Safety Assessment of Cetethyl Morpholinium Ethosulfate

Abstract

Cetethyl Morpholinium Ethosulfate is a quaternary salt used as an antistatic agent and as a surfactant in several hair care products. The concentration at which this ingredient is used is unknown, although data reported in 1984 indicated a maximum concentration of 1%. In an inhalation toxicity study, the approximate lethal concentration of Cetethyl Morpholinium Ethosulfate was 0.403 mg/ml. This ingredient was shown to be a severe ocular irritant in an animal study. No other safety test data on this ingredient was available. These data were clearly insufficient to support the safety of Cetethyl Morpholinium Ethosulfate in cosmetics. Data available on Morpholine were summarized, but these data themselves were insufficient to support safety. The data needed in order to complete the safety assessment of Cetethyl Morpholinium Ethosulfate include: methods of manufacture and impurities, especially nitrosamines; current concentration of use; skin penetration, if there is significant skin penetration, then both a 28-day dermal toxicity study to assess general skin and systemic toxicity and a reproductive and developmental toxicity study are needed; two genotoxicity studies, at least one in a mammalian system, if positive, then a two-year dermal carcinogenesis study using NTP methods may be needed; UV absorption data, if significantly absorbed, then photosensitization data are needed; dermal irritation and sensitization; and ocular toxicity, if available.

INTRODUCTION

Cetethyl Morpholinium Ethosulfate is a quaternary ammonium salt that is used as an antistatic agent and as a surfactant in shampoos, hair conditioners, and permanent waves. The following is a review of the available safety data on this ingredient, including data on the component Morpholine, which was reviewed by the CIR Expert Panel in an earlier safety assessment.

CHEMISTRY

Definition and Structure

Cetethyl Morpholinium Ethosulfate (CAS No. 78-21-7) is the quaternary salt that conforms to the formula:

\[
\left[ \begin{array}{c} \text{O} \\ \text{CH}_2 \text{N} \text{CH}_2 \text{CH}_3 \\ \text{(CH}_2\text{)}_{14}\text{CH}_3 \\ \text{CH}_3\text{CH}_2\text{OSO}_3^- \end{array} \right]^+ 
\]

Other names for this ingredient include: Cetyl Ethyl Morpholinium Ethosulfate; 4-Ethyl-4-Hexadecylmorpholinium Ethyl Sulfate; Morpholinium, 4-Ethyl-4-Hexadecyl- Ethyl Sulfate; and Quaternium-25 (Wenninger and McEwen, 1993).

Chemical and Physical Properties

Cetethyl Morpholinium Ethosulfate is available commercially as either a 92+% amber, waxy solid or as a 35% clear amber solution. The pH of the
solid product ranges from 3.0-5.0, but the pH of the solution ranges from 5.0-5.5. Solid Cetethyl Morpholinium Ethosulfate gels at 40°C and liquefies at 75°C. The viscosity of a 35% solution is 6.5 cps, and the specific gravity at 25°C is 1.02 (CTFA, no date).

Method of Manufacture

Cetethyl Morpholinium Ethosulfate is prepared by the quaternization of N-cetyl morpholine with diethyl sulfate (CTFA, no date).

COSMETIC USE

Cetethyl Morpholinium Ethosulfate is used as an antistatic agent and as a surfactant for its emulsifying properties in hair care products (Wenninger and McEwen, 1992). The product formulation data submitted to the Food and Drug Administration (FDA) in 1996 reported that Cetethyl Morpholinium Ethosulfate is used in a total of 13 formulations (Table 1) (FDA, 1996). The concentrations used in these formulations are not known since concentration of use values are no longer reported to the FDA by the cosmetic industry (Federal Register, 1992). However, data submitted in 1984 indicated that this ingredient was used at concentrations up to 1% in hair conditioners (FDA, 1984). There was no listing for permanent waves or shampoos.

ANIMAL TOXICOLOGY

Acute Toxicity

A mixture containing 25% Cetethyl Morpholinium Ethosulfate (with 50% isobutyl stearate and 25% stearate ethyl) was inhaled by male albino ChR-CD rats (initial body weight 226-286 g) five times for 4-h each time, followed by a 14-day observation period (particle size not given). Each group had six rats. Group 1 (0.224 mg/l) rats had no clinical signs of toxicity; other groups had slight weight loss, abnormally red ears, paws, and tails, extensive hair loss, abnormal sounds during respiration, gasping, nasal and oral discharge, and redness in the genital region. Within three weeks after exposure, hair loss stopped. Two of six rats died in each of Groups 2 and 3 (0.403 and 0.674 mg/l, respectively). Three of six rats died in Group 4 (1.148 mg/l), four of six rats died in Group 5 (1.328 mg/l), and six of six rats of Group 6 (5.315 mg/l) died. All fatalities occurred in the first six days after exposure. The 4-h approximate lethal concentration (ALC) was 0.403 mg/m³ (Haskell Laboratory, 1977).

| TABLE 1 |
| COSMETIC PRODUCT FORMULATION DATA ON CETETHYL MORPHOLINIUM ETHOSULFATE (FDA, 1996) |

<table>
<thead>
<tr>
<th>Product Category</th>
<th>Total No. Formulations in Category</th>
<th>Total No. of Formulations Containing Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair conditioners</td>
<td>715</td>
<td>5</td>
</tr>
<tr>
<td>Permanent waves</td>
<td>434</td>
<td>7</td>
</tr>
<tr>
<td>Shampoos (non-coloring)</td>
<td>972</td>
<td>1</td>
</tr>
<tr>
<td>1996 Total</td>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

2
**Ocular Irritation**

Atlas Chemical Industries, Inc. (1964) evaluated the primary irritation of Cetyl Morpholinium Ethosulfate to the ocular conjunctival mucosa of New Zealand White rabbits (1.6-2.1 kg). A 0.1 ml volume of undiluted Cetyl Morpholinium Ethosulfate (35% active ingredient in water) was instilled into the conjunctival sac of nine eyes. Six eyes were left unwashed and the remaining three were washed with 20 ml water at body temperature 2 sec. after instillation. Each eye was then observed for a one-hour period after application of the test chemical at 24, 48, 72, and 96 hours, as well as at 7 days. Cetyl Morpholinium Ethosulfate was extremely irritating to the unwashed eyes; in five of six, the iris could not be viewed because of the extensive corneal damage. The three washed eyes also had severe irritation and researchers suggested that Cetyl Morpholinium Ethosulfate should be labeled as hazardous to the eye (Atlas Chemical Industries, Inc., 1964).

**SAFETY ASSESSMENT OF MORPHOLINE**

The CIR Expert Panel issued a Final Report on the safety of Morpholine in 1987. The following is a summary of the data reviewed by the Panel and the conclusion it reached (Elder, 1989).

Morpholine is a heterocyclic secondary amine (Estrin et al., 1982) that is readily nitrosated (International Agency for Research on Cancer [IARC], 1978; National Research Council, 1983). The potential exists for the formation of N-nitrosomorpholine when Morpholine is used in formulations. N-Nitrosomorpholine is carcinogenic in laboratory animals (IARC, 1978).

Morpholine was largely unmetabolized by rats (Mallar and Heidelberger, 1957; Tanaka et al., 1978; Sohn et al., 1982; Ohnishi, 1984), dogs (Rhodes and Case, 1977), and rabbits (Van Stee et al., 1981), whereas 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 (Sohn et al., 1982).

The oral LD₅₀ of Morpholine was between 1.05 and 1.63 g/kg for rats (Shea, 1939; Smyth et al., 1954; 1956; 1970) and 0.9 g/kg for guinea pigs (Shea, 1939). Unneutralized solutions of Morpholine caused severe corneal necrosis (Carpenter and Smyth, 1946; Smyth et al., 1954), but upon neutralization Morpholine was not injurious to rabbit eyes (Grant, 1974). Undiluted Morpholine was corrosive to the skin of rabbits (Reinhardt and Britelli, 1981; Texaco, Inc., 1985), but a mascara formulation containing 1% Morpholine was non-irritating (CTFA, 1977a).

In studies of acute and short-term dermal toxicity, deaths of guinea pigs and rabbits were caused by undiluted/unnitrosated Morpholine and diluted/unnitrosated Morpholine, respectively. In both cases, the skin was necrotic (Shea, 1939).

Short-term oral administration of Morpholine at various doses caused swelling, congestion, and necrosis of various organs in rats and guinea pigs. At higher dosages, deaths occurred in both species (Shea, 1939).

The most severe toxic effects observed in acute inhalation studies of Morpholine were irritation to the eyes and nose and increased respiratory rate (Ivanov et al., 1973; National Research Council, 1981; 1983).

In short-term inhalation studies with rats, irritation of the mucous membranes and an increased respiratory rate were observed. Nasal lesions, as well as red, white, and dark foci in the lungs, were noted (Conaway et al., 1984a). In another study, the weight, residual volume, and total capacity of the lungs were decreased (Takezawa and Lam, 1978). In another study, an increase in chromosomal aberrations was observed in rat bone cells but these results have not been confirmed (Migukina, 1973).

Chronic inhalation studies in rats and guinea pigs produced the observations that the exposure to Morpholine caused changes in nervous system activity, arterial blood pressure, and peripheral blood indices at both high and low concentrations. No changes were observed 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 one month after the Morpholine exposure ended. At lower concentrations of Morpholine, a decrease in the size of lymph nodules in the spleen was noted, but this effect was not observed one month after exposure had ended. Chromosomal aberrations were noted at both dosages, but those of the low dosage were not significantly greater than the control rate (Migukina, 1973; National Research Council, 1981; 1983).

Nitrosation of Morpholine produces N-nitrosomorpholine (IARC, 1978), 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 NO₂ increased the incidence of pulmonary adenomas in mice; N-nitrosomorpholine was present in the lungs of mice exposed to both Morpholine and atmospheric NO₂ (Van Stee et al., 1983).

Endogenous formation of N-nitrosomorpholine in humans has not been demonstrated, but the presence of N-nitrosopropylene in human urine suggests that nitrosation does occur in humans. Morpholine can be nitrosated in human gastric juice in the presence of nitrites (Zeibarth, 1974; Ohshima and Bartsch, 1981). Morpholine was a weak positive mutagen in LS178 mouse lymphoma assay, in BALB/3T3 malignant cell transformation and fibroblast transformation assays (Conaway et al., 1982; Texaco Chemical Co., 1982), and in sister chromatid exchange assays (Texaco Chemical Co., 1982). Morpholine was negative for mutagenicity in the Ames test with and without activation by S9 rat liver fraction (Spanggord et al., 1982); a modification of this same test also produced negative results (National Research Council, 1981). At non-toxic doses, Morpholine did not increase the rate of DNA repair in rat hepatocytes (Conaway et al., 1984b). 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 (Edwards et al., 1979). Results from other host-mediated assays were the same (Zeigler and Legator, 1971; Braun et al., 1977). 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 (Inui et al., 1979). Pyrolysates of Morpholine at 500 and 600°C were mutagenic in the Ames test (Ohe, 1982).

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 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 (Shank and Newberne, 1976). In mice treated with Morpholine and nitrites, pulmonary adenoma was the most common neoplasm (Greenblatt et al., 1971).

Addition of sodium ascorbate had an inhibitory effect on the carcinogenicity of the Morpholine-nitrite combination. An increase was observed 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 a consequence of the reduced incidence of hepatic neoplasms. The increase in gastric neoplasms after the administration of sodium ascorbate was observed in both rats and mice (Greenblatt et al., 1971; Mirvish, 1982).

In humans, Morpholine was a cutaneous, ocular, and mucous membrane irritant, and a skin sensitizer (Greenberg et al., 1954; Hawley, 1971; Sax, 1979). Morpholine was absorbed through the skin, by which route it was highly toxic; the toxicity diminished when Morpholine vapor was diluted to less than 25% (Hawley, 1971; Sax, 1979; American Conference of Governmental Industrial Hygienists, Inc., 1980). Ocular irritation from Morpholine vapor could lead to corneal edema, a lesion resulting in "hazy" or "halo" vision (Texaco, Inc., 1985).

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 (CTFA, 1977b).

In its Discussion, the CIR Expert Panel indicated that it is aware of reports 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 nitroamines. Therefore, the Expert Panel could not conclude that Morpholine is safe without additional data regarding the formation of N-nitrosomorpholine under conditions of use. The Expert Panel issued a formal conclusion of insufficient data. The type of information required was either analytical in-use data regarding the formation of N-nitrosomorpholine or an appropriate risk assessment (Elder, 1989).

SUMMARY

Cetethyl Morpholinium Ethosulfate is the quaternary salt that serves as an antistatic agent and surfactant in cosmetic products. It is prepared by the quaternization of N-cetyl morpholine with diethyl sulfate. In 1996, the cosmetic industry reported to the FDA that Cetethyl Morpholinium Ethosulfate was used in 13 formulations.

Data on Cetethyl Morpholinium Ethosulfate regarding absorption, metabolism, mutagenicity, carcinogenicity, or clinical safety were not available. Little data were available on toxicity of Cetethyl Morpholinium Ethosulfate. This report, therefore, presented data on Morpholine which are applicable to Cetethyl Morpholinium Ethosulfate.

The 4-h approximate lethal concentration of a mixture containing 25% Cetethyl Morpholinium Ethosulfate in rats was 0.403 mg/m². Cetethyl Morpholinium Ethosulfate at a concentration of 35% was severely irritating and caused extensive corneal damage to the eyes of rabbits.

Morpholine is a heterocyclic secondary amine that is readily nitrosated. N-Nitrosomorpholine, a carcinogen in laboratory animals, can be formed when Morpholine is used in formulations.

Morpholine was largely unmetabolized by rats, dogs, and rabbits, but was extensively metabolized in guinea pigs. In rats and hamsters, most of the administered Morpholine was excreted unchanged in the urine.

The acute oral LD₅₀ of Morpholine in rats was 1.05-1.63 g/kg. Morpholine was toxic in laboratory animals by oral, dermal, and inhalation routes. Unneutralized Morpholine also caused severe corneal damage in rabbits.

Morpholine with nitrites caused carcinogenic responses in long-term feeding studies. Lesions were attributed to the formation of carcinogenic nitroamines, not Morpholine itself.

Morpholine was a cutaneous, ocular, and mucous membrane irritant and skin sensitizer in humans. It was readily absorbed through human skin. Cosmetic formulations containing Morpholine have not produced irritation or sensitization reactions.

DISCUSSION

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 Procedures, the Expert Panel informed the public of its decision that the data on Cetethyl Morpholinium Ethosulfate were not sufficient for determining whether this ingredient, under relevant conditions of use, was either safe or unsafe. The Panel released an Insufficient Data Announcement on March 5, 1996, outlining the data needed to assess the safety of Cetethyl Morpholinium Ethosulfate. No data or comments were received during the 90-day public comment period. Additional data
needed to make a safety assessment are: (1) methods of manufacture and impurities, especially nitrosamines; (2) concentration of use; (3) skin penetration; if there is significant skin penetration, then both a 28-day dermal toxicity study to assess general skin and systemic toxicity and a reproductive and developmental toxicity study are needed; (4) two genotoxicity studies, at least one in a mammalian system; if positive, then a two-year dermal carcinogenesis study using NTP methods may be needed; (5) UV absorption data; if significantly absorbed, then photosensitization data are needed; (6) dermal irritation and sensitization; and (7) ocular toxicity, if available. To assess safety in rinse-off products, the data needs are primarily nos. 1, 2, 5, 6, and 7.

**CONCLUSION**

The CIR Expert Panel concludes that the available data are insufficient to support the safety of Cetethyl Morpholinium Ethosulfate for use in cosmetic products.

**ACKNOWLEDGEMENT**

Rebecca S. Johnson, Scientific Analyst and Writer, prepared this report.

**REFERENCES**


Cosmetic, Toiletry, and Fragrance Association (CTFA). (No date) Unpublished data submitted by CTFA. Cosmetic ingredient chemical description of Cetethyl Morpholinium Ethosulfate.


* Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, N.W., Suite 310, Washington, D.C. 20036.


