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6

Final Report on the Safety Assessment of Morpholine

Morpholine is used in cosmetic products as a surfactant and emulsifier at concentrations up to 5%.

Morpholine is metabolized in guinea pigs but was not significantly metabolized in rats, dogs, or rabbits. Dermal LD_{50}s in rabbits ranged between 0.3 and 1.2 g/kg. The oral LD_{50}s in rats were between 1.1 and 1.6 g/kg; in guinea pigs the oral LD_{50} was 0.9 g/kg.

In studies of acute and short-term dermal toxicity Morpholine as an undiluted and unneutralized solution or as a diluted and unneutralized solution applied daily to the skin of guinea pigs and rabbits, respectively, caused the deaths of the test animals within 2 weeks. In both cases, the skin was necrotic.

Unneutralized solutions of Morpholine caused severe corneal necrosis, but upon neutralization Morpholine was not injurious to rabbit eyes.

In short-term inhalation studies (in rats) with varying concentrations of Morpholine, the effects observed included irritation of the mucous membranes and an increased respiratory rate. Chronic inhalation studies of Morpholine in rats and guinea pigs reported changes in the nervous system activity and arterial and peripheral blood pressure. At high concentrations Morpholine produced swelling of the alveolar cells and atrophy of lymphoid elements in the spleen. At lower concentrations a decrease in the size of the lymph nodules in the spleen was noted.

Morpholine was a weak positive mutagen in L5178 mouse lymphoma assay, in BALB/3T3 malignant cell transformation and fibroblast transformation assays, and in sister chromatid exchange assays, but was negative in the Ames test with and without metabolic activation. At nontoxic doses Morpholine did not increase the rate of DNA repair in rat hepatocytes. Results of other mutagenic assays varied according to the system used.

Nitrosation of Morpholine produces *N*-nitrosomorpholine, which has been mutagenic in a variety of test systems. Simultaneous exposure of laboratory animals to Morpholine and nitrites has caused a number of different cancers. A carcinogenic response was produced in rats in a long-term feeding study of Morpholine in which nitrites were present in the diet.

In humans, Morpholine is absorbed and is considered to be a skin and eye irritant, as well as a skin sensitizer. A formulation containing 1% Morpholine indicated that the ingredient was neither an irritant nor sensitizer.

Morpholine is not considered to be an animal carcinogen. It reacts easily with nitrosating agents, resulting in the formation of *N*-nitrosomorpholine. Under conditions of use, it is highly unlikely that Morpholine is totally free of carcinogenic nitrosoamines. Without quantitative data regarding the formation of *N*-nitrosomorpholine under conditions of use, it cannot be concluded that Morpholine is safe for use in cosmetic products.

INTRODUCTION

The following report is a literature review on the chemistry, use, and toxicology of Morpholine. Reviews on Morpholine also have been published by Reinhardt and Brittelli,[1] Texaco,[2] Nieneker,[3] and the National Research Council.[4,5] Studies dealing with the nitrosation of Morpholine to form *N*-nitrosomorpholine, a potent animal carcinogen, have been reviewed by the International Agency for Research on Cancer.[6] Quantitative data on the formation of *N*-nitrosomorpholine under conditions of use were unavailable for the safety assessment of Morpholine as a cosmetic ingredient.

CHEMISTRY

Definition and Structure

Morpholine is the heterocyclic secondary amine that conforms to the formula shown in Figure 1.[7]

Morpholine (CAS No. 110-91-8) is also known as tetrahydro-1,4-oxazine, tetrahydro-2H-1,4-oxazine, diethylene oximide, diethyleneimide oxide, and diethylene imidoxide.[7-9]

Properties

Morpholine is a clear, hygroscopic liquid with a characteristic amine odor. It is soluble in water, methanol, ethanol, benzene, acetone, ether, castor oil, ethylene glycol, 2-hexanone, linseed oil, turpentine, and pine oil. It is insoluble in concentrated sodium hydroxide solutions.[8,10,11] Additional chemical and physical data are presented in Table 1.

The solvent power of Morpholine was reported to exceed that of benzene, pyridine, and dioxane.

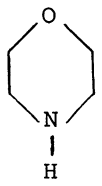


FIG. 1. Structure of Morpholine.

TABLE 1. Chemical and Physical Data for Morpholine

		Reference
Physical form	Colorless, volatile, alkaline, oily liquid	1
Odor	Amine odor	1
Formula	C_4H_9NO	15
Molecular weight	87.12	11, 16, 17
Assay	99.0% minimum (for cosmetic use)	10
	98% (technical grade)	15
Vapor density	1.1 at 38°C	1
	3.0 (air = 1)	14, 16
Vapor pressure	6.6 mmHg at 20°C	15, 17
	7.0 mmHg at 20°C	9, 14
	8.0 mmHg at 20°C	1
	10 mmHg at 23°C	16
Refractive index	1.4537–1.4547 at 20°C	10
	1.4540	8
	1.4545 at 20°C	2
	1.4548 at 20°C	11
Specific gravity	0.998 at 25°/25°C	16
	0.996–1.000 at 25°/25°C	10
	1.0005 at 20°C	11
	1.001 at 20°C/20°C	2, 18
	1.0017 (H_2O = 1)	14
	1.007 at 20°C	8, 17
Boiling point	126.0°C minimum	19, 10
	128.3	9, 11, 14
	128.9	8, 15–17
	130.0 maximum	10, 19
Melting point	−4.9°C	8, 11, 15, 17
	−7– −5°C	1
Viscosity	2.23 centipoises at 20°C	8, 14, 15
Surface tension	37.5 dynes/cm at 20°C	2, 8
Dipole moment	1.58 debyes	2, 8
pH	11.2 (undiluted)	14
	11.4 (25% in aqueous solution)	19
	11.36–11.54 mEq/g	10
pK_b	5.64	2
Flash point		
Open cup	38°C (100°F)	8, 15, 16
Closed cup	35°C (95°F)	9, 14, 17
Autoignition temperature	310°C (590°F)	14, 15, 6
Explosive limits in air	Lower 1.8%	14, 17
	Upper 11%	9
Weight per gallon	8.34 lbs at 20°C	15
Weight	1 ml weighs 1 g	18
1 mg/liter	281 ppm	1
1 ppm	3.56 mg/m^3	1
Arsenic (as As)	3 ppm maximum	10
Lead (as Pb)	20 ppm maximum	10
Conductivity	6 mho/cm × 10^{10}	2
Dielectric constant	7.176 esu	2
	7.33	12
Heat capacity	41.6 cal/mol/deg 25°C	2
Heat of vaporization	9510 cal/mol (45–129°C)	2
Density	0.999 g/cc at 20°C	2
Flammability	Flammable	1

Source: American Conference of Governmental Industrial Hygienists.

TABLE 2. pH of Aqueous Solutions of Morpholine[2]

Concentration of Morpholine (wt. %)	*pH*
0	7.0
0.001	8.8
0.01	9.4
0.1	10.0
1.0	10.6
10.0	11.2

The vapor pressures of aqueous solutions of Morpholine are very close to that of water alone, and this property is made use of where solutions of constant alkalinity are required.[12] The pHs of aqueous solutions of Morpholine are presented in Table 2.

The chemical reactivity of Morpholine is centered on the nitrogen of the secondary amino group, the oxygen being present as an ether oxygen and the carbons all saturated.[12] The ether oxygen improves its solvent properties and is responsible for Morpholine being a weaker base than the simple amines.[3] Morpholine readily forms salts with inorganic acids, including CO_2, and with organic carboxylic acids, anhydrides, chlorides, and esters.[12] It reacts with fatty acids to form soaps.[13] The compound is highly reactive with strong oxidizers.[9,14]

In humans, the odor threshold of Morpholine is 0.01 ppm.[20]

The infrared spectrum of Morpholine has been published.[10]

Method of Manufacture

One method of producing Morpholine is based on the dehydration of diethanolamine by a strong acid shown in Figure 2.[12,13] Acids that can be used in this process include oleum, concentrated sulfuric acid, and concentrated hydrochloric acid. A molar excess of acid is used generally at a temperature over 150°C. The crude acidic reaction mixture is neutralized by the addition of an alkali to give an aqueous solution of Morpholine. Purified Morpholine is recovered from the aqueous solution by an extraction using either an organic solvent or concentrated aqueous alkali followed by distillation. Production of Morpholine by this procedure is undesirable because of the large amount of inorganic waste.[13] Since technical diethanolamine contains triethanolamine, industrial Morpholine contains *N*-hydroxyethylmorpholine.[21]

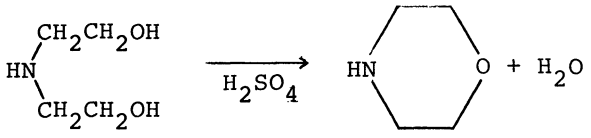


FIG. 2. Dehydration of diethanolamine to produce Morpholine.

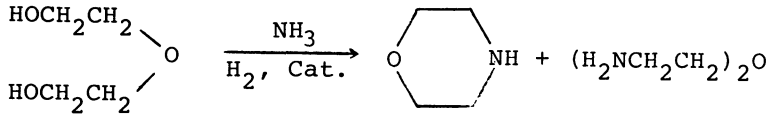


FIG. 3. Reaction of diethylene glycol with ammonia to form Morpholine.

Morpholine also can be prepared by the reaction of diethylene glycol with ammonia. The two react in the presence of hydrogen and a hydrogenation catalyst at a temperature between 150 and 400°C and a pressure of 30 to 400 atmospheres[12,13] (Fig. 3).A large number of hydrogenation catalysts can be used in this process. Among the possible catalysts are those containing one or more of the following metals: copper, nickel, cobalt, chromium, molybdenum, manganese, platinum, palladium, rhodium, and ruthenium. Excess ammonia is removed from the crude reaction mixture by a stripping operation. The Morpholine is then recovered by fractional distillation to give a product of about 97.8% purity. The compound 2-(2-aminoethoxy)ethanol [CAS No. 929-06-6] is a byproduct of the process.[12,13]

Morpholine also can be made from *bis*(2-chloroethyl)ether and anhydrous ammonia by heating the reactants in a suitable solvent in a closed vessel to 50°C for 24 h:[12,13] (Fig. 4).After the excess ammonia is vented, the product is filtered from ammonium chloride, and distilled to separate the Morpholine from the solvent and any unreacted chloroether. The conversion is 80% based on ether consumed, and the yield is 30% based on the bis(2-chloroethyl)ether present.[12,13]

Impurities

Known impurities in cosmetic grade Morpholine include arsenic (3 ppm maximum) and lead (20 ppm maximum).[10] When Morpholine is prepared from diethanolamine, *N*-hydroxyethylmorpholine may also occur as an impurity. *N*-hydroxyethylmorpholine is mutagenic and possibly carcinogenic.[22]

Because Morpholine is readily nitrosated, the potential exists for contamination of cosmetic products with *N*-nitrosomorpholine (NMOR), which is a potent carcinogen in animals.[6]

Commercially available cosmetics and toiletries bought on the open market in the Federal Republic of Germany were analyzed for nitrosamines and *N*-nitrosomorpholine (NMOR). As indicated in Table 3, NMOR was found in

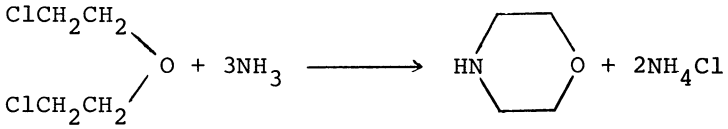


FIG. 4. Reaction of *bis*(2-chloroethyl)ether and anhydrous ammonia to form Morpholine.

TABLE 3. *N*-Nitrosomorpholine (NMOR) in Toiletry Articles and Cosmetics[23]

	Number of samples	Number positive	Concentration (μg/kg; ppb)	
			Maximum	Average
Shampoos	45	13	640	133
Color toners	7	—	—	—
Hair conditioners	16	—	—	—
Foam baths	7	—	—	—
Shower gels	9	4	380	145
Cream and oil baths	8	2	440	—
Cosmetic bath additives	5	—	—	—
Children's shampoos	5	1	230	—
Children's bath and skin care products	8	6	360	80
Body lotions and rubs	6	—	—	—
Face tonics, cleaners, and masks	29	—	—	—

13 of 45 shampoos ($\leq$ 640 ppb), in 4 of 9 shower gels ($\leq$ 380 ppb), in 2 of 8 cream and oil baths (440 ppb), in 1 of 5 children's shampoos (230 ppb), and in 6 of 8 children's bath and skin care products ($\leq$ 360 ppb).[23] In another report,[24] NMOR was detected at concentrations between 48 and 1,240 ppb in 7 mascara products (Table 4).

Data are not available to show that the Morpholine used in cosmetic formulations is free of nitrosomorpholine; it is not known whether the NMOR found in the cosmetic preparations was formed in the cosmetics.

TABLE 4. Analytical Results for *N*-Nitrosomorpholine[a][24]

Sample no.	Product name/lot no.	N-Nitrosomorpholine (ng/g)[b]	Percentage recovery[c]
DCST-84-2029	Mascara black/none	48	67.5
		75	
DCST-84-2030	Mascara deep brown/none	116	63.5
		89	
DCST-84-2031	Mascara dark brown/02AA	336	73.2
		332	93.3
DCST-84-2032	Mascara black/24A	99	72.8
		108	74.4
DCST-84-2033	Mascara dark black/none	1190[d]	90.3
		1240[d]	96.4
DCST-84-2034	Conditioning mascara brown/none	118	77.6
		118	93.7
DCST-84-2035	Conditioning mascara black/none	214	81.8
		207	79.8

[a]Results confirmed by GC-MS or other techniques.
[b]*N*-nitrosomorpholine expressed in ng/g (ppb), corrected for recovery.
[c]*N*-nitrosopiperidine was added as an internal standard at the 50–100 ng/g level.
[d]*N*-nitrosodimethylamine was also detected in this sample, with concentration at 13 and 16 ng/g, respectively.

Analytical Methods

Analytical methods for the separation and/or determination of Morpholine include ion chromatography,[25] mass spectrometry,[26] thermogravimetric analysis,[27] spectrophotometry,[28] colorimetric analysis,[29] and gas chromatography.[30–32] Gas chromatography coupled with a thermal energy analyzer may also be used to determine traces of NMOR resulting from the *N*-nitrosation of Morpholine. The detection limit of this method is 2 ng/g.[33]

High-performance liquid chromatography has been used for the separation of Morpholine and its metabolites (*N*-hydroxymorpholine, *N*-methylmorpholine *N*-methylmorpholine *N*-oxide) from biological fluids and tissue preparations.[34] Morpholine also may be detected in such biological samples as urine, feces, blood, plasma, bile, and tissue by gas and gas–liquid chromatography.[35,36]

Chemical Reactions

Because of the chemical inertness of ethers in general, most of the chemical reactions of Morpholine involve the secondary amino group of the compound. With inorganic acids, Morpholine forms salts, and with organic acids it forms either the salt or the amide. Like most secondary amines, Morpholine reacts with carboxylic acids and their anhydrides, chlorides, and esters to give the corresponding morpholides. Reactions of Morpholine with isocyanates give the corresponding substituted ureas. Mannich bases undergo amine exchange when heated with Morpholine; the amine function of certain amides can also be caused to exchange with Morpholine. Phenols, ketones, nitroalkanes, and amides react with Morpholine and formaldehyde to give corresponding 4-morpholinylmethyl compounds. Two molecules of Morpholine condense with an aldehyde to form a 4,4′-alkylidenedimorpholine. The addition of formic acid to a mixture of Morpholine and carbonyl compounds leads to reductive alkylation of the Morpholine (Leuckart-Wallach reaction). In addition to the Mannich and Leuckart-Wallach reactions, Morpholine can be alkylated and arylated to form various derivatives. Like most secondary amines, Morpholine can be linked by alpha- and beta-unsaturated ketones, esters and nitriles. The heterocyclic amine also reacts with alkylene oxides to yield the corresponding 2-(4-morpholino)alkanols. Morpholine is used in the Willgerodt-Kindler reaction to convert aryl alkyl ketones to ω-aryl fatty acid amides. Morpholine may also react with oxidizing agents, undergo direct chlorination, and form complexes with metallic halides. Upon exhaustive methylation, thermal decomposition may result in rupture of the Morpholine ring (Hofmann degradation)[2] with the release of fumes containing nitrogen oxides.[37]

The chemical reactions of Morpholine are reviewed in detail elsewhere.[2,3,12,13]

Nitrosation Potential

Morpholine, and certain other amines, and amides are readily nitrosated to form nitrosamines and nitrosamides, respectively. According to the National

Research Council:[5]

> Of the approximately 300 *N*-nitroso compounds that have been tested, 85% of the 209 nitrosamines (including *N*-nitrosomorpholine) and 92% of the 86 nitrosamides have been shown to produce cancer in laboratory animals.[38,39] *N*-Nitroso compounds are readily formed by the interaction of nitrosating agents (nitrous acid, oxides of nitrogen, certain organic nitro compounds, and other nitroso compounds) and secondary or tertiary amines and amides. The amines and amides may be nitrosated to nitrosamines and nitrosamides under acidic, neutral, or alkaline conditions. Atmospheric NO_2 may also participate in the nitrosation of amines in aqueous solution.[40]

Morpholine is a secondary amine and is readily nitrosated by nitrous acid. As with other secondary amines, the nitrosation is acid-catalyzed and proceeds most rapidly at a pH of 3.4,[41,42] but some nitrosation of secondary amines occurs at a pH as high as 6.[43,44] The nitrosation rate is proportional to the morpholine concentration and the square of the nitrite concentration, according to the following equation, where the brackets refer to molar concentration:

$$\text{rate} = k[\text{amine}][\text{nitrite}]^2.$$

The k value, units in $(M^{-2})(s^{-1})$, varies in different amines and is 0.0017 for dimethylamine, 0.42 for morpholine, 83 for piperazine, and 250 for *N*-methylaniline. Hence, morpholine is nitrosated with moderate ease. The nitrosation of amines is catalyzed by such anions as iodide, bromide, and thiocyanate, and this reaction follows a different equation.[42]

USES

Noncosmetic

Morpholine has a wide range of applications, including use as a chemical intermediate; antioxidant; wax and polish emulsifier; corrosion inhibitor; solvent for resins, waxes, casein, and dyes; boiler additive; intermediate in the production of rubber accelerators; and intermediate in the production of optical brighteners for detergents.[2,8,15,17,45] Morpholine also is used as a polymerization inhibitor, a catalyst for certain chemical reactions, gelling agent in preparation of catalysts, separating agent for various purification procedures, to stabilize thermally cellulose materials, and a preservative for book paper.[2] The metric tons of Morpholine used for various applications are listed in Table 5.

Federal regulations permit use of Morpholine in several direct and indirect food additive applications. Title 21 Part 172.235 of the Code of Federal Regulations[46] allows certain fatty acid salts of Morpholine to be used as components of protective coatings applied to fruits and vegetables. For this direct food additive use, Morpholine concentrations may not exceed that

TABLE 5. Uses of Morpholine[4]

Uses	*Amount used (metric tons/yr)*	*Percentage of total*
Rubber chemicals	3,600	33
Corrosion inhibitors	2,700	25
Optical brighteners	1,100	10
Alkyl morpholines	1,100	10
Waxes and polishes	900	8
Exports	800	7
Miscellaneous uses	800	7

"reasonably required to produce its intended effect." Indirect food additive applications for Morpholine include use as a corrosion inhibitor for steel or tinplate used in food containers,[47] a defoaming agent used in the manufacture of paper and paperboard for food packaging,[48] as a component of adhesives used in food packaging materials,[49] and as a defoaming agent in animal glue used in food packaging materials.[50] Morpholine may not exceed 10 ppm as a boiler additive in steam that will contact food.[51]

Although the Food and Drug Administration allows use of Morpholine as a direct and indirect food additive, one of the largest U.S. commercial producers of Morpholine recommended in a letter to its customers in July, 1980 that this compound not be used in food applications. This recommendation was reported to be based on data suggesting that Morpholine could be weakly mutagenic.[5,52]

Morpholine derivatives are used as insecticides, fungicides, herbicides, anesthetics, analgesics, and antiseptics. Various Morpholine compounds also are used as plasticizers, viscosity improvers, antioxidants, corrosion inhibitors, photographic developing agents, and ink eradicators. In the textile industry, Morpholine derivatives are used as lubricants, emulsifiers, adjuvants, whitening agents, and softening agents.[2,8,53] Morpholine fatty acid salts are used as surface active agents and emulsifiers.[8]

The various uses of Morpholine and Morpholine derivatives are reviewed in more detail elsewhere.[2,3,12,13]

Cosmetic

Morpholine is used in cosmetic products as a surfactant and emulsifier.[54]

Data submitted to the Food and Drug Administration (FDA) in 1981 by cosmetic firms participating in the voluntary cosmetic registration program indicated that Morpholine was used in a total of 38 cosmetic products. Product types formulated with this compound included eyeliner, eye shadow, mascara, and skin care preparations. The greatest use of Morpholine was in mascara (32 products). Reported concentrations of Morpholine in these products ranged from $\leq 0.1\%$ (4 products), > 0.1–1% (17 products), to > 1–5% (17 products).[55,56] Data from the Federal Republic of Germany suggest that Morpholine is used as an ingredient in shampoos and bath products.[23]

TABLE 6. Product Formulation Data for Morpholine[57]

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (percentage)*		
			> 1–5	*≥ 1*	*> 0.1–1*
Eye makeup remover	77	29	18		11
Eye, face, or body preparations other than eye makeup removers	1264	2		2	
1986 Totals		31	18	2	11

Data on the use of Morpholine in cosmetic products supplied to the FDA in 1986 (Table 6) indicated that the greatest use of Morpholine was in eye makeup removers, at concentrations of > 0.1–1% and > 1–5%.[57]

Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators must conform to the format of concentration ranges and product categories as described in Title 21 Part 720.4 of the Code of Federal Regulations.[58] Since certain cosmetic ingredients are supplied to the formulator at less than 100% concentration, the concentration reported by the formulator may not necessarily reflect the actual concentration found in the finished cosmetic product; the actual concentration in such an instance would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of a "concentration range" provides opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing a 2–10-fold error in the assumed ingredient concentration.

The ocular region and the skin are the areas directly exposed to cosmetic products containing Morpholine. The potential also exists for Morpholine-containing products to come in contact with the conjunctiva and cornea. Cosmetics formulated with Morpholine may be applied repeatedly over the course of many years.

Morpholine is listed in Annex II of the European Economic Community (EEC) Cosmetics Directive. Annex II contains those compounds that may not be used in cosmetic formulations.[59] Morpholine's inclusion on this list was based on its potential for skin, eye, and mucous membrane irritation, its hepatoxicity, and its occurrence on the German SCRIBAS list of compounds that may only be used in prescription pharmaceuticals.[60]

BIOLOGY

Effect on Enzyme Activity

Twenty male Wistar rats were exposed intermittently to 300 ppm Morpholine vapor 6 h/day, 5 days/week for 4–15 weeks. A second group of rats served as untreated controls. Acetylcholine esterase activity in the gluteal muscle of treated rats was elevated after 8 weeks; however, this activity

returned to that of control activities by the 12th–15th week. The gluteal muscle activities of succinate dehydrogenase and creatine kinase did not differ from that of controls over the 15 week study. In spinal cord axons, acetylcholine esterase activity was comparable to that of controls throughout the study. Succinate dehydrogenase activity was elevated in spinal cord axons by week 15.[61]

Kung and Wilson[62] investigated the effect of Morpholine on pulmonary enzymic activity and the pulmonary uptake and metabolism of 5-hydroxytryptamine (5-HT). Measurement of the accumulation of 5-HT by pulmonary tissue was used "to assess possible pulmonary toxicity" resulting from Morpholine exposure. Male rats were exposed by inhalation to 450 ppm Morpholine 6 h/day, 5 days/week for 5 weeks. Slices of lung were prepared and incubated with ^{14}C-5-HT (1.9 μM) at 37°C for periods up to 30 mins. Significantly higher tissue concentrations of ^{14}C-5-HT were found in preparations of lungs from exposed rats compared to controls. Further studies suggested that the increased tissue concentration of 5-HT was due to an effect of metabolism rather than the transport of 5-HT into the cell. Morpholine also inhibited monoamine oxidase activity. The authors suggested that the accumulation of Morpholine in the lungs and other tissues known to have high activities of monoamine oxidase may be of toxicologic importance.

Tombropoulos et al.[63] evaluated a number of biochemical variables as possible indices of early pulmonary injury resulting from exposure to Morpholine. Rabbits were exposed by inhalation to 250 ppm of Morpholine, 6 h/day for 31 days. Animals were killed either immediately after exposure or 48 h postexposure. The lungs were lavaged with 50 ml of ice cold saline and the lavage fluid centrifuged to separate the cellular supernatant fractions. The following enzyme activities were determined in both fractions: α-mannosidase (α-M), β-N-acetylglucosaminidase (β-NAG), and acid and alkaline phosphatases. In addition, the binding of wheat germ agglutinin and *Ricinus communis* agglutin-120 by pulmonary macrophages was studied. The α-mannosidase activity of the macrophages was increased by 300% in the males and 170% in females exposed to Morpholine. The increase was significant in animals killed immediately after exposure. Acid phosphatase activity was significantly increased up to 200% of control activity. β-NAG activity was also increased. The binding of lectins to macrophages was decreased in exposed animals compared to controls. The majority of the changes measured returned to control values by 2 days postexposure. It was concluded that exposure of rabbits to Morpholine by inhalation induced enzyme changes in pulmonary lavage fluids that may be related to pulmonary damage.

α-Mannosidase and acid phosphatase were induced in alveolar macrophages from the lungs of rabbits following exposure to Morpholine (minimum purity = 99%). The induction was observed after inhalation of Morpholine vapor (250 ppm 6 h/day, 5 days/week for 33 exposures) or when macrophages were cultured in the presence of Morpholine (2.3 mM). In vivo, maximum induction of α-mannosidase and acid phosphatase in female rabbits was 1.7-fold and 2-fold, respectively, and in male rabbits, 3-fold and unchanged, respectively. In vitro, maximum hydrolase induction of α-mannosidase and acid phosphatase in macrophages from male rabbits was 1.4-fold and

1.3-fold, respectively, and from female rabbits, 1.3-fold and 1.5-fold, respectively. The induction of acid hydrolases in vitro was rapid, reaching a maximum within 4 h in alveolar macrophages from male rabbits and within 8 h in those from females. The induction was dose dependent.[64] Similar results were reported in a preliminary study by the same authors.[65]

Metabolism, Excretion, and Distribution

Morpholine was excreted primarily unchanged in the urine. In a number of studies it has been reported that Morpholine was not readily metabolized in the rat,[35,66–68] dog,[69] and rabbit.[70] In contrast, Sohn et al.[67] reported that Morpholine was extensively metabolized by *N*-methylation and *N*-oxidation in the guinea pig. Morpholine was metabolized to a much smaller extent by *N*-methylation in the rat and hamster.[67] Reports also suggested that Morpholine was preferentially distributed to the kidneys in rabbits[70] and to the intestines in rats.[35] These studies are reviewed below.

Oelschlager and Temple[71] reviewed the role of *N*-oxidation in the metabolism of various morpholine-containing drugs. They suggested that Morpholine may undergo metabolic modification by oxidation of the carbon atom adjacent to the nitrogen, oxidation of the β carbon atom, or oxidation of the nitrogen atom.

Following intraperitoneal injection of radioactive Morpholine, 87% of the administered dose was excreted in the urine of rats within 24 h. No radioactivity was detected in the expired air.[66]

Six young adult C.F.E. strain rats, about 2 months old and weighing approximately 175 g, were dosed by oral intubation with 50 mg of [G^{3}H]Morpholine in 1 ml of water. These rats, three male and three female, were housed in glass metabolism cages and were allowed feed and water ad libitum. The urine, feces, and expired gases were collected every 24 h for 4 days. Up to 73.5% of the radioactive compound was excreted in the urine within the first day. A maximum of 1.9% of the radioactivity was found in the feces. An average of 4.2% of the radioactivity was exhaled as tritiated water, indicating that some of the morpholine was completely metabolized.[72]

Morpholine (250 mg/kg) and morpholine oleate (125 mg/kg) were given orally to rats. Approximately 92% and 77% of administered Morpholine and morpholine oleate, respectively, were excreted unchanged in the urine within 48 h. Only 1.42% and 6.58% of Morpholine and morpholine oleate, respectively, were excreted in the feces over a period of 156 h. After 24 h, the distribution of administered Morpholine in the stomach, intestines, liver, kidneys, and blood were 1.10%, 0.49%, 0.05%, 0.05%, and 0.64%, respectively.[68]

Sprague Dawley rats, Syrian golden hamsters, and Strain II guinea pigs were injected intraperitoneally with 125 mg/kg ^{14}C-Morpholine dissolved in saline. Following the single exposure, the animals were placed in metabolism cages and urine was collected for a 24 h period. The 0–24 h urine samples examined by high-performance liquid chromatography (HPLC) contained unmetabolized Morpholine and smaller amounts of *N*-methylmorpholine-*N*-oxide. This latter metabolite formed a considerable portion (20–46%) of the total excreted radioactivity in guinea pigs, but only a small fraction (1–6%) in

rats and hamsters. A second group of guinea pigs was given a similar intraperitoneal injection of ^{14}C-Morpholine as above. Four hours after dosing, the animals were killed. Extracts of liver, kidneys, and spleen, analyzed by HPLC, contained Morpholine, *N*-hydroxymorpholine, *N*-methylmorpholine-*N*-oxide, and *N*-methylmorpholine.[34]

Urinary metabolites and blood plasma concentrations of Morpholine (purity = 99.2%) were examined in three rodent species: the Sprague-Dawley rat, the Syrian golden hamster, and the strain II guinea pig. Marked differences were noted between the guinea pig and the other two species with respect to plasma concentrations as well as the metabolism of Morpholine. After intraperitoneal administration of 125 mg/kg ^{14}C-Morpholine (50 μCi) per animal, the blood plasma half-lives in the rat, hamster, and guinea pig were 115, 120, and 300 minutes, respectively. In all three species, approximately 80% of the radioactivity was excreted in the urine within 24 h. Nonmetabolized ^{14}C-Morpholine formed up to 99% of the urinary radioactivity in the rat and hamster; however, a significant portion of the dose (approximately 20%) appeared in the guinea pig urine as *N*-methylmorpholine-*N*-oxide. This metabolite of Morpholine was confirmed by thin-layer chromatography, gas chromatography, and mass spectrometry. Preliminary results also indicated that in the rat, 1.5% of the administered dose was excreted in the feces and approximately 0.5% was expired through the lungs as $^{14}CO_2$. No comparable data were available on the excretion in feces or exhaled air for the hamster or guinea pig.[67]

The excretion and distribution of Morpholine were studied in male Wistar rats by means of chemical analysis and radioassay. For the chemical assay, Morpholine-HCl was given orally and by i.v. injection in single doses of 500 mg/kg and 250 mg/kg, respectively. For the radioassays, ^{14}C-Morpholine was administered in a single dose of either 200 mg/kg (orally) or 150 mg/kg (i.v.). Morpholine-HCl was eliminated rapidly after oral and i.v. administration. In the chemical assay, greater than 85% of the dose appeared in the urine during the first 24 h, and 90% of the dose was excreted over a period of 3 days. Smaller amounts (3–10% over 5 days) were excreted in feces. Similar results were obtained by radioassay. Over 5 days, 80–90% of the radioactivity was excreted in urine, and 3–10% in feces. Analysis of the pooled urine by thin-layer chromatography indicated that Morpholine was excreted mainly unchanged. ^{14}C-Morpholine accumulated primarily in muscle and intestine regardless of route of administration, with significant quantities remaining in the intestine for 12 h. Elimination of radioactivity from other organs, tissues, and blood was rapid after both oral and i.v. administration. Radioactivity in blood at 6 h was about half the activity at 2 h. Morpholine had the lowest affinity for adipose tissue. Among the organs examined for distribution of ^{14}C-Morpholine were the brain, heart, liver, lungs, spleen, kidneys, intestines, muscle, testes, and stomach.[35]

A dose of 5 mmol/kg of ^{14}C-Morpholine (purity > 98%) was administered by i.v. injection to male New Zealand rabbits. Ninety percent of the administered dose was excreted unchanged in the urine. Radioactivity was "preferentially distributed" to the kidneys. Thirty minutes after the single i.v. exposure, concentrations of ^{14}C-Morpholine in the renal cortex and medulla were 6.6

TABLE 7. Accumulation of Morpholine in Brain and Perirenal Fat of Wistar Rats[a](61)

Exposure period (weeks)	*Morpholine concentration (nmol/g)*	
	Brain	*Perirenal fat*
4	221 ± 31	40 ± 14
8	478 ± 41	104 ± 51
12	416 ± 72	61 ± 22
15	503 ± 92	37 ± 15

[a]Rats exposed intermittently to 300 ppm Morpholine vapor 6 h/day, 5 days/week for 4–15 weeks.

and 15.3 times, respectively, the concentration in the blood. The concentration of ^{14}C-Morpholine in the lungs and liver 30 min after injection was approximately twice the concentration in the blood. An average of 0.6% and 43% of the dose was excreted in the bile and urine, respectively, during the first 3 h following injection. No significant binding of ^{14}C-Morpholine to serum proteins was detected.(70)

^{14}C-Morpholine was administered to dogs by an unspecified route. The total radioactivity in the blood after 24 h was 0.31–0.63% of the administered dose. Urinary excretion of radioactivity as a percentage of dose was 81–85%. Seventy to 80 percent of the urinary radioactivity was accounted for as Morpholine, with no other detectable metabolites. The half-life of the radioactive metabolites in whole blood samples and plasma was 22–28 and 13 days, respectively. The authors suggested that little of the tetrahydro-oxazine ring of Morpholine was converted to low-molecular-weight fragments (possibly glyoxylate) and incorporated into tissue components.(69)

Twenty male Wistar rats were exposed intermittently to 300 ppm (12.5 μmol/L) Morpholine vapor 6 h/day, 5 days/week for 4–15 weeks. The animals were killed after 4, 8, 12, or 15 weeks, and brain and perirenal fat samples taken. The specimens were analyzed for Morpholine content by gas chromatography. Weight gain of treated animals was similar to that of non-treated controls. Concentrations of Morpholine in the brain increased towards the end of the exposure period. Morpholine concentrations in fat were 5–14 times less than those in the brain (Table 7). The authors suggested that increases in the Morpholine content of the brain with continued exposure may indicate saturation of the metabolic clearance of Morpholine.(61)

TOXICOLOGY

Acute Toxicity

Oral

Smyth et al.(73) reported an acute oral LD50 for Morpholine of 1.05 g/kg (confidence range: 0.95–1.16 g/kg). The LD50 was determined by intubation of dosages in a logarithmic series to unfasted groups of five female Carworth-Wistar rats. For a period of 14 days following the single oral dose, the animals were observed for mortality. The LD50 was estimated by the methods of Thompson(74) and Weil.(75)

TABLE 8. Reported LD50 Values for Morpholine

Animal	*Strain*	*Sex*	*Application*	*LD50 (g/kg)*	*Reference*
Mice	Carworth Farm	M	i.p.	0.413 ± 0.026	79
Mice	dd	—	i.p.	1.35	80
Mice	—	M,F	Inhalation	LC50 = 1450 ppm, 1900 ppm, respectively	81
Rabbits	New Zealand albino	M	Dermal	0.31–0.81	73
Rabbits	—	—	Dermal	0.500	14
Rabbits	—	—	Dermal	1.21	1
Rats	Carworth–Wistar	F	Oral	1.05 ± 0.1	73
Rats	—	—	Oral	1.42–1.44	76
Rats	—	—	Oral	1.61–1.63	77
Rats	—	—	Oral	1.6	18
Rats	—	M,F	Inhalation	LC50 = 2250 ppm, 2150 ppm, respectively	81
Guinea Pigs	—	—	Oral	0.9	18

The acute oral LD50 in rats was 1.43 ml/kg[76] and 1.62 ml/kg.[77]

In an early study,[18] single doses of undiluted and unneutralized Morpholine ($\geq$ 98% purity) were "fed" to rats and guinea pigs. Doses ranging from 0.1 g/kg to 10 g/kg were "not tolerated" by either species. Those receiving the higher dosages did not survive "the time necessary to administer the liquid"; the others died immediately after dosing. Hemorrhages into the stomach and small intestines were the most frequent observations; some animals had nasal hemorrhages.

In a second study, Morpholine (purity $\geq$ 98%) was diluted with 4 parts water and then given orally to 57 rats and 33 guinea pigs. For rats, the LD50 was 1.6 g/kg. All deaths occurred within 2 or 3 days of dosing. The most common observation in rats given "large doses" (unspecified) was hemorrhage of the stomach; however, animals given 1.6 g/kg had no gross pathologic changes. For guinea pigs, the minimum lethal dosage was 0.9 g/kg. Signs in those guinea pigs that eventually died after doses of Morpholine included "complete collapse," prostration, diarrhea, and hemorrhage of the stomach.[18]

A single 5.0 g/kg dose of a cosmetic containing 1% Morpholine and prepared as a 25% suspension in corn oil was given to 5 fasted Harlan Wistar rats. There was no indication of toxicity during the 7 days of the study.[78]

The acute LD50 values for Morpholine are summarized in Table 8.

Dermal

Smyth et al.[73] reported an acute dermal LD50 for Morpholine of 0.50 ml/kg (0.31–0.81 ml/kg). The LD50 was estimated by using groups of four male New Zealand albino rabbits according to the method described by Draize et al.[82] The test material was applied as a single dose to the clipped skin of the trunk beneath an impervious plastic film. Approximately one-tenth of the body surface of the animal was in contact with the test material. The plastic film was removed after 24 h and the animals were observed thereafter for 14 days.

Shea[18] assessed the dermal toxicity and skin absorption of Morpholine in two investigations using rabbits. In the first investigation, undiluted and unneutralized Morpholine was applied as a single 0.9 g/kg dose to the

clipped skin of the midsection of seven rabbits. Two of the seven rabbits died. These two animals had blackened necrotic epidermis and an inflamed edematous dermis at the site of application, and severe burns of the underlying organs. The five surviving rabbits had severe burns at the site of treatment. In the second investigation, unneutralized and diluted Morpholine (one part Morpholine, two parts water) was applied at a daily dosage of 0.9 g/kg to the clipped skin of the midsection of a second group of seven rabbits. All rabbits died before the eleventh dose. Necrosis of the treated skin, and inflammation and congestion of the underlying organs, were evident upon gross examination. Microscopic lesions of the liver included necrosis, congestion, cloudy swelling, fibrosis, and fatty change. The kidneys had an "abnormal amount of secretion" in the tubules, and the spleen was congested. The lungs and stomach were normal under nontreated sites; however, these organs were "friable" when under the area of direct Morpholine application. The skin was necrotic and congested after the ninth and tenth applications. The heart appeared normal on microscopic examination.

Inhalation

Lam and Van Stee[81] reported that the inhalation LC50 values for morpholine in male and female rats were 2250 and 2150 ppm, respectively. In male and female mice, the LC50 values were 1450 and 1900 ppm, respectively. No other details were available.

Rats were exposed by inhalation for 4 h to Morpholine at 260, 40, or 3 mg/m^3. Morpholine toxicity was evaluated by measuring respiratory rate, lung weight, and uptake of stain by lung tissue. To measure the degree of lung staining, a 1% solution of neutral red stain was injected into the tail vein after the animals were exposed to Morpholine. The rats were killed 1 and 10 min later. Subsequently, 0.5 g sections of the lungs were removed to extract the stain. Measurements at 1 and 10 mins corresponded to points of maximum accumulation and secretion of the dye, based on observations in preliminary experiments. In undamaged cells, the dye was taken up, stored as a granule, and then removed. Damaged cells lost the ability to collect the stain in a granule; instead, the nucleus and cytoplasm became stained by diffusion. At 260 mg/m^3, the investigators observed an increase in respiratory rate, but no effect on the weight of the lungs. The exposed rats also retained a greater amount of stain than did controls after 1 and 10 min. Rats exposed to a Morpholine dosage of 40 mg/m^3 eliminated less stain from pulmonary tissue after 10 mins than did controls. No changes in respiratory rate or lung weight were observed at dosages of 40 or 3 mg/m^3. The investigators reported the test for stain removal from pulmonary tissue to be the most sensitive indicator of irritation.[4,5,83]

Ivanov and Germanova[84] reported that the minimum irritating value of Morpholine for rats exposed to Morpholine containing air for 4 h was 2.6 times higher than the values for human subjects.

Results of additional acute inhalation studies are summarized in Table 9.

Intraperitoneal

Four male Carworth Farm mice were given 350 mg/kg of Morpholine by single intraperitoneal injection. Relative to untreated controls, treated mice

TABLE 9. Effects of Acute Inhalation Exposure to Morpholine

Species	Concentration ppm	Concentration mg/m^3	Exposure time (h)	Effects	References
Rat	"Pure vapor"		4	4/6 deaths; all animals had irritation of the eyes, nose and extremities. Yellow fur observed	37
Rat	12,000	—	8	No deaths	14
Rat	8,497	29,740	8	1/6 deaths; survivors had normal weight gain. No gross lesions at necropsy	85, 37
Rat	8,000	28,000	8	0/6 deaths during the 14 days following the single exposure	73
Rat	6,734	23,569	4	0/12 deaths; signs of irritation and stained hair, but normal weight gain during the 14 day observation period. No gross lesions at necropsy	84, 37
Rat	6,285	22,000	1	Lacrimation, rhinitis, inactivity	86
Rat (male)	2,250	—	—	LC50 = 2,250 ppm	81
Rat (female)	2,150	—	—	LC50 = 2,150 ppm	81
Rat	—	3, 40, 260	4	260 mg/m^3 increased respiratory rate, but no effect on lung weight. No changes in lung weight or respiratory weight at 40 or 3 mg/m^3	83, 4, 5
Mouse	1,391	4,869	—	LC50 = 1,391 ppm	87
Mouse (male)	1,450	—	—	LC50 = 1,450 ppm	81
Mouse (female)	1,900	—	—	LC50 = 1,900 ppm	81

had no changes in erythrocyte or leukocyte counts. An intraperitoneal LD50 of 413 ± 26 mg/kg was estimated. Surviving mice were necropsied 72 h after the single injection. No gross or microscopic lesions were noted in the lymph nodes, bone marrow, lungs, kidneys, or gastrointestinal tract of treated mice. "Questionable damage" was observed in the spleen, and the testes had "mild damage." The earliest change in the spleen was usually hypocellularity of the red pulp. Testicular changes included nuclear pyknosis, mitotic arrest, disruption of the spermatogenic layers, and desquamation and fusion of spermatogenic cells.[79]

Morpholine was administered by i.p. injection to groups of dd strain mice (six animals/group). A single dose of 500 mg/kg caused sedation and bradypnea. A single Morpholine dose of 100 mg/kg produced piloerection. An intraperitoneal LD50 of 1350 mg/kg was estimated.[80]

Short-Term Toxicity

Oral

Male rats were administered Morpholine in the diet at dosages of either 323, 93.1 or 27.6 mg/kg/day for 4 weeks. An increase in weight of adrenal glands and a lower mean body weight gain were observed in animals treated with 323 mg/kg (3500 ppm). Lower dosages (93.1 or 27.6 mg/kg) had no

apparent adverse effects. Histopathologic evaluation was not mentioned; however, no gross lesions were found at necropsy.[1,86]

Shea[18] evaluated the short-term oral toxicity of Morpholine (purity ≥ 98%). The compound was administered for 30 consecutive days by stomach tube at dosages of 0.8, 0.16, and 0.32 g/kg to three groups of rats (20 rats per group). Morpholine was "diluted so that approximately 2 ml was delivered at each dose." Observations of rats treated with 0.8 g/kg included lethargy, weight loss, "shock," intense irritation of the intestinal tract, and congestion of the gastric mucosa. Microscopic changes in the liver included cloudy swelling, marked congestion, hemorrhage, necrosis, and an increase in Kupffer and connective tissue cells. The kidneys showed tubular degeneration, swelling, and necrosis with desquamation of the necrotic epithelium. The spleens of the 0.8 g/kg group animals were congested and had increased hemosiderin deposits. Necrosis of the mucosal epithelium and congestion was observed in the stomach. The lungs had congestion and alveolar cell desquamation. Ten rats of the 0.8 g/kg groups survived to the 20th dose whereas only one rat survived to the end of the 30 day study.

In rats exposed to 0.32 g/kg, 12 rats survived all 30 doses. Only 8 of these 12 surviving animals gained weight. Gross lesions included congestion of the stomach and lungs. At microscopic examination, the tissues from five rats of the 0.32 g/kg group had similar changes as in the 0.8 gm/kg group, "but to a lesser degree." Of 20 rats exposed to 0.16 g/kg, 12 survived. No gross lesions were noted. Microscopic changes were assessed in three rats of this group. Changes in the liver included congestion, swelling, and a marked increase in Kupffer and connective tissue cells. The kidneys had congestion and cloudy swelling, with areas of necrosis and epithelial cell desquamation. The lungs had a few foci of congestion, and the stomach had necrosis of the mucosa. The spleen appeared normal.

The short-term toxicity of Morpholine also was evaluated in guinea pigs. The compound was administered orally for 30 days at dosages of 0.45, 0.18, 0.09, or 0.0 g/kg to 4 groups of guinea pigs. The control group had 6 animals; each treatment group had 20 guinea pigs. Signs in animals fed 0.45 g/kg included prostration, sneezing, and coughing. Only three animals of the high-dose group gained weight, and only four survived the study. Gross lesions were "congestion and dark organs." Microscopic lesions of the liver included congestion, cloudy swelling, an increase in the number of Kupffer cells, areas of necrosis, and fatty change. The renal lesions included necrotic tubules, cloudy swelling of tubular epithelium, and congestion. At microscopic examination, the lungs were congested and had thickened pleura. Necrosis was observed in the gastric mucosa; after the 20th dose, no mucosa remained. Of 20 guinea pigs given 0.18 g/kg, 8 survived. "Irritation" was the "chief gross pathology." Microscopic lesions were the same as in the 0.45 g/kg group, "but of less severity." Seventeen guinea pigs of the 0.09 g/kg group survived. Weight gains of this group were greater than those of the control group, and no gross lesions were observed at necropsy. Microscopic changes included cloudy swelling of both the renal tubules and hepatocytes. The spleen, lungs, and stomach of animals in the low-dose group appeared normal.[18]

Dermal

Shea[18] also evaluated the dermal toxicity and skin absorption of Morpholine in two studies using guinea pigs. In the first study, undiluted neutralized Morpholine (adjusted to pH 7.0 with sulfuric acid) was applied daily for 30 days to the clipped skin of the midsection of three guinea pigs. Dosages of 0.9, 0.18, and 0.27 g/kg were applied (one animal/dose). The three animals gained weight during the period of application. No gross or microscopic lesions were observed, except that the skin was thickened at the site of application. In the second study, undiluted, unneutralized Morpholine was applied daily at a dosage of 0.9 g/kg to the clipped skin of the midsection of seven guinea pigs. No animal survived beyond the 13th application. All animals lost weight and were moribund before death. Gross lesions were necrotic and edematous skin at the treated sites, with congestion of the underlying organs. Microscopic lesions of the skin included edema of the dermal layer and necrosis and sloughing of the epidermis. In the liver, Kupffer cells were increased, and the hepatic changes included hemorrhage, cirrhosis, and fatty degeneration. Renal lesions were tubular necrosis, hemorrhage, excessive secretion into renal tubules, congestion, tubular swelling, and thickening of the capsule of Bowman. The spleen appeared normal.

Inhalation

The subchronic inhalation toxicity of Morpholine (purity $\geq$ 99%) was assessed by Conway et al.[21] Sprague-Dawley rats were exposed to chamber concentrations of either 0, 25, 100, or 250 ppm 6 h/day, 5 days/week for 13 weeks. Treated and control groups each consisted of 20 males and 20 females (40 rats per group). Ten rats of each sex and at each dosage were necropsied at 7 weeks; the remaining animals were necropsied at the end of week 13. No animals died during the 13 week exposure. Irritant effects of Morpholine exposures were generally noted in the 250 ppm group; a reddish discharge or crust was observed around the nose, mouth, and eyes after the first week. Salivation also was observed. Slight rapid breathing was noted in all experimental groups, but not in the control group. Body weight gains for the 100 ppm females were greater than those of the control group after weeks 2, 3, and 6–13, and the mean weight of the 250 ppm males was consistently lower than the mean weight of male controls during weeks 6–13. No other treated animals differed from controls in body weight. At the end of weeks 7 and 13, gross lesions of the lungs were red, white, and dark foci on the pleural surface, which "were possibly treatment related." Lung sections of all rats killed after 7 weeks contained early lesions of chronic murine pneumonia. By week 13, these lesions had increased in severity in the 250 ppm group. Some of the livers of treated rats had rounded margins, but the liver/body weight ratios were not significantly increased, and there were no compound-related histomorphologic alterations. Rats exposed to 250 ppm had focal erosion and focal squamous metaplasia of the mucosa of the maxilloturbinates; effects were observed in 6 of 10 males and 2 of 10 females after week 7. There also was a sporadic increase of secretions in the Harderian gland sections. Almost all rats of the 250 ppm group necropsied after 13 weeks had comparable

changes; the lesions, which were increased in incidence and severity, involved the nasal septum and anterior nasal cavities in addition to the nasoturbinates and maxilloturbinates. Focal necrosis involved the mucosa and necrotic cell debris was observed in the nasal cavity of two females of the 100 ppm group, but not in males exposed to 100 ppm. The investigators suggested that the lesions of the nasal mucosa, nasal septum, maxilloturbinates, and nasoturbinates in rats exposed to 250 and 100 ppm were entirely consistent with the irritating properties of Morpholine. Nasal lesions were not observed in rats from the 25 ppm group or the control group. Hepatic and renal lesions were observed in all experimental groups, but these lesions were not considered to be compound related since they occurred in the animals of the control group as well. No significant compound-related effects were noted in treated groups with respect to organ weights, or results of hematologic tests, clinical chemistry analysis, or urinalysis. Tests of the urine included pH, specific gravity, measurement of glucose, albumin, and occult blood levels, volume, and microscopic examination of sediment. Blood samples were evaluated for hematocrit, hemoglobin, red and white blood cell count, and differential leukocyte count. Clinical chemistry parameters examined included total protein, albumin, A/G ratio, calcium, sodium, potassium, alkaline phosphatase, total bilirubin, blood urea nitrogen, glucose, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, inorganic phosphorus, total cholesterol, total lipids, and triglycerides. Organs and tissues examined at necropsy included brain, adrenal glands, lungs, heart, liver, spleen, kidneys, testes, ovaries, and nasal cavity.

Rats were exposed by inhalation to either 2000 ppm Morpholine 4 h/day for 4 days, or 450 ppm Morpholine 4 h/day, 5 days/week for 30 days. Body weight, lung weight, residual volume, total lung capacity, and the "single breath diffusing capacity of the lungs for carbon monoxide" were measured daily in the 2000 ppm group and on the 30th day in the 450 ppm group. The rate of body weight gain was less in both of the treated groups compared to the control rats. Lung weight, residual volume, and total lung capacity were increased, and diffusion capacity of the lungs was decreased independent of body weight in rats exposed to 2000 ppm Morpholine. In rats inhaling 450 ppm Morpholine, residual volume and total lung capacity were increased independently of body weight. The investigators concluded that in both exposure groups Morpholine had a toxic effect on the lungs of the rat independent of body weight changes.[88]

Lam and Van Stee[81] reported that rats were exposed by inhalation to 450 ppm Morpholine 6 h/day, 5 days/week for 8 weeks. Animals were killed weekly to evaluate morphologic changes in the lungs and other major organs. Morpholine produced "changes to the sensory areas... such as the eyes and nose." Feed consumption and body weight gains were depressed and the organ to body weight ratios were increased for the lungs and kidneys. No other details were available.

Inhalation of Morpholine at 8 mg/m^3 (2.3 ppm) for 4 h/day, 5 days/week for 2 months resulted in reversible hypotension and leukopenia in rats. The author also found an increase in the number of chromosomal aberrations of bone marrow cells at this concentration and suggested a maximal permissible

concentration of 0.5 mg/m^3 for Morpholine.[89] These data have not been confirmed.

An unspecified number of rats were exposed by inhalation to 18,000 ppm Morpholine (purity ≥ 98%). Exposures were 8 h a day for 5 days. Irritation of the eyes, nose, and thoracic walls was observed. After the first day one rat died. The kidneys and liver were congested. A second rat, which died on the third day, had pulmonary and renal congestion. A third rat died on the fourth day and had degeneration of the epithelial cells of renal tubules. Three rats died after termination of exposure on the fifth day. Thickened alveoli, emphysematous areas, necrosis of renal tubules, and areas of degeneration, fatty change and necrosis of the liver were noted.[18]

Chronic Toxicity: Inhalation

A chronic inhalation study of Morpholine was conducted in "60 Sprague-Dawley rats/sex/group" at exposure concentrations of 0, 10, 50, and 150 ppm for 6 h per day, 5 days/week for 104 weeks. Body weight gains, organ weights, hematologic and clinical chemistry findings were normal in the exposed groups and comparable to the control animals. There were no exposure-related adverse changes observed in the liver, kidneys, brain, intestines, lungs, or any other internal organ or tissue. It was reported that the incidences of neoplasms were comparable among all groups, including the control group, and were typical of the strain and age of the rats. Adverse effects observed during the study included irritation of the eyes, nose, and skin. Eyes showed corneal changes (keratitis) at the highest exposure concentration of 150 ppm. Near the end of the study, corneal opacities were observed. Cataracts were noted in a few rats, but these findings were not believed to be significant. A sex-related increased incidence of retinal degeneration was noted in the high-exposure females. The observed ocular and nasal lesions were consistent with the irritating properties reported for Morpholine.[14] Further details of this unpublished study are not currently available.

Rats and guinea pigs were exposed by inhalation to 70 or 8 mg/m^3 of morpholine for 4 h/day, 5 days/week for 4 months. The investigators examined peripheral blood, lungs, liver, and kidneys, measured nervous system activity (by an undefined summary-threshold index), arterial pressure, and respiratory rate, and looked for chromosomal aberrations in bone marrow cells in anaphase and telophase. The results of the study are summarized in Table 10. Morpholine had its most damaging effect on the spleen. Exposure to 70 mg/m^3 of morpholine resulted in destruction of the lymphoid cells. This effect was not reversible 1 month after exposure to Morpholine ended.[4,5,89]

Ocular Irritation

Severe corneal necrosis was observed in a range-finding study in which the eyes of albino rabbits were treated with 40% Morpholine in either propylene glycol, water, or deodorized kerosene. The dosage of the test solution was 0.005 ml; however, the number of applications and the number of animals tested were not reported.[73,90]

TABLE 10. Effects of Chronic Inhalation Exposure to Morpholine on Rat and Guinea Pig[4,5,89]

Concentration	*Index of effect*	*Species*	*Effect*
70 mg/m^3	Nervous system activity	Rat	Initial increase, followed by return to control levels
		Guinea pig	Initial decrease; increase by end of 4 months
	Arterial pressure	Rat	Initial increase; decrease by 2nd month
	Peripheral blood	Rat	Increase in hemoglobin and red blood cell count; decrease in leukocytes at 1st and 4th months
		Guinea pig	Decrease in hemoglobin and leukocyte
	Electrocardiogram	Rat	No change
	Organ function	Rat	No changes in liver, kidneys, and testes
		Guinea pig	No changes in kidneys, and testes; change in liver function
	Morphology	Rat, guinea pig	Swelling of alveoli and atrophy of respiratory lymphatics; atrophy of lymphoid elements of the spleen even in animals killed 1 month after exposure ended
	Mutagenesis	Rat	Increase in number of chromosomal aberrations resulting from fragmentation
8 mg/m^3	Nervous system activity	Rat	Increase through 1st month of exposure
	Arterial pressure	Rat	Decrease by 2nd month
	Peripheral blood	Rat	Decrease in lymphocytes at 2nd month
		Rat, guinea pig	No changes in liver function
	Organ function	Rat	No changes in liver function
	Morphology	Rat, guinea pig	Decrease in size of lymph nodes of spleen; effect not observed in animals killed 1 month after exposure ended
	Mutagenesis	Rat	Increased in number of chromosomal aberrations although not significantly greater than spontaneous rate

Following neutralization with hydrochloric acid, Morpholine (0.02 M in water) had no injurious effect on the eyes of rabbits "when applied continuously for 10 min." The corneal epithelium had previously been removed to facilitate penetration of the compound. Morpholine was also neutralized with salicylic acid to form morpholine salicylate. The irritative action of 10–20% aqueous solutions of morpholine salicylate on rabbit eyes was considerably less than from unneutralized Morpholine.[91] No other details were available.

Morpholine caused moderate ocular injury with ulceration of the conjunctiva and clouding of the cornea when instilled as a single dose into the eyes of rabbits. A score of 7 on a scale of 1–10 was recorded at the 24 h evaluation.[91] No other details were given.

Albino rabbits, three of each sex, were used in both a dermal irritation study and an ocular irritation study using a mascara composite containing 1% Morpholine. Into one eye of each rabbit was instilled 0.1 ml of the cosmetic daily for 14 days. Before the application of the cosmetic each day, the eye was evaluated for ocular irritation. There was a slight redness of the conjunctiva for the duration of the study; this cleared within 24 h of the last treatment. A

sodium fluorescein dye test performed at the conclusion of the study indicated no abnormalities of the corneal or iris membranes.[78]

Draize irritation scores of 80–110 (maximum score = 110) were reported for undiluted Morpholine, indicating that the compound was corrosive to the rabbit eye.[14] No other data were available. Another report indicated that the Draize irritation score in rabbits for undiluted Morpholine (0.1 ml) was 67.7/110, indicating severe ocular irritation.[1]

Skin Irritation

Undiluted Morpholine was applied in a single 0.01 ml dose to the clipped skin of five albino rabbits. Necrosis was observed within 24 h.[73] It should be noted that the author only reported the most severe reaction in the group of rabbits and not the reaction of each rabbit. Therefore, the number of rabbits with dermal necrosis could not be ascertained.

Skin irritation scores for Morpholine in rabbits were 6.6–8.0 (maximum score = 8.0), indicating "corrosive" effects on the skin.[14]

Another report indicated that 0.5 ml of undiluted Morpholine was "corrosive" to rabbit skin.[1]

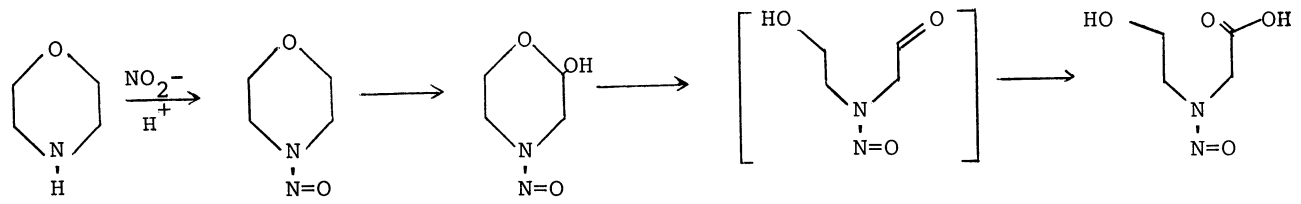
To the normal or abraded skin of six albino rabbits, three of each sex, was applied 0.5 ml of a mascara composite containing 1% Morpholine. The application was made daily for 14 days, and the animals were collared to prevent them from cleaning the treated sites. At the end of the 14 day treatment period no dermal toxicity or irritation was observed. All of the rabbits gained an average of 190 g during this study.[78]

IN VIVO FORMATION OF *N*-NITROSOMORPHOLINE

As a weak base, Morpholine is easily nitrosated by nitrous anhydride; the reaction rate is pH dependent. Weak bases are more susceptible to nitrosation because they are more likely to exist in an un-ionized state at physiological pH. Nitrosation of Morpholine produced *N*-nitrosomorpholine (NMOR), a potent animal carcinogen.[6]

In studies with mice, Morpholine administered orally and nitrogen dioxide given by inhalation reacted to form NMOR,[98] which resulted in an increase in pulmonary adenomas.[70] Small amounts of NMOR were present in mice exposed to Morpholine and atmospheric NO_2.[99] It is not known whether NMOR was formed in vivo on exposure to NO_2, or whether NO_2 exposure produced a nitrosating agent in vivo that reacted with Morpholine in tissue to produce NMOR.[100,101] The endogenous formation of NMOR in rats gavaged with Morpholine and nitrite was demonstrated by the identification in the urine of the NMOR metabolite, *N*-nitroso(2-hydroxyethyl)glycine[102] (Fig. 5).

Groups of three to four mice were given Morpholine by gavage and were subsequently exposed to nitrogen dioxide at concentrations of 0.2–50 ppm for up to 4 h. The powdered extracts of whole mouse bodies were obtained after the mice had been frozen and pulverized in liquid nitrogen, and NMOR



Morpholine N-Nitrosomorpholine N-Nitroso(2-Hydroxyethyl)-Glycine

FIG. 5. NMOR metabolite *N*-nitroso(2-Hydroxyethyl)-Glycine

concentrations were determined. NMOR yields were nitrogen dioxide concentration and time dependent. Smaller amounts of NMOR were found when mice were exposed to nitrogen dioxide, given Morpholine, and then immediately powdered. Similar smaller amounts were observed when Morpholine was added to powdered mice that had been exposed to nitrogen dioxide prior to being powdered. Only very small amounts of NMOR were observed in mice given Morpholine and exposed only to air. NMOR was undetectable in mice not given Morpholine or exposed to nitrogen dioxide only.[98]

Mirvish[103] repeated the experiment by Iqbal et al.,[98] but instead used a method of analyzing for NMOR that prevented NMOR formation after the mice had been powdered. He concluded that the NMOR found in the powdered mice in the previous experiment was an artifact. He found that a nitrosating agent was formed in vivo from nitrogen dioxide and that it produced NMOR during analysis of the powdered mice. Mirvish[103] gavaged rats with Morpholine, exposed them to nitrogen dioxide, and did not find NMOR in the rat bodies. However, rats gavaged with Morpholine and sodium nitrite contained large amounts of NMOR.

In another experiment, Mirvish et al.[101] found that the nitrosating agent formed upon exposure of mice to nitrogen dioxide in an inhalation chamber could be extracted with ether from the aqueous homogenate of the whole animal. This ether extract was capable of *N*-nitrosating Morpholine. About 88% of the nitrosating agent formed upon exposure of mice to nitrogen dioxide was located in the skin, one-third of which was in the hair. It was later found that the major nitrosating agent formed in the skin after exposure to NO_2 is cholesteryl nitrite.[104]

In a study by Van Stee et al.,[99] groups of male mice were exposed to nitrogen dioxide 3–6 h each day for 5 days, or were gavaged with 1 g/kg Morpholine, or were exposed to nitrogen dioxide and also were gavaged with Morpholine. The researchers used the analytical method of Mirvish[103] as well as another method utilizing a different means of preventing artifactual NMOR production. The findings of these researchers were contrary to those of Mirvish. NMOR was found in the bodies of mice exposed to nitrogen dioxide and Morpholine, but not in those exposed to either chemical alone. NMOR was found in the whole animals and in the intestinal tract (one-third of that found) but not in the heart plus lungs. Coadministration of sodium ascorbate or α-tocopheryl acetate had no effect on the amount of NMOR in any tissue. The researchers concluded that there was in vivo formation of significant quantities of NMOR.

The endogenous formation of NMOR has so far not been demonstrated in humans. This may be explained by the fact that NMOR was quickly metabolized by α-hydroxylation and the uptake of Morpholine was too small to yield detectable quantities of the NMOR metabolite *N*-nitroso(hydroxyethyl)-glycine in the urine. However, the in vivo formation of the nonmetabolizing *N*-nitrosoproline (NPRO) and its identification in human urine[105] strongly suggested that upon inhalation of NO_x or ingestion of *N*-nitrosating agents (NO_2^- and organic nitrosating compounds) all readily nitrosatable amines, especially Morpholine, can be endogenously converted to *N*-nitrosamines. This has been shown by increased formation of NPRO in humans who were

on a diet rich in nitrite,[105] and in cigarette smokers who inhaled NO_x as a smoke constituent.[106–109] The fact that thiocyanate catalyzed endogenous *N*-nitrosation[23] was important because thiocyanate was the major detoxification product of hydrogen cyanide, which was ingested with certain food items, and also inhaled as a constituent of tobacco smoke. There was further proof of endogenous nitrosations in that ingestion of ascorbic acid, an inhibitor of *N*-nitrosation, was successful in preventing the endogenous formation of NPRO in smokers.[106]

A study was undertaken to determine the possibility of the nitrosation of Morpholine in the human stomach. Morpholine was 100% nitrosated under the optimal conditions of pH 3, temperature 37°C, reaction time 6 h , and amine and nitrite concentrations of 0.02 mol/L and 0.04 mol/L, respectively. When the reaction was carried out in human gastric juice (at a less favorable pH) for a shorter time period (to approximate conditions in the human stomach), nitrosation of the Morpholine did occur, leading the author to conclude that there is a potential cancer risk involved in the consumption of amines and nitrites.[110]

GENOTOXICITY

Morpholine was weakly mutagenic without S9 fraction in the L5178 mouse lymphoma assay, and caused significant increases in the number of type III foci in the BALB/3T3 malignant cell transformation assay. The compound was tested at five different dosages (0.001–0.3 μl/ml). Relative to controls, cell survival was 49% or greater. In the BALB/3T3 mouse fibroblast transformation assay, a definite dose–response relationship was observed.[2,111] Morpholine also was reported "slightly positive" in the sister chromatid exchange test; no other details were available.[2]

Because this heterocyclic amine was weakly mutagenic in the BALB/3T3 in vitro transformation assay, sister chromatid exchange assay and the mouse lymphoma forward mutation assay, a major U.S. producer of the compound recommended in 1980 that Morpholine not be used in foods.[52]

Morpholine was evaluated by the plate incorporation assay of the *Salmonella*/mammalian microsome mutagenicity test described by Ames et al.[112] Five Morpholine dosages (10–5000 μg) and five *Salmonella typhimurium* strains (TA1535, TA1537, TA1538, TA98, TA100) were tested, both in the presence and absence of S9 liver fraction from Aroclor-1254-induced rats. No mutagenicity was observed.[113]

"Practical-grade" Morpholine was tested in vitro for mutagenicity in *Salmonella* strains TA98, TA100, TA1535, and TA1537. The investigators used a preincubation modification of the *Salmonella*/microsome test using Aroclor-induced rat and Syrian hamster liver S9. No mutagenicity was observed either with or without S9 at dosages ranging up to 10.0 mg/plate.[4]

Morpholine (purity = 99.2%) and a series of morpholine derivatives were evaluated for the induction of DNA repair in rat hepatocyte primary culture/DNA repair assay. Cellular toxicity was observed at the highest

Morpholine dosage examined (1 mg/ml); however, no induction of DNA repair occurred at nontoxic Morpholine concentrations (0.0001–0.1 mg/ml). Two animal metabolites of Morpholine, *N*-methylmorpholine oxide and *N*-hydroxymorpholine, also did not induce DNA repair at the nontoxic concentrations tested (0.0001–10 mg/ml and 0.0001–1.0 mg/ml, respectively). A putative metabolite of Morpholine, 3-Morpholone, also was inactive; however, the chemical intermediate *N*-hydroxyethylmorpholine induced DNA repair in the dosage range 1–5 mg/ml. It was concluded that genotoxicity of substituted Morpholines was a function of the substituent moiety rather than Morpholine itself.[22]

Morpholine and sodium nitrite were tested for mutagenicity in the intrahepatic host-mediated assay using female CD-1 mice. *Salmonella typhimurium* 1530 was injected intravenously into the mice 10 min prior to oral intubation of Morpholine or sodium nitrite. The mice were killed at specified times and the bacteria were subsequently recovered from the liver and scored for histidine revertants. No mutagenic activity above background was observed when Morpholine (4, 8, 20, 40 μg/g) or sodium nitrite (120 μg/g) was intubated alone. However, when administered sequentially in combination with sodium nitrite (120 μg/g), Morpholine (40 μg/g) caused an increase in reversion frequency that was 58 times the background. The lowest Morpholine dosage tested (4 μg/g) in combination with sodium nitrite (120 μg/g) caused a nine-fold increase in histidine reversions over the background. The investigators estimated that under acidic conditions (pH 3.4) approximately 12–19% of the administered Morpholine doses were nitrosated to NMOR in the presence of excess nitrite. Gavage of sodium thiocyanate (120 μg/g), a known catalyst of nitrosation, enhanced NMOR formation as measured by mutagenicity. Gavage of ascorbic acid (120 or 360 μg/g), a known inhibitor of nitrosation, caused a dosage-related inhibition of NMOR mutagenicity.[96]

Morpholine was assessed for mutagenicity in a second host-mediated assay. Male Swiss albino mice were given intraperitoneal injections of *Salmonella typhimurium* G-46. This treatment was followed by oral or intramuscular administration of up to 500 mg/kg Morpholine. Three hours after Morpholine exposure, the mice were killed and the bacteria in peritoneal exudates were examined for genetic mutations. Morpholine was inactive as a mutagen when given by oral or intramuscular routes of administration.[114]

Braun et al.[95] treated NMRI mice with Morpholine concentrations of 1450, 2175, or 2900 μmoles/kg by gavage in the intraperitoneal host-mediated assay. No mutagenic activity was noted in the indicator organism, *Salmonella typhimurium* TA 1950. In the same study, oral administration of equimolar concentrations of sodium nitrite and Morpholine (1450, 2175, 2900 μmol/kg) produced a mutagenic response. This mutagenic response was observed only when the sodium nitrite was administered either concurrently with Morpholine or 10 min after Morpholine. Oral administration of sodium nitrite 10 min prior to Morpholine produced no mutagenic response. At concentrations up to 2900 μmol/kg, sodium nitrite by itself did not produce mutagenic activity.

The transplacental mutagenesis of products formed in the stomach of Syrian golden hamsters administered sodium nitrite and Morpholine was

studied by Inui et al.[97] Pregnant hamsters were given either Morpholine (500 mg/kg) or Morpholine (500 mg/kg) plus sodium nitrite (500 mg/kg) by stomach tube on day 11 or 12 of pregnancy. The amounts of NMOR recovered from the stomachs of the hamsters treated with Morpholine plus sodium nitrite ranged from a high of 1.94 mg after 1 h to 0.59 h after treatment. Twenty-four hours after treatment, the hamster embryos were excised and examined for chromosomal aberrations, micronuclear formation, morphologic or malignant transformation, and drug resistance mutation. Cells exposed in utero to Morpholine alone had no increases in chromosomal aberrations, micronuclei, 8-azaguanine- or ouabain-resistant mutants, or transformation rates. The number of 8-azaguanine- and oubain-resistant colonies was markedly increased after administration of Morpholine plus sodium nitrite; the embryonic fibroblasts also had a marked increase in frequency of micronucleation and a slight increase in chromosome aberrations. Treatment with Morpholine plus sodium nitrite also caused morphologic or malignant transformation of fetal cells. After cultivation in vitro, cells from some transformed colonies produced tumors when inoculated into the cheek pouch of golden hamsters. Orally administered *N*-nitrosomorpholine (positive control) had the same transplacental biological actions on embryonic fibroblasts. Morpholine alone was "ineffective" as a transplacental mutagen.

Biochemical cytogenetic (bone marrow cells) and mutagenic determinations (dominant lethal test) were performed in rats receiving chronic dietary administration of sodium nitrite and Morpholine given separately or in combination. No dominant lethal or cytogenetic effects were noted in rats receiving 1% dietary sodium nitrite for 31 weeks, or 1% dietary sodium nitrite for 15 weeks followed by the combination of 1% sodium nitrite and 0.5% Morpholine for 16 more weeks. The surviving rats in the latter group had hepatic neoplasms and a lower protein and RNA content and higher DNA content than the nontreated rats.[115]

Migukina[89] exposed rats by inhalation to Morpholine at 8 or 70 mg/m^3 for 4 months and reported a dosage-related increase in chromosomal aberrations.

Results of two studies indicated that Morpholine was negative in the "promoting activity test."[116,117] In the first of these studies,[116] Morpholine in water was administered in a single oral dose to Sprague Dawley rats. Forty-eight hours later, the animals were killed and the adrenal glands removed and prepared for microscopic examination. The number of adrenocortical mitoses was then determined and compared to those of controls. The "predictive" test described by Danz[116] was based on the observation that some carcinogens cause a mitotic stimulation of adrenocortical epithelia in vivo. This effect was postulated to be "brought about by the emergence of humoral growth stimulators, which are evidenced in restorative regeneration and therefore accounts for the promoting activity of carcinogens."[116] No details were available for the second study.[117]

Morpholine (10 μg) was pyrolyzed at 300, 400, 500, and 600°C for 3 min, and the pyrolysates were assayed for mutagenicity according to the method of Ames.[112,118] Rat liver microsomal fraction (S9 mix) was used to provide metabolic activation. Pyrolysates at 500° and 600°C were mutagenic in *Salmonella typhimurium* strains TA98 and TA100.[119]

CARCINOGENICITY

Shank and Newberne[94] investigated the carcinogenicity of Morpholine and sodium nitrite in a two generation feeding study. Pregnant Sprague-Dawley CD rats and pregnant Syrian golden hamsters were fed, from the time of conception, an agar-gel diet containing various concentrations of Morpholine and/or sodium nitrite, or *N*-nitrosomorpholine. When the F_0 females littered, rats and hamsters of both sexes were randomly selected from the F_1 generation for carcinogenicity studies; for the rat studies an F_2 generation was derived from the F_1 dams. The studies were terminated with the killing of surviving F_2 rats at week 125 and of 110-week-old hamsters. Dietary concentrations and tumor incidences for the experimental groups are summarized in Tables 11 and 12. Hepatocellular carcinoma and sarcomas of the liver and lungs were the most common tumors observed in the rats. The neoplasms induced by nitrite and Morpholine were morphologically similar to those induced by NMOR. High concentrations of nitrite and Morpholine together were carcinogenic to rats. When the Morpholine concentration was reduced and the nitrite concentration remained high, the incidence of hepatic cell carcinoma decreased with a linear dose–response relationship. With high Morpholine concentration and decreasing nitrite concentrations, the number of hepatic tumors was sharply reduced. No hepatic or pulmonary tumors were observed in the control group, although other tumors were seen in group 3. The high concentration of Morpholine alone was either weakly carcinogenic or nitrite from an unknown source was present. Animals in group 3 also developed two malignant gliomas, which are rare in this rat species. In the rats fed the high nitrite concentration alone, there was a high incidence of tumors of the lymphoreticular system and a large number of animals developed tumors other than hepatocellular neoplasms and angiosarcomas. The researchers suggested that Morpholine itself may be a hazardous compound and that it is likely that nitrosation occurs in the stomachs of rats. In 16 hamsters fed the high dietary concentrations of nitrite and Morpholine together, 5 hepatic cell carcinomas and 1 pulmonary cystadenoma were observed. This regimen was carcinogenic; other diets produced fewer tumors. Hamsters were more resistant to tumor induction by nitrite and Morpholine than were rats. Similar results were described by these same investigators in earlier reports.[120,121]

Groups of seven female Sprague-Dawley rats were fed sodium nitrite and Morpholine concurrently in the diet at concentrations of 0.5% each for 8 weeks and were observed until they died. Equal numbers of animals received either 0.5% Morpholine alone or 0.5% sodium nitrite alone for 8 weeks. All animals that received Morpholine plus nitrite developed hepatocellular adenomas and, with one exception, hepatocellular carcinomas. Two animals had hemangioendotheliomas of the liver, and one animal had a hepatic tumor diagnosed as a cyst-adenocarcinoma. Renal adenoma was found in one animal. Morpholine or nitrite given alone did not induce tumors.[122]

Groups of 20 female and 20 male Swiss mice were given 0.08 g/L NMOR in the drinking water for life. Corresponding groups received 6.33 g/kg Morpholine in the diet concurrently with 1.0 g/L sodium nitrite in the drinking water. Controls were treated with the same concentrations of Mor-

TABLE 11. Incidence of Tumors Among Rats Fed Experimental Diets[94]

	Dietary level (ppm)				*Incidence (percentage) of*					*Age at death with liver carcinoma (wk)*	
Test group	*Sodium nitrite*	*Morpholine*	*N-Nitrosomorpholine*	*No. of rats*[a]	*Liver cell carcinoma*	*Liver angiosarcoma*	*Lung angiosarcoma*	*Other angiosarcoma*	*Metastases from liver to lung*	*First death*	*Median*[b]
1	0	0	0	156	0	0	0	0	—	—	—
2	1000	0	0	96	1	0	0	1	0	123	—
3	0	1000	0	104	3	0	2	1	0	68	—
4	1000	1000	0	159	97	14	23	1	49	19	38
5	1000	50	0	117	59	5	6	0	17	47	111
6	1000	5	0	154	28	12	8	1	7	24	—
7	50	1000	0	109	3	2	1	0	0	89	—
8	5	1000	0	172	1	2	1	1	0	64	—
9	50	50	0	152	2	1	1	1	0	65	—
10	5	5	0	125	1	2	2	1	0	88	—
11	0	0	5	128	58	15	9	1	22	53	106
12	0	0	50	94	93	21	20	1	58	30	56

[a] F_1 and F_2 generations combined.
[b] Age (in weeks) by which 50% of the population had died with hepatocellular carcinoma.

TABLE 12. Incidence of Tumors Among Hamsters Fed Experimental Diets[94]

Test group	Dietary level (ppm)			No. of animals	Liver cell carcinomas		No. of angiosarcomas	No. and type of other tumors
	Sodium nitrite	Morpholine	N-nitroso morpholine		No.	%		
1	0	0	0	23	1	4	4	0
2	1000	0	0	30	0	0	1 (sc)	0
3	0	1000	0	22	0	0	0	0
4	1000	1000	0	16	5	31	0	1 lung cyst adenoma
5	1000	50	0	32	0	0	0	1 malignant lymphoma 1 keratinizing acanthoma of stomach 1 adrenal adenoma
6	1000	5	0	40	0	0	0	1 liver cyst adenoma
7	50	1000	0	22	0	0	1 (spleen)	1 tumor of adrenal glomerulosa
8	5	1000	0	19	0	0	0	0
9	50	50	0	30	0	0	1 (spleen)	1 malignant lymphoma
10	5	5	0	40	0	0	0	1 malignant lymphoma with leukemia 1 ovarian adenocarcinoma 1 cyst adenoma of bile duct
11	0	0	5	35	0	0	0	0
12	0	0	50	18	1	6	1 (liver)	0

pholine or sodium nitrite alone or left untreated. At 40 weeks, all survivors were killed. Of those treated with NMOR, 16 of 22 animals (73%) developed pulmonary adenomas (total number, 106); in addition, 4 hepatic cell carcinomas and 1 papillary adenoma of the bile duct were observed. Of those that received concurrent administration of Morpholine and sodium nitrite, 20 of 35 (57%) had a total of 41 pulmonary adenomas. Treatment with sodium nitrite alone or with Morpholine alone produced no effects in the mice when compared with untreated controls.[92] In the same study,[92] high levels of dietary sodium ascorbate (vitamin C) given concurrently with Morpholine and sodium nitrite either diminished the incidence of neoplasms or increased the induction period. When 5.75, 11.5, or 23.0 g/kg of dietary sodium ascorbate were administered to A strain mice that also received 6.33 g/kg of Morpholine and 2.0 g/L of sodium nitrite in their drinking water, a 72–89% inhibition of adenoma formation was observed.[92]

Pulmonary adenomas were induced in mice fed (2–6 g/kg in the diet) Morpholine, piperazine, *N*-methylaniline, methylurea, and ethylurea and given drinking water containing sodium nitrite (1 g/L) for 6 months. The feeding of dimethylamine and nitrite under the same conditions did not induce tumors. When the piperazine concentration was kept constant and nitrite concentrations were varied, the pulmonary adenoma yield was approximately proportional to the nitrite concentration squared. The addition of sodium ascorbate to the diets decreased tumor numbers. When Morpholine was fed to the mice and the drinking water contained sodium nitrite, hepatic cell tumors were produced and were attributed to the in vivo production of NMOR. The

addition of sodium ascorbate decreased the number of hepatic cell tumors, but gastric papillomas and carcinomas were observed. The latter were not seen in the mice treated with Morpholine and sodium nitrite without sodium ascorbate. It was suggested that the sodium-ascorbate-treated mice did not die early of hepatic tumors and, therefore, lived long enough to develop NMOR-induced gastric tumors. The experiment was repeated; in the second trial Morpholine and sodium nitrite and sodium ascorbate may have induced acanthosis and hyperkeratosis of the squamous portion of the stomach.[103]

Groups of 40 male MRC Wistar rats were treated for 2 years either with Morpholine (10 g/kg food) together with sodium nitrite (3 g/L drinking water) or with *N*-nitrosomorpholine (0.15 g/L drinking water). In both cases, a group of rats were given sodium ascorbate (22.7 g/kg food) in addition to these treatments. When ascorbate was present in the diet, the hepatic tumors induced by Morpholine plus nitrite had a longer induction period (93 vs. 54 weeks), a slightly lower incidence (49% vs. 65%), and no metastases to the lungs, indicating that ascorbate had reduced the in vivo formation of *N*-nitrosomorpholine. Sodium ascorbate did not alter hepatocellular tumor induction by the preformed *N*-nitrosomorpholine. Of those treated with Morpholine, nitrite, and ascorbate, 21 of 39 animals developed gastric tumors (14 squamous-cell papillomas, 7 squamous-cell carcinomas).[93]

Groups of 35 male CD-1 mice were exposed to nitrogen dioxide (1–2 ppm in air, 6 h/day, 5 days/week for 30 weeks) and/or Morpholine (0.1% in drinking water for 30 weeks). Control mice received water and conditioned air. Pulmonary concentrations of Morpholine averaged 23.5 ± 27.8 μg/g of tissue (wet weight) during the first 15 weeks of exposure. The observed rates of pulmonary adenomas in the various groups were: controls (3.8%; 1 of 26); NO_2 (3.8%; 1 of 26); Morpholine (7.1%; 2 of 28); and NO_2 plus Morpholine (21.2%; 7 of 33). Results of statistical analyses suggested that the combination of nitrogen dioxide (NO_2) plus Morpholine increased the probability of the occurrence of pulmonary adenoma.[123]

A chronic inhalation study of Morpholine was conducted in "60 Sprague-Dawley rats/sex/group" at exposure concentrations of 0, 10, 50, and 150 ppm for 6 h/day, 5 days/week for 104 weeks. Body weight gains, organ weights, hematologic findings, and clinical chemistry results were normal in the exposed groups and comparable to the control animals. There were no exposure-related adverse changes observed in the liver, kidneys, brain, intestines, lungs, or any other internal organ or tissue. The incidences of neoplasms were comparable among all groups, including the control group, and were typical of the strain and age of the rats.[14] Further details of this unpublished study are not currently available.

CLINICAL ASSESSMENT OF SAFETY

Skin, Eye, and Mucous Membrane Irritation

Occlusive patch testing, using the Shelanski/Jordan repeat insult procedure, and in-use testing of two mascara products containing 1% Morpholine were undertaken using 320 women between the ages of 18 and 65. The in-use testing was structured so that 50 women used the charcoal mascara once daily

for 4 weeks, while another 50 women did the same with blue mascara. In addition, 100 women used mascara, lash conditioner, and eye makeup remover daily, with half of the subjects using each color of mascara. The other 100 women did not use either of the mascara products, but they were involved in the patch testing of these products.[124]

Following the patching procedure listed above, the products to be tested were placed on occlusive patches, which were then placed on the cleaned area of the subjects' backs. The patches remained in place for 24 h and were then removed. The sites were then graded according to the method of Alexander A. Fisher, M.D. This procedure was repeated every Monday, Wednesday, and Friday for $3\frac{1}{2}$ weeks (a total of 10 applications). There was a 10 day–2 week rest period following the tenth application, after which the subjects were again patched, with the patches remaining in place for 48 h. After the 48 h, the sites were again scored. One week to 10 days after this the subjects were again patched for 48 h, and the sites were read, as before, after the removal of the patches. Twenty-four hours after this reading, a final reading was done.[124] The conclusion drawn from this study was that neither of the mascara products containing 1% Morpholine was a primary irritant nor were they contact sensitizers.[124]

Of the 320 subjects patch tested with charcoal mascara, 314 had no reaction. The remaining six subjects had varying reactions. The researchers concluded that the irritation experienced by these six subjects was either nonspecific or due to the occlusive patching procedure.[124]

As with the patch test results with charcoal mascara, 314 of the 320 subjects tested with blue mascara had no reactions. The conclusion reached by the researchers with regard to the six subjects who did have reactions was the same as that reached for the reactions of the subjects tested with charcoal mascara: the irritation was either nonspecific or due to the patch testing procedure.[124]

Any complaints associated with the in-use testing of the products were few and were due to improper usage of those products, which then resulted in the product entering the eye and causing mild irritation.[124]

Secondary references reported that Morpholine is a cutaneous, ocular, and mucous membrane irritant, and a skin sensitizer.[15,16,19] Windholz[8] reported that Morpholine was "corrosive" to human skin, and Hawley[1971] reported that the compound was absorbed through the skin. Morpholine also was reported as moderately toxic by ingestion and inhalation,[15,16] and highly toxic by the dermal route.[16]

A manufacturer of Morpholine reported that Morpholine was "extremely irritating" to the ocular mucosa, and that the substance has the potential to cause "permanent eye injury." The vapor may cause transient corneal edema (hazy vision). It was reported that Morpholine was "corrosive" to the skin, and could be "expected to cause severe skin damage with burns and blistering." It also was indicated that "toxic effects due to skin absorption" were possible, and that the compound "may cause irritation" of the upper respiratory tract.[14]

The American Conference of Governmental Industrial Hygienists[17] reported that concentrated Morpholine readily permeates the skin. They noted

that the undiluted compound was very irritating to the eyes, and there was a moderately high degree of hazard by skin contact. The hazard diminished as the product was diluted to less than 25% with water. This group also reported that industrial use of Morpholine has resulted in instances of respiratory tract irritation.

Exposure Limits

The American Conference of Governmental Industrial Hygienists[17,125] has adopted for Morpholine a "threshold limit value–time weighted average" (TLV–TWA) and a "threshold limit value–short term exposure limit" (TLV–STEL) of 20 ppm (70 mg/m^3) and 30 ppm (105 mg/m^3), respectively. The TLV–TWA is defined as the airborne concentration for a normal 8-h workday and a 40-h workweek to which nearly all workers may be repeatedly exposed without adverse effect. The TLV–STEL is the maximal airborne concentration to which workers can be exposed for a period up to 15 min without causing irritation, chronic or irreversible tissue change, or necrosis. It was stated that a TLV of 20 ppm would be low enough to prevent irritation and harmful effects on the eyes and vision. The threshold limit values for Morpholine also carried a "skin" notation, indicating that skin absorption can be an important route of exposure. The threshold limit values are used as guides in the control of health hazards, and are not intended to be used to differentiate between safe and unsafe concentrations.

West Germany and the USSR have adopted workplace limits for Morpholine of 20 ppm and 0.13 ppm, respectively.[17]

SUMMARY

Morpholine is a heterocyclic secondary amine; it is a clear liquid with a characteristic amine order. Morpholine is a solvent and solutions of Morpholine are basic, though Morpholine itself is a weak base. Known impurities in Morpholine may include traces of arsenic and lead, and sometimes *N*-hydroxyethyl-morpholine. Since Morpholine is readily nitrosated, the potential exists for the formation of *N*-nitrosomorpholine when Morpholine is used in formulations. *N*-nitrosomorpholine is carcinogenic in laboratory animals. Morpholine has a wide variety of industrial applications, and it has been approved as an indirect food additive in various categories by the FDA. In cosmetics, Morpholine is used as a surfactant and an emulsifier. It is used most often in mascara, eyeliners, eyeshadows, and skin care preparations; in these uses, it may be in contact with the skin and eye for extended periods of time.

Morpholine was largely unmetabolized by rats, dogs, and rabbits, while in guinea pigs it was extensively metabolized by *N*-methylation and *N*-oxidation. Most of the Morpholine administered to rats and hamsters was excreted unchanged in the urine.

The LD50 values of Morpholine in various types of laboratory animals have been reported. In mice, the i.p. LD50 was between 0.413 and 1.35 g/kg, depending on the strain of mouse. Dermal LD50s in rabbits ranged between

0.31 and 1.21 g/kg, and oral LD50s in rats were between 1.05 and 1.63 g/kg. In guinea pigs the oral LD50 was 0.9 g/kg.

Unneutralized solutions of Morpholine caused severe corneal necrosis, but upon neutralization Morpholine was not injurious to rabbit eyes.

Morpholine in its undiluted form was corrosive to rabbit skin, while a mascara formulation containing 1% Morpholine was nonirritating to rabbit skin.

In studies of acute and short-term dermal toxicity Morpholine as an undiluted and unneutralized solution or as a diluted and unneutralized solution applied daily to the skin of guinea pigs and rabbits, respectively, caused the deaths of the test animals within 2 weeks. In both cases, the skin was necrotic.

In rats, the short-term oral administration of Morpholine at various dosages caused swelling, congestion, and necrosis of various organs. At higher dosages, the rats died. The results in guinea pigs were similar to those in rats.

The greatest effects observed upon acute inhalation exposure to Morpholine were irritation to the eyes and nose and increased respiratory rate.

In short-term inhalation studies (in rats) with varying concentrations of Morpholine, the effects observed included irritation of the mucous membranes and an increased respiratory rate. Nasal lesions, as well as red, white, and dark foci in the lungs, were noted. In another study using rats, the weight, residual volume, and total capacity of the lungs were increased, while the diffusing capacity of the lungs was decreased. Another author reported an increase in chromosomal aberrations in rat bone cells but these results have not been confirmed.

Chronic inhalation studies in rats and guinea pigs led to the observations that the exposure to Morpholine caused changes in the nervous system activity (rats and guinea pigs), the arterial blood pressure, and the peripheral blood (rats) at both high and low concentrations. There were no changes in the functions of the liver, kidneys, and testes, with the exception of liver function in guinea pigs at the higher dosage. At the higher concentration of Morpholine, the lesions included swelling of the alveolar cells and atrophy of lymphoid elements in the spleen; these effects were still obvious 1 month after the Morpholine exposure had ended. At the lower concentration of Morpholine a decrease in the size of the lymph nodules in the spleen was noted, but this effect was not observed 1 month after exposure had ended. Chromosomal aberrations were noted at both dosages, but those at the lower dosages were not significantly greater than the control rate.

Nitrosation of Morpholine produces *N*-nitrosomorpholine, which has been mutagenic in a variety of test systems. Simultaneous exposure of laboratory animals to Morpholine and nitrites has caused a number of different cancers. Exposure to Morpholine combined with the inhalation of NO_2 increased the incidence of pulmonary adenomas in mice; *N*-nitrosomorpholine was present in the lungs of mice exposed to both Morpholine and atmospheric NO_2.

Endogenous formation of *N*-nitrosomorpholine in humans has not been demonstrated, but the presence of *N*-nitrosoproline in human urine suggests that nitrosation does occur in humans. Morpholine can be nitrosated in human gastric juice in the presence of nitrites.

Morpholine was a weak positive mutagen in L5178 mouse lymphoma assay, in BALB/3T3 malignant cell transformation and fibroblast transformation assays, and in sister chromatid exchange assays. Morpholine was negative for mutagenicity in the Ames test in both the presence and absence of the S9 rat liver fraction; a modification of this same test also produced negative results. At nontoxic dosages Morpholine did not increase the rate of DNA repair in rat hepatocytes. In the intrahepatic host-mediated assay with mice Morpholine alone was negative for the production of revertants, but when Morpholine and sodium nitrite were administered in combination, reversions were significantly increased. Results from other host-mediated assays were the same. In a transplacental mutagenesis study in hamsters, the combination of Morpholine and sodium nitrite caused an increase in micronucleation and chromosome aberrations in embryonic fibroblasts; morphologic or malignant transformations of fetal cells were also noted. Pyrolysates of Morpholine at 500 and 600°C were mutagenic in the Ames test.

A carcinogenic response was produced in rats in a long-term feeding study of Morpholine in which nitrites were present in the diet. It was suggested that the Morpholine was nitrosated in the stomachs of the test animals. Morpholine and nitrites were also carcinogenic to hamsters, although hamsters appeared to be more resistant than rats to the carcinogenic effects. The most common neoplasms reported in rats were sarcomas, adenomas, and carcinomas of the liver. Other neoplasms of the liver were also noted. In mice treated with Morpholine and nitrites, pulmonary adenomas were the most common neoplasms.

Addition of sodium ascorbate had an inhibitory effect on the carcinogenicity of the Morpholine–nitrite combination. There was an increase in the incidence of gastric neoplasms in test animals fed diets with added sodium ascorbate (with Morpholine and nitrites present); this was attributed to increased longevity, or as a consequence of a reduced incidence of hepatic neoplasms. The increase in gastric neoplasms after the administration of sodium ascorbate was observed in both rats and mice.

In humans, Morpholine was a skin, eye, and mucous membrane irritant, and a skin sensitizer. Morpholine was absorbed through the skin, by which route it was highly toxic; the toxicity diminished when Morpholine was diluted to less than 25%. Eye irritation from Morpholine vapor may lead to corneal edema, a condition resulting in "hazy" or "halo" vision.

Results of a patch test using a panel of human subjects with a mascara formulation containing 1% Morpholine indicated that the mascara was not an irritant or sensitizer.

DISCUSSION

The Panel is aware of reports indicating that Morpholine is an occupational irritant and sensitizer; however, cosmetic formulations containing Morpholine have produced neither irritation nor sensitization.

Morpholine is not considered to be an animal carcinogen. It reacts easily with nitrosating agents resulting in the formation of *N*-nitrosomorpholine.

Under conditions of use, it is highly unlikely that Morpholine is totally free of carcinogenic nitrosoamines. The Expert Panel cannot conclude that Morpholine is safe without quantitative data regarding the formation of *N*-nitrosomorpholine under conditions of use.

Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the CIR Procedures, the Expert Panel informed the public of its decision that the data on Morpholine are insufficient to determine whether this ingredient, under each relevant condition of use, is either safe or not safe. The Panel released a "Notice of Insufficient Data Announcement" on June 29, 1987 outlining the data needed to assess the safety of Morpholine. The type of information required was either analytical in-use data or an appropriate risk assessment.

No response to the Notice of Insufficient Data was received within an appropriate time period.

CONCLUSION

The safety of this ingredient has not been documented and substantiated. The CIR Expert Panel cannot conclude that Morpholine is safe for use in cosmetic products until such time that the appropriate safety data have been obtained and evaluated.

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REFERENCES

1. REINHARDT, C.F., and BRITTELLI, M.R. (1981). Heterocyclic and miscellaneous nitrogen compounds. Morpholine. Cited in: Clayton, A.D., and Clayton, F.E. (eds.). *Patty's Industrial Hygiene and Toxicology*, 3rd ed., vol. 2. New York: John Wiley and Sons, pp. 2693–96.
2. TEXACO CHEMICAL CO. (1982). Technical brochure on Morpholine. Available from Texaco, Inc., White Plains, New York.
3. NIENEKER, D.L. (1967). Morpholine. *Kirk-Othmer Encyclopedia of Chemical Technology*, 2nd ed. **13**, 659–70.
4. NATIONAL RESEARCH COUNCIL (1981). *Selected Aliphatic Amines and Related Compounds: An Assessment of the Biological and Environmental Effects*. Washington, DC: National Academy Press. NTIS No. PB83-133066.
5. NATIONAL RESEARCH COUNCIL (1983). An assessment of the health risks of morpholine and diethylaminoethanol. Prepared for the Army Medical Research and Development Command by the Committee on Toxicology Under Contract DAMD-17-82-C-3028. NTIS No.: PB85-122661.
6. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC). (1978). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 17. Some *N*-Nitroso Compounds. Lyon: International Agency for Research on Cancer, pp. 263–80.

7. ESTRIN, N.F., CROSLEY, P.A., and HAYNES, C.R. (1982). *CTFA Cosmetic Ingredient Dictionary*, 3rd ed. Washington, DC: The Cosmetic, Toiletry and Fragrance Association.
8. WINDHOLZ, M. (1983). *The Merck Index*, 10th Ed. Rahway, NJ: Merck and Co., p. 899, no. 6137.
9. MACKISON, F.W. (September, 1978). *Picket Guide to Chemical Hazards*. Washington, DC: U.S. Dept. of H.E.W. NIOSH/OSHA, p. 136–37.
10. ESTRIN, N.F., HAYNES, C.R., and WHELAN, J.M. (1982). *CTFA Compendium of Cosmetic Ingredient Composition. Specifications/Spectra*. Washington, DC: Cosmetic, Toiletry and Fragrance Association.
11. WEAST, R.C. (1982). *CRC Handbook of Chemistry and Physics*, 63rd ed. Boca Raton, FL: CRC Press, p. C-378.
12. BROWN, A.R. (1966). Morpholine: Its properties and uses. Manufact. Chem. Aerosol News **37**(12), 50–2.
13. MJOS, K. (1978). Cyclic amines. In: Grayson, M., and EcKroth, D. (eds.). *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed., vol. 2. New York: John Wiley and Sons, pp. 295–308.
14. TEXACO, INC. (1985). Unpublished data submitted to CIR by Texaco, Inc. Executive Summary: Chronic Inhalation Study of Morpholine in Rats. Letter to Jonathon T. Busch (Cosmetic Ingredient Review) from Mary Jane Von Allmen (Sr. Project Safety Administrator). Texaco, Inc., Research Environment and Safety Dept., October 29, 1985.
15. HAWLEY, G.G. (1971). *The Condensed Chemical Dictionary*, 8th ed. New York: Van Nostrand Reinhold, p. 596.
16. SAX, I.N. (1979). *Dangerous Properties of Industrial Materials*, 5th ed. New York: Van Nostrand Reinhold, p. 841.
17. AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS, INC. (1980). Morpholine. In: *Documentation of the Threshold Limit Values*, 4th ed. Cincinnati, OH, p. 291.
18. SHEA, T.E., JR. (1939). The acute and subacute toxicity of Morpholine. J. Ind. Hyg. Toxicol. **21**(7), 236–45.
19. GREENBERG, L.A., LESTER, D., and HAGGARD, H.W. (1954). *Handbook of Cosmetic Materials*. New York: Interscience Publishers, p. 191.
20. AMOORE, J.E., and HAUTALA, E. (1983). Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J. Appl. Toxicol. **3**(6), 279–90.
21. CONAWAY, C.C., COATE, W.B., and VOELKER, R.W. (1984). Subchronic inhalation toxicity of morpholine in rats. Fund. Appl. Toxicol. **4**, 465–72.
22. CONAWAY, C.C., TONG, C., and WILLIAMS, G.M. (1984). Evaluation of morpholine, 3-morpholinone, and N-substituted morpholines in the rat hepatocyte primary culture/DNA repair test. Mutat. Res. **136**(2), 153–7.
23. SPIEGELHALDER, B., and PREUSSMANN, R. (1984). Contamination of toiletries and cosmetic products with volatile and nonvolatile N-Nitroso carcinogens. J. Cancer Res. Clin. Oncol. **108**, 160–3.
24. THERMO ELECTRON CORPORATION (April 30, 1985). Identification of Nitrosamines and Nitrosating Agents in Cosmetic Products—II. Quarterly Report to Food and Drug Administration, FDA contract no. 223-84-2052, 90 pp.
25. GILBERT, R., RIOUX, R., and SAHEB, S.E. (1984). Ion chromatographic determination of morpholine and cyclohexylamine in aqueous solutions containing ammonia and hydrozine. Anal. Chem. **56**(1), 106–9.
26. SINGER, G.M., and LIJINSKY, W. (1976). Naturally occurring nitrosatable compounds. I. Secondary amines in food stuffs. J. Agric. Food Chem. **24**(3), 550–3.
27. LORANT, B. (1977). Thermogravimetric determination of basic and intermediary substances in cosmetics. Seifen. Ole. Fette. Wachse **103**(14), 393–6.
28. KARWEIK, D.H., and MEYERS, C.H. (1979). Spectrophotometric determination of secondary amines. Anal. Chem. **51**(2), 319–20.
29. BURENKO, T.S., ZHURAVLEV, E.G., and MIKLASHEVICH, T.A. (1977). Determination of morpholine in air. Gig. Tr. Prof. Zabol. **3**, 55–6.
30. NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (1977). Morpholine. In: *NIOSH Manual of Analytical Methods*, 2nd ed., vol. 3. Cincinnati, OH: U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, pp. S150-1–S150-9.
31. BIANCHI, A., and MUCCIOLI, G. (1978). Gas chromatographic determination of morpholine in the presence of isopropanol, toluene, and xylene in the work atmosphere. Ann. Ist. Super. Sanita. **14**(3), 441–5.

32. HAMANO, T., MITSUHASHI, Y., and MATSUKI, Y. (1981). Glass capillary gas chromatography of secondary amines in foods with flame photometric detection after derivatization with benzenesulfonyl chloride. Agric. Biol. Chem. **45**(10), 2237–44.
33. BRUNNEMANN, K.D., SCOTT, Y.C., and HOFFMANN, D. (1982). *N*-nitrosomorpholine and other volatile *N*-nitrosamines in snuff and tobacco. Carcinogenesis **3**(6), 693–6.
34. SOHN, O.S., FIALA, E.S., CONWAY, C.C., and WEISBURGER, J.H. (1982). Separation of morpholine and some of its metabolites by high-performance liquid chromatography. J. Chromatogr. **242**(2), 374–80.
35. TANAKA, A., TOKIEDA, T., NAMBARU, S., OSAWA, M., and YAMAHA, T. (1978). Excretion and distribution of morpholine salts in rats. J. Food. Hyg. Soc. **19**(3), 329–34.
36. TOMBROPOULOS, E.G. (1979). Micromethod for the gas chromatographic determination of morpholine in biological tissues and fluids. J. Chromatogr. **164**(1), 95–9.
37. CARPENTER, C.P. (1983). Morpholine. In: *Encyclopedia of Occupational Health and Safety*. pp. 1406–7.
38. SHANK, R.C., and MAGEE, P.N. (1981). Toxicity and carcinogenicity of *N*-nitroso-compounds. In: Shank, R.C. (ed.), *Mycotoxins and N-Nitroso Compounds: Environmental Risks*, vol. 1. Boca Raton, FL: CRC Press, pp. 185–217.
39. NATIONAL RESEARCH COUNCIL, COMMITTEE ON NITRITE AND ALTERNATIVE CURING AGENTS IN FOOD. (1981). *The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds*. Washington, DC: National Academy Press.
40. CHALLIS, B.C., FINE, D.H., GOFF, E.U., HOFFMAN, G.A., and SHUKER, D.E.G. (1982). Amine nitrosation and nitrosation by gaseous nitrogen dioxide. In: Bartsch, H., Oneil, I.K., Castegnaro, M., and Okada, M. (eds.). *N-Nitroso Compounds: Occurrence and Biological Effects*. IARC Scientific Publication No. 41. Lyon, France: IARC, pp. 11–20.
41. RIDD, J.H. (1961). Nitrosation, diazotisation, and deamination. *Q. Rev. Chem. Soc.* **15**, 418–441.
42. MIRVISH, S.S. (1975). Formation of *N*-nitroso compounds: Chemistry, kinetics, and *in vivo* occurrences. Toxicol. Appl. Pharmacol. **31**, 325–51.
43. TURNEY, T.A., and WRIGHT, G.A. (1959). Nitrous acid and nitrosation. Chem. Rev. **59**, 497–513.
44. MIRVISH, S.S. (1970). Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. J. Natl. Cancer Inst. **44**, 633–39.
45. WING, E.G. (1980). Development and analysis of noncorrosive aqueous alcohol formulations for use with the "Aquasol" valve. Soap Cosmet. Chem. Spec. 56(5):40, 40A, 40B, 50.
46. CODE OF FEDERAL REGULATIONS. (April 1, 1984). Title 21 Part 172.235. Coatings, films and related substances. Morpholine.
47. CODE OF FEDERAL REGULATIONS. (April 1, 1984). Title 21 Part 178.3300. Corrosion inhibitors used for steel or tinplate.
48. CODE OF FEDERAL REGULATIONS. (April 1, 1984). Title 21 Part 176.210. Defoaming agents used in the manufacture of paper and paperboard.
49. CODE OF FEDERAL REGULATIONS. (April 1, 1984). Title 21 Part 175.105. Substances for use only as components of adhesives. Adhesives.
50. CODE OF FEDERAL REGULATIONS. (April 1, 1984). Title 21 Part 178.3120. Animal glue.
51. CODE OF FEDERAL REGULATIONS. (April 1, 1984). Title 21 Part 173.310. Boiler water additives.
52. MONAGHAN, P.R. (1980). Letter dated July 14, 1980 to Morpholine customers: Results of recent tests conducted by Texaco indicate that morpholine may be weakly mutagenic. Belaire, TX: Texaco Chemical Company, 2 pp. [As cited in National Research Council, 1983, p. 11].
53. EVANS, E. (1971). Systemic fungicides in practice. Pestic. Sci. **2**(5), 192–6.
54. RINZLER, C.A. (1977). *Cosmetics: What the Ads Don't Tell You*. New York: Thomas Y. Crowell.
55. FOOD AND DRUG ADMINISTRATION. (Dec. 22, 1981). Cosmetic product formulation data: Ingredients used in each product category. Computer printout. Washington, D.C.
56. FOOD AND DRUG ADMINISTRATION. (Jan. 5, 1982). (a) Frequency of Trade Name Ingredients in the Cosmetic Product File, and (b) Frequency of Common, Usual, or Chemical Names in the Cosmetic Product File. Computer printout. Washington, D.C.
57. FOOD AND DRUG ADMINISTRATION. (1986). Cosmetic product formulation data: Ingredients used in each product category. Washington, D.C.
58. CODE OF FEDERAL REGULATIONS. (April 1, 1984). Title 21 Part 720.4 Voluntary filing of cosmetic product ingredient and cosmetic raw material composition statement. Information requested about cosmetic products.
59. EUROPEAN ECONOMIC COMMUNITY (EEC). (1986). The EEC Cosmetics Directive. Updated version —incorporating all amendments until 15th June 1986. J. Dupuis, ed. Annex II, No. 344.

60. DUPUIS, J. (1987). Personal communication. COLIPA.
61. SAVOLAINEN, H., and ROSENBERG, C. (1983). Morpholine vapor inhalation and interactions of simultaneous nitrite intake: Biochemical effects on rat spinal cord axons and skeletal muscle. Arch. Toxicol. **53**(2), 143–50.
62. KUNG, H.C., and WILSON, A.G.E. (1978). Effect of morpholine on the pulmonary clearance of 5-hydroxytryptamine. Fed. Proc. **37**(3), 222.
63. TOMBROPOULOS, E.G., VAN STEE, E.W., WILSON, A.G.E., and HOOK, G.E.R. (1979). Tissue distribution of inhaled morpholine and its effects on lung lavages. Fed. Proc. 38/3, no. 1873.
64. TOMBROPOULOS, E.G., KOO, J.O., GIBSON, W., and HOOK, G.E.R. (1983). Induction of morpholine of lysosomal α-mannosidase and acid phosphatase in rabbit alveolar macrophages in vivo and in vitro. Toxicol. Appl. Pharmacol. **70**(1), 1–6.
65. TOMBROPOULOS, E.G., KOO, J.O., GIBSON, W., and HOOK, G.E.R. (1982). The induction of α-mannosidase in alveolar macrophages by morpholine. Fed. Proc. 41/5, no. 8506.
66. MALLER, R.K. and HEIDELBERGER, C. (1957). Cancer Res. 17:284. [As cited in Oelschlaeger and Temple, 1978, p. 115.]
67. SOHN, O.S., FIALA, E.S., CONWAY, C.C., and WEISBURGER, J.H. (1982). Metabolism and disposition of morpholine in the rat, hamster, and guinea pig. Toxicol. Appl. Pharmacol. **64**(3), 486–91.
68. OHNISHI, T. (1984). Studies on mutagenicity of the food additive Morpholine (fatty acid salt). Jpn. J. Hyg. **39**(4), 729–48.
69. RHODES, C., and CASE, D.E. (1977). Non-metabolite residues of ICI 58,834 (viloxazine). Studies with ^{14}C-morpholine, ^{14}C-ethanolamine and ^{14}C-glyoxylate. Xenobiotica **7**(1–2), 112 (Abstract).
70. VAN STEE, E.W., WYNNS, P.C., and MOORMAN, M.P. (1981). Distribution and disposition of morpholine in the rabbit. Toxicology **20**(1), 53–60.
71. OELSCHLAEGER, H., and TEMPLE, D.J. (1978). The role of *N*-oxidation in the metabolism of morpholine containing drugs. In: Gorrod J.W. (ed.). *Biological Oxidation of Nitrogen.* Proc. Int. Symp., 2nd meeting. Amsterdam: Elsevier/North-Holland Biomedical Press, pp. 71–82.
72. GRIFFITHS, M.H. (1968). The metabolism of *n*-triphenylmethylmorphine in the dog and rat. Biochem. J. **108**, 731–740.
73. SMYTH, H.F., CARPENTER, C.P., WEIL, C.S., and POZZANI, U.C. (1954). Range-finding toxicity data. List V. Arch. Ind. Hyg. Occup. Med. **10**:61–8.
74. THOMPSON, W.R. (1947). Use of moving averages and interpolation to estimate median-effective dose. Bacteriol. Rev. **11**, 115–45.
75. WEIL, C.S. (1952). Tables for convenient calculation of median-effective dose and instructions in their use. Biometrics **8**, 249–63.
76. SMYTH, H.F., WEIL, C.S., WEST, J.S., and CARPENTER, C.P. (1969). An exploration of joint toxic action: Twenty-seven industrial chemicals intubated in rats in all possible pairs. Toxicol. Appl. Pharmacol. **14**, 340–7.
77. SMYTH, H.F. WEIL, C.S., WEST, J.S., and CARPENTER, C.P. (1970). An exploration of joint toxic action, II. Equitoxic versus equivolume mixtures. Toxicol. Appl. Pharmacol. **17**, 498–503.
78. COSMETIC, TOILETRY AND FRAGRANCE ASSOCIATION (CTFA). (May 3, 1977). Industry submission of unpublished data. Acute oral and subacute dermal and ocular irritation of mascara composite containing Morpholine. (3-11-1).
79. LANDING, B.H., GOLDIN, A., NOE, H.A., and GOLDBERG, B. (1949). Systemic pathological effects of nitrogen mustards, and a comparison of toxicity, chemical structure, and cytotoxic effect, with reference to the chemotherapy of tumors. Cancer, November: 1055–66.
80. KASE, Y., MIYATA, T., and YUIZONO, T. (1967). Pharmacological activities of alicyclic amines. I. Comparison of pharmacological activities of piperidine with those of other amines. Jpn. J. Pharmacol. **17**(3), 475–90.
81. LAM, H.F., and VAN STEE, E.W. (1978). A reevaluation of the toxicity of morpholine. Fed. Proc. **37**(3), 679 (Abstract No. 2459).
82. DRAIZE, J.H., WOODARD, G., and CALVERY, H.O. (1944). Methods for study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. **82**, 377–90.
83. IVANOV, N.G., GERMANOVA, A.L., KLYACKINA, A.M., MAKSIMOV, G.G., and POZDNYAKOV, V.S. (1973). Comparative evaluation of the methods of finding the irritating action of industrial toxins and the calculations of their maximum permissible concentrations in the air of a work zone. In: Letavet, A.A. and Sanotskiy, I.V. (eds.). *The Toxicology of New Industrial Chemical Substances*, Issue No. 13,

p. 19–28. Moscow: USSR Academy of Medical Sciences (Translated from the Russian). [As cited In National Research Council, 1981, pp. 105–107.]
84. IVANOV, N.G., and GERMANOVA, A.L. (1973). Comparative sensitivity of animals and man to the effects of irritant toxins. In: Latavet, A.A., and Sanotskiy, I.V. (eds.). *The Toxicology of New Industrial Chemical Substances*. Issue No. 13. Moscow: USSR Academy of Medical Sciences, pp. 36–41 and abstract. [As cited in: National Research Council, 1983, pp. 2–3.]
85. INTERNATIONAL LABOUR OFFICE. (1972). In: *Encyclopedia of Occupational Health and Safety*, Vol. 2. Geneva, Switzerland: International Labour Office, pp. 915–916. [As cited in National Research Council, 1981a; 1983.]
86. INDUSTRIAL BIO-TEST LABORATORIES, INC. (1970). Bio-Fax Data Sheet: Morpholine. Northbrook, IL: Industrial Bio-Test Laboratories, Inc. [As cited in: National Res. Council, 1981; 1983, and Reinhardt and Brittelli, 1981.]
87. ZAEVA, G.N., TIMOFIEVSKAYA, L.A., BAZAROVA, L.A., and MIGUKINA, N.V. (1968). Comparative toxicity of a group of cyclic imino compounds. Toksikol. Nov. Prom. Khim Veshchestv **10**, 25–35. Chem. Abstract 71:47804w, 1969. [As cited in: National Research Council, 1981a; 1983].
88. TAKEZAWA, J., and LAM, H.F. (1978). Toxic effect of morpholine on rat lungs. Fed. Proc. **37**(3), 247. Abstract.
89. MIGUKINA, N.V. (1973). Evaluation of the danger of morpholine during chronic action. In: Letavet, A.A., and Sanotskiy, I.V. (eds.). *The Toxicology of New Industrial Chemical Substances*, Issue No. 13, pp. 87–94. Moscow: USSR Academy of Medical Sciences. (Translated from the Russian) [As cited in: National Research Council, 1981a; 1983].
90. CARPENTER, C.P., and SMYTH, H.F. (1946). Chemical burns of the rabbit cornea. Am. J. Ophthalmol. **29**, 1363–72.
91. GRANT, W.M. (1974). *Toxicology of the Eye*, 2nd ed. Springfield, IL: Charles C. Thomas, pp. 722–3.
92. GREENBLATT, M., MIRVISH, S., and SO, B.T. (1971). Nitrosamine studies. Induction of lung adenomas by concurrent administration of sodium nitrite and secondary amines in Swiss mice. J. Natl. Cancer Inst. **46**, 1029–34.
93. MIRVISH, S.S., PELFRENE, A.F., GARCIA, H., and SHUBIK, P. (1976). Effect of sodium ascorbate on tumor induction in rats treated with morpholine and sodium nitrite, and with nitrosomorpholine. Cancer Lett. **2**, 101–8.
94. SHANK, R.C., and NEWBERNE, P.M. (1976). Dose–response study of the carcinogenicity of dietary sodium nitrite and morpholine in rats and hamsters. Food Cosmet. Toxicol. **14**, 1–8.
95. BRAUN, R., SCHONEICH, J., and ZIEBARTH, D. (1977). *In vivo* formation of *N*-nitroso compounds and detection of their mutagenic activity in the host-mediated assay. Cancer Res. **37**(12), 4572–79.
96. EDWARDS, G., WHONG, W.Z., and SPECINER, N. (1979). Intrahepatic mutagenesis assay: A sensitive method for detecting *N*-nitrosomorpholine and *in vivo* nitrosation of morpholine. Mutat. Res. **64**(6), 415–23.
97. INUI, N., NISHI, Y., TAKETOMI, M., MORI, M., YAMAMOTO, M., YAMADA, T., and TANIMURA, A. (1979). Transplacental mutagensis of products formed in the stomach of golden hamsters given sodium nitrite and morpholine. Int. J. Cancer **24**(3), 365–72.
98. IQBAL, Z.M., DAHL K., and EPSTEIN, S.S. (1980). Role of nitrogen dioxide in the biosynthesis of nitrosamines in mice. Science **207**, 1475–77.
99. VAN STEE, E.W., SLOANE, R.A., SIMMONS, J.E., and BRUNNEMANN, K.D. (1983). *In vivo* formation of *N*-nitrosomorpholine in CD-1 mice exposed by inhalation to nitrogen dioxide and by gavage to morpholine. J. Natl. Cancer Inst. **70**(2), 375–79.
100. MIRVISH, S.S., ISSENBERG, P., and SAMS, J.P. (1981). *N*-nitrosomorpholine synthesis in rodents exposed to nitrogen dioxide and morpholine. In: Scanlan, R.A., and Tannenbaum, S.R. (eds.). *N-Nitroso Compounds*. ACS Symposium Series 174. Washington, DC: American Chemical Society.
101. MIRVISH, S.S., SAMS, J.P., and ISSENBERG, P. (1983). The nitrosating agent in mice exposed to nitrogen dioxide: Improved extraction method and localization in the skin. Cancer Res. **43**, 2550–54.
102. HECHT, S.S., and MORRISON, J.B. (1984). A sensitive method for detecting *in vivo* formation of N-nitrosomorpholine and its application to rats given low doses of morpholine and sodium nitrite. Cancer Res. **44**, 2873–77.
103. MIRVISH, S.S. (1982). *In vitro* formation of *N*-nitroso compounds: Formation from nitrite and nitrogen dioxide and relation to gastric cancer. Banbury Report **12**:227–41.
104. MIRVISH, S.S., BABCOCK, D.M., DESHPANDE, A.D., and NAGEL, D.O. (1986). Identification of cholesterol as a mouse skin lipid that reacts with nitrogen dioxide to yield a nitrosating agent and of

cholesteryl nitrite as the nitrosating agent produced in a chemical system from cholesterol. Cancer Letters. **31**, 97–104.
105. OHSHIMA, H., and BARTSCH, H. (1981). Quantitative estimation of endogenous nitrosation in humans by monitoring *N*-nitrosoproline excreted in urine. Cancer Res. **41**, 3658–62.
106. HOFFMANN, D., and BRUNNEMANN, K.D. (1983). Endogenous formation of *N*-nitrosoproline in cigarette smokers. Cancer Res. **43**, 5570–74.
107. LADD, K.F., NEWMARK, H.L., and ARCHER, M.C. (1984). *N*-nitrosation in smokers and nonsmokers. J. Natl. Cancer Inst. **73**, 83–7.
108. TSUDA, M., NUTSUMA, J., SATO, S., HIRAYAMA, T., KAKIZOE, T., and SUGIMURA, T. (1986). Increase in the levels of *N*-nitrosoproline, *N*-nitrosothioproline, and *N*-nitroso-2-methylthioproline in human urine by cigarette smoking. Cancer Letts. **30**, 117–24.
109. LU, S-H., OSHIMA, H., FU, H-M, TIAN,Y., LI, F-M., BLETTNER, M., WAHRENDORF, J., and BARTSCH, H. (1986). Urinary excretion of N-nitrosoamino acids and nitrate by inhabitants of high- and low-risk areas for esophageal cancer in Northern China: Endogenous formation of nitrosoproline and its inhibition by vitamin C. Cancer Res. **46**, 1485–91.
110. ZEIBARTH, D. (1974). Studies on the nitrosation of secondary amines in buffer solutions and in human gastric juice. Arch. Geschwulstforsch. **43**(1), 42–51.
111. CONAWAY, C.C., MYHR, B.C., RUNDELL, J.O., and BRUSICK, D.J. (1982). Evaluation of morpholine, piperazine, and analogs in the L5178Y mouse lymphoma assay and BALB/3T3 transformation assay. Environ. Mutagen. **4**, 390 (Abstract).
112. AMES, B.N., McCANN, J., and YAMASAKI, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. **31**, 347–64.
113. SPANGGORD, R.J., MORTELMANS, K.E., GRIFFIN, A.F., and SIMMON, V.F. (1982). Mutagenicity in *Salmonella typhimurium* and structure–activity relationships of wastewater components emanating from the manufacture of trinitrotoluene. Environ. Mutagen. **4**, 163–79.
114. ZEIGER, E., and LEGATOR, M. (1971). Mutagenicity of *N*-nitrosomorpholine in the host-mediated assay. Mutat. Res. **12**(4), 469–71.
115. SAURO, F., FRIEDMAN, L., and GREEN, S. (1973). Biochemical, mutagenic, and pathological effects of nitrosamines in rats. Toxicol. Appl. Pharmacol. **25**, 449 (Abstract No. 27).
116. DANZ, M., URBAN, H., SCHMIDT, A., and ZIEBARTH, D. (1978). A possible short-term prediction of potential carcinogenicity of chemical compounds *in vivo* by means of a promoting activity test (PAT). Exp. Pathol. **16**, 109–120.
117. AMLACHER, E., and ZIEBARTH, D. (1979). Effectiveness in the carcinogenicity prescreening: A partial comparison of the bacterial mutagenicity test (Ames), the thymidine incorporation inhibiting screening system (Amlacher) and the promoting activity test (Danz). Arch. Geschwulstforsch. **49**(6), 490–4.
118. AMES, B.N., DURSTON, W.E., YAMASAKI, E., and LEE, F.D. (1973). Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. **70**, 2281–85.
119. OHE, T. (1982). Mutagenicity of pyrolysates from guanidine, ureide, secondary amines, and polyamines found by the Salmonella/mammalian-microsome test. Mutat. Res. **101**(3), 175–87.
120. NEWBERNE, P.M., and SHANK, R.C. (1973). Induction of liver and lung tumors in rats by the simultaneous administration of sodium nitrite and morpholine. Food Cosmet. Toxicol. **11**(5), 819–25.
121. SHANK, R.C., and NEWBERNE, P.M. (1973). Induction of tumors by simultaneous feeding of nitrite and morpholine. Toxicol. Appl. Pharmacol. **25**(3), 448 (Abstract No. 25).
122. SANDER, J., and BUERKLE, G. (1969). Induction of malignant tumors in rats by simultaneous feeding of nitrite and secondary amines. Z. Krebsforsch. **73**(1), 54–66.
123. VAN STEE, E.W., BOORMAN, G.A., and HASEMAN, J.C. (1980). Pulmonary adenomas in mice exposed to NO_2 by inhalation and morpholine by ingestion. Pharmacologist **22**(3), 158 (Abstract, no. 13).
124. CTFA. (May 3, 1977). Industry submission of unpublished data. Repeated insult patch testing and in-use testing of mascaras containing one percent Morpholine (3-11-2).
125. AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS, INC. (1981). TLVs: Threshold limit values for chemical substances in workroom air adopted by ACGIH for 1981, pp. 3, 23.