Health Saf. 2024 Jun;9(2):Doc034. https://doi.org/10.34865/mb11091e9_2ad

Manuscript completed:
04 May 2021

Publication date:
28 Jun 2024

MAK value (2021)	**5 ml/m³ (ppm) ≙ 18 mg/m³**
Peak limitation (2021)	**Category I, excursion factor 1**
Momentary value (2021)	**10 ml/m³ (ppm) ≙ 36 mg/m³**
Absorption through the skin	–
Sensitization	–
Carcinogenicity	–
Prenatal toxicity (2021)	**Pregnancy Risk Group C**
Germ cell mutagenicity	–
BAT value	–
CAS number	110-91-8
Molar mass	87.12 g/mol
1 ml/m³ (ppm) ≙ 3.615 mg/m³	**1 mg/m³ ≙ 0.277 ml/m³ (ppm)**

Note: Reaction with nitrosating agents can result in the formation of carcinogenic *N*-nitrosomorpholine, see List of MAK and BAT Values Section III "Amines which form carcinogenic nitrosamines on nitrosation" (DFG 2021).

The first documentation for morpholine dates from 1974 (Henschler 1974, available in German only). In addition, supplements are available for all end points (Greim 1996, available in German only) and for peak limitation (Greim 2000, available in German only). Cited unpublished toxicological studies from companies have been made available to the Commission.

The most sensitive end point is the local effect of morpholine on the upper respiratory tract. To derive MAK values for substances that cause local effects on the upper respiratory tract or the eyes, the Commission has been using a method (Brüning et al. 2014) since 2014 that compares sensory irritation in humans and histopathological changes in rodents.

Effects in Humans

There are no new data available.

As described in the documentation of 1974 (Henschler 1974), nasal mucosal irritation was observed in humans after exposure to 12 000 ml morpholine/m³ for 1 minute, coughing occurred after 1 to 1.5 minutes.

In an unpublished study cited in a secondary source, testing of black or blue mascara containing 1.0% morpholine according to the Shelanski-Jordan test scheme did not provide any evidence of a contact-sensitizing effect. The study was performed with 320 women as follows: induction by occlusive 24-hour patch testing on 3 days per week for a total of 10 applications; 1st challenge with occlusive 48-hour patches after an interval of 10–14 days, reading after the removal of the patches; 2nd challenge as for the 1st challenge 7–10 days after the 1st challenge, reading after the removal of the patches and 24 hours later. Six of the 320 subjects produced irritant effects that were regarded by the authors as nonspecific or a result of the occlusive patch testing procedure (CIREP 1989). Due to the incomplete documentation and the lack of information on the other components of the mascaras as well as the low morpholine concentration of 1.0%, the reactions of the 6 test subjects cannot be attributed to irritant or sensitizing effects caused by the morpholine contained in these cosmetics. This study is therefore not included in the evaluation.

Animal Experiments

There are no new data available.

Repeated exposure

In the supplement from 1996 (Greim 1996), the following inhalation studies are described (see Table 1). The 7 and 13-week studies in Sprague Dawley rats yielded a NOAEC (no observed adverse effect concentration) of 25 ml morpholine/m³, as focal necrosis with cell debris occurred in the nasal cavity of 2 females at 100 ml/m³ (Conaway et al. 1984). In a 2-year study in Sprague Dawley rats, no systemic effects were found at 50 ml/m³, but irritation with findings in the nasal turbinates occurred, which was severe at the highest concentration tested of 150 ml/m³. Focal necrosis was observed in the nasal mucosa of 8 of the 120 animals exposed to 50 ml/m³ (6 ♂, 2 ♀); in the control animals, this occurred in 1 female animal that died prematurely. Squamous metaplasia was observed in 7 exposed male animals and 3 control animals (not statistically significant), and neutrophil infiltration was found in 10 exposed female animals compared with in 6 control animals with the same findings. The NOAEC was 10 ml/m³ (Harbison et al. 1989). The margin between the low and the middle concentration with a factor of 5 was relatively large, and the findings at 50 ml/m³ occurred only in a few animals with a low, sometimes not statistically significant, incidence. It can therefore be assumed that the concentration of 50 ml/m³ is close to the beginning of the concentration–effect curve, and the NAEC (no adverse effect concentration) is probably well above 10 ml/m³.

A benchmark calculation based on the incidences of focal necrosis in the males in the 2-year study (Harbison et al. 1989) using the "bayesian model-averaging function" of the US EPA BMDS software (US EPA 2020), yielded a $BMDL_{05}$ (lower confidence limit of the benchmark concentration for a 5% increase in incidence) of 19.78 ml/m³. Performing this calculation for both sexes together yielded a $BMDL_{05}$ of 34.65 ml/m³ (Table 3). This concentration represents the calculated NAEC.

Tab. 1 Findings after repeated inhalation of morpholine in rats

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 10 ♂, 10 ♀	7 weeks, 0, 25, 100, 250 ml/m³, 6 hours/day, 5 days/week, purity 99%	**100 ml/m³**: **NOAEC**; **250 ml/m³**: nose: focal ulceration and necrosis with cell debris in respiratory epithelium of septum, nasal turbinates and maxilloturbinates, necrotic and focal squamous metaplasia in 6/10 ♂ and 1/10 ♀, metaplasia without necrosis in 1/10 ♀, necrosis and inflammation in anterior nasal cavity in 2/10 ♂, eye: secretion from Harderian glands in 5/20 ↑, lungs: perivascular and peribronchial lymphoid hyperplasia and focal pneumonia in all animals, in some cases congestion and focal accumulation of alveolar macrophages	Conaway et al. 1984
rat, Sprague Dawley, 10 ♂, 10 ♀	13 weeks, 0, 25, 100, 250 ml/m³, 6 hours/day, 5 days/week, purity 99%	**25 ml/m³ and above**: intermittent rapid breathing, red, white and dark spots on pleural surface of lungs, **NOAEC**; **100 ml/m³**: focal necrosis with cell debris in nasal cavity in 0/10 ♂ and 2/10 ♀; **250 ml/m³**: in the first week of exposure, sporadic reddish discharge or crust around eyes and nose, nose: focal necrosis of the squamous epithelium of the anterior nasal cavity in 8/10 ♂ and 3/10 ♀, necrosis of nasal septal epithelium in 5/10 ♂ and 5/10 ♀, focal necrosis of the respiratory epithelium lining the maxilloturbinates in 10/10 ♂ and 8/10 ♀ with cell debris in 7/10 ♂ and 8/10 ♀ and focal squamous metaplasia in 9/10 ♂ and 9/10 ♀, lung: pneumonia, lesion number and severity ↑ compared with 7-weeks result	Conaway et al. 1984
rat, Sprague Dawley, 70 ♂, 70 ♀, interim examination week 53 in 10 animals per group	104 weeks, 0, 10, 50, 150 ml/m³, 6 hours/day, 5 days/week, purity 99.16%	**10 ml/m³**: **NOAEC**; **50 ml/m³**: incipient irritation in nasal turbinates: infiltration of neutrophils, focal squamous metaplasia, necrosis (for incidences, see Table 2); **150 ml/m³**: irritant effects: localized wounds and crusting of blood around the eyes, nostrils, face, body and skin, with necrosis, chromodacryorrhea, the infiltration of neutrophils, and squamous metaplasia and necrosis ↑ (for incidences, see Table 2)	Harbison et al. 1989

NOAEC: no observed adverse effect concentration

Tab. 2 Incidence of findings in the nasal cavity of rats after exposure to morpholine for 2 years (Harbison et al. 1989)

	Concentration [ml/m³]			
	0	**10**	**50**	**150**
number of preterm deaths[a)]				
(preterm deaths) ♂	19	16	27	25
(preterm deaths) ♀	25	33	28	25
number at end of study				
(week 105) ♂	40	44	33	32
(week 105) ♀	35	27	32	35
neutrophilic infiltrate				
(preterm deaths) ♂	2/19	3/16	3/27	11/25
(preterm deaths) ♀	4/25	5/33	8/28	18/25
(week 105) ♂	3/40	2/44	3/33	16/32
(week 105) ♀	2/35	3/27	2/32	23/35
focal squamous metaplasia				
(preterm deaths) ♂	1/19	0/16	4/27	18/25
(preterm deaths) ♀	2/25	0/33	0/28	15/25
(week 105) ♂	2/40	1/44	3/33	28/32
(week 105) ♀	1/35	0/27	2/32	27/35
necrosis in paranasal sinuses				
(preterm deaths) ♂	0/19	0/16	3/27	8/25
(preterm deaths) ♀	1/25	0/33	2/28	17/25
(week 105) ♂	0/40	0/44	3/33	12/32
(week 105) ♀	0/35	0/27	0/32	18/35

[a)] moribund and dead animals

Tab. 3 Results of benchmark calculation for the incidences of focal necrosis in male and female rats in the 2-year study (Harbison et al. 1989).

Benchmark dose				
BMD	49.44877385			
BMDL	34.64901075			
BMDU	64.191176			
Model averaging - individual models				
Model	**Posterior probability**	**BMD**	**BMDL**	**BMDU**
Dichotomous Hill	0.151933195	44.62621808	33.0902763	57.21311
Gamma	0.065500074	40.45526236	29.5444563	51.91852
Logistic	0.02077114	59.06313658	49.2581561	69.93533
Log-Logistic	0.159018301	44.98900026	33.0777973	58.10891
Log-Probit	0.082365961	46.95630223	35.9532461	58.46766
Model averaging - individual models				
Model	**Posterior probability**	**BMD**	**BMDL**	**BMDU**
Multistage	0.000109746	25.48044026	18.6802559	32.79422
Probit	0.39050716	55.99147975	46.3205919	66.6695
Quantal Linear	3.4174E-05	16.50523022	13.4767424	20.52242

BMD: benchmark dose; BMDL: lower confidence limit of the BMD; BMDU: upper confidence limit of the BMD

Local effects on skin and mucous membranes

Skin

Application of 0.5 ml undiluted morpholine to the dorsal skin of Vienna White or New Zealand White rabbits caused severe inflammation and tissue necrosis after less than 1 hour (ECHA 2020).

Eyes

In a test carried out according to OECD Test Guideline 405 in the rabbit eye, 0.1 ml of undiluted morpholine caused necrosis in and around the eyes after application for 30 seconds; morpholine was corrosive after an application period of 24 hours (ECHA 2020).

Sensitization

Sensitizing effects on the skin

In a modified Buehler test already described in the supplement from 1996 (Greim 1996), 10 male Hartley guinea pigs were induced with 5% morpholine in petrolatum. For this purpose, 0.1 g of morpholine was applied occlusively to the shaved neck for a period of 24 hours, 3 times per week, for a total of 2 weeks. In deviation from OECD Test Guideline 406, only 10 instead of 20 animals were used and induced by occlusive application 6 times instead of 3 times for 24 hours instead of 6 hours. The 24-hour occlusive challenge with 0.1%, 0.5% or 2% morpholine in petrolatum was carried out 2 weeks after the last induction treatment. None of the animals in the treated or control groups produced a response. Of the 10 animals in each group sensitized to 4,4′-dithiodimorpholine or morpholinyl mercaptobenzothiazole and subsequently exposed to up to 2% morpholine, 3 animals and 1 animal, respectively, reacted (Wang and Suskind 1988). These reactions are most likely not cross-reactions, but nonspecific reactions in the animals that were markedly sensitized to the other 2 substances.

Sensitizing effects on the airways

There are no data available.

In vitro data and structure–activity relationships

When incubated with the amino acids lysine, cysteine or glycine for up to 168 hours at room temperature, morpholine, unlike 2-mercaptobenzothiazole (which was tested in parallel), did not react with any of the amino acids (Wang and Tabor 1988).

The structure-activity tool DEREK (Deductive Estimation of Risk from Existing Knowledge) did not predict a skin sensitizing effect for morpholine (NIOSH 2017).

Reproductive and developmental toxicity

Fertility

In an extended one-generation study according to OECD Test Guideline 443 (see Table 4; BASF SE 2021), male and female Wistar rats were given daily gavage doses of morpholine hydrochloride (purity 99.4%) in water at doses of 0, 60, 200 or 600 mg/kg body weight and day (0, 42.3, 141 and 423 mg morpholine/kg body weight and day). The hydrochloride was used to achieve a higher level of systemic exposure than possible with the irritant morpholine. Control animals received water daily by gavage. F0 animals were treated for at least 10 weeks prior to mating and then mated within the respective dose group. The F1 animals were assigned to 2 different cohorts (1A and 1B).

No substance-related mortality or adverse clinical signs indicative of systemic toxicity occurred. Transient salivation for a short period of time after gavage administration was observed in almost all F0 and F1 animals in the middle and high dose groups. As a reason for this, it was suggested that the morpholine hydrochloride solution had a bad taste or caused a local effect in the upper digestive tract. The food and water consumption of the F0 and F1 animals in the middle and high dose groups were increased. The body weight gains of the animals in the high dose group were increased during some periods of the study. Female animals were affected more than males. These findings were not regarded as adverse effects or as signs of systemic toxicity.

The haemoglobin and haematocrit values were decreased in the parental animals of the high dose group (dose equivalent to 423 mg morpholine/kg body weight and day) compared with the values in the control group, indicating mild anaemia. In addition, increased urea levels in both sexes, decreased total protein and albumin levels in the male F0 animals, and increased triglyceride levels in the female F0 animals were signs of altered protein and lipid metabolism. The increased potassium and inorganic phosphate and decreased chloride concentrations in both sexes of the F0 and F1 generations, and the decreased sodium values in the female animals of the F0 generation and in both sexes of the F1 generation indicated metabolic acidosis. Haemoglobin was found in the urine of the male animals of the F0 and F1 generations of the high dose group, the cause of which could not be determined. In the high dose group, a statistically significant increase in the mean absolute and relative liver and kidney weights was observed in the F0 generation and in the terminal body weights in female F0 animals. These findings were considered to be probably substance-related because a dose–response relationship was evident and they occurred also in the F1 generation in cohort 1A; in cohort 1B, increased absolute and relative liver weights and increased terminal body weights were observed only in the females. A slight increase in the mean absolute liver weights was noted in the female F0 animals in the middle dose group and was considered to be probably substance-related as similar changes occurred in the F1 generation in cohort 1B. However, there was no histopathological correlate in the liver and kidneys, and the body weights were within the range of historical control values. Thus, the organ weight changes in the F0 generation were considered substance-related but not adverse. In the F1 generation 1A cohort, the statistically significant increases in the mean absolute and relative liver and kidney weights were considered treatment-related, as were the statistically significant increases in the mean absolute and relative liver weights and the mean absolute kidney weights in cohort 1B. The statistically significant increase in terminal body weights was a consistent change in all the generations and cohorts studied (F0, F1 with cohorts 1A and 1B) and was considered to be treatment-related but not adverse.

No treatment-related histopathological abnormalities occurred in the F0 and F1 animals (cohorts 1A and 1B, histopathological examination was not performed in cohort 1B). All other findings in the internal organs examined occurred only in single cases in F1 cohort 1A or were equally distributed across the control and treatment groups and were not treatment-related.

Clinical examination did not reveal any evidence that morpholine hydrochloride affected fertility or reproductive performance in the F0 animals up to the highest dose tested.

The results for oestrous cycle, mating behaviour, pregnancy and parturition, lactation and weaning were similar between the rats of all groups and the values were within the range of the historical control values of the test laboratory. This was also the case for the gross and histopathological findings in the female reproductive organs, and their weights, in the F0 and F1 generations. There was no difference in the differential ovarian follicle counts between the high dose group and the control group in the F1A female animals.

In the males of the F0 and F1A generations, a slight but statistically significant increase in abnormal sperms in the cauda epididymis was seen in the high dose group; in the middle dose group, the number of abnormal sperms was increased and sperm motility was decreased in only one F1A animal. Histopathologically, an increase in the incidence and severity of tubular degeneration in the testis and epididymis of male F0 animals was observed in the high dose group; this effect was minimal in the middle dose group. One male F0 animal exhibited multinucleated giant cells in the testis, which indicates that germ cells were no longer supported by the Sertoli cells. In the epididymis of middle and high dose F0 animals, cell debris was increased in incidence and severity in a dose-dependent manner (2/25 minimal and 8/25 minimal to mild, respectively). The findings occurred also in male F1A animals in the middle and high

dose groups, but to a lesser extent (2/20 minimal and 4/20 minimal, respectively). Although there were no effects with regard to fertility in the males, these findings are considered adverse.

No signs of perinatal or postnatal toxicity were observed in the F1 animals up to puberty; histopathology, thyroid hormones, anogenital distance and the presence of nipples/areolae were examined. The timing of vaginal opening and preputial separation were within the normal range of the controls.

In this extended one-generation study, the NOAEL (no observed adverse effect level) for systemic toxicity for the parental animals was 200 mg morpholine hydrochloride/kg body weight and day (141 mg morpholine/kg body weight and day), because at the LOAEL (lowest observed adverse effect level) of 600 mg/kg body weight and day (423 mg morpholine/kg body weight and day) there were clinical pathological findings indicative of mild anaemia, altered protein and lipid metabolism, and metabolic acidosis (BASF SE 2021).

Based on the increased incidence of males with tubular degeneration in the testes and the subsequent decreased sperm count and decreased sperm motility at and above 200 mg morpholine hydrochloride/kg body weight and day, the NOAEL for fertility in the male rats is 60 mg morpholine hydrochloride/kg body weight and day (42.3 mg morpholine/kg body weight and day). The NOAEL for fertility in the female rats is 600 mg/kg body weight and day (423 mg morpholine/kg body weight and day), the highest dose tested. Morpholine hydrochloride did not affect the reproductive ability of affected male rats nor caused effects on female reproductive organs. The NOAEL for perinatal and postnatal toxicity in F1 animals up to puberty is 600 mg morpholine hydrochloride/kg body weight and day (423 mg morpholine/kg body weight and day), the highest dose tested (BASF SE 2021).

Tab. 4 Extended one-generation study with gavage administration of morpholine hydrochloride (BASF SE 2021)

Species, strain, number per group	Exposure	Findings
rat, Wistar, 25 ♀, 25 ♂	0, 60, 200, 600 mg **morpholine hydrochloride**/kg body weight and day (0, 42.3, 141, 423 mg **morpholine**/kg body weight and day), gavage, purity 99.4%, dissolved in water, OECD Test Guideline 443	**42.3 mg morpholine/kg body weight**: **NOAEL for ♂ fertility;** **141 mg morpholine/kg body weight**: **NOAEL for systemic toxicity in parent animals,** F0 animals: salivation, liver weights ↑ (absolute +7%, relative +4%) and kidney weights ↑ (absolute +6%, relative +3%; considered not to be adverse), ♂: multinucleated giant cells ↑ in seminiferous tubules in 1/25 animals, degeneration of seminiferous tubules in 3/25 animals ranging from minimal to mild, cell debris in epididymis minimal in 2/25 animals, F1 animals: cohort 1A: salivation, liver and kidney weights ↑ (considered not to be adverse), ♂: percentage of abnormal sperm ↑ and sperm motility ↓ in 1/20 animals, cell debris in epididymis in 2/20 animals ranging from minimal to mild; **423 mg morpholine/kg body weight and day**: **NOAEL perinatal und postnatal toxicity up to puberty,** **NOAEL for ♀ fertility,** F0 animals: ♀ and ♂: salivation, liver weights ↑ (absolute +24%, relative +18%) and kidney weights ↑ (absolute +17%, relative +11%; considered not to be adverse), urea, potassium, inorganic phosphate ↑, chloride ↓, ♂: cholesterol ↑, percentage of blood in urine ↑, percentage of abnormal sperm ↑, degeneration of seminiferous tubules in 13/25 animals from minimal to severe, cell debris in epididymis in 8/25 animals from minimal to mild, ♀: haemoglobin and haematocrit ↓, triglycerides ↑, total protein, albumin, sodium ↓, F1 animals: cohort 1A: ♀ and ♂: salivation, liver and kidney weights ↑ (considered not to be adverse), potassium and inorganic phosphate ↑, sodium ↓, ♂: percentage of blood in urine ↑, urine volume ↓, degeneration of seminiferous tubules in 4/20 animals from minimal to mild, cell debris in epididymis in 4/20 animals minimal, no effects: F0 animals, F1 animals: oestrous cycle, mating behaviour, pregnancy and parturition, lactation and weaning, weights of female reproductive organs, differential follicle count in ovary in F1A females, offspring: litter parameters, perinatal toxicity, organs (weight and histopathology), sexual organs and sexual maturation (preputial separation and vaginal opening)

NOAEL: no observed adverse effect level

Developmental toxicity

Prenatal studies

The results of 2 prenatal developmental toxicity studies according to OECD Test Guideline 414 are shown in Table 5.

In a developmental toxicity study in pregnant Crl:WI[Han] Wistar rats (BASF SE 2009), morpholine hydrochloride (purity 97%) was administered at doses of 0, 75, 250 or 750 mg/kg body weight and day (morpholine doses of 0, 52.9, 176 and 529 mg/kg body weight and day) by gavage in water from gestation days 6 to 19. The control group was given water by gavage. All rats had implantation sites. Doses of 250 and 750 mg/kg body weight and day caused mild regenerative anaemia and an increase in absolute liver weights in the dams. In addition, at 750 mg/kg body weight and day, the mean food consumption was reduced by 13% from gestation days 6 to 10 and body weight gains were reduced by 41% from gestation days 6 to 8. At this dose, also blood urea levels were increased, and there were indications of the impairment of liver cells and liver cell metabolism (increased alanine aminotransferase activity, increased bilirubin and cholesterol concentrations). Gavage doses of morpholine hydrochloride had no effect on gestational and litter parameters (conception rate, number of corpora lutea, total number of implantations, number of resorptions, number of live foetuses, sex ratio, preimplantation and postimplantation losses). There were also no effects on foetal body weights. Morpholine hydrochloride did not cause malformations, or external or soft tissue variations and did not affect the overall incidence of skeletal variations. Foetal findings were primarily slight increases in delayed ossifications in the middle and high dose groups.

The increase in the incidence of the findings "delayed ossification of the parietal bone with unchanged cartilage" and "wavy ribs" was statistically significant in the middle and high dose groups; although there was no dose–response relationship, the incidences were consistently outside the range of historical control data. In the low dose group, the former finding was likewise significantly increased, but was within the historical control range. The authors considered these delays in ossification to be transient phenomena that are completely reversible in the postnatal period. Therefore, they considered these findings to be secondary to maternal toxicity and not relevant for developmental toxicity. The incidences of 3 skeletal variations, namely supraoccipital holes, incomplete ossification of the skull and non-ossified sternum (all with intact underlying cartilage, segment not specified) were increased with statistical significance. Of these, only the increased incidence of incomplete ossification of the skull in the high dose group slightly exceeded the historical control range. Again, these ossification delays were considered secondary to maternal toxicity and not relevant for developmental toxicity. Therefore, the authors established the NOAEL for developmental toxicity in rats to be 750 mg/kg body weight and day (529 mg morpholine/kg body weight and day). Statistically significant haematological changes occurring in dams at 250 mg morpholine hydrochloride/kg body weight and day (176 mg morpholine/kg body weight and day) and above (BASF SE 2009) were regarded as indications of maternal toxicity. However, the Commission considers the delays in ossification at and above 250 mg morpholine hydrochloride/kg body weight and day to be adverse and has set the NOAEL for developmental toxicity and maternal toxicity in rats at 75 mg morpholine hydrochloride/kg body weight and day. This is equivalent to 52.9 mg morpholine/kg body weight and day.

In a developmental toxicity study, groups of 25 pregnant New Zealand White rabbits (BASF SE 2020) were given gavage doses of morpholine hydrochloride (purity 99.4%) of 0, 20, 70 or 210 mg/kg body weight and day (morpholine doses of 0, 14.1, 49.4 and 148 mg/kg body weight and day) in water from gestation days 6 to 28. The control group received drinking water by gavage. Per group, 21 to 24 dams had implantation sites. On gestation day 29, 414 blood samples were collected from the ear veins of all dams in addition to the OECD test guideline requirements. In the high dose group of 210 mg/kg body weight and day, the dams exhibited hypochromic microcytic anaemia in the form of decreases in the haemoglobin and haematocrit levels and the mean corpuscular haemoglobin content and mean corpuscular volume. In addition, the mean food consumption was reduced with statistical significance by up to 34% from gestation days 6 to 13 and by 14% from gestation days 6 to 28 compared with the control values. There was a decrease in body weight gains between gestation days 6 and 11 and between gestation days 6 and 28 (59% less than the control value), which was statistically significant. There was a statistically significant decrease in body weights on gestation day 6 compared with the initial weights (–28.5 g; control group: +62.8 g).

Morpholine hydrochloride had no effect on gestational and litter parameters (conception rate, number of corpora lutea, total number of implantations, number of resorptions, number of live foetuses, sex ratio, preimplantation and postimplantation losses) up to the highest dose. There were also no effects on foetal body weights. Morpholine hydrochloride doses of 210 mg/kg body weight and day induced soft tissue malformations (atresia of the aortic arch) in 2 foetuses from 2 litters. In addition, the incidences of 3 skeletal variations (misshapen sacral vertebrae; supernumerary 13th rib, cartilage present; unossified talus, cartilage present) were increased with statistical significance. The incidences of soft tissue malformations and skeletal variations at 210 mg morpholine hydrochloride/kg body weight and day (148 mg morpholine/kg body weight and day) were outside the historical control range, which indicates a treatment-related effect. Concurrent maternal toxicity was present in the form of hypochromic microcytic anaemia. The NOAEL for developmental toxicity and maternal toxicity for rabbits is thus 70 mg/kg body weight and day, which corresponds to 49.4 mg morpholine/kg body weight and day (BASF SE 2020).

Generation study

In the extended one-generation study in accordance with OECD Test Guideline 443 described in the Section "Fertility", the NOAEL for systemic toxicity in the parent animals was 200 mg/kg body weight and day (141 mg morpholine/kg body weight and day; see Table 4). At 600 mg/kg body weight and day (423 mg morpholine/kg body weight and day), clinical pathology yielded findings indicating marginal anaemia, altered protein and lipid metabolism, and metabolic acidosis. There were no signs of perinatal or postnatal toxicity in the F1 animals up to puberty. The timing of vaginal opening and preputial separation were within the control range. The NOAEL for perinatal toxicity in the F1 offspring was 600 mg morpholine hydrochloride/kg body weight and day, the highest dose tested, which corresponds to 423 mg morpholine/kg body weight and day (BASF SE 2021).

Tab. 5 Prenatal developmental toxicity studies with gavage administration of morpholine hydrochloride

Species, strain, number per group	Exposure	Findings	References
rat, Crl:WI[Han] Wistar, 25 ♀	**GD 6–19**, 0, 75, 250, 750 mg **morpholine hydrochloride**/kg body weight and day (0, 52.9, 176, 529 mg **morpholine**/kg body weight and day); gavage, purity 97%, dissolved in water, examination on gestation day 20, OECD TG 414	**52.9 mg morpholine/kg body weight**: **NOAEL developmental and maternal toxicity**: dams: absolute liver weights ↑ (+6.5%); **176 mg morpholine/kg body weight**: dams: mild, regenerative anaemia: number of red blood cells ↓ (–4% compared with controls), haemoglobin ↓ (–4% compared with controls), haematocrit ↓ (–3% compared with controls), number of reticulocytes ↑ (+29% compared with controls), absolute liver weights ↑ (+9.7%), foetuses: ossification delays ↑ and incidences for some localizations outside the historical control range; **529 mg morpholine/kg body weight**: dams: mild, regenerative anaemia: number of red blood cells ↓ (–4% compared with controls), haemoglobin ↓ (–4% compared with controls) and haematocrit ↓ (–4% compared with controls), number of reticulocytes ↑ (+29% compared with controls), absolute liver weight ↑ (+9. 9%), food intake ↓ (–13% GD 6–10) and body weight gains ↓ (–41% GD 6–8), increased liver metabolism (ALT activity and urea, bilirubin, cholesterol ↑), salivation on GD 12–19 (8/25 animals) for several minutes following substance administration, foetuses: ossification delays ↑ and with some incidences outside historical control values, incomplete ossification (% incidence parietal bone: 13.5, 27.1, 34.9, 34.9; historical controls: 16.5 [9.0–27.6] and % incidence of wavy ribs: 11.9, 12.4, 24.3, 24.5; historical controls: 7.5 [1.0–18.0]); no effect on litter parameters (number of live foetuses, sex ratio, pre-implantation and post-implantation losses), foetal weights, malformations, external or soft tissue variations and no effect on overall incidence of skeletal variations	BASF SE 2009

Tab. 5 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rabbit, New Zealand White, 25 ♀	**GD 6–28**, 0, 20, 70, 210 mg **morpholine hydrochloride**/kg body weight and day (0, 14.1, 49.4, 148.2 mg **morpholine**/kg body weight and day), gavage, purity 99.4%, dissolved in water, examination on gestation day 29, OECD TG 414	**49.4 mg morpholine/kg body weight**: **NOAEL developmental and maternal toxicity;** **148.2 mg morpholine/kg body weight**: dams: hypochromic, microcytic anaemia: haemoglobin and haematocrit ↓, MCH and MCV ↓, food consumption ↓ (GD 6–13 by up to 34% and GD 6–28 by 14%), body weight gains ↓ (GD 6–11 and GD 6–28 by 59%), body weights ↓ on GD 6 (–28.5 g; controls: +62.8 g), foetuses: malformation: aortic arch atresia 2/25 (rare finding), historical controls: 2/3415 foetuses (0.058%) in 2/381 litters (0.52%) [0.0–0.5%], skeletal variations: misshapen sacral vertebrae (10.5% in mean foetuses/litter, historical controls: 4.9% [1.9–8.6]), supernumerary 13th rib (78.8% mean foetuses/litter, historical controls: 57.7% [47.0–70.9]), incomplete ossification of talus (4.1% mean foetuses/litter, historical controls: 1.3% [0.0–2.6])	BASF SE 2020

ALT: alanine aminotransferase; GD: gestation day; MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; NOAEL: no observed adverse effect level

Manifesto (MAK value/classification)

The most sensitive end point is the local effect of morpholine on the upper respiratory tract in humans and animals.

MAK value. There are no suitable human data available from which a MAK value can be derived. In a 2-year study in Sprague Dawley rats, the NOAEC was 10 ml/m^3; at 50 ml/m^3, irritant effects occurred in the nasal mucosa and focal necrosis was found in 8 of the 120 animals (6 ♂, 2 ♀) (Harbison et al. 1989); this concentration therefore represents the beginning of the concentration–effect relationship. The NAEC is likely to be well above 10 ml/m^3. A benchmark calculation based on the incidences of focal necrosis in male animals (6/60) (see Section "Animal Experiments") yielded a $BMDL_{05}$ (lower confidence limit of the benchmark concentration for a 5% increase in incidence) of 19.78 ml/m^3.

The value of 19.78 ml/m^3 was extrapolated to humans (1:3) (Brüning et al. 2014), which yielded a concentration of 6.6 ml/m^3 and, using the preferred value approach, a MAK value of 5 ml morpholine/m^3.

From the NOAEC of 25 ml/m^3 determined in the 13-week study in rats (Conaway et al. 1984), an airborne concentration limit of 4 ml/m^3 is calculated (extrapolation of data from animal experiments to humans (1:3), increase in the effects with time (1:2)); this further supports the derived MAK value.

Peak limitation. Due to the local effects of morpholine, assignment to Peak Limitation Category I has been retained. Since the MAK value of 5 ml/m^3 is very close to the extrapolated NAEC of 6.6 ml/m^3, an excursion factor of 1 has been set for peak limitation.

A momentary value of 10 ml/m^3 has been set for morpholine in analogy to that for other aliphatic amines with a MAK value of 5 ml/m^3 (for example ethylamine, methylamine).

Prenatal toxicity. Due to the lack of developmental toxicity studies, morpholine was previously assigned to Pregnancy Risk Group D (Greim 1996).

In a prenatal developmental toxicity study in Wistar rats carried out according to OECD Test Guideline 414 with gavage administration (BASF SE 2009), there was a statistically significant change in haematological parameters in the dams and the incidences of delayed ossification were increased in a non-dose-dependent manner in the foetuses, both effects starting at 250 mg morpholine hydrochloride/kg body weight and day (176 mg morpholine/kg body weight and day). The incidences of the findings "incomplete ossification of the parietal bone with unchanged cartilage" and "wavy ribs" in the foetuses were outside the respective historical control ranges. These findings were slightly increased in frequency at 75 mg/kg body weight and day (52.9 mg morpholine/kg body weight and day), but were within the

historical control range. Therefore, the NOAEL for prenatal developmental toxicity and maternal toxicity in the rat is 75 mg morpholine hydrochloride/kg body weight and day (52.9 mg morpholine/kg body weight and day).

In a prenatal developmental toxicity study in New Zealand White rabbits carried out according to OECD Test Guideline 414 with gavage administration, the soft tissue malformation "aortic arch atresia" was found in 2 foetuses from 2 litters at 210 mg morpholine hydrochloride/kg body weight and day (148 mg morpholine/kg body weight and day). The incidences of skeletal variations "misshapen sacral vertebrae", "supernumerary 13th rib" and "unossified talus (cartilage present)" were likewise increased with statistical significance compared with the control values. Concurrent pronounced maternal toxicity was present in the form of hypochromic microcytic anaemia, decreased food consumption and decreased body weight gains (GD 6–11 and GD 6–28 by 59%). Thus, the NOAEL for developmental toxicity and maternal toxicity is 70 mg/kg body weight and day (49.4 mg morpholine/kg body weight and day) for rabbits (BASF SE 2020).

In an extended one-generation study in Wistar rats carried out according to OECD Test Guideline 443 with gavage administration (BASF SE 2021), there were no signs of perinatal and postnatal toxicity in the F1 animals up to puberty. The NOAEL for systemic toxicity in the parental animals was 200 mg morpholine hydrochloride/kg body weight and day (141 mg morpholine/kg body weight and day) because clinical pathology yielded findings at 600 mg morpholine hydrochloride/kg body weight and day indicating mild anaemia, altered protein and lipid metabolism, and metabolic acidosis. The NOAEL for perinatal toxicity is 600 mg morpholine hydrochloride/kg body weight and day (423 mg morpholine/kg body weight and day), the highest dose tested (BASF SE 2021).

The following toxicokinetic data are taken into consideration for the extrapolation of the NOAELs for prenatal developmental toxicity and for perinatal toxicity in the rat of 52.9 and 423 mg morpholine/kg body weight and day, respectively, and the NOAEL for prenatal developmental toxicity in the rabbit of 49.4 mg morpholine/kg body weight and day to a concentration in workplace air: the species-specific toxicokinetic correction values for the rat and rabbit (1:4 and 1:2.4), the daily exposure of the rats in the one-generation study in comparison with the 5 days per week exposure at the workplace (7:5), the assumed oral absorption (100%), the body weight (70 kg) and respiratory volume (10 m^3) of the person, and the assumed 100% absorption by inhalation. The concentrations calculated from this are 25.7 ml/m^3 (92.6 mg/m^3), 288 ml/m^3 (1036 mg/m^3) and 40 ml/m^3 (144 mg/m^3), respectively, corresponding to 5 times, 58 times and 8 times the MAK value of 5 ml morpholine/m^3 (18 mg/m^3).

For the rat and rabbit, the 5 and 8-fold margins between the NOAELs for developmental toxicity and the MAK value are not sufficiently large, but the true NAELs (no adverse effect levels) may be higher. Relative to the corresponding LOAELs of 250 and 210 mg morpholine hydrochloride/kg body weight and day (after toxicokinetic conversion: 86 and 120 ml morpholine/m^3, respectively) in the rat and rabbit, the margins to the MAK value of 5 ml morpholine/m^3 are 17 and 24-fold, respectively. Concurrent pronounced maternal toxicity occurred in the form of 59% lower body weight gains (GD 6 to 28) and hypochromic microcytic anaemia in rabbits and mild regenerative anaemia in rats. The studies were conducted with the non-irritant hydrochloride in order to be able to administer the maximum amount of morpholine by gavage to the animals. However, the oral hydrochloride dose does not correlate with the actual workplace exposure to morpholine vapour. The corresponding morpholine concentrations in air would lead to significant irritant effects, at least in the range of the LOAECs (lowest observed adverse effect concentrations). This means that such a high dose could not be tested via inhalation.

The 58-fold margin between the NOAEL for perinatal toxicity in the rat and the MAK value is sufficiently large.

Overall, there are sufficient data available to assign morpholine to Pregnancy Risk Group C at a MAK value of 5 ml/m^3.

Absorption through the skin. Data for percutaneous absorption are not available. Model calculations for a highly diluted and thus presumably no longer corrosive 0.5% aqueous morpholine solution yielded absorbed amounts of between 2.5 mg (IH SkinPerm; Tibaldi et al. 2014) and 9.7 mg (Fiserova-Bergerova et al. 1990) under standard conditions (exposure for 1 hour, 2000 cm^2 of skin). In the 2-year inhalation study in rats, the systemic NOAEC was 50 ml/m^3 (180 mg/m^3). Taking into consideration the increased respiratory volume at the workplace (1:2), the extrapolation of data from animal experiments to humans (1:2), the 100% absorption by inhalation and a respiratory volume of 10 m^3, a

systemically tolerable amount of 450 mg is obtained. In comparison, the calculated dermal absorption from a diluted solution is low, so that morpholine has not been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Reliable clinical data in humans and experimental studies in animals or in vitro studies with positive results are not available for skin-sensitizing effects of morpholine. Findings of sensitizing effects of morpholine on the airways have likewise not been described. Morpholine has therefore not been designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

References

BASF SE (2009) Morpholine hydrochloride – prenatal developmental toxicity study in Wistar rats oral administration (gavage) OECD 414. Project No 30R0448/07052, 2009, Ludwigshafen: BASF SE, unpublished

BASF SE (2020) Morpholine hydrochloride – prenatal developmental toxicity study in New Zealand White rabbits oral administration (gavage) OECD 414. Project No 40R0448/07R065, 2020, Ludwigshafen: BASF SE, unpublished

BASF SE (2021) Morpholine hydrochloride – extended one-generation reproduction toxicity study in Wistar rats oral administration (gavage) OECD 443. Project No 90R0448/07R069, 2021, Ludwigshafen: BASF SE, unpublished

Brüning T, Bartsch R, Bolt HM, Desel H, Drexler H, Gundert-Remy U, Hartwig A, Jäckh R, Leibold E, Pallapies D, Rettenmeier AW, Schlüter G, Stropp G, Sucker K, Triebig G, Westphal G, van Thriel C (2014) Sensory irritation as a basis for setting occupational exposure limits. Arch Toxicol 88(10): 1855–1879. https://doi.org/10.1007/s00204-014-1346-z

CIREP (Cosmetic Ingredient Review Expert Panel) (1989) Final report on the safety assessment of morpholine. J Am Coll Toxicol 8(4): 707–748. https://doi.org/10.3109/10915818909010528

Conaway CC, Coate WB, Voelker RW (1984) Subchronic inhalation toxicity of morpholine in rats. Fundam Appl Toxicol 4(3 Pt 1): 465–472. https://doi.org/10.1016/0272-0590(84)90204-5

DFG (Deutsche Forschungsgemeinschaft), editor (2021) List of MAK and BAT Values 2021. Maximum Concentrations and Biological Tolerance Values at the Workplace. Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, report 57. Düsseldorf: German Medical Science. https://doi.org/10.34865/mbwl_2021_eng

ECHA (European Chemicals Agency) (2020) Morpholine (CAS Number 110-91-8). Registration dossier. Joint submission, first publication 02 Mar 2011, last modification 23 May 2020. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/13364, accessed 19 Oct 2020

Fiserova-Bergerova V, Pierce JT, Droz PO (1990) Dermal absorption potential of industrial chemicals: criteria for skin notation. Am J Ind Med 17(5): 617–635. https://doi.org/10.1002/ajim.4700170507

Greim H, editor (1996) Morpholin. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 23rd issue. Weinheim: VCH. Also available from https://doi.org/10.1002/3527600418.mb11091d0023

Greim H, editor (2000) Morpholin. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 30th issue. Weinheim: Wiley-VCH. Also available from https://doi.org/10.1002/3527600418.mb11091d0030

Harbison RD, Marino DJ, Conaway CC, Rubin LF, Gandy J (1989) Chronic morpholine exposure of rats. Fundam Appl Toxicol 12(3): 491–507. https://doi.org/10.1016/0272-0590(89)90023-7

Henschler D, editor (1974) Morpholin. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 3rd issue. Weinheim: VCH. Also available from https://doi.org/10.1002/3527600418.mb11091d0003

NIOSH (The National Institute for Occupational Safety and Health) (2017) Skin notation (SK) profile: morpholine [CAS No. 110-91-8]. Cincinnatti, OH: NIOSH. https://www.cdc.gov/niosh/docket/review/docket153c/pdfs/eid-tr-sk-morpholine-03242015.pdf, accessed 17 Dec 2020

Tibaldi R, ten Berge W, Drolet D (2014) Dermal absorption of chemicals: estimation by IH SkinPerm. J Occup Environ Hyg 11(1): 19–31. https://doi.org/10.1080/15459624.2013.831983

US EPA (US Environmental Protection Agency) (2020) Benchmark Dose Software (BMDS). Version 3.2. https://www.epa.gov/bmds/benchmark-dose-software-bmds-version-312-download, accessed 07 Oct 2020

Wang X, Suskind RR (1988) Comparative studies of the sensitization potential of morpholine, 2-mercaptobenzothiazole and 2 of their derivatives in guinea pigs. Contact Dermatitis 19(1): 11–15. https://doi.org/10.1111/j.1600-0536.1988.tb02861.x

Wang X, Tabor MW (1988) Studies of the reactivity of morpholine, 2-mercaptobenzothiazole and 2 of their derivatives with selected amino acids. Contact Dermatitis 19(1): 16–21. https://doi.org/10.1111/j.1600-0536.1988.tb02862.x