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Final Report on the Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone

Methylisothiazolinone and Methylchloroisothiazolinone (MI/MCI) are heterocyclic organic compounds that are used in cosmetics as a broad spectrum preservative system.

MI/MCI was absorbed after oral administration and then was excreted in the urine or feces; storage in the tissues was minimal. Up to 62% of a single percutaneous dose was bound to the site of application 24 hours after exposure. The MI/MCI-CG bound to the skin had a 13.1-day half-life.

MI/MCI was moderately to highly toxic to rats, and highly toxic to rabbits when administered orally, and moderately toxic when applied dermally. MI/MCI was not a cumulative ocular irritant when tested at 55 ppm. The dermal irritation of MI/MCI was concentration dependent but nonirritating to rabbit skin at 560 ppm concentrations; this nonirritating concentration is well above the maximum recommended use concentration.

No treatment-related effects were observed in rats which received MI/MCI in oral doses up to 24.4 mg/kg/day for 2 weeks. Doses of MI/MCI up to 2.8 mg/kg/day applied dermally to rabbits, 5 days per week for 3 weeks, produced moderate irritation at the application site but no systemic toxicity. Dermal application of MI/MCI at doses up to 0.4 mg/kg/day for 3 months produced no systemic toxicity in rabbits. No toxicologically significant treatment-related effects were observed in rats or dogs at doses up to 30 and 28 mg/kg/day, respectively. The result of genotoxic testing of MI/MCI varied with the assay used. Dermal application of 400 ppm MI/MCI-CG, 3 times per week for 30 months, had no local or systemic tumorigenic effect in male mice.

MI/MCI administered by gavage to pregnant rabbits and rats at doses up to 13.3 mg/kg/day was toxic to the dam, embryo, and fetus; the compound was not teratogenic.

MI/MCI is a sensitizer however, the concentration of MI/MCI in cosmetic products which produced sensitization varies. The available human sensitization test data at concentrations of 50 ppm and above are not in agreement. MI/MCI-CG was not a sensitizer or photosensitizer at a concentration of 15 ppm.

It is concluded that Methylisothiazolinone/Methylchloroisothiazolinone may be safely used in "rinse-off" products at a concentration not to exceed 15 ppm and in "leave-on" cosmetic products at a concentration not to exceed 7.5 ppm. The stated

safe use concentration refers to a mixture containing 23.3% Methylisothiazolinone and 76.7% Methylchloroisothiazolinone.

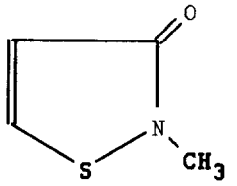
INTRODUCTION

This review on the safety of use of Methylisothiazolinone and Methylchloroisothiazolinone includes all the published data, as well as unpublished data submitted to CIR by interested individual cosmetic ingredient suppliers and formulators. Most of the data were developed prior to the start of the review. Other data cited were developed and submitted during the review in response to specific concerns expressed by the CIR Expert Panel.

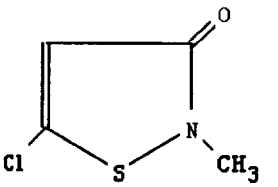
CHEMISTRY

Definition and Structure

Methylisothiazolinone and Methylchloroisothiazolinone are the CTFA adopted names for the heterocyclic organic compounds that conform to the formulae:[1,2]



Methylisothiazolinone



Methylchloroisothiazolinone

Other names for Methylisothiazolinone (CAS No. 2682-20-4) include 2-methyl-3[^{2}H]isothiazolone and 2-methyl-4-isothiazolin-3-one. Methylchloroisothiazolinone (CAS No. 26172-55-4) also is known as 5-chloro-2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-3[^{2}H]isothiazolone.[1,3]

Both Methylisothiazolinone and Methylchloroisothiazolinone are the active ingredients in a family of commercial microbiocides and preservatives under the trade names Kathon-CG, Kathon-886, Kathon-WT, and Kathon-LX.[4] Frequently, these two isothiazolinones (or a mixture of these two compounds) are often referred to in the literature by trade name.[1] To avoid use of proprietary names in this report, Kathon-CG and Kathon-886 will be referred to as MI/MCI-CG and MI/MCI-886, respectively. Although only MI/MCI-CG is used to formulate cosmetics, data on MI/MCI-886 has been included for completeness.

Composition for Cosmetic Use

Methylisothiazolinone and Methylchloroisothiazolinone are supplied to cosmetic manufacturers in the form of a commercial biocide product, MI/MCI-CG.[3] The

[1]Kathon is a registered tradename of the Rohm and Haas Company of Philadelphia.[3]

composition of MI/MCI-CG is presented in Table 1. The product is an aqueous solution containing 0.35% Methylisothiazolinone and 1.15% Methylchloroisothiazolinone (total active ingredients [a.i.] = 1.50%). Magnesium salts (23.0%) are present in the product as stabilizers.[5] In this evaluation, all concentrations are cited as parts per million (ppm) of active MI/MCI-CG unless otherwise stated.

Properties

MI/MCI-CG is readily miscible in water, lower alcohols, glycols, and other hydrophilic organic solvents.[3] Chemical and physical properties of this commercial product are presented in Table 1.

Methylchloroisothiazolinone and Methylisothiazolinone have melting points of 52–55°C and 47–50°C, respectively.[6,7] Methylisothiazolinone has a boiling point of 93°C.[7]

The nuclear magnetic resonance and ultraviolet (UV) absorption spectral data for Methylisothiazolinone and Methylchloroisothiazolinone are given in Table 2 and indicate that these compounds do not absorb light in the ultraviolet (UVB) band. Mass spectra for Methylisothiazolinone and Methylchloroisothiazolinone are given by Bruze et al.[2]

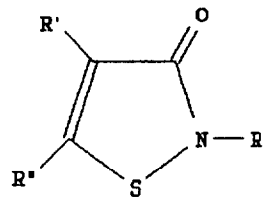
Table 1. Composition, Chemical, and Physical Properties of MI/MCI-CG[a]

Composition	
Active ingredients	
Methylisothiazolinone (MI)	0.35%
Methylchloroisothiazolinone (MCI)	1.15%
	1.50%
Inert ingredients	
Magnesium salts[a]	23.0%
Water	75.5%
	98.5%
Chemical and Physical Properties	
Appearance	Clear liquid
Color	Light amber
Odor	Mild
Specific gravity at 20°C	1.19
Density (lb/gal)	9.9
pH (as supplied)	3.5
Active ingredient content (%)	1.5
Viscosity at 23°C	5.0 cp (± 0.2 cP)
Freezing point	−18 to −21.5°C
Miscibility	Miscible with water, lower alcohols, glycols, and other hydrophillic organic solvents
Compatibility	Reported to be biologically and physically compatible with emulsifiers, proteins, and anionic, nonionic, and cationic surfactants. The active ingredients may be inactivated by amines, mercaptans, sulfides, and sulfites
Stability	Reported to be stable for at least 1 year at ambient temperature, and for at least 6 months at 50°C

[a]Reported by Wright et al.[8] as magnesium nitrate.

Source: Ref. 3.

TABLE 2. NUCLEAR MAGNETIC RESONANCE AND ULTRAVIOLET ABSORPTION SPECTRAL DATA FOR MI AND MCI



				Chemical shifts[a,b]			Coupling constant (Hz)	UV (Methanol)	
Compound	R	R'	R"	R	R'	R"	$J_{4,5}$	λ max (mμ)	log ε
MI	CH_3	H	H	3.27(s)	6.05(d)	7.98(d)	6.0	278	3.87
MCI	CH_3	H	Cl	3.25(s)	6.20(s)			277	3.82

[a]NMR spectra were determined in deuterated chloroform solution, with tetramethylsilane as an internal reference.

[b]The multiplicity of the absorption is shown in parentheses: s—singlet; d—doublet.

Source: Ref. 7.

The sulfur atom of *N*-substituted isothiazolones such as Methylisothiazolinone and Methylchloroisothiazolinone is electrophilic and reacts with nucleophiles.[9] Monte et al.[10] reported that Methylchloroisothiazolinone can interact with the sulfhydryl group of enzymes and other proteins causing cleavage of its ring structure. No other details were reported.

Results of a photolysis study indicated that both Methylchloroisothiazolinone and Methylisothiazolinone are readily photolyzed to other products by the action of ultraviolet (UV) radiation. A 48% reduction in the content of Methylchloroisothiazolinone and a 61% reduction in Methylisothiazolinone content occurred following irradiation of each isothiazolinone in aqueous solution with lamps having the intensity and UV spectrum of natural sunlight.[11] The length of exposure was 48 hours. In a separate study, it was observed that 80% of Methylchloroisothiazolinone [1000 ppm (0.1%) in aqueous solution] underwent degradation following 24 hours of UV exposure.[12] The photolysis products in these studies were not identified.

The rate of hydrolysis of Methylchloroisothiazolinone at low concentrations [~1 ppm (0.0001%)] increases with increasing pH, increasing temperature, and to a limited extent, increasing ionic strength of buffer. The compound is stable under acidic conditions, but the "rate of disappearance" from aqueous solution increases by a factor of about 2000 from pH 4.5 to 11. As the temperature increases from 7 to 40°C, the "rate of disappearance" from aqueous solution of Methylchloroisothiazolinone increases by one to two orders of magnitude.[11]

While the free bases Methylchloroisothiazolinone and Methylisothiazolinone are unstable, their shelf lives may be markedly extended by the formation of adducts with calcium or magnesium salts.[5,11] This formation presumably occurs through the oxygen of the carbonyl group.[11] MI/MCI-CG will remain stable for one year at ambient temperature, and for at least six months at 50°C.[3]

Method of Manufacture/Analytical Methods

Methylisothiazolinone and Methylchloroisothiazolinone can be prepared by the methods described by Lewis et al.,[7] using the chlorine-induced cyclization of 3,3'-dithiodipropionamides. Methylisothiazolinone is also formed as a by-product (25% yield) of the synthesis of Methylchloroisothiazolinone.[11]

MI/MCI-CG has been determined using thin-layer chromatography (TLC) with UV[13] or other methods of detection[14] as well as high performance liquid chromatography (HPLC).[2,15] Gas chromatography coupled with mass spectrometry was used for the analysis of MI/MCI-CG and the identification of Methylisothiazolinone and Methylchloroisothiazolinone.[2,16]

Impurities

In its petitions for approval of a mixture of Methylchloroisothiazolinone and Methylisothiazolinone as an antimicrobial agent in food packaging materials, Rohm and Haas reported that a carcinogenic impurity, dimethylnitrosamine (DMN), was formed as a reaction by-product at very low concentrations in the reaction mixture. Analytical methods were developed to measure the DMN at low concentrations. Hence a new manufacturing process using a specific reactant, methyl-3-mercaptopropionate, is now stipulated to limit the presence of DMN to concentrations ranging from 0.1 to 0.8 ppm of the additive in 39 commercial batches analyzed. The Food and Drug Administration (FDA)[16] conducted a risk assessment and calculated that the petitioned uses combined with the currently regulated use as a slimicide would result in a concentration of DMN less than 0.18 ppt of the daily diet. They estimated, based on a daily diet of 3 kg of food, that the daily intake of DMN would be less than 0.54 ng per person. The petitions were therefore approved with the stipulation that the compounds are manufactured from methyl-3-mercaptopropionate.[17] See also section entitled "Use-Noncosmetic."

USE

Cosmetic

Methylisothiazolinone and Methylchloroisothiazolinone are used in cosmetics in the form of a commercial biocide, MI/MCI-CG. As noted earlier in Table 1, MI/MCI-CG is an aqueous solution containing 23% magnesium salts and the two active ingredients, Methylchloroisothiazolinone (1.15%) and Methylisothiazolinone (0.35%). The product is supplied to cosmetic manufacturers and formulators as a 1.5% active aqueous solution. MI/MCI-CG is used in cosmetics and toiletries as a broad-spectrum preservative, and is reported to be effective against both gram-negative and gram-positive bacteria, as well as fungi and yeast.[3] The antimicrobial was used in Europe prior to use in the U.S.[4] In 1980, approximately 55,000 and 20,000 tons of cosmetic products were formulated with MI/MCI-CG in Europe and the U.S., respectively.[4,8]

The chemical supplier of MI/MCI-CG has recommended use of its product in cosmetics at concentrations ranging from 0.02 to 0.1% as supplied [3–15 ppm (0.0003–0.0015%) a.i.].[3] The European Economic Community[18] established a directive permitting use in cosmetics of a 3:1 mixture of Methylchloroisothiazolinone

and Methylisothiazolinone at concentrations up to 0.003% (30 ppm). In response to an increased concern on the sensitization potential of this compound, the directive was amended and the maximum permitted concentration was lowered from 30 ppm to 15 ppm.[19]

Rastogi[20] reported that MI/MCI-CG was detected in 11 of 22 cosmetic products investigated (6/9 shampoo, 4/9 skin cream, 1/3 hair balm, and 0/1 body lotion). The concentration of MI/MCI-CG varied from 0.8 to 15 ppm.

Subsequently, Rastogi[20] analyzed 156 of the most commonly used cosmetic products in Denmark for MI/MCI-CG. Sixty-six (42%) of these MI/MCI-containing products were rinse-off products, and 15 were leave-on products. Of these 66 products, 49 were found to have concentration levels of < 10 ppm, MI/MCI-CG 14 had concentrations of 10–15 ppm, and 3 contained > 15 ppm.

As approved by FDA and the EEC, the ratio of MCI to MI in MI/MCI-CG should be 3:1. HPLC analysis revealed that 15 of the 66 rinse-off products and 11 of the 15 leave-on products had a "disturbed MCI:MI ratio." The author suggests that this latter finding is a result of reactions of MCI and/or MI with other cosmetic ingredients within a given product. Accordingly, the cosmetic products that contain MI/MCI-CG rather than MI/MCI itself should be assayed for their allergenic potential.

Data submitted to the Food and Drug Administration (FDA) in 1986[21] by cosmetic firms participating in the voluntary cosmetic registration program, indicated that MI/MCI-CG, Methylisothiazolinone, and Methylchloroisothiazolinone were ingredients used in 381 cosmetic products (only the combined total was given) (Table 3). Products formulated with these materials included hair and shampoo formulations (53%), skin care preparations (41%), bath products (2%), eye and facial makeup

Table 3. Product Formulation Data for MI/MCI-CG

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*	
			>0.1-1	*≤0.1*
Eye and facial makeup preparations	874	8	1	7
Hair conditioner and other hair preparations, including hair coloring preparations	1725	79	6	73
Hair shampoos (noncoloring)	838	124	2	122
Bath soaps and other foaming detergent bath preparations	581	8	2	6
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	729	33	7	26
Face, body, and hand skin care preparations (excluding shaving preparations)	2165	95	24	71
Other skin care preparations	978	29	15	14
Suntan preparations	243	5	2	3
1986 Totals		381	59	322

Source: Ref. (21).

preparations (2%), and suntan preparations (1%). The majority of these products (85%) contained MI/MCI-CG, Methylisothiazolinone, or Methylchloroisothiazolinone at reported concentrations of $\leq 0.1\%$, with the remaining products (15%) containing these materials in the concentration range of > 0.1 to 1.0%.[21]

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.[22] Since certain cosmetic ingredients are supplied to the formulator at less than 100% concentration (in this case a concentration of 1.5%), the concentration reported by the formulator may not necessarily reflect the actual concentration found in the finished cosmetic product; the actual concentration would be a fraction of that reported to the FDA. Data 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 two- to ten-fold error in the assumed ingredient concentration.

The skin, hair, and scalp are the areas directly exposed to cosmetic products formulated with Methylisothiazolinone and Methylchloroisothiazolinone. The potential also exists for these isothiazolinones to come in contact with the eye through the use of shampoos formulated with these materials and through the use of eye makeups.

Noncosmetic

Research into the chemistry of isothiazolinones in the early 1960s led to the development of a number of commercial antimicrobial products currently in use.[3] These products, which contain Methylisothiazolinone and Methylchloroisothiazolinone as the active ingredients, are used in a variety of applications including mildewcides for leather and fabric; antibiofoulants and slimicides for cooling towers, paper mills, and oil recovery applications; microbiocides for swimming pool water; and preservatives for metal working fluids, emulsion polymers, latex paints, cutting oils, jet and heating fuels, and household cleaning products.[3,4,11,23]

A 3:1 mixture of Methylchloroisothiazolinone and Methylisothiazolinone (as calcium chlorides) has been approved as an antimicrobial agent to control slime in the manufacture of paper and paperboard products that contact food. A limitation of 2.5 lbs per ton of dry weight fiber was stipulated.[24]

More recently, FDA has approved the safe use of 3:1 mixture of Methylchloroisothiazolinone and Methylisothiazolinone as an antimicrobial agent for polymer latex emulsions in adhesives[25] and in paper coatings[26] which contact food. The mixture must be manufactured from methyl-3-mercaptopropionate to minimize the formation of the carcinogenic impurity dimethylnitrosamine and may contain magnesium nitrate at a concentration equivalent to the isothiazolone active ingredients (wt/wt). The use of this mixture in paper coatings is limited to a concentration not to exceed 50 ppm (0.005%) (based on the isothiazolone active ingredients) in the coating formulation. In reaching its decision, the FDA established an acceptable daily intake of 0.24 mg per person. The estimated cumulative dietary exposure to these ingredients resulting from proposed uses as well as the regulated use as a slimicide would not exceed 0.04 mg per person per day.[17]

BIOLOGY

Fate in the Environment

Modes and rates of dissipation of Methylchloroisothiazolinone calcium chloride and Methylisothiazolinone calcium chloride were determined over a range of conditions likely to occur in the environment. In aquatic and terrestrial environments, degradation of both compounds at concentrations near 1 ppm was observed to occur rapidly by hydrolytic, photochemical, and biological action. Hydrolysis increased with increasing pH and increasing temperature. Adsorption by soil or river silt was not significant; however, adsorption and subsequent metabolism to CO_2 by certain aquatic ferns was rapid. "The decomposition of both isothiazolinones by several chemical and biological mechanisms appears to ensure the compounds will not persist in the environment."[11]

Krzeminski et al.[12] subsequently identified the major degradative pathway in the environment for the calcium chloride salts of both Methylchloroisothiazolinone and Methylisothiazolinone (Fig. 1). In eight systems covering chemical, biochemical, and photochemical aspects of environmental degradation,[2] the disappearance of the two compounds was rapid with both compounds generating a similar distribution of degradation products, both qualitatively and quantitatively. The principal degradative pathway involved dissociation of calcium chloride, ring opening, loss of chlorine and sulfur, and subsequent formation of *N*-methylmalonamic acid. The degradation then proceeded through malonamic, malonic, acetic, and formic acids to carbon dioxide. Other products along the degradative pathway were tentatively identified as 5-chloro-2-methyl-4-isothiazolin-1-oxide, *N*-methylglyoxylamide, ethylene glycol, and urea.

Voets et al.[27] also measured the degradation of Methylisothiazolinone and Methylchloroisothiazolinone in synthetic sewage and in a mineral solution under both aerobic and anaerobic conditions. Substantial degradation (80–100%) was observed in the organic medium under aerobic conditions; no residual toxicity was noted. No degradation was noted under anaerobic conditions. The investigators stated that these compounds are probably metabolized by a mixed flora because no single bacterium utilizing them as a carbon source could be isolated.

Antimicrobial Activity

MI/MCI-CG possesses broad-spectrum antimicrobial activity. The results of "minimum inhibitory concentration" tests against a variety of microorganisms are available in the review article by Law et al.[3]

Zeelie and McCarthy[28] found that the minimum inhibitory concentration of MI/MCI-CG against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* was 30 $\mu g/cm^3$. In their study, propyl gallate and *t*-butyl hydroquinone potentiated the antimicrobial activity of MI/MCI-CG against all three organisms, whereas butylated hydroxyanisole potentiated the antimicrobial activity of the biocide against *S. aureus* only.

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[2] The eight systems include: (1) an activated sludge system, (2) a river/water system, (3) an acetone-water (30:70 v/v system), (4) a basic hydrolysis system, (5) a photolysis system, (6) rat urine, (7) extract of rat feces, and (8) extract of aquatic plants.[12]

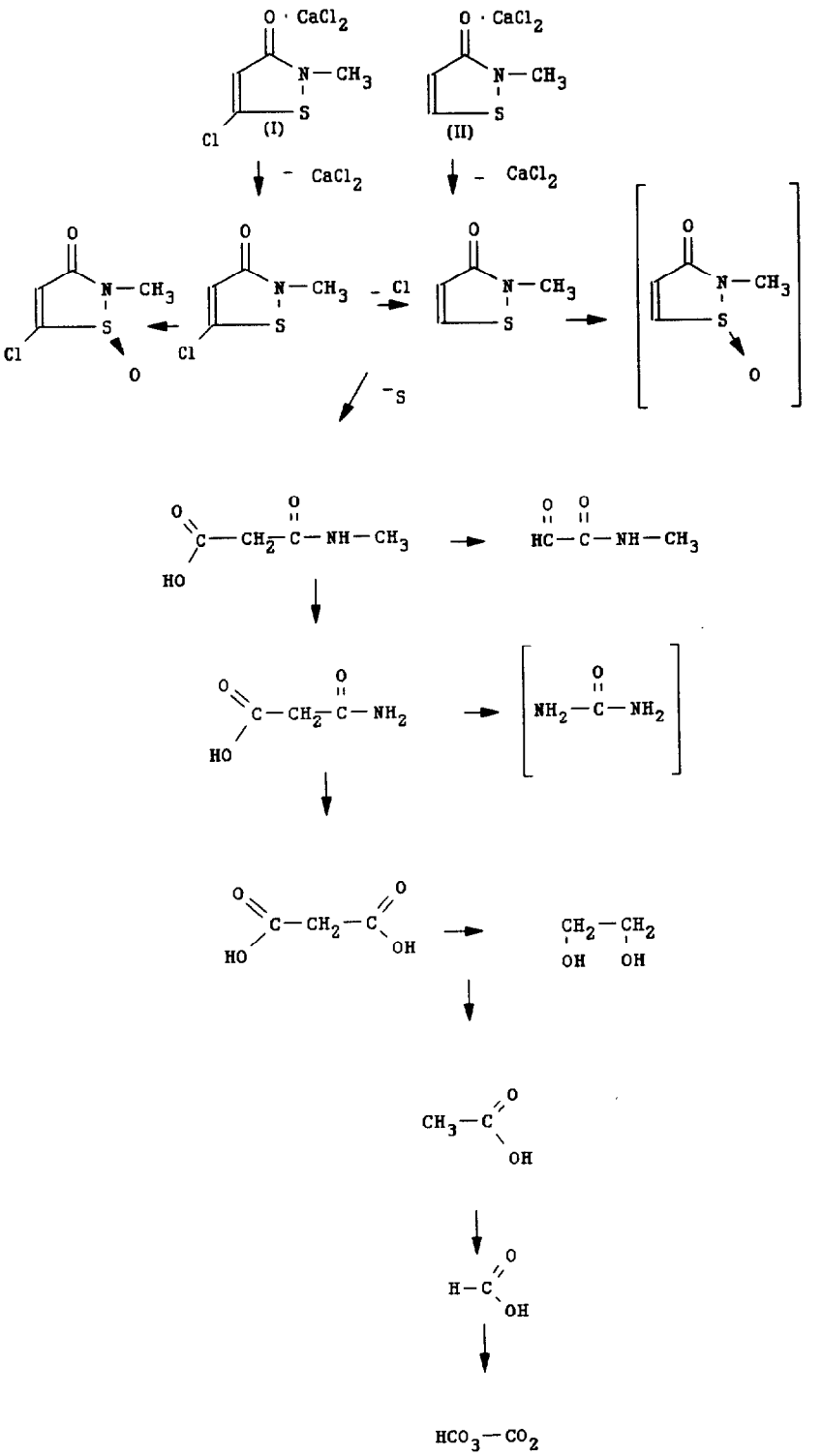

FIG. 1. Major degradative pathway of the calcium chloride salts of Methylchloroisothiazolinone (I) and Methylisothiazolinone (II) (bracketed structures are postulated) Ref. 12.

Synergistic antibacterial activity was produced by combination of MI/MCI-CG and imidazolidinylurea against some gram-negative bacteria, one gram-positive species, *Sarcina lutea*, as well as *C. albicans* and *Aspergillus versicolor*. The synergism for *C. albicans* was as much as four-fold. There was no synergism against *S. aureus*, *Streptococcus faecalis*, or *Bacillus subtilis*. The individual antibacterial properties and synergism were pH independent.[29]

MI/MCI-CG is used as an antimicrobial agent over the pH range typically encountered in cosmetic and toiletry products. Although Methylisothiazolinone and Methylchloroisothiazolinone are both biologically functional in terms of antimicrobial activity, the chlorinated molecule is the more active of the two. The antimicrobial activity of Methylisothiazolinone and Methylchloroisothiazolinone may be inactivated by amines, mercaptans, sulfides, and sulfites.[3]

For an evaluation of the efficacy of MI/MCI-CG as an antimicrobial agent in typical cosmetic formulations and raw materials, the reader is referred to the review article by Law et al.[3]

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The absorption, distribution, and excretion of MI/MCI-886 (stabilized with calcium chloride) was evaluated after oral administration to Wistar rats. Two pairs of male and female adult rats received an aqueous solution of MI/MCI-886 by gavage for 7 consecutive days. One pair of rats received MI/MCI-886 with [^{14}C]Methylchloroisothiazolinone (^{14}C- in carbon positions 4 and 5; specific activity of 0.76 μCi/mg) and nonradioactive Methylisothiazolinone at a dose of 2.1 mg/rat/day; whereas, the other pair of rats received MI/MCI-886 with [^{14}C]Methylisothiazolinone (^{14}C- in carbon positions 4 and 5; specific activity of 0.95 μCi/mg) and nonradioactive Methylchloroisothiazolinone at a dose of 0.64 mg/rat/day. Each rat was housed in a separate metabolism cage. Every 24 hours just before dosing, expired air, urine, and feces were collected. These samples, together with the tissues and organs obtained at necropsy, were analyzed for radioactivity. Complete metabolism to carbon dioxide was slight (1.5% or less) and storage in tissues was minimal (2.1% or less). Analysis of 25 organs and tissues indicated that ^{14}C was almost uniformly distributed in the animals, with the largest residues (several ppm) found in the digestive and excretory organs. The lowest concentrations were found in the brain, spinal cord, and gonads (0.12–0.5 ppm). Most of the ^{14}C residue was excreted with a half-life of < 1 day, with approximately 87 to 93% of the administered dose being recovered in the urine or feces. Although Methylisothiazolinone was metabolized or eliminated at a slightly faster rate than Methylchloroisothiazolinone, little difference was found in the manner in which rats metabolized the two compounds. Also, no apparent significant difference was found in the metabolism of either compound between male and female rats. The investigators concluded that [^{14}C]MI/MCI-886 was appreciably absorbed following oral administration to rats with small but detectable amounts distributed in the tissues.[11,30]

The absorption and disposition of MI/MCI-CG was studied in Sprague-Dawley rats after intravenous (i.v.) or dermal administration of the compound with ^{14}C in the carbonyl carbon of either Methylchloroisothiazolinone (specific activity 10.47 mCi/g) or Methylisothiazolinone (specific activity 13.72 mCi/g). [^{14}C]Methylchloroisothiazolinone MI/MCI-CG was rapidly distributed to the blood, liver, kidneys, and testes

following an i.v. dose of 0.8 mg/kg (60 μCi/kg) administered over a 10–20 second period to 24 male rats via the femoral vein. The total recovery of radioactivity ranged from 94 to 111%. The ^{14}C radioactivity in the plasma was rapidly eliminated while the concentration of radioactivity in the blood remained constant at 3 ppm (μg/g) from 6 to 96 hours after administration and comprised 29% of the dose. The investigators suggested that the persistence of ^{14}C radioactivity in the blood (terminal component half-life of 17 days) may indicate that the radioactivity was bound to erythrocyte macromolecules such as hemoglobin and was eliminated slowly during normal erythrocyte clearance (half-life of 14 days in the rat) by the liver and spleen. The elimination of radioactivity from the tissues examined (liver, kidneys, and testes) was biphasic, with a terminal half-life of > 4 days. The concentration of radioactivity was slightly higher in the kidneys than in the liver at each sample time, whereas the 0.03–0.05 ppm concentration in the testes was 10 times lower than in the liver. By 96 hours, the feces, urine, and exhaled carbon dioxide had accounted for 35, 31, and 4% of the dose, respectively.[31]

For the dermal absorption study, 64 male rats were divided into five groups and were administered single 24-h topical applications of 0.2 ml of an aqueous solution containing either 500, 1000, 2000, or 4000 ppm (0.05, 0.1, 0.2, or 0.4%) a.i. [^{14}C]Methylchloroisothiazolinone MI/MCI-CG or 2000 ppm (0.2%) a.i. [^{14}C]Methylchloroisothiazolinone MI/MCI-CG. An additional 12 rats were given four consecutive 24-hour applications of 0.2 ml of 500 or 1000 ppm (0.05 or 0.1%) [^{14}C]Methylchloroisothiazolinone MI/MCI-CG. The solutions were applied to the skin in a glass ring (10.2 cm^2) on the dorsal lumbar region. The percent absorption was calculated as the difference between the amount applied and the amount washed off the skin 24 hours after dosing. The percutaneous absorption of [^{14}C]Methylchloroisothiazolinone MI/MCI-CG ranged from 89 to 94% over the applied concentration range of 500 to 4000 ppm (0.05–0.4%) and was 13% greater than that of [^{14}C]Methylisothiazolinone MI/MCI-CG (82%) at 2000 ppm (0.2%). The systemic bioavailability of MI/MCI-CG was substantially less; approximately one-half of the absorbed MI/MCI-CG was associated with the skin at the application site 24 hours after application. Elimination of the total ^{14}C radioactivity from the application site had a half-life of 13.1 days; the investigators suggested this was due to the normal desquamation of epithelial cells. Since the half-life of MI/MCI-CG applied to the skin was 13.1 days, repeated applications could result in an accumulation of the preservative at the site of application. The authors noted that the actual plateau concentration on the skin would depend upon the amount applied and the application interval. As the applied concentration of [^{14}C]Methylchloroisothiazolinone MI/MCI-CG increased, the relative amount of radioactivity associated with the skin decreased, whereas that in the excreta increased. This indicated a greater systemic penetration at the higher concentrations. The amounts of radioactivity found were in the following order: whole blood > plasma > kidneys > liver > testes. Small amounts of radioactivity were found in the testes [< 2 ppb (0.0000002%)] and blood [24 ppb (0.0000024%)] 28 days after the single dermal application.

Consecutive applications of the radioactive biocide did not affect the proportion of the dose absorbed from the skin, although the proportion excreted was higher than after a single application of an equivalent amount of radioactive MI/MCI-CG. Consecutive applications of only the higher dose also resulted in lower concentrations of blood radioactivity. Urinary excretion of the total ^{14}C of either Methylchloroisothiazolinone (~ 9%) or Methylisothiazolinone (~ 17%) was substantially greater than the fecal

excretion (~ 3% for each). These observations indicate the absorption, distribution, and elimination of radioactive MI/MCI-CG involve dose-dependent and saturable processes.[30,31]

MI/MCI-886 with ^{14}C in either the Methylchloroisothiazolinone (C4 and C5) or the Methylisothiazolinone (C4 and C5) isomer was evaluated for absorption in male Sprague-Dawley rats using dermal, oral by gavage, and intravenous routes of exposure. A range-finding study was conducted first with MI/MCI-CG (1.5% a.i.), with radioactivity in the carbonyl carbon of the Methylchloroisothiazolinone isomer (specific activity 10.47 μCi/mg). Doses of 25, 250, and 2500 ppm a.i. MI/MCI-CG were applied in an aqueous solution to the shaved backs of groups of two male rats by means of a pipette and glass ring. Sites were wiped with an aqueous soap solution immmediately after application or at the end of seven days. For the definitive study, aqueous [^{14}C]Methylchloroisothiazolinone MI/MCI-886 (14.6% a.i.) having a specific activity of 38.40 μCi/mg was applied at doses of 2.5 (4 rats) or 25 ppm (11 rats) dermally, 25 μg/kg orally (8 rats), and 25 μg/kg intravenously (4 rats). Aqueous [^{14}C]Methylisothiazolinone MI/MCI-886 (14.5% a.i.) having a specific activity of 49.55 μCi/mg was similarly administered. Dermal application sites were wiped with water either immediately or 6 hours after application, and the wipes analyzed for radioactivity. Urine and feces were collected from all animals at intervals while whole blood was collected from those rats dermally or orally dosed. Plasma was collected only from those rats in the range-finding study. At termination, ring washes and application site skins from the dermally dosed rats were collected. All of the samples taken were analyzed for radioactivity (Table 4). The proportions of [^{14}C]Methylchloroisothiazolinone MI/MCI-886 systemically absorbed were 38 and 27% after 6 h dermal doses of 2.5 to 25 ppm, respectively. The proportions of [^{14}C]Methylisothiazolinone MI/MCI-886 systemically absorbed were 43 and 26% at dermal doses of 2.5 and 25 ppm, respectively. The percentage of the dermal dose absorbed decreased with increasing doses from 2.5 to 25 ppm, although the quantity of MI/MCI systemically absorbed increased in approxi-

Table 4. Results of Absorption Study with MI/MCI-CG and MI/MCI-886 in Rats

				Percent of Recovered Activity			
Labelled isomer	*Route*	*Dose*	*Peak blood conc. (ppm)*	*Extreta*[a]	*Wipe & ring wash*	*Appl. site skin*	*Percent absorption*[b]
Methylchloroisothiazolinone	IV	25 μg/kg	ND[c]	100	—	—	(100)
	Oral	25 μg/kg	0.098	100	—	—	62
	Dermal	2.5 ppm	ND	38	4	59	38
	Dermal	25 ppm	0.075	27	1	72	27
	Dermal	250 ppm	0.007	29	3	68	29
	Dermal	2500 ppm	1.445	50	3	46	50
Methylisothiazolinone	IV	25 μg/kg	ND	100	—	—	(100)
	Oral	25 μg/kg	0.222	100	—	—	90
	Dermal	2.5 ppm	ND	44	2	54	43
	Dermal	25 ppm	0.195	26	2	73	26

[a]Excreta = urine (u) + feces (f) + uf wash + cage wash.

[b]Percent absorption for oral administration and dermal application = absorption amounts relative to absorption from i.v. administration (normalized to 100% recovery for i.v. administration).

[c]ND = Not determined.

Source: Ref. 32.

mately a dose-dependent fashion. The major portion of the dermal dose of MI/MCI was quickly bound to the application site skin and was not systemically absorbed. The excretion pattern was qualitatively different and the peak whole blood concentration was disproportionately greater after a dermal dose of 2500 ppm than after doses of 250 ppm and less, leading the investigators to conclude that nonlinear kinetics apply after dermal application. The ^{14}C derived from MI/MCI and/or its metabolites had a strong affinity for binding to erythrocytes. Methylchloroisothiazolinone- and Methylisothiazolinone [^{14}C]MI/MCI-886 were similar in their percent dermal absorption, binding to application sites and excretion patterns as well as percent excreted following i.v., oral, and dermal administration. However, Methylisothiazolinone [^{14}C]MI/MCI-886 produced greater blood concentrations after dermal or oral administration and a 45% greater relative absorption after oral administration than Methylchloroisothiazolinone [^{14}C]MI/MCI-886. Comparison of the results from the range-finding study and the definitive study indicated no significant difference in the percent absorption of [^{14}C]MI/MCI after a dermal dose left on the skin for 7 days and a dose wiped off 6 h after application.[32]

A study was conducted to compare the [^{14}C]metabolite profiles following oral and dermal dosing of MI/MCI-886 in male rats. The design of the study was based on results of a previous dermal/oral absorption study[32] in which most of the ^{14}C from an oral dose of MI/MCI-886 was excreted over 24 h, while a significant amount of the ^{14}C from a dermal dose was excreted over 48 h. Three experiments were conducted; experiments A and B were to provide a large, pooled urine and feces sample for development of a high-performance liquid chromatography (HPLC) analytical method for separation and structure identification of individual metabolites, while experiment C was to provide individual excreta samples from rats dosed orally or dermally for comparison of metabolite profiles between dosing routes and comparison of metabolite elution times with those of synthetic standards. In experiment A, 6 male rats were given a 6.25 mg/kg dose by gavage of an aqueous solution of 2500 ppm a.i. Methylchloroisothiazolinone [^{14}C]MI/MCI-886. In experiment B, three male rats were given a similar dose of Methylisothiazolinone [^{14}C]MI/MCI-886. Each isomer was radioactive in the 4 and 5 positions; Methylchloroisothiazolinone and Methylisothiazolinone [^{14}C]MI/MCI-886 had specific activities of 38.4 and 49.55 mCi/g, respectively. The urine and feces of these rats were collected for 24 h. In experiment C, groups of 4 male rats were given an oral dose, as above, of either [^{14}C]MI/MCI-886 or a dermal dose of 1.67 mg/kg of aqueous 2500 ppm a.i. MI/MCI-886 with ^{14}C in either isomer. Urine and feces from those rats dosed dermally were collected at 6, 24, and 48 hours while excreta from those dosed orally were collected at 6 and 24 hours only. Rats were then killed and the blood and skin application sites collected. Blood, urine, and feces were analyzed for ^{14}C. Oral dosing of MI/MCI-886 with ^{14}C in either isomer was followed by the rapid excretion of ^{14}C in the urine (50–77%) and feces (23–54%) by 24 h. Dermal application of MI/MCI-886 with ^{14}C, in either isomer, was followed by a much slower elimination of ^{14}C, with most of the radioactivity (20–28%) appearing in the urine by 48 h and only a minimal amount in the feces (1–2%). The profiles of urinary metabolites following oral or dermal dosing of Methylchloroisothiazolinone [^{14}C]MI/MCI-886 were qualitatively similar. Differences appeared only in the relative amounts of specific metabolites. Similar results were obtained in a study with Methylisothiazolinone [^{14}C]MI/MCI-886. Each profile provided evidence of at least 16 radioactive metabolites. Metabolites identified included *N*-methyl malonamic acid, malonic acid, and malonamic acid. Based on co-chromatography with synthetic standards and chromatographic behavior,

the urinary metabolites were small polar organic acids. Neither parent isomer was detected unchanged in the urine. Reactivity studies were also conducted *in vitro* with MI/MCI-886 and thiol reagents. These indicated that reduction and ring opening may account for the *in vivo* formation of the small organic acids derived from MI/MCI-886. Studies with [^{3}H]radioactive glutathione and MI/MCI-886 ^{14}C in either isomer revealed no conjugate formation.[33]

The dermal absorption of [^{14}C]MI/MCI-886 (specific activity of 0.81 mCi/g) was evaluated by analyzing blood samples from two adult female rabbits. The hair was clipped from the dorsal surface of each rabbit and the skin of one was abraded. Each rabbit was treated on two different sites with 0.5 ml of the test solution containing 100 ppm (0.01%) a.i. Occlusive patches were employed and left in place for 24 h and then removed and the procedure repeated for three consecutive days. Blood samples were collected from the marginal ear vein at 0, 2, 4, 7, 24, 28, 48, and 55 h and assayed for radioactivity. No radioactivity was detectable in the blood samples (sensitivity of testing = 4.5 ppb MI/MCI-886).[30]

The dermal absorption of radioactive MI/MCI was evaluated *in vitro* using freshly excised adult male rat (Crl:CD^{R}BR) skin sections mounted in Franz diffusion cells. A series of eight studies was conducted. Most of the bathing solutions contained gentamcin to control bacterial growth. MI/MCI (14.6/14.5% a.i.) had ^{14}C in the 4 and 5 positions of either the Methylchloroisothiazolinone (specific activity of 4.22 mCi/g) or the Methylisothiazolinone isomer (specific activity of 1.73 mCi/g). A single 35 µl aqueous sample of MI/MCI with ^{14}C in either isomer was applied to the skin at concentrations of 25 or 2500 ppm. At various times after application, the skin sections were wiped with cotton swabs moistened with distilled water and the wipes, skin, and bathing solutions were analyzed for ^{14}C. The ^{14}C found both in or bound to the skin as well as that penetrating the skin into the bathing solution was considered to be bioavailable. The ^{14}C derived from Methylchloroisothiazolinone-radioactive MI/MCI was 99 and 117% bioavailable 3 and 6 h after application of 225 and 2500 ppm, respectively. Ninety percent of the radioactivity remained in the skin. The ^{14}C derived from Methylisothiazolinone[^{14}C]MI/MCI was 3 to 27% bioavailable within 3 to 6 h after application of either 25 or 2500 ppm. Maximum bioavailability was approximately 80% and was reached within 48 to 96 h. At 96 h, more ^{14}C from Methylisothiazolinone [^{14}C]MI/MCI had penetrated the skin than from Methylchloroisothiazolinone [^{14}C]MI/MCI. In TLC and HPLC analyses of the bathing solutions, none of the radioactivity represented the intact parent isomers. The investigators noted that the Franz diffusion cell system is a valid model for estimating the relative bioavailability of MI/MCI in different matrices and that the use of the Methylchloroisothiazolinone-labelled isomer would provide a worse-case estimate of the bioavailability of MI/MCI.[34]

TOXICOLOGY

Aquatic and Avian Toxicity

Mallak and Brunker[23] reported that the LC_{50} (median lethal concentration) of MI/MCI-886 in trout and sunfish was 0.14 mg/L and 0.54 mg/L, respectively. The LC_{50} values were based on an exposure period of six days.

Krzeminski et al.[11] reported that a 3:1 mixture of Methylchloroisothiazolinone and Methylisothiazolinone was moderately toxic to *Lepomis machrochirus* (Bluegill

sunfish). Storage of the two isothiazolinones was minimal in the tissues and viscera of fish exposed continuously to sublethal concentrations of the mixture (0.02, 0.12, 0.80 ppm) for periods of 2 to 8 weeks. The isothiazolinones were rapidly excreted by the fish when the microbiocidal mixture was removed from the water system.

MI/MCI-886 was toxic to both fresh and marine fish species with LC_{50} ranging from 100 to 540 ppb a.i. LC_{50} for shellfish ranged from 14 ppb (0.0000014%) a.i. in bay mussels larvae, to 59 ppm (0.0059%) a.i. in fiddler crabs.[30]

MI/MCI-886 was toxic to avian species. The acute oral LD_{50} of MI/MCI-886 in Bobwhite quail was determined to be 85 and 97 mg a.i./kg in two different tests. Bobwhite quail and Peking Duck had an 8-day dietary LC_{50} of > 60 and > 100 mg a.i./kg/day, respectively.[30]

Acute Toxicity

Oral

MI/MCI-CG and MI/MCI-886 were evaluated for acute oral toxicity in rats in eight tests. These products were tested as received or as diluted solutions. The LD_{50} rates for females were 45 and 64 mg/kg a.i., while those for males were 40, 41, 45, 50, 56, 57, 64, and 78.5 mg/kg a.i. These are classified as moderately to highly toxic by the Hodge and Sterner system of classification.[35] The actual product MI/MCI-CG had an LD_{50} of 3350 mg/kg, classified as slightly toxic. The major signs of toxicity in these tests were those associated with severe gastric irritation, lethargy, and ataxia.[30]

MI/MCI-886 was evaluated for acute oral toxicity in 16 female New Zealand white rabbits. Administered as a 10% solution in methylcellosolve, the LD_{50} was 30 mg/kg a.i. The major signs of toxicity were decreased motor activity and respiration and signs associated with severe gastric irritation.[30]

Dermal

MI/MCI-CG and MI/MCI-886 were evaluated for acute dermal toxicity in seven tests using New Zealand white rabbits. These products were tested as received or as diluted solutions. The dermal LD_{50} rates were > 4.5, > 75, > 75, 87, 94 (abraded), 112 (intact), and 130 mg/kg a.i.[30] These values (with the exclusion of the 4.5 mg/kg value) are classified as moderately toxic by the Hodge and Sterner system of classification.[35]

Intraperitoneal

MI/MCI-886 was tested for acute intraperitoneal (i.p.) toxicity in Wistar rats. Administered in water, the i.p. LD_{50} ratings for males and females were 4.6 and 4.3 mg a.i./kg, respectively. The major sign of toxicity was decreased motor activity and the principal lesion was peritonitis.[30]

Inhalation

MI/MCI-886 was evaluated for acute inhalation toxicity in six tests using rats. MI/MCI-886 was tested as received or in aqueous solution. The inhalation levels of LC_{50} were variously reported as > 0.15, 0.2 (males), 0.2 (females), > 0.65, 0.672, > 1.3, > 1.4 (females), and < 1.4 (males) mg a.i./L air. The major signs of toxicity were marked dyspnea and salivation and death, and the principal lesions included pulmonary congestion, edema, and hemorrhages.[30] The actual product MI/MCI-CG had an LC_{50} of > 4.6 mg/L air (air saturated with solution containing 10 times greater content of active ingredients than MI/MCI-CG).[3]

Irritation

Chorioallantoic Membrane

MI/MCI-CG and MI/MCI-886 were evaluated for irritation potential in the Hen's Egg Chorioallantoic Membrane Test. On day 10 of incubation, the shells of White Leghorn eggs were scratched around the air cell and then pared off. The vascular chorioallantoic membrane was subsequently exposed by removing the inner egg membrane. The test substance was then dropped onto the membrane in a volume of 0.2 ml. Four eggs were tested at each concentration of test material. Two eggs treated with the vehicle only served as controls. Following application of the test substance, the chorioallantoic membrane, the blood vessels (including the capillary system), and the albumen were examined and scored at 0.5, 2, and 5 minutes after treatment for irritant effects (hyperemia, hemorrhage, coagulation). At later observation times, the lesions were similar. The numerical time-dependent scores were summed to give a single numerical value indicating the irritation potential of the test material. The mean value of four tests made possible an assessment of irritation by a classification scheme analogous to the Draize categories. MI/MCI-886 and MI/MCI-CG, with active concentrations of 15.0 and 1.5%, respectively, were described as strong irritants. MI/MCI-CG tested at 0.3 and 0.075% a.i. produced moderate and slight irritation, respectively. At 0.03% a.i., MI/MCI-CG was nonirritating. Hyperemia, hemorrhages and coagulation were noted at higher concentrations. These corrosive effects were comparable to *in vivo* results[36] based on Draize eye irritation tests.[37]

Ocular

MI/MCI-886 and MI/MCI-CG were evaluated for ocular irritation in eight Draize or modified Draize tests using albino rabbits. MI/MCI-886 ranging in concentration from 1.1 to 14% a.i. and MI/MCI-CG with a 1.5% a.i. concentration were corrosive when tested as supplied. Aqueous dilutions of MI/MCI-886 with concentrations of 0.056% a.i. were nonirritating; 0.28% a.i. was slightly to moderately irritating; 0.56 and 1.7% a.i. were moderately to severely irritating; and 2.8 and 5.6% a.i. were severely irritating (corrosive).[30]

The cumulative ocular irritation of MI/MCI-886 was evaluated using six male rabbits. A 0.1 ml sample of an aqueous dilution of MI/MCI-886 containing 56 ppm (0.0056%) a.i. was instilled into the conjunctival sac of one eye of each rabbit every 15 minutes for 2 hours. This procedure was repeated daily, five days a week for four weeks. Six other rabbits received the vehicle (tap water with 1 ppm available chlorine) as controls. Sporadic and mild conjunctivitis was observed in both groups. MI/MCI-886, at an active concentration of 56 ppm (0.0056%), was not an eye irritant.[30]

Dermal

MI/MCI-CG and MI/MCI-886 were evaluated for dermal irritation in nine tests using New Zealand white rabbits. Occlusive patches were used and sites were both intact and abraded. MI/MCI-886, as supplied at active concentrations ranging from 1.1 to 13.7%, was severely irritating as indicated by the Primary Irritation Indices (PII) ranging from 6.8 to 8.0 (max 8), respectively. MI/MCI-CG, with an a.i. concentration of 1.5%, was severely irritating with PIIs of 7.3 and 7.5. Aqueous dilutions of MI/MCI-886 were tested with the following results: a concentration of 0.056% a.i. was nonirritating; 0.28% a.i. was moderately irritating (PII = 3.16); 0.56% a.i. was severely irritating (PII = 6.3); 5.6% a.i. was corrosive to rabbit skin.[30]

Short-Term Toxicity

Oral

MI/MCI-886 was administered in the diet to groups of 5 male and 5 female rats for two weeks. Concentrations administered were 0, 7.3, 22.4, 74, and 224 ppm a.i.; equivalent to 0, 0.82, 2.5, 8.2, and 24.4 mg/kg/day a.i. No treatment-related effects were observed during the study or at necropsy.[30]

MI/MCI-886 was similarly administered in the diet to groups of Beagle dogs consisting of one male and one female. Administration continued for 2 weeks at concentrations of 28, 84, 280, and 840 ppm a.i.; equivalent to 1.2, 4.3, 15, and 29 mg/kg/day a.i. for the males and 1.3, 3.5,12, and 38 mg/kg/day a.i. for the females. A slight decrease in feed consumption was noted at the two greater doses in both males and females. The high-dose male had an increased hematocrit value, the two higher dose females had decreased leukocyte counts, and a slight decrease in blood glucose was noted in both the high dose male and female. No other treatment-related effects were observed during the study or at necropsy.[30]

Dermal

MI/MCI-886 was evaluated for dermal toxicity using groups of 10 male and 10 female albino rabbits (only the control group had 5 males and 5 females). Occlusive patches containing a 0.1% aqueous solution of MI/MCI-886 were applied to both intact and abraded skin daily, 5 days a week for three weeks. The concentrations applied were 0, 0.56, and 2.8 mg/kg/day a.i. All of the treated animals had moderate dermal irritation at the application site. No systemic toxicity was noted at necropsy or microscopic examination of the kidneys and liver.[30]

Inhalation

MI/MCI-886 was evaluated for inhalation toxicity using groups of 10 male rats. The rats were exposed 6 hours daily, 5 days a week for two weeks to an aerosolized aqueous solution of MI/MCI-886 yielding concentrations of 0, 0.03, 0.07, and 0.13 mg/L of air a.i. A decreased weight gain was noted in animals of the mid- and high-dose groups. One and two rats from the low- and high-dose groups, respectively, died during the study; lesions included pulmonary hemorrhages, swollen livers, and "possible" chronic passive congestion. These effects were considered treatment-related. The no-observable-effect-level (NOEL) was < 0.03 mg/L of air a.i.[30]

Subchronic Toxicity

Oral

MI/MCI-886 was administered in the diet to groups of 15 male and 15 female rats for three months. The concentrations in the diets were 0, 44.8, 146, and 448 ppm a.i. (equivalent to approximate doses of 0, 3, 10, and 30 mg/kg/day a.i.). The doses were adjusted during the study to assure a constant intake of MI/MCI-886. No rats died during the study. The treated rats had a slightly increased incidence of alopecia and skin scabbing when compared with control rats. Dose-related increases in absolute and relative adrenal gland weights were noted in the females, while the high-dose males had a slight but significant increase in serum glutamic oxalocetic transaminase (SGOT) activities. No treatment-related lesions were found at necropsy or microscopic exami-

nation. Therefore, the increased adrenal gland weights and SGOT values were considered of no toxicological significance.[30]

MI/MCI-886 was administered in the diet to groups of 4 male and 4 female beagle dogs for three months. Concentrations administered were 0, 84, 280, and 840 ppm a.i. (equivalent to approximate does of 0, 3, 9, and 28 mg/kg/day a.i.). No treatment-related effects were noted. Hematologic, clinical chemistry, and urinalysis values were normal. No lesions were found at gross and microscopic examination. No treatment-related toxicity was associated with the administration of MI/MCI-886 to dogs for three months at concentrations up to 28 mg/kg/day a.i.[30]

MI/MCI-886 was administered in the drinking water at concentrations of 0, 25, 75, and 225 ppm a.i. (equivalent to 0, 3, 8, and 20 mg/kg/day a.i.) to groups of 25 male and 25 female rats for 13 weeks. Of the two control groups, one received only tap water and the other received tap water containing all of the inorganic ions present in MI/MCI-886 (9% $MgCl_2$, 15% $Mg(NO_3)_2$, and 0.6% $KBrO_3$) at a concentration equivalent to that of the high-dose group. At the end of 13 weeks, 15 rats/gender/group were killed for necropsy, and the organs weighed. The remaining 10 rats/gender/group were maintained on the appropriate drinking solutions for two more weeks prior to mating for the reproductive phase of the study (see Teratogenicity). No rats died during the study. Compound-related decreases in body weight and feed consumption were not considered toxicologically significant. Water consumption was significantly decreased in all treatment groups. At necropsy at the end of the toxicity and reproductive phases, no treatment-related changes were found. A significant decrease in globulin and an increase in A/G ratios was noted in the high-dose males and the ion control group. A significant decrease in total protein was also noted at the high dose. SGOT activities were significantly increased in the females. Relative weights of the liver and kidneys were significantly increased for the male and female rats of the high-dose group, respectively. Slight gastric irritation was found in 7/15 males and 5/15 females of the high-dose group, a change not seen in the low- or mid-dose groups or in either of the control groups. MI/MCI-886 had a NOEL of 75 ppm a.i. (equivalent to 6.28 and 10.8 mg/kg/day a.i. for males and females, respectively) and a minimal effect level of 225 ppm (16.3 and 24.7 mg/kg/day for males and females, respectively) when administered in the drinking water for 13 weeks.[30]

Dermal

MI/MCI-886 was evaluated for dermal toxicity using groups of 6 male and 6 female New Zealand white rabbits. Dermal applications of 1 ml/kg were applied daily, 5 days per week for 13 weeks to both intact and abraded skin. An aqueous dilution of MI/MCI-886 was administered at concentrations of 0, 100, 200, and 400 ppm a.i. (equivalent to 0, 0.1, 0.2, and 0.4 mg/kg/day). Deaths occurred in all treatment groups: 3/12, 5/12, and 4/12 from the low, mid, and high doses, respectively. These were attributed to endemic respiratory disease which may have been aggravated by the stress of treatment with MI/MCI-886, a known irritant. No control animals died. A dose-related dermal irritation consisted of slight to severe erythema and very slight edema at all concentrations. No treatment-related lesions were found at necropsy or microscopic examination. The investigators concluded that dermal application of MI/MCI-886 at concentrations up to 400 ppm for 13 weeks produced no systemic toxicity in rabbits.[30]

Sensitization, Photosensitization, and Phototoxicity

The commercial biocide, MI/MCI-886, was evaluated for production of delayed contact dermatitis in guinea pigs. The undiluted commercial product was an aqueous solution which contained a mixture of Methylchloroisothiazolinone and Methylisothiazolinone in a ratio of 3:1, respectively, (total a.i. = 14.4%) with $MgCl_2$ (9%) and $Mg(NO_3)_2$ (16%) present as stabilizers. Various aqueous dilutions of the product were prepared, and the final concentrations of the two isothiazolinone active ingredients were confirmed by high-pressure liquid chromatography. The patch test procedures described by Ritz and Buehler[38] were employed. For the induction phase, 0.4 ml doses of the diluted product were applied under occlusive patches to the clipped backs of Hartley guinea pigs. The patches were held in place by a rubber "dental dam." Induction concentrations ranged from 20 to 2000 ppm. Three, 6-h applications were made per week for three consecutive weeks for a total of nine induction exposures. The treated sites were rinsed with water following application of the test materials. Twelve to 15 days after the last induction dose, the animals were challenged with 0.4 ml of the diluted product by means of an occlusive patch. The challenge concentrations ranged from 20 to 2000 ppm. Control guinea pigs also were challenged with the diluted product at the same concentrations. Approximately 24 hours after the challenge exposure, the backs of the guinea pigs were depilated with a commercial hair remover. The treated sites were graded for skin erythema 2 to 5 hours after depilation and 48 hours after challenge. The EC_{50} values for induction and "elicitation" of delayed contact dermatitis were estimated by probit analysis as described by Finney.[39] The EC_{50} was defined as the concentration at which delayed contact dermatitis was seen in 50% of the population (Table 5). No skin erythema was observed in the control guinea pigs. The incidence of delayed contact dermatitis was dependent on the induction concentration. At a challenge concentration of 2000 ppm, 1/20, 2/15, 9/15, 10/10, and 20/20 guinea

Table 5. Incidence of Delayed Contact Dermatitis in Guinea Pigs Induced and Challenged by Various Concentrations of MI/MCI Biocide

Induction treatment	*Induction concentration (ppm a.i.)*[a,b]	*Incidences of Delayed Contact Dermatitis Challenge Concentration (ppm a.i.)*[a–c]								
		2000	*1000*	*500*	*250*	*200*	*100*	*50*	*25*	*20*
Noninduced control	0	0/20		0/10		0/10		0/30	0/10	
MI/MCI biocide[d]	2000	20/20	2/2	1/2	1/2	2/10				0/10
	1000		4/5	3/5		3/15		0/20		
	500	10/10		3/10			0/10			
	100	9/15					1/15			
	50	2/15				1/15	0/15	0/15		
	25	1/20				0/20	0/20		0/20	

[a]Dosage volume = 0.4 ml/patch.

[b]a.i. = active ingredients.

[c]The number of animals that responded at either 24 or 48 hours after the challenge exposure over the total number of animals challenged in that group.

[d]MI/MCI biocide = commercial product containing Methylisothiazolinone (MI) and Methylchloroisothiazolinone (MCI).

Source: Ref. 5.

pigs "responded" when treated with 25, 50, 100, 500, and 2000 ppm, respectively. The incidence of delayed contact dermatitis also was dependent on the challenge concentration. At an induction concentration of 1000 ppm, 0/20, 3/15, 3/5, and 4/5 guinea pigs "responded" when challenged with 50, 200, 500, and 1000 ppm a.i., respectively. The investigators suggested that a "no response concentration zone" was indicated by the data. The reported "no response zone" corresponded to induction (I) and challenge (C) active ingredient concentrations of: 2000 (I) and 20 (C) ppm; 1000 (I) and 50 (C) ppm; 500 (I) and 100 (C) ppm; 50 (I) and 100 (C) ppm; and 25 (I) and 200 (C) ppm. The estimated EC_{50} for induction in guinea pigs challenged with 2000 ppm was 88 ppm a.i., with 95% confidence limits of 66–145 ppm a.i. The calculated EC_{50} for "elicitation" (sensitization) in guinea pigs induced with 1000 ppm a.i. was 429 ppm a.i., with 95% confidence limits of 272–995 ppm. The authors reported: (1) the potential of MI/MCI-886 to cause delayed contact dermatitis was dependent on both the induction and challenge concentrations; (2) the number of induction doses may be an important factor in demonstrating the sensitization potential of MI/MCI-886 and; (3) there is a "no response concentration" at which the biocide product can be used without concern for clinically significant delayed contact dermatitis.[5,30]

MC/MCI-886 was evaluated for skin sensitization using a modified Buehler technique. Groups of 10 guinea pigs (strain not specified) were treated with two 5-hour occlusive patches containing concentrations of 1400, 4200, and 14,000 ppm a.i. The control group was treated with water. The high dose produced irritation after a single application; minimal irritation was noted at the application site in the low- and mid-dose groups. Two weeks after the second induction application, the animals were challenged with an aqueous dilution of MI/MCI-886 containing 420 ppm. Twelve days later, the animals were rechallenged with 1400 ppm. The first challenge produced no reactions. Rechallenge produced sensitization reactions in 4/10, 7/10, and 6/10 animals in the low-, mid-, and high-dose groups, respectively.[30]

Methylisothiazolinone and MI/MCI-886 were evaluated for delayed contact hypersensitivity using a modified Buehler technique. Groups of 20 Hartley guinea pigs were induced with occlusive patch applications of aqueous solutions of either 16,000 ppm Methylisothiazolinone or 2000 ppm MI/MCI-886 (these were the highest nonirritating concentrations of each respective substance). Patches were applied 6 hours daily, three days per week for three weeks. After each 6-hour exposure, the application sites were washed. Following a two-week nontreatment period, the test groups and a noninduced control group were challenged with the same induction concentrations. Methylisothiazolinone and MI/MCI-886 clearly produced delayed contact hypersensitivity in 16/20 and 20/20 guinea pigs, respectively. These animals were subsequently rechallenged to evaluate possible cross-reactions, a "threshold" concentration for the elicitation of sensitization, and the persistence of hypersensitivity. Those animals induced with Methylisothiazolinone did not respond to challenge with either 160 or 1,600 ppm Methylisothiazolinone; however, they did respond to challenge with 2000 ppm MI/MCI-886. The "threshold" for elicitation of sensitization was between 1,600 and 16,000 ppm for Methylisothiazolinone. Those animals treated with MI/MCI-886 responded positively to challenge with 200 and 2000 ppm MI/MCI-886 but not to 20 ppm MI/MCI-886 or 16,000 ppm Methylisothiazolinone. The "threshold" for elicitation of sensitization was between 20 and 200 ppm for MI/MCI-886. After a nontreatment period of 28 to 35 days, those animals treated with MI/MCI-886 responded positively to challenge with concentrations of MI/MCI-886 ranging from 250 to 2000 ppm. Thus, MI/MCI-886 induced sensitization persisted in the guinea pig for at least 35 days.[30]

An aqueous solution of MI/MCI-886 containing 56 ppm a.i. was evaluated for sensitization in 10 albino guinea pigs using the maximization procedure of Magnusson-Kligman. No reactions were observed 24 and 48 hours after challenge. The investigators concluded that MI/MCI-886, at a concentration of 56 ppm, was not a skin sensitizer under these test conditions.[30]

No incidence of delayed contact dermatitis was observed when MI/MCI-CG was applied to the skin of guinea pigs at induction and challenge concentrations of 1500 ppm. The induction phase consisted of one application per week for three weeks. The number of animals used and whether the sites had occlusive patches were not stated (private communication to P.K. Chan).[40]

MC/MCI-886 was evaluated for irritation, sensitization, phototoxicity, and photosensitization using groups of 8 guinea pigs. A range-finding test was conducted to determine the maximum nonirritating and nonphototoxic concentrations. Single applications of graded dilutions of MI/MCI-886 were made to the shaved backs of each animal. In one group, the sites were irradiated from 35 cm for 15 minutes with a 275 W General Electric sunlamp. The highest nonphototoxic/nonirritating concentration was 1400 ppm. This concentration was then used for the sensitization and photosensitization tests. Two test groups of 8 guinea pigs each were treated with applications of 0.5 ml of an aqueous dilution containing 1400 ppm MI/MCI-886 four times per week for two weeks. The application sites did not have occlusive patches. After a 10–14-day nontreatment period, both groups were challenged with 420 ppm and rechallenged with 1400 ppm; one group was also irradiated (as previously described) during each challenge phase. No phototoxic reactions were observed. No sensitization or photosensitization reactions were observed upon challenge with 420 ppm. On rechallenge with 1400 ppm, 7/8 guinea pigs in each group had reactions indicative of sensitization; severity of the reactions was the same in both groups. The investigators concluded MI/MCI-886 was neither phototoxic nor photosensitizing, but was a sensitizer under these test conditions.[30]

GENOTOXICITY

Wright et al.[8] found that the commercial biocide, MI/MCI-886, was mutagenic in three different studies. The biocide contained (by weight): 10% Methylchloroisothiazolinone, 3.4% Methylisothiazolinone, 9% magnesium chloride, and 15% magnesium nitrate in aqueous solution. In the first of the three studies, MI/MCI-886 was evaluated in a plate-incorporation assay by means of the method described by Ames et al.[41] Preliminary tests indicated that MI/MCI-886, in the absence of S-9 mix, was mutagenic to *Salmonella typhimurium* strain TA100, but not to strains TA1535, TA1537, or TA98. *S. typhimurium* TA100 was therefore assayed in plate-incorporation tests in order to obtain dose-response curves. Three separate experiments were performed, each using one plate per dose of MI/MCI-886, which was diluted in sterile water to achieve the desired concentration. In the first experiment, assays were performed in the dose range of 0 to 40 nl MI/MCI-886 (0 to 4.36 μg a.i./plate) in the presence and absence of liver S-9 mix from phenobarbital-treated rats. In two other experiments, S-9 mix was omitted. Positive controls consisted of spot-tests with methyl methanesulfonate and 2-aminofluorene. Reproducible linear dose-response curves in all three experiments were obtained where MI/MCI-886 was tested in the absence of S-9 mix. A mean slope of 2.69 ± 0.28 revertants per ng of active ingredients indicated that

one (or both) of the biologically active ingredients of MI/MCI-886 was a potent mutagen. If Methylchloroisothiazolinone was the mutagen, this slope would be equivalent to 533 revertants per nmole; the corresponding value for Methylisothiazolinone being 1227 revertants per nmole. Addition of S-9 fraction diminished, but did not eliminate the mutagenicity of MI/MCI-886, reducing the slope to 38 and 87 revertants per nmole for Methylchloroisothiazolinone and Methylisothiazolinone, respectively. In the absence of S-9, MI/MCI-886 was toxic above a dose of 20 nl per plate (2.69 μg/plate). The reduction of the mutagenic effect of MI/MCI-886 by S-9 mix was accompanied by a reduction of its toxicity, since a linear dose-response curve for mutagenicity was obtained up to and including a dose of 5.36 μg/plate, double the value obtained in the absence of S-9 mix. The results of the genotoxicity testing are presented in Table 6.

The mutagenicity of MI/MCI-886 demonstrated in the previous investigation was confirmed in a second plate incorporation assay by Wright et al.[8]

In this second study, MI/MCI-886 was assayed for mutagenicity in *S. typhimurium* TA100 and *Escherichia coli* WP2uvrA(p) by the method described by Venitt and Crofton-Sleigh.[42] In the first of two experiments, MI/MCI-886 was diluted 1:10,000 in deionized water and then assayed in the dose range of 1 to 2 nl/plate (134 to 2680 ng a.i. per plate). The assay was performed with and without the addition of S-9 mix from the livers of Aroclor 1254-induced rats. In the second experiment, S-9 was not used and the dose range was 0.1–1.0 nl/plate (13.4–134 ng a.i. per plate). Three plates per dose were used at each dose in both experiments. Sodium azide was used as a positive control, yielding slopes of 755 and 1109 (mutants per μg) for *E. coli* WP2uvrA(p) and *S. typhimurium* TA100, respectively. In the absence of S-9, toxic effects in both species were observed at doses of 0.134 μg/plate and above. The addition of S-9 extended the observed toxicities to 1.34 μg/plate and above.

In the third study by Wright et al.,[8] MI/MCI-886 was assayed for mutagenicity in the absence of an exogenous activation system in two separate fluctuation tests using the method of Gatehouse.[43] The bacterial strains *S. typhimurium* TA100 and *E. coli* WP2uvrA(p) were employed, and positive controls consisted of 4-chloromethylbiphenyl for TA100 and potassium dichromate for *E. coli*. Reproducible linear dose-response curves were obtained for both bacterial species, with the *Salmonella* strain being about 1.8 times more sensitive to the mutagenic effects of MI/MCI-886 than the *Escherichia* strain. Negative mutagenic results were obtained in a single experiment using TA98 (data not published).

MI/MCI-886 containing 10.1% (w/w) Methylchloroisothiazolinone was mutagenic in the plate incorporation assay. The biocide dissolved in dimethylsulfoxide (DMSO) was evaluated without S-9 mix using *S. typhimurium* strain TA100 according to the methods described by Ames et al.[41] Product doses of 1.0, 2.0, 5.0, 10, 20, and 50 μg/ml produced a mean number of revertants per plate of 0, 742.0, 1050, 592, 189.7, and 134.0, respectively. The positive control agent, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, also was mutagenic in TA100 without S-9 mix; the vehicle control was nonmutagenic.[44]

MI/MCI-CG was mutagenic in the Ames assay. Solutions of the commercial product were prepared in 17 concentrations ranging from 1.0 μg to 10.0 mg/0.1 ml by dilution of the concentrated product with DMSO. Aliquots of 0.1 ml/plate were then used to test each solution for mutagenesis according to the method of Ames et al.[41] *S. typhimurium* strain TA100 was used both with and without addition of liver S-9 fraction from Aroclor-treated rats. The positive controls used for the tests with and without S-9

activation were 2-aminoanthracene and sodium azide, respectively. All tests were run in duplicate and the incubated plates were examined for toxicity (the point at which the growth of the test organism was inhibited by the antibacterial agent). Without S-9 activation, toxicity prevented the evaluation of MI/MCI-CG concentrations ≥ 80 μg/plate (a.i. = 1.2 μg/plate). The bacteriostatic effect of the product was ameliorated considerably by S-9 activation. Approximately 25 times as much active ingredient per plate (30 μg) after microsomal activation was required to produce the degree of toxicity observed without activation. MI/MCI-CG produced statistically significant increases in the number of revertants/plate at concentrations ranging from 0.30 to 15.0 and 0.03 to 0.75 μg a.i./plate with and without S-9 activation, respectively. The results with S-9 activation indicated that, on the basis of concentration in top agar, the combined MI/MCI-CG active ingredients had a mutagenicity "about equal" to that of the positive control, 2-aminoanthracene. Without S-9 activation, mutagenicity was markedly increased with MI/MCI-CG having approximately seven times the mutagenicity of sodium azide. Without S-9 activation, the mutagenicity first became significant when the active ingredients of MI/MCI-CG reached a concentration of 0.01 ppm of top agar (0.03 μg a.i./plate). This concentration was a thousand times less than the manufacturer's maximum recommended usage level in cosmetics of 3–15 ppm. The reduction in mutagenicity with the addition of S-9 fraction may be explained by the fact that MI/MCI-CG contains two active ingredients, with Methylchloroisothiazolinone interacting with the sulfhydryl group of enzymes and other proteins causing cleavage of the ring structure. According to the investigators, ring cleavage by S-9 proteins may reduce the toxic and mutagenic potential of Methylchloroisothiazolinone, allowing measurement of the mutagenicity of Methylisothiazolinone.[10]

Methylisothiazolinone and Methylchloroisothiazolinone were each evaluated for clastogenic activity in the mouse micronucleus test. Male C57B1/6J mice were given two consecutive 250 mg/kg doses of the test material by intraperitoneal injection. Doses were administered 24 hours apart and were equivalent to 50 to 80% of the intraperitoneal LD_{50}. Five hundred polychromatic erythrocytes were examined from each animal, and the incidence of micronuclei/1000 cells was scored at both 24 and 48 h. The ratio of polychromatic erythrocytes to mature erythrocytes also was determined as a measure of cytotoxicity. Results indicated that Methylisothiazolinone, Methylchloroisothiazolinone, and *N,N*-dinitrosopentamethylenetetramine (negative control) were negative for clastogenic activity at both sampling times. The system positive control, cyclophosphamide, gave a statistically significant increase in micronuclei. In bone marrow cells treated with Methylisothiazolinone or Methylchloroisothiazolinone, the ratio of polychromatic erythrocytes to mature erythrocytes did not deviate from the normally expected range. The authors concluded that although the negative results confirmed previous bone marrow cytogenic investigation on MI/MCI-886 (quoted by Wright et al.),[8] their own findings must be treated with some reservation since no chemical class control was known for the two thiazolones tested. They suggested that genotoxic chemicals with complex metabolism *in vivo* or that are highly organotropic may not register a positive result in an *in vivo* assay in which only one organ is sampled.[45]

During product development of the MI/MCI biocide, the manufacturer conducted an Ames test and a cytogenetics test, both at Litton Bionetics, 1976 and 1973, respectively. The Ames test was conducted using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 as well as *Saccharomyces cerevisiae* strain D-4 with MI/MCI-886 (a.i. of 14%) at concentrations of 0.00005 to 0.1 μl product/plate. Each strain was tested with and without metabolic activation. MI/MCI-886 produced

Table 6. Genotoxicity of Methylisothiazolinone and Methylchloroisothiazolinone

Compound	Test	Results		Reference
		w/S-9	*w/o S-9*	
MI/MCI-886 (13.4% a.i.[a])	Ames assay			
	S. typhimurium TA98	—	(−)	8
	S. typhimurium TA100	(+)	(+)	
	S. typhimurium TA1535	—	(−)	
	S. typhimurium TA1537	—	(−)	
MI/MCI-886 (13.4% a.i.)	Plate incorporation assay			8
	S. typhimurium TA100	(+)	(+)	
	E. coli WP2uvrA(p)	(+)	(+)	
MI/MCI-886 (13.4% a.i.)	Fluctuation test			8
	S. typhimurium TA100	—	(+)	
	S. typhimurium TA98	—	(+)	
	E coli WP2uvrA(p)	—	(−)	
MI/MCI-886 (10.1% MCI)	Ames assay			44
	S. typhimurium TA100	—	(+)	
MI/MCI-CG (1.5% a.i.)	Ames assay			10
	S. typhimurium TA100	(+)	(+)	
MI	Mouse micronucleus test for clastogenic activity	(−)		45
MCI		(−)		
MI/MCI-886 (14% a.i.)	Ames assay			30
	S. typhimurium TA98	(−)	(−)	
	S. typhimurium TA100	(−)	(−)	
	S. typhimurium TA1535	(−)	(−)	
	S. typhimurium TA1537	(−)	(−)	
	S. typhimurium TA1538	(−)	(−)	
	Saccharomyces cerevisiae D4	(−)	(−)	
MI/MCI-886 (14% a.i.)	Cytogenetics test for chromosomal aberrations in rat	(−)		30
MI/MCI-886 (15% a.i., 2 different lots)	Ames test			46
	S. typhimurium TA98	(−)	(−)	
	S. typhimurium TA100	(−)	(+)	30
	S. typhimurium TA1535	(−)	(−)	
	S. typhimurium TA1537	(−)	(−)	

MI	Ames assay				46
	S. typhimurium TA98	(−)		(−)	
	S. typhimurium TA100	(−)		(−)	30
	S. typhimurium TA1535	(−)		(−)	
	S. typhimurium TA1537	(−)		(−)	
MCI	Ames assay				46
	S. typhimurium TA98	(−)		(−)	
	S. typhimurium TA100	(−)		(+)	30
	S. typhimurium TA1535	(−)		(−)	
	S. typhimurium TA1537	(−)		(−)	
MI/MCI-886 (15% a.i.)	Gene mutation assay using a mouse lymphoma cell line	(+)		(+)	46 30
MI/MCI-886 (17.2% a.i.)	Gender-linked recessive lethal test with *Drosophila melanogaster* (injection and oral routes)		(−)		46 30
MI/MCI-CG (1.5% a.i.)	Unscheduled DNA synthesis using rat hepatocytes		(−)		46
MI/MCI-886 (15% a.i.)	*In vivo* cytogenetics assay (for chromosomal aberrations) using mice		(−)		46 30
MI/MCI-886 (15% a.i.)	Assay to detect induced cell transformation in the mouse embryo fibroblast cell line C3H 10T1/2		(−)		46 30
MI/MCI-CG (1.5% a.i.)	*In vitro* chromosomal aberration test using Chinese hamster lung fibroblasts		(−)		30
MI/MCI-886 (16% a.i.)	*In vivo* cytogenetics assay using mice	—		(−)	30
MI/MCI-886 (a.i. not specified)	Mutagenicity test using L5178Y mouse lymphoma cell line	—		(+)	30
MI/MCI-886 (a.i. not specified)	DNA binding				38
	in vitro with mouse lymphoma cell line	No DNA binding detected.			
	in vivo with rat testicular DNA	No DNA binding Detected.			

[a]a.i = active ingredients

inhibition of growth at the high dose of 0.1 μl/plate (0.014 μl a.i./plate). A slight increase in the number of revertants as compared to controls was seen at 0.05 μl/plate (0.007 μl a.i./plate) with TA100 without metabolic activation; however, this was not confirmed in a repeat test. No other increases were observed; MI/MCI-886 was not mutagenic under these test conditions.[30]

For the cytogenetics test, MI/MCI-886 (in 0.5% Methocel) was administered by gavage at concentrations of 0, 0.28, 2.8, and 28 mg a.i./kg daily for 5 days to groups of 5 Sprague-Dawley rats. A positive control group was administered triethylene melamine. No chromosomal aberrations were found in the bone marrow specimens of any of the treated or negative control animals; chromosomal aberrations were seen in 35% of the cells from the positive control group. MI/MCI-886 did not induce chromosomal changes in rat bone marrow cells under the conditions of this assay.[30]

Although MI/MCI-886 was not mutagenic or clastogenic in these two tests, subsequent personal communication indicated that the biocide induced an increase in revertants in *S. typhimurium* strain TA100. This was confirmed in the Rohm and Haas laboratories and led to an extensive evaluation of the mutagenic potential of this biocide.[46]

Four lots of the biocide were used for the series of studies: lots A (MI/MCI-886), B (MI/MCI-886), C (MI/MCI-886), and D (MI/MCI-CG) containing 15, 15, 17.2, and 1.5% a.i., respectively. The first evaluation was an Ames test using *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 with or without metabolic activation. Without a metabolizing system, MI/MCI-886 was very toxic to all strains and had a steep dose response. Metabolic activation shifted the toxic response to higher concentrations. A statistically significant increase in revertants was noted for TA100 without metabolic activation at concentrations of 0.099 to 0.198 and 0.099 to 0.495 μg a.i./plate for Lots A and B, respectively. Purified samples of Methylisothiazolinone and Methylchloroisothiazolinone were also tested in the Ames assay. Without metabolic activation, Methylchloroisothiazolinone inhibited growth in all strains and significantly increased the number of revertants in TA100 at concentrations of 0.20, 0.25, and 0.30 μg a.i./plate and in two of three trials at 0.10 μg a.i./plate. Methylisothiazolinone induced no mutagenic activity in any strain with or without activation although it did inhibit the growth of TA100 at concentrations of 25 μg a.i./plate and above (without S-9), a concentration 25 to 50 times higher than that observed with Methylchloroisothiazolinone.[30,46]

The second test was a gene mutation assay using mouse lymphoma cell line L5178Y ($T/K^{\pm}$) with or without metabolic activation. Test concentrations of MI/MCI-886 (Lot A) were selected to range from nontoxic to 10% relative growth. MI/MCI-886 had an extremely steep toxicity curve; the addition of an activation system shifted the toxicity to a 10-fold higher concentration. MI/MCI-886 significantly increased the mutant frequencies by three to five times background at concentrations of 0.198 and 0.297 μg a.i./ml without activation and by 2–10 times background at concentrations of 2.97 to 5.94 μg a.i./ml with activation.[30,46]

A gender-linked recessive lethal test using *Drosophilia melanogaster* was conducted by both injection and oral administration of MI/MCI-886 (Lot C). Canton-S wild-type males were fed either 86 (LC_{30} at 72 h) or 52 μg a.i./ml or were injected with 0.3 μl of an aqueous solution of 258 μg a.i./ml (equivalent to 77 ng a.i.; LC_{30} at 24 h). They were then mated with virgin Basc females. The number of lethals in the progeny of the treated males was comparable to the number obtained with the control males; MI/MCI-886 was not mutagenic under the conditions of this *in vivo* test.[30,46]

The potential of MI/MCG-CG (Lot D) to induce unscheduled DNA synthesis was measured by autoradiography in primary cultures of adult rat hepatocytes by the method of Williams[47,48] with modifications by Probst et al.[49] MI/MCI-CG and two positive controls, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and 2-acetylaminofluorene, were dissolved and serially diluted in DMSO; dilutions of DMSO served as the negative control. Primary cultures were incubated for 20 hours with 0.00375 to 7.5 μg a.i./ml MI/MCI-CG. Cytotoxicity was observed at concentrations of MI/MCI-CG above 0.75 μg a.i./ml. MI/MCI-CG did not induce unscheduled DNA synthesis in the cultured rat hepatocytes.[46]

An *in vivo* cytogenetics assay was conducted using groups of 8 male Charles River CD-1 mice. MI/MCI-886 (Lot A) was administered orally in sterile water at concentrations of 1.5, 6, and 15 mg a.i./kg on an acute basis and at a concentration of 15 mg a.i./kg on a short-term (daily for 5 days) basis. Mice were killed at 6, 24, and 48 hours after the single dose and 6 hours after the last multiple dose. The bone marrow cells from the femurs were examined for chromosomal aberrations. MI/MCI-886 at the highest concentration tested (15 mg a.i./kg) did not induce an increase in chromosomal aberrations at either 6, 24, or 48 hours after the single dose or 6 hours after the last multiple dose. The number of scorable metaphases from the treated mice was decreased at 48 hours so the mice exposed to 6 mg/kg were examined; no significant increase in chromosomal aberrations was noted. The incidence of chromosomal aberrations in both treated and negative controls (water solvent) groups was within historical control values for Charles River CD-1 mice.[30,46]

The potential of MI/MCI-886 (Lot A) to induce cell transformation was evaluated using the mouse embryo fibroblast cell line C3H 10T1/2 (no metabolic activation). Test concentrations ranged from 0.0099 to 0.16 μg a.i./ml with a yield of 98–33% survival relative to control cells. Negative (untreated) and positive (dimethylbenzanthracene) controls were used. A single plate with type III foci was seen in the untreated control group; MI/MCI-886 did not induce any type III transformed foci in the 113 treated plates.[30,46]

With the cumulative results of this series of tests, Scribner et al.[46] noted that the steep dose–response toxicity curve made the detection of a mutagenic response difficult. The mutagenic activity of Methylchloroisothiazolinone but not Methylisothiazolinone would suggest that the former was responsible for the mutagenic activity of the MI/MCI biocide. Although the biocide induced point mutations in *S. typhimurium* TA100 and in mouse lymphoma L5178Y cells, it was in the absence of metabolic activation. With activation, no mutagenicity was observed in TA100 and a concentration 10 times higher was needed to produce an effect in the mouse lymphoma cells. This, together with the fact that the biocide induced no unscheduled DNA synthesis in primary hepatocytes, no point mutations in *Drosophila* and no chromosomal aberrations in mouse bone marrow cells, led the investigators to conclude that the MI/MCI biocide appears to be detoxified by animal systems and is unlikely to produce a mutagenic effect in animals. MI/MCI biocide also did not induce transformed foci in the C3H 10T1/2 cell transformation assay, which generally is considered a more direct indicator of carcinogenesis than the point mutation assays. Scribner et al.[46] noted that the potential for heritable genetic effects in humans was limited by the small quantities of MI/MCI biocide available to germ cells under expected exposure conditions. They estimated that at a use concentration of 15 ppm MI/MCI biocide in cosmetics, 1.4 kg of cosmetics would have to be applied to the skin with 100% absorption, equal distribution, and no detoxification in order to obtain a concentration in the germ cells

equivalent to that which produced a detectable mutagenic effect in mammalian cells in culture. They concluded that the MI/MCI biocide should not pose a hazard under normally accepted use conditions.

The potential of MI/MCI-CG (1.5% a.i.) to induce chromosomal aberrations was evaluated *in vitro* in Chinese hamster lung fibroblasts. Concentrations ranging from 0.03 to 8 μg/ml product (equivalent to 0.00045 to 0.12 μg/ml) were tested; concentrations of 1 to 8 μg/ml MI/MCI-CG (0.015 to 0.12 μg/ml) were toxic. No significant increases in the number of chromosomal aberrations were noted at the remaining concentrations when compared to the vehicle control. The positive control group, *N*-methyl-*N'*-nitrosoguanidine, produced a significant increase in chromosomal effects. MI/MCI-CG did not induce chromosomal aberrations under the conditions of this test.[30]

The potential mutagenicity of MI/MCI-886 was evaluated using an *in vivo* cytogenetic test. MI/MCI-886 was administered as a single oral dose to groups of 5 male Crj:CD-1 mice at concentrations of 0, 3, 9, and 30 mg/kg. A fifth group received 6 mg/kg once daily for five consecutive days. Animals receiving single and multiple doses were killed 30 and 6 hours after administration, respectively. Smears of bone marrow cells from the femur of each animal were prepared and examined for micronuclei. No increase in the frequency of bone marrow micronucleated erythrocytes was noted in the treated animals when compared with the water controls. MI/MCI-886 was considered nonmutagenic.[30]

The potential of MI/MCI-886 to bind to DNA was evaluated *in vitro* with the L5178Y mouse lymphoma cell line and *in vivo* using rat testicular DNA. The mutagenicity of MI/MCI-886 was also tested. Lymphoma cells treated for 4 hours with 0.3 μg/ml of [^{14}C]MI/MCI-886 had a viability of 17 to 37%. Total DNA recovery was independent of cell survival and indicated recovery of DNA from both lysed and viable cells. No radioactivity was found in the DNA after *in vitro* treatment with 0.2 to 0.4 μg/ml of [^{14}C]MI/MCI-886 (detection limit of one molecule per 160,000 nucleotides). Concurrent treatment of cells with 0.3 μg/ml of nonradioactive MI/MCI-886 produced an increase in mutations at the thymidine kinase locus to four times background. To evaluate the DNA binding *in vivo*, 0.2 ml of a solution containing 2000 ppm [^{14}C]MI/MCI-886 was applied to the shaved backs of Sprague-Dawley rats in two studies. Total testicular radioactivity 24 hours after application averaged 0.007 and 0.019 ppm in the two respective experiments. The testicular DNA was isolated and analyzed for ^{14}C. No radioactivity was detected bound to the DNA with a detection limit of one molecular per 670,000 nucleotides. At least 99% of the ^{14}C in the rat testes was not associated with the DNA.[30]

The data obtained in absorption studies using water, acetone:water (75:25, w/w) or acetone as the vehicles indicated that when a single dose of [^{14}C]MI/MCI, or a pulse dose after preapplication of nonradioactive material, the use of acetone:water vehicle resulted in a slightly greater amount of ^{14}C activity in the skin than when administered in water. There was no significant difference between the vehicle used when multiple treatments were made. The incomplete solubility of MI/MCI in acetone (100%) affected absorption and was considered not to be an appropriate vehicle. It is concluded that the data from the absorption studies and the existing genotoxic data are sufficient to conclude that a DNA binding study is not necessary.[29]

The preceding summary of data from mutagenic assays on MI/MCI-CG contains both positive and negative results. Positive results were observed in the Ames assay with strain TA100.[8,44,10,46,30] Positive mutagenic results were also obtained when MI/MCI-CG was assayed in the L5178Y mouse lymphoma cell line.[30,46] The Environmen-

tal Protection Agency (EPA) concluded that bacterial test systems (for mutagenicity) are not appropriate for assessing the mutagenic potential of microbiocides in mammalian systems.[50] The EPA Scientific Advisory Committee for the Federal Insecticide, Fungicide and Rodenticide Act also advised[51] on October 25, 1983, that ". . . responses to chemicals or conditions of unknown or unverified mutagenicity in L5178Y cannot be concluded, with a sufficient degree of certainty to be evidence of mutagenicity or of potential hazard." The committee stated that ". . . the L5178Y assay is not recommended for EPA's preferred test for mutation in cultured mammalian cells."

CARCINOGENICITY

MI/MCI-CG (2.67% as supplied) was evaluated for dermal oncogenicity in a mouse skin painting study. A 25 µl sample of the biocide solution in distilled water containing 400 ppm was applied topically three times per week for 30 months to the dorsal skin of 40 male Charles River CD-1 mice. A positive control group of 40 male mice was similarly treated with 1000 ppm 3-methylcholanthrene in acetone. The negative control group was painted with tap water. All mice were shaved three days prior to the initiation of dosing and weekly throughout the study. Sites were moistened with distilled water prior to each application. Applications were made with a centaur pipette and a 25 µl disposable tip. All mice were necropsied. Tissues and organs microscopically examined from all mice in the treated and negative control groups included the skin, liver, lungs, heart, kidneys, spleen, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bone with marrow, and all tissues with gross lesions. The percent survival in the water control group was greater than that of the MI/MCI-CG-treated mice for a period of time in the mid and latter stages of treatment; at 24 months, the survival rate was 67.5% (27/40) for controls and 32.5% (13/40) for MI/MCI-CG-treated mice. However, there was no statistically significant difference in survival at 30 months as 7/40 treated mice (17.5%) and 10/40 negative control mice (25%) survived the length of the study. All of the positive control mice died within 16 months. MI/MCI-CG-treated skin had brown staining, epidermal necrosis, eschar, hyperplasia, hyperkeratosis, dermal inflammation, and increased dermal collagen. Two masses, one hemangiosarcoma and one hemangioma, were also noted at the MI/MCI-CG-treatment sites. The mouse with the hemangiosarcoma at the application site also had an hemangiosarcoma in the liver. These neoplasms were not considered treatment-related as similar vascular neoplasms were seen in the spleen, liver, and skin of the tail of three water control mice. No masses were found at the application site in the water control mice. All positive controls developed squamous cell carcinomas at the site of application within 6 months. There was no indication of a treatment-related increase of neoplasms either systematically or locally in mice treated with MI/MCI-CG. The investigators concluded that 30 months of cutaneous application of MI/MCI-CG at a concentration of 400 ppm (0.04%) a.i. had no local or systemic tumorigenic effect in male mice.[30,52]

Teratogenicity and Reproductive Toxicity

MI/MCI-886 (in aqueous solution) was administered by gavage to groups of 15 pregnant Dutch belted rabbits on days 6 through 18 of gestation at doses of 0, 1.5, 4.4, and 13.3 mg/kg/day a.i. There were two control groups, one received distilled water

and the other received a magnesium–water solution. MI/MCI-886 was maternally toxic; 5/15, 12/15, and 14/15 dams died at the low, mid, and high doses, respectively. Signs of toxicity included ataxia, diarrhea, and severe gastric irritation. At Cesarean section of the surviving dams, a decrease in the number of live fetuses, and an increase in the number of resorption sites and postimplantation losses were observed. No visceral or skeletal malformations were found in the fetuses from any of the treated groups. The investigators concluded MI/MCI-886 was not teratogenic but was embryotoxic and fetotoxic if administered at doses that were highly toxic to rabbits.[30]

MI/MCI-886 (in aqueous solution) was administered by gavage to groups of 25 pregnant Sprague-Dawley rats on days 5 through 15 of gestation at doses of 1.5, 4.5, and 15 mg/kg/day a.i. The control groups received distilled water. MI/MCI-886 was maternally toxic; 1/25, 2/25, and 3/25 dams died at the low-, mid-, and high-dose levels, respectively. Signs of toxicity included wheezing, alopecia, and gastric irritation. No treatment-related effects were noted in any of the reproductive parameters of the surviving dams and fetuses. Upon visceral examination, two exencephalic fetuses, one in the control group and one in the mid-dose group, were observed. No significant anomalies were found upon skeletal examination. The investigators concluded that MI/MCI-886 administered to rats at dosages up to 15 mg/kg/day a.i. was not teratogenic.[30]

MI/MCI-886 was administered in the drinking water to groups of 10 male and 10 female Charles River rats for 15 weeks. Concentrations administered were 0, 25, 75, and 225 ppm (equivalent to 0, 3, 8, and 20 mg/kg/day). Rats within the same dose groups were then mated. Maternal health as well as fetal health up to day 21 after delivery were monitored. No adverse effects on fertility, reproduction, fetal survival, or fetal health were observed.[30]

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation and Sensitization

Predictive Tests

A Lanman–Maibach repeated insult patch test (RIPT) was conducted to evaluate the highest nonirritating concentration of MI/MCI-886. Aqueous dilutions of MI/MCI-886 containing concentrations ranging from 6.25 to 800 ppm were applied to the back of each of 11 subjects daily for 5 consecutive days. Occlusive patches were applied for 23 h and the sites were examined for irritation upon removal. Each subject was also patched with low and high irritant control substances. MI/MCI-886 was a strong irritant at 400–800 ppm, a slight irritant at 200 ppm, and essentially nonirritating at 100 ppm. Six subjects were sensitized to MI/MCI-886: one at 12.5 ppm, two at 25 ppm, two at 50 ppm, and one at 100 ppm. MI/MCI-886 was considered a skin sensitizier; however, the threshold concentration of induction could not be determined as the subjects were exposed to such high concentrations.[30]

A modified Draize RIPT study was conducted using 196 human volunteers.[53] Six induction exposures at 150 ppm MI/MCI-CG in petrolatum were followed by four induction exposures at 300 ppm (in water). Of the 196 human subjects, 7 had delayed contact sensitivity (5 at 2+ and 2 at 3+; 0–4 scale) to the challenge of 150 ppm MI/MCI-CG. The 7 subjects who had positive reactions were retested, approximately 30 days later, at 7.5, 15, 75, and 150 ppm MI/MCI-CG. Two subjects reacted again to 75 and 150 ppm, but not to 7.5 or 15 ppm.

A follow-up use test of shampoos containing MI/MCI-CG at concentrations of 25, 75, or 150 ppm was conducted on 4 of the 7 who had positive reactions in the RIPT. Each of these four participants reacted to the shampoo containing 25 ppm, two reacted at 75 ppm, and four at 150 ppm. The author cautioned against the extrapolation of the "rinse-off" use test data to "leave-on" use.

Maibach[54] conducted a series of three 21-day cumulative irritancy assays as well as a Draize sensitization study to evaluate the appropriate diagnostic patch-testing techniques for MI/MCI-CG. These were conducted with graded dilutions of MI/MCI-CG prepared in water or in petrolatum containing 2.5% polysorbate 85 to assist solubility. In the cumulative assays, occlusive patches each containing 0.2 ml were applied to the same site on the upper arm or back daily 5 times per week for a total of 21 applications. Sites were scored prior to each successive application on a scale of 0–4. In the first study, 13 subjects were each tested with aqueous dilutions of MI/MCI-CG at concentrations of 1, 10, 15, 25,and 50 ppm. No signs of irritation were observed in any of the 13; a rechallenge with 50 ppm 2 weeks later was negative for sensitization. In the second study, 12 subjects were each tested with aqueous dilutions of MI/MCI-CG at concentrations of 100, 200, and 300 ppm. No significant irritation was observed at 100 ppm, while four subjects had cumulative scores of 3.5–14 and 4.5–15.5 at 200 and 300 ppm, respectively. The volunteer with the strongest reaction also had a score of 4 at 100 ppm. The volunteer and two others reacted to a challenge with 100 ppm 2 weeks later and were considered sensitized. In the third phase of the study, 14 subjects were tested with 25, 50, and 100 ppm in the petrolatum. With the exception of the volunteer mentioned above, no reactions were noted. Patches containing either petrolatum, 2.5% polysorbate in petrolatum, or 100 ppm MI/MCI-CG in aqueous solution were applied as controls.

For the Draize study, occlusive patches containing 0.2 ml of the test material were applied to the same site on the upper back or arm of each subject for 48 or 72 hours three times per week for three weeks. Sites were scored upon patch removal. Ninety-six and 104 subjects were treated with 50 and 100 ppm, respectively. Of those subjects treated with 50 ppm, none had any evidence of sensitization during induction or challenge; however, one of 52 had an equivocal response when rechallenged with 100 ppm. A positive response was seen during induction and challenge in 2 of the 104 subjects patched with 100 ppm although one was suspected of having been sensitized during a previous study. No positive responses were seen in 80 subjects tested with 100 ppm in petrolatum. The investigator concluded that MI/MCI-CG has low irritancy potential at the concentrations recommended for use in hair and skin preparations. The potential for irritation appears to be dose-related and increases significantly at concentrations 10 to 15 times that used in cosmetics. He suggested that 100 ppm was a useful diagnostic concentration.[53]

Cardin et al.[55] conducted a series of 13 prophetic RIPTs using a total of 1450 subjects to assess the dose–response of MI/MCI-CG. The induction period consisted of occlusive patches (saturated with either 0.3 or 0.5 ml of the test material) applied to the outer aspect of the upper arm on Mondays, Wednesdays, and Fridays for three consecutive weeks. Two weeks after the final induction, duplicate challenge patches were applied (1 to each arm). All patches were left in place for 24 hours and scored at 48 and 72 hours (induction) or 96 hours (challenge) on a scale of 0–5. MI/MCI-CG was tested in aqueous solution, in aqueous dilutions of prototype rinse-off products, and in a prototype body lotion at concentrations of 5 to 20 ppm (Table 7). No signs of induction or elicitation of delayed sensitization were seen at concentrations of isothiazolinone of

less than 12.5 ppm. Three subjects developed reactions suggestive of delayed sensitization: one tested with 12.5 ppm in a 0.1% aqueous solution and two tested at 20 ppm in water. A rechallenge of these subjects with the same test materials produced inconclusive results. All were negative to testing with the two controls, water, and the shampoo without MI/MCI-CG. However, their hypersensitivity was confirmed by a second rechallenge using 100 ppm aqueous isothiazolinone. The authors noted that these three subjects subsequently participated without incident in the provocative product use testing reported by Weaver et al.[56]

In the analysis of the results of their study, Cardin et al.[55] referred to unpublished screening tests with human cadaver skin in which 10% of the applied [^{14}C]isothiazolinone was detected on or in the skin after 1- and 2-minute exposures followed by rinsing (simulating rinse-off product use). After a 20-minute exposure followed by rinsing, 40% of the applied dose remained on or in the skin. They calculated that the effective exposure to the isothiazolinone mixture from use of rinse-off products was no greater than 1/133 of the highest ineffective dose used in testing (10 ppm). Considering the lowest induction concentration for the isothiazolinones was approximately 13 ppm under the repeated occlusive conditions of this test, and the results of the use challenge and threshold-diagnostic patch-testing program previously reported,[56] the investigators concluded that as much as 5 ppm active isothiazolinone ingredients in a rinse-off product would not be likely to cause allergic dermatoses.

A combined RIPT and arm dip test was conducted on 10 naive human volunteers and 2 subjects previously sensitized to MI/MCI-886. MI/MCI-886 was dissolved in water to give a concentration of 56 ppm. In the RIPT, the solution was applied under occlusive patches 24 hours a day, 5 days per week, for four consecutive weeks (20 induction exposures). Following two weeks of nontreatment, each volunteer was challenged for 24 h with the same solution. Arm immersion tests were run simultaneously on the same subjects. Their arms were dipped into the test solution twice daily for 15 min, 5 days per week, for 4 weeks. After two weeks of nontreatment, the volunteers immersed their arms once more. No skin irritation or sensitization was observed in any of the subjects.[30]

In a Draize RIPT using 18 volunteers, an aqueous solution of MI/MCI-886 containing 25 ppm was applied under occlusive patches 24 hours per day, 3 days per week, for 3 consecutive weeks (9 induction exposures). After two weeks of nontreat-

TABLE 7. RESULTS OF MI/MCI-CG PROPHETIC THRESHOLD TESTING[55]

Isothiazolinone active concentrations on patch	*Vehicle and concentration*	*No. of tests*	*No. of subjects tested*	*Subjects Sensitized*	
				No.	*%*
5 ppm	Hair conditioner, 10% aq.	1	104	0	0
	Shampoo, 0.1% aq.	2	197	0	0
	Liquid soap, 3% aq.	1	115	0	0
6 ppm	Shampoo, 0.25% aq.	1	103	0	0
10 ppm	Hair conditioner, 3.3% aq.	1	112	0	0
	Liquid fabric softener, 12.5% aq.	1	163	0	0
	Body lotion, as is	2	152	0	0
	Distilled water	1	175	0	0
12.5 ppm	Shampoo, 0.1% aq.	1	84	1	1.2
15 ppm	Body lotion, as is	1	200	0	0
20 ppm	Water	1	45	2	4.4

ment, each subject was challenged for 24 hours with another patch containing the same concentration of the preservative. None of the subjects had primary irritation. One subject had reactions indicative of sensitization; this subject gave a positive response when rechallenged 6 weeks later. The investigators concluded that 25 ppm MI/MCI-886 induced contact sensitization in one of 18 subjects.[30]

Nine subjects volunteered for treatment with MI/MCI-CG in a diagnostic threshold patch test. The procedures outlined by the International Contact Dermatitis Group and the North American Contact Dermatitis Group were employed.[57] Occlusive patches with filter pads saturated with aqueous solutions containing 1, 2, 5, 10, 15, 25, 50, and 100 ppm MI/MCI-CG were applied to the skin for 48 hours. Evaluations of the treated sites were made at 49, 96, and 168 hours. None of the nine panelists had skin reactions to 1,2, 5, 10, or 15 ppm MI/MCI-CG; however, MI/MCI-CG concentrations of 25, 50, and 100 ppm produced skin sensitization in 1/9, 6/9, and 9/9 subjects, respectively. The authors concluded that MI/MCI-CG is capable of causing delayed hypersensitivity in humans, provided exposure conditions are sufficiently exaggerated.[56]

RIPTs were conducted with cosmetic formulations, metal working fluids, and acrylic emulsions to evaluate skin sensitization to the active ingredients in MI/MCI-CG and MI/MCI-886 (Table 8). Sensitization was observed in 6/10 individuals exposed to 560 ppm and 6/142 individuals exposed to 56 ppm. No sensitization was noted in 20 individuals exposed to 70 ppm.[30]

Schwartz et al.[58] conducted two double-blind studies to evaluate the safety of MI/MCI-CG as a preservative in "leave-on" body lotions. The studies consisted of pre- and post-use phase diagnostic patch testing with 100 ppm MI/MCI-CG and 13 weeks of daily use of either the test lotion with 15 ppm MI/MCI-CG or a control lotion without MI/MCI-CG. A total of 100 subjects (72 test, 28 control) in California and 109 subjects (88 test, 21 control) in Florida completed the studies. The initial diagnostic patch was occlusive and any subject with a positive reaction was excluded. During the use phase, the lotions were applied daily to the arms, legs, and trunk. No adverse reactions were noted during this phase in the California study; two reactions (one control, one test) were noted in the Florida study but were not product-related. The second diagnostic patch (semiocclusive) was applied two weeks later; all subjects were negative in California while one positive reaction in a control subject was noted in the Florida study. Two weeks later all subjects were rechallenged with occlusive patches; again all subjects were negative with the exception of the same control subject which had a positive reaction to the first challenge. The investigators suggested that this subject may have been sensitized by the initial diagnostic application of MI/MCI-CG. The investigators concluded that MI/MCI-CG, at an effective concentration for preservation and under realistic use conditions for a "leave-on" body lotion, presented little, if any, risk of adverse effect.

Skin sensitization to a shampoo containing 9 ppm MI/MCI-CG was assessed in a 3-month in-use study conducted in three different laboratories. All subjects were pretested with a 24 or 48 h semiocclusive patch containing 7.5 ppm MI/MCI-CG. No reactions indicative of irritation or sensitization were observed. A total of 179 subjects shampooed their hair for 90 consecutive days with the shampoo product containing MI/MCI-CG while 69 subjects shampooed their hair with a control shampoo not containing MI/MCI-CG. Two and 4 weeks after the induction period the subjects were challenged and rechallenged with concentrations of 12.5 and 27 ppm, respectively. Occlusive challenge patches were left in place for 24 h (one lab) or 48 h (two labs). Blood and urine samples were also collected and analyzed. No clinical significant

Table 8. Results of Unpublished Repeated Insult Patch Tests with Cosmetic Formulations, Metal Working Fluids, and Acrylic Emulsions Containing MI/MCI-886/CG[30]

Products	*MI/MCI-886/CG (ppm active ingredients)*	*No. of subjects*	*Results*
Nonionic ointment (occluded)[a]	0	10	0/10 sensitized; no irritation
	56	10	2/10 sensitized; moderate to severe irritation
	560	10	6/10 sensitized; severe irritation
	28	10	No sensitization; no irritation
Anionic hand lotion (occluded)	0	50	0/50 sensitized; 21/50 skin fatiguing
	56	50	4/50 sensitized; 20/50 skin fatiguing
Rechallenge	42	4 sensitized	2/4 sensitized
		6 nonsensitized	0/6 sensitized
Rechallenge	28	4 sensitized	1/4 sensitized
		6 nonsensitized	0/6 sensitized
Rechallenge 1 month later	0	2	No sensitization; no irritation
	5.6		
	11.2		
	16.7		
	22.4		
Anionic hand lotion (occluded)	28	10	No sensitization; no irritation
Nonionic lotion (occluded)	28	10	No sensitization; 5/10 with slight to moderate irritation
Metal working fluids (occluded)	0	10	No sensitization; no irritation
	14	10	No sensitization; no irritation
	28	10	No sensitization; no irritation
	56	10	No sensitization; no irritation
	0	10	No sensitization; no irritation
	42	10	No sensitization; 1/10 skin fatiguing; no primary irritation
	70	10	No sensitization; 1/10 skin fatiguing; no primary irritation
	0	10	No sensitization; no irritation
	42	10	No sensitization; 1/10 skin fatiguing; no primary irritation
	70	10	No sensitization; 1/10 skin fatiguing; no primary irritation
Acrylic emulsions (unoccluded)	56	50	No sensitization; 2/50 with slight irritation
	56	12	No sensitization; no irritation
	56	10	No sensitization; no irritation
	28	50	No sensitization; 2/50 with transient papular lesions not considered related to treatment
Rechallenge at 2, 3 and 4 months to determine duration of sensitization (occluded)		4 sensitized (to 56 ppm MI/MCI-886)	No reactions at 2 mos 1/4 and 2/4 previously sensitized individuals reacted to all materials containing MI/MCI at 3 and 4 mos, respectively
		6 nonsensitized	0/6 nonsensitized subjects had a reaction

Table 8. Results of Unpublished Repeated Insult Patch Tests with Cosmetic Formulations, Metal Working Fluids, and Acrylic Emulsions Containing MI/MCI-886/CG (Continued)

Products	*MI/MCI-886/CG (ppm active ingredients)*	*No. of subjects*	*Results*
Nonionic lotion	0		Sensitization induced by 56 ppm MI/MCI-886 may be appreciably reduced several mos after the initial sensitization period
	56		
Anionic lotion	0		
	56		
Metal working fluid	56		
MI/MCI-886 (stabilized w/$Mg(NO_3)_2$)	56		
MI/MCI-886 (aqueous)	56		
Water	0		

[a]Study conditions

irritation or sensitization was observed in any of the subjects. Hematological, clinical chemistry, and urinalysis values were normal. The investigators concluded that the shampoo containing 9 ppm MI/MCI-CG was not an irritant or a sensitizer under the conditions of these tests.[30]

A generic skin care lotion containing 15 ppm MI/MCI-CG was tested on more than 250 adult male and female volunteers in a Shelanski RIPT.[59,60] Prior to the study, seven volunteers were disqualified because each showed evidence of sensitization to MI/MCI-CG. A "control" lotion containing three different preservatives, 0.125% MDM hydantoin, 0.15% methylparaben, and 0.1% propylparaben is also included in the study. During the 3-week induction period, 0.2 ml of the test lotion was applied to each subject four times per week. The fourth week was used either as a make-up week for subjects missing one of the induction tests and/or as a nontreatment period for those who had received the total 12 patch treatment series. The test lotion containing 15 ppm MI/MCI-CG was used for the four challenge patches applied at 24 h intervals during the fifth week (or sixth week for those who made up a missed application during the fourth week). In this challenge, the 0.2 ml test solution was applied to a previously untreated site and occluded in a manner similar to the patches applied during the induction phase of the study.

During the induction phase, erythema was observed on skin sites of 18/252 subjects treated with the lotion containing 15 ppm MI/MCI-CG. During the challenge phase, 13/244 subjects who completed the induction patch series responded to the lotion containing 15 ppm MI/MCI-CG; 7 of these 13 subjects received a graded response of 4 (0–7 scale). The remaining 6 individuals had a response of 1. Of the 7 subjects who had a response of 4 during the first challenge phase, 5 were available for a second challenge with 100 ppm MI/MCI-CG 2–3 months after the first challenge. Unlike the initial challenge in which the test site was covered by an occlusive Webril patch on an impermeable plastic film, this rechallenge was occluded for 48 hours with Finn Chambers. A grade 4 response was observed in 4/5 subjects, with the remaining subject having no response. Of the 7 subjects who had a grade 4 response during the first

challenge, 6 were available for rechallenge with 25, 50, and 100 ppm MI/MCI-CG. The procedure was the same as used for the second 100 ppm MI/MCI-CG challenge. Positive reactions were observed in 6/6 subjects tested with 50 and 100 ppm; 2/6 responded to the 25 ppm MI/MCI-CG.[59]

Two of three subjects who had a response of 4 during the induction phase, but not during the challenge phase, were also rechallenged with 100 ppm MI/MCI-CG. No response was observed in these two subjects. Two subjects who did not have a positive response during either the initial induction or challenge phase were rechallenged with 100 ppm MI/MCI-CG. Each had a grade 4 response at 72 and 96 hours post-exposure. Subsequently, these two subjects were rechallenged with 25, 50, and 100 ppm MI/MCI-CG. Each had a grade 4 response 96 hours after being rechallenged.[59]

A supervised in-clinic use test[61] was conducted using 24 individuals who had exhibited some degree of a skin reaction to a previously tested lotion containing 15 ppm MI/MCI-CG.[59] Twenty-six control volunteers were also included in the follow-up study. The lotion was identical to that previously tested.[59] Approximately 0.2 ml of the lotion was gently applied onto an area approximately 1 × 2 inch on the antecubital space of the left arm of each subject. A total of 15 applications were made over a three-week time period. During week 3, a slight amount of the lotion was applied to a discrete 1 × 2 inch area on the submandibular area on the face and neck of each subject daily for the last five treatment periods. The areas were treated again after 72 hours and observed for an additional 4 days. The investigator reported that "none of the subjects had maculopapular eruptions indicative of allergic contact dermatitis at the application sites." Nonerythematous folliculitis indicative of a comedogenic presence was seen only in the antecubital flexure area in each of four subjects. These four subjects had previously had positive patch test reactions to MI/MCI-CG. (*Note:* In the original study,[59] 0.2 ml of the test lotion containing 15 ppm MI/MCI-CG was applied to a 2 × 2 cm^2 occlusive Webril patch (4 cm^2); in this study,[22] 0.2 ml test lotion was applied to a 1 × 2 inch area (12.9 cm^2) without an occlusive patch.)

An RIPT of an aqueous solution containing 15 ppm MI/MCI-CG was conducted using 109 volunteers.[62] An initial 24-hour sensitization patch containing 0 or 75 ppm MI/MCI-CG was conducted to eliminate previously sensitized individuals. There was an irritation reaction to the control solution without preservative, but none to the solution containing 75 ppm MI/MCI-CG. The induction phase of the study consisted of nine consecutive 24 h applications under an occlusive patch of a solution containing 0 or 15 ppm MI/MCI-CG over a 3-week time period. The patches were removed by the subjects after 24 hours of exposure. The patch sites were read at 48 hours after the Monday and Wednesday applications, and 72 hours after the Friday application. After a 2-week nontreatment period, the subjects were challenged with the test solution. There were no indications of sensitization to the control lotion or the lotion containing 15 ppm MI/MCI-CG in any of 98 subjects who completed the study. Concurrent with the testing of the lotion containing 15 ppm MI/MCI-CG, a sensitization assay of the same lotion containing 0.25% glydant, 0.15% methylparaben, and 0.10% propylparaben was conducted in the testing program. Sensitization was produced by this preservative system in the same test population.

An RIPT using 433 subjects, of which 394 completed the testing program, was conducted to clarify the sensitization potential of 15 ppm MI/MCI-CG.[63] Of the total subjects who were enrolled, each had tested negatively to prescreen single test application of 100 ppm MI/MCI-CG. The test subjects were divided into one group of 221 controls (205 completed the study) who were patch tested with water and another group of 212 subjects (189 completed the study) who were patch tested with 15 ppm

MI/MCI-CG. Each subject received a patch containing 0.2 ml of either water or MI/MCI-CG on a patch (Johnson and Johnson New Super Stick Coverlet) applied to the upper portion of the scapular back. After the first patches, new patches were applied during the week at 48 h intervals and 72 h intervals on weekends until 10 insult patches had been applied. If a single severe reaction was observed during the induction phase, a 4+ on a 0-4 scale, the induction phase was terminated and the subjects rested for 10–14 days.

These subjects were then challenged with water, 15 ppm MI/MCI-CG, or 100 ppm MI/MCI-CG in a manner similar to the induction patches with the exception that the 100 ppm subjects were patch tested with Finn Chambers on Scanpor; Blender-in tape kept the Scanpor in place. All other subjects who completed the full 10 patch induction phase were treated in a similar manner. During the induction phase, 35/205 of the water controls gave at least one positive response (three at 1, seven at 2, thirteen at 3, and twelve at the maximum value of 4). Likewise, in the 15 ppm MI/MCI-CG test group 42/189 had at least one positive response during the induction phase of the test program (fourteen at 1, nine at 2, five at 3, and fourteen at the maximum response value of 4). Two from the control group gave a positive response upon challenge; none of the subjects of the 15 ppm test group responded to the 15 ppm challenge. Two subjects from the 15 ppm induction group and one subject from the control induction group responded to the 100 ppm challenge. The reason for the large number of positive responses reported during the induction phase for the water control group was not explained; *aquagenic urticaria* was suggested as a possible reason.

A second RIPT at 7.5 ppm MI/MCI-CG was also conducted by Rohm and Haas.[64] Both the 184 water control subjects and the 184 MI/MCI-CG test subjects who completed the program were patched using an occlusive plastic chamber (Hilltop, Cincinnati, OH) held in place with paper tape (Scanpore, Hargeplaster, Oslo, Sweden). With the exception of the method used to cover the test sites, this testing program paralleled that of the 15 ppm study[63] but was performed at a different testing laboratory. Unlike the 15 ppm MI/MCI-CG study which reported a large number of positive responses during the induction phase for both the control and the MI/MCI-CG groups, this did not occur in either the control or the MI/MCI-CG test group. No confirmed sensitization reactions were reported in the control; one subject in the 7.5 ppm test group gave a confirmed positive allergic dermatitis response to the 100 ppm challenge, but not to the 7.5 ppm challenge patch. The tap water used in both the 15 and the 7.5 ppm was from the same source. The water in the 7.5 ppm study was tested during the test program and did not contain MI/MCI-CG.[65]

Summaries of unpublished RIPTs on four different types of cosmetic formulations are available.[66] The eight separate RIPT studies using conditioners containing MI/MCI-CG were as follows: 30 ppm using 51 people, 3.0 ppm using 52 people, 7.5 ppm using 55 people, 7.5 ppm using 52 people, 12.0 ppm using 51 people, 12.0 ppm using 57 people, 12.0 ppm using 48 people, and 12.0 ppm using 44 people. Two RIPT studies on hair sprays were as follows: 7.5 ppm using 52 people and 7.5 ppm using 50 people. RIPT studies on eight gel formulations were conducted using 12 ppm MI/MCI-CG using the following number of people per group: 52, 45, 46, 51, 49, 51, and 51. Three separate RIPT studies on three mousse products containing 7.5, 12.0, and 12.0 ppm were tested individually on 53, 53, and 56 people, respectively. The test material was applied three times per week and covered with occlusive patches for 24 hours, then removed for a 24–48 h period before site observation and reapplication. No evidence of skin sensitization or allergic contact dermatitis was observed in any of the 21 separate studies.

Two cosmetic formulations containing 0.18 ppm MI/MCI-CG were tested in a modified Shelanski RIPT on 200 volunteers. Although each formulation was a mild irritant, they were not sensitizers.[67,68] Additional product formulations were also separately tested, each using a modified Shelanski RIPT procedure. A lotion containing 7.5 ppm MI/MCI-CG was tested using 108 subjects;[69] a cream containing 7.5 ppm MI/MCI-CG was tested using 102 subjects;[70] a cream containing 3.0 ppm MI/MCI-CG was tested using 54 subjects;[71] two bath gels containing 15 ppm MI/MCI-CG were tested separately using 50 subjects each;[72,73] a lotion containing 6 ppm MI/MCI-CG was tested using 102 subjects;[74] a lotion containing 7.5 ppm MI/MCI-CG was tested using 100 subjects;[75] and a lotion containing 7.5 ppm MI/MCI-CG was tested using 103 subjects.[76] Although there was some evidence of irritation in subjects tested with the two gels, there was no evidence of sensitization from any of the nine products tested.

Twenty-eight different formulations, each containing 7.5 ppm MI/MCI-CG, were tested in 11 RIPT studies using 2335 healthy subjects.[77] Each subject received three applications of the test formulation on Monday, Wednesday, and Friday for three weeks. Application sites were covered by occlusive patches between each application. Following a two-week nontreatment period, a challenge application of 7.5 ppm MI/MCI-CG was applied under an occlusive patch and scored at 24 and 48 hours after removal. Of the total 2335 subjects tested with 7.5 ppm MI/MCI-CG, 31 (1.3%) of the subjects "exhibited reactions which the investigators interpreted as being related to allergic sensitization." One separate panel of 216 subjects received initial applications of 100 ppm MI/MCI-CG in water. By the time the second occlusive patch was evaluated, 63 of the 216 subjects had a 2+ or greater reaction using a scale of 0–4. The remaining induction and challenge applications of MI/MCI-CG were made at a concentration of 50 ppm under semiocclusive patches. Forty of the 216 subjects were considered sensitized and 23 of those sensitized were in the group of 63 that had severe reactions by the second induction reading. None of the 40 sensitized subjects reacted to a concurrent patch test with a sunscreen containing 7.5 ppm MI/MCI-CG, although three additional subjects had sensitivity reactions to the sunscreen product. The 40 subjects sensitized to aqueous MI/MCI-CG were not included in the total number of subjects sensitized (31/2335). The 31 positive responses were tallied as individual subjects within each of the 11 panels who responded to one or more patches. In a panel of 212 subjects, each subject receiving three separate patches of different formulations containing 7.5 ppm MI/MCI-CG, 14 positive reactions occurred. There were eight positive responses in a panel of 223 subjects patch tested with two separate formulations containing 7.5 ppm MI/MCI-CG. There were three positive reactions in a panel of 55 subjects in which each subject received only one patch containing 7.5 ppm MI/MCI-CG. There were no responses reported in a panel of 217 subjects who were each patch tested with five separate formulations containing 7.5 ppm MI/MCI-CG. Thus the clustering of positive reactions within a panel does not appear to be directly related to the number of individual formulations tested on each subject, but may due be to the differences in the specific formulations, all of which contained 7.5 ppm MI/MCI-CG.

Several authors have reported contact allergic reactions to isothiazolinones other than Methylisothiazolinone and Methylchloroisothiazolinone, including: (1) 2-*n*-octyl-4-isothiazolin-3-one;[78–80] (2) 1,2-benzisothiazolin-3-one;[80–85] and (3) 3-ethylamino-1,2-benziso-thiazole hydrochloride.[86] The common molecular feature in all of these chemical agents is the isothiazoline ring. Pilger et al.[6] have suggested that while different side chains on the specific isothiazoline compounds may modify their

physical and chemical characteristics, any substance containing the isothiazoline ring system may be a potential sensitizing agent. The potential for cross-reactivity between the various isothiazolinones has not yet been fully evaluated.[4]

Provocative Tests

The International Contact Dermatitis Research Group and The North American Contact Dermatitis Group have cooperated in an extensive study to define the sensitization risk associated with use of MI/MCI-CG in cosmetics and toiletries. Over 7000 patients were patch tested with an aqueous solution containing 100 ppm MI/MCI-CG. The incidence of positive patch test reactions was 0.58%.[4]

Bjorkner et al.[87] reported the results of studies conducted in two different clinics in which patients were patch tested with MI/MCI-886 or MI/MCI-CG. The number of patients, the active ingredient concentration, and the types of skin reactions for these studies are summarized in Table 9. Allergic skin reactions were observed at ingredient concentrations of 1000 ppm (8/36 subjects; 22.2%), 300 ppm (16/460 subjects; 3.5% and 27/516 subjects; 5.2%), 250 ppm (10/170 subjects; 5.9%), and 100 ppm (4/210; 1.9%). No allergic skin reactions were observed at 7 ppm. Of 40 patients patch tested simultaneously with 1000 ppm and 300 ppm, 10 (25%) had skin irritation reactions to 1000 ppm (0.1%). No skin irritation was noted at 300 ppm. In the various studies, skin biopsies were taken from treated sites having irritant or allergic reactions. The skin had focal necrosis in the upper epidermis, but no spongiosis or lymphocytic infiltrate in the dermis. Skin with an allergic reaction had spongiosis in the epidermis and a lymphocytic infiltrate in the dermis; however, no focal necrosis was observed. The investigators suggested their results preclude the conclusion that MI/MCI-CG is safe as a preservative in cosmetics and toiletries.

Bjorkner et al.[87] reported the results of a study in which 34 patients were patch tested with MI/MCI-CG or serial dilutions of MI/MCI-CG. Active ingredient concentrations of 10, 30, 100, 250, and 300 ppm caused positive reactions in 2, 8, 10, 17, and 24 subjects, respectively. The authors observed that in the literature, 100 ppm MI/MCI-CG was recommended as the routine patch test concentration; however, they noted that an active ingredient concentration of 100 ppm, patch test results were negative in 50% of the cases. These authors reported that MI/MCI-CG was the second most common contact sensitizer in their clinics.

Table 9. Results of Patch Tests with MI/MCI-886 and MI/MCI-CG[87]

Clinic	*Test material*	*Active ingredient concentration (ppm)*	*No. of patients tested*	*Number of Patients with Reactions*[a]		
				A	*I*	*F*
Malmo	MI/MCI-886	1000	36	8	0	0
Malmo	MI/MCI-CG	300	460	16	0	4
Lund	MI/MCI-CG	300	516	27	0	4
Lund	MI/MCI-CG	250	170	10	0	2
Lund	MI/MCI-CG	100	210	4	0	0
Lund1	MI/MCI-CG	7	2006	0	0	0
Malmo	MI/MCI-CG	1000	40	0	10	5
		300		0	0	5

[a]A = allergic skin reaction; I = irritant skin reaction; F = "flare-up" skin reaction.

In a use test, an unspecified preparation containing 15 ppm MI/MCI-CG was applied on a double-blind basis twice a day for up to 7 days to the antecubital areas of patients who had previously been sensitized to MI/MCI-CG. Of the 13 patients tested, 7 (54%) developed a mild dermatitis associated with the preservative mixture containing 15 ppm MI/MCI-CG. The preparation without MI/MCI-CG elicited no skin reactions.[87]

De Groot et al.[4] noted that the concentration of the active ingredients in MI/MCI-CG was too low to elicit positive patch test reactions when the cosmetic antimicrobial was tested "as is." They also observed that the concentration adequate for patch testing may be lower in petrolatum than in an aqueous solution, since patients they tested had stronger positive patch test reactions to 100 ppm MI/MCI-CG in petrolatum than to an aqueous solution containing the preservative. MI/MCI-CG was an important source of cosmetic allergy in the Netherlands, where two of the three most popular moisturizing creams contain this preservative. These authors recommended that MI/MCI-CG be added to routine cosmetic screening trays.

One hundred and seventy-nine dermatitis patients with suspected cosmetic allergies were patch tested with various fragrance materials and preservatives, including 150 ppm MI/MCI-CG in petrolatum. On the basis of a history of these 179 patients, 56 (31.2%) suffered or had suffered from "atopic disease." The incidence of atopy in the general population was estimated at approximately 20%. Patch test reactions to 1% MI/MCI-CG in petrolatum were evaluated after 48 and 72 hours. A total of 6 positive reactions (3.4%) to the preservative were reported.[88]

Two consecutive cohorts of 656 and 653 patients in 1985/1986 and 1986/1987, respectively, were patch tested with 100 ppm MI/MCI-CG as well as 26 other common allergens. Patches were applied using Finn chambers with standard allergen concentrations and the sites were scored at 48 and 72 h and graded on a scale of 0 to 3+. The prevalence of MI/MCI-CG sensitivity for 1985/1986 and 1986/1987 was 0.8% and 1.1%, respectively; the difference in prevalence between the two cohorts was not statistically significant. For 1985–1987, the overall prevalence of MI/MCI-CG sensitivity was 0.9%. The rate of sensitization to MI/MCI-CG was measured in 212 patients with negative patch tests by retesting after 6 to 15 months; the mean rate of sensitization was 1/2280 patient months or 0.5% of a population/year. The investigators noted that the number of patients (212) was small and not consecutive and therefore the rate of sensitization found could only be considered as an approximation. Forty-five patients having a negative reaction to MI/MCI-CG were retested four weeks later. No reactions were produced, indicating that the rate of sensitization by patch testing with 100 ppm MI/MCI-CG was low. The investigators suggested that the small and stable prevalence of MI/MCI-CG sensitivity and the low rate of new sensitization were reflective of a slight potential for sensitization.[89]

Hannuksela[90] reported a rapid increase in MI/MCI-CG allergy in Finland (Table 10). In unselected dermatological patients, the number of positive reactions to 100 ppm MI/MCI-CG increased from 0% in 1983 to 4.6% in 1986. Repeated open application tests were performed with creams containing either 7 or 15 ppm MI/MCI-CG; 5 of 10 reacted positively to the 7 ppm cream and 1 of 2 reacted positively to the 15 ppm cream. Only 2 of these 6 positive reactors tested negative to 100 ppm MI/MCI-CG; in later testing, one of the two tested positive to 200 ppm MI/MCI-CG. Eighteen patients who had responded positively to 100 ppm MI/MCI-CG were patched with serial dilutions of MI/MCI-CG. At concentrations of 10, 25, 50, and 100 ppm MI/MCI-CG, the numbers of positive reactors were 1, 4, 10, and 18, respectively. In 22 of the total 35 positive cases, the apparent cause of "Kathon dermatitis" was a popular Finnish

Table 10. Provocative Patch Test Results with 100 ppm of MI/MCI-CG[90]

Year	No. Tested	Positive Reactions	
		No.	%
1983 June-Sept.	167	0	0
1984 Jan.-Dec.	260	3	1.2
1985 Jan.-Apr.	292	2	0.7
1985 May-Aug.	151	1	0.7
1985 Sept.-Dec.	306	13	4.2
1986 Jan.-Mar.	285	14	4.9

In 1984, the patients were suspected of being allergic to a preservative. Other patients were unselected eczema patients routinely tested.

moisturizing cream containing 19 ppm a.i. Methylisothiazolinone and Methylchloroisothiazolinone. The cream entered the market at the beginning of 1984, but in the autumn of 1985 the amount of MI/MCI-CG was reduced to 7 ppm and subsequently, parabens were substituted as the preservative.

De Groot and Bruynzeel[91] reported that the addition of MI/MCI-CG (100 ppm aqueous a.i.) to the European standard series in 1986 had produced, by March 31, 1987, positive reactions in a total of 36/587 dermatitic patients in their two clinics. Of the 36 patients with positive reactions, 27 were definitely relevant. All of the 27 had been using cosmetic products containing MI/MCI-CG at concentrations of 12 ppm or less. Thirteen patients had applied the cosmetics to healthy skin (especially the eyelids and face), while 14 had applied the products to already damaged skin. When use of the suspected cosmetic was discontinued, the dermatitis generally cleared in those with healthy skin and usually improved, although it did not heal completely, in those with the damaged skin. The area affected in these patients included the face (22), the hands (11), and the neck and arms (8). In the De Groot clinic, MI/MCI-CG ranked third among several ingredients in the induction of positive reactions. In the opinion of the investigators, MI/MCI-CG should be included in the European standard series.

Two studies were conducted in France to evaluate the sensitization potential of MI/MCI-CG in aqueous solution at a concentration of 6 ppm. A modified Shelanski RIPT was used on 55 patients having a history of allergic dermatitis (34), nonallergic dermatoses (22), or other illness (10). No irritation or sensitization was noted; four patients had transient skin discoloration. The second test was an epicutaneous test for irritation and sensitization (methods not specified) conducted using 50 patients. No sensitization or irritation was produced by MI/MCI-CG.[30]

Ninety-eight patients with contact dermatitis of the face were tested for sensitization to MI/MCI-CG at a concentration of 100 ppm in water using Finn chambers and Scanpor. The test material was applied to the back of each patient with occlusive patches (length of time not specified). Sites were examined at 48 and 72 hours; 6/98 had a positive reaction. None of these patients reacted to tests with their own cosmetic or toiletry products. The investigators suggested that the recommended concentration of MI/MCI-CG in cosmetics probably was too low to induce a patch test response to the cosmetic.[92]

Among 1511 contact dermatitis patients patch tested with 100 ppm MI/MCI-CG in aqueous solution, 13 (0.8%) had positive skin reactions (one of which was classified as an "irritant" reaction). Of the 13 reactors, 8 were re-evaluated by retest with the same

test substance two weeks later. All 8 subjects had positive patch test reactions. The degree of skin sensitivity was further investigated in 11 of the initial 13 reactors by a provocative use test with various cosmetic lotions containing 7.7 to 15.5 ppm MI/MCI-CG. Applications of the lotions formulated with MI/MCI-CG were made daily for 5 days to one elbow flecture. None of the 11 patients developed skin reactions to the products, including the 8 subjects who had demonstrated positive skin reactions at retesting. The investigators concluded that a positive patch test reaction to 100 pm (0.01%) does not initiate eczema after exposure to MI/MCI-CG at the low concentrations (reported as 3-15 ppm) used in cosmetic products.[93]

Weaver et al.[56] conducted a diagnostic provocative use test to determine the skin sensitivity of humans to consumer products containing MI/MCI-CG. Eighteen subjects who had a known skin hypersensitivity to MI/MCI-CG (confirmed through positive reactions to diagnostic patch testing with an aqueous solution containing 100 ppm) were given various prototype products to use in place of their regular brands for periods of three or six weeks. These products included a liquid soap (5 ppm), shampoo (4 ppm), hair conditioner (5 ppm), liquid fabric softener (6 ppm), and bath and shower foam (5 ppm). In all but one instance, the panelists used multiple product types concurrently. At least one of the test products was used at least once daily. No allergic skin reactions resulted from use of the five products (4–6 ppm). The investigators suggested that there was a very transient exposure by consumers to concentrated rinse-off personal care products. These rinse-off products are diluted with water essentially immediately to provide much lower concentrations. The resulting use concentrations of these products typically range from less than 5% to not more than 20%, depending upon the product being considered. Therefore, the typical in-use exposure to isothiazolinones from these rinse-off products was about 1 ppm. The authors also suggested that testing under typical use conditions demonstrated the uneventful use of MI/MCI-CG at the concentrations required for effective preservation of rinse-off products and that the use of these products "pose at most an extremely small risk of eliciting clinical dermatoses even among consumers who are allergic to this preservation mix."

Bruze et al.[2] conducted a test to determine the contact sensitizer in MI/MCI-CG. A total of 516 patients were routinely patched with MI/MCI-CG in water at a concentration of 300 ppm from May to December of 1984. In 1985, 170 patients were routinely patched with 250 ppm MI/MCI-CG. Twenty-two patients with contact allergy to MI/MCI-CG traced in this way participated in the study. Six other subjects who had been actively sensitized to MI/MCI-CG participated also. The subjects were patch tested with serial dilutions of MI/MCI-CG containing 10, 30, 100, and 300 ppm, as well as with five chromatographically separated fractions. The fractions were dissolved in water/methanol and patch tested at concentrations corresponding to those of the respective fraction in test preparations of MI/MCI-CG. Of the group of 22, the number of positive reactions at 10, 30, 100, and 300 ppm were 1, 3, 9, and 22 for MI/MCI-CG; 1, 5, 11, and 22 for Fraction IV; and 0, 0, 1,and 2 for Fraction II, respectively. One subject reacted to all five fractions. The one subject reacting to 10 ppm of Fraction IV also reacted to 100 ppm of Fraction II. Of the group of six actively sensitized, the numbers of positive reactions at 10, 30, 100, and 300 ppm were 0, 2, 4, and 6 for MI/MCI-CG, and 0, 2, 5, and 6 for Fraction IV. No reactions were produced by the other three fractions. There were no statistical differences in the strength of the reactions. Furthermore, 18 patients were patch tested with equal concentrations of Fractions II and IV (225 ppm; equal to the concentration of Fraction IV in MI/MCI-CG 300 ppm). Fraction IV elicited

positive reactions in all 18 while four had reactions to Fraction II. Mass spectrometry and nuclear magnetic resonance spectrometry were used to analyze the structures of Fractions II and IV; Fraction II was determined to be Methylisothiazolinone and Fraction IV to be Methylchloroisothiazolinone. The investigators concluded that Methylchloroisothiazolinone was the principal contact sensitizer in MI/MCI-CG, but that Methylisothiazolinone was also a sensitizer, as two subjects reacted to a concentration of 75 ppm. They suggested that the two reactions to Methylisothiazolinone may be cross-reactions to Methylchloroisothiazolinone. They stated that a difference in sensitizing potential could not be deduced from the results of the patch test using equal concentrations of the two, as the greater response to Methylchloroisothiazolinone may produce primary sensitization to this ingredient as it is present in MI/MCI-CG at a concentration three times that of Methylisothiazolinone. These same investigators also reported that they have conducted predictive studies (in press) using guinea pigs under equivalent conditions and have found both ingredients to be sensitizers, Methylchloroisothiazolinone being the more potent.[94] Similar results were reported in human studies in which additional data indicated human sensitization to a dichlorinated Methylisothiazolinone.[95]

De Groot et al.[96] reported that 81 of the 1620 patients tested in the Netherlands had allergic contact dermatitis to MI/MCI-CG. Of these, 46% had become sensitized by using cosmetics containing the preservative. Nearly all of the cosmetic products identified as the cause of the dermatitis were leave-on products.

In a study of 119 patients suffering from contact dermatitis related to the use of cosmetics, De Groot et al.[97] reported that the most important cosmetic allergen in this study was MI/MCI-CG. Of 119 patients, 33 reacted positively to this ingredient.

Pasche and Hunziker[98] report that of the 420 patients tested with 100 ppm MI/MCI-CG, 23 (5.5%) had positive reactions. Threshold patch testing was performed on 12 of these patients at MI/MCI-CG concentrations of 7, 15, 25, 50, and 100 ppm. The reaction sites were reduced below 25 ppm; however, a slight positive reaction was obtained in two patients at concentrations of 7 ppm. Other authors have reported positive reactions below 25 ppm.[90]

De Groot and Herxheimer[99] reviewed the prevalence rates of sensitization in patient populations that were tested with MI/MCI-CG in various countries. The authors noted that for those patients whose positive skin reactions were related to the use of cosmetic formulations containing MI/MCI-CG, most cases were associated with the use of "leave-on" cosmetic products. The authors concluded that the use of MI/MCI-CG in "leave-on" cosmetic products should be prohibited; however, the use of the ingredient at low concentrations in "rinse-off" products does not carry an appreciable risk of contact allergy.

In Germany, among 671 consecutive patients patch tested using the ICDRG procedures at 100 ppm, 23 (3.43%) had a positive reaction to MI/MCI-CG.[100]

Fransway[101] reported that for the 1983–1986 period, 13 of 365 patients (3.6%) had positive allergic reactions when tested with 100 ppm MI/MCI-CG. The percent positive responses decreased during 1986–1987 to 20 of 655 (3.1%) and to 7 of 358 (2.0%) for those tested from 1987–1988. The author cautioned against the removal of MI/MCI-CG from all "leave-on" products until the discrepancies in prevalence of sensitivity to MI/MCI-CG and the significance of positive skin test responses are more fully understood.

The preliminary results from an international multicenter study to determine the frequency of sensitizations to MI/MCI-CG in a clinical population was reported.[59] The

results from patch testing 3645 patients with 100 ppm MI/MCI-CG in Europe and 506 in the United States indicated a sensitization incidence of 2.9% in Europe and 1.6% in the United States. A follow-up report on 949 subjects tested in the United States indicated that a total of 1.9% had positive responses.[61] To determine a possible threshold level of skin sensitivity to MI/MCI-CG, 103/114 patients who had positive responses in the initial challenge were rechallenged at 25, 50, and 100 ppm MI/MCI-CG. Thirteen percent were negative to all three challenge levels; 87% were positive (33% at 100 ppm, 28% at 50 ppm, and 26% at 25 ppm). A provocative use test using 96 subjects who were positive to MI/MCI-CG was also conducted on two lotions, one with 15 ppm MI/MCI-CG and a control without MI/MCI-CG. After daily use for one week, 63% were negative to both the MI/MCI-CG lotion and the negative control. Of the 33 patients who had discordant reactions, 88% were positive to MI/MCI-GG at 15 ppm.

Foussereau[102] reported that 1.11% (6/540) patients had an allergic response to an aqueous solution containing 100 ppm MI/MCI-CG. The study was conducted in Strasbourg from November 17, 1986 to August 29, 1988. Of the 6 cases of allergy to MI/MCI-CG, five were also positive to nickel (15% of the total patients tested were allergic to nickel). Cosmetics used by 5 of the 6 subjects who had positive reactions to MI/MCI-CG were available and were analyzed for MI/MCI-CG. Cosmetics used by each of those five positive subjects contained MI/MCI-CG at concentrations lower than 15 ppm. This reported data on the amount of MI/MCI-CG in cosmetics used in France were consistent with that reported by Rastogi[20] for Denmark.

The North American Contact Dermatitis Group patch tested over 1100 patients with MI/MCI-CG at a concentration of 100 ppm in aqueous and/or petrolatum-based vehicles. There were 13 positive responses to the aqueous phase and to the petrolatum base. Three of the patients reacted to both phases for overall response rate of 1.7%. The authors reviewed the available relevant data as it related to patient advice and noted that ". . . it may be an overstatement to recommend that avoidance of all material containing MI/MCI-CG will be truly necessary, particularly for wash-off products containing MI/MCI-CG at low concentrations. . . ."[103]

Lewis and Moss[104] reported that statistical variation could explain reported patient sensitization rates as high as 2.48%. However, rates as high as 4 and 7% may be due to a specific factor in the environment.

Photosensitization and Phototoxicity

An aqueous solution of MI/MCI-CG was evaluated for sensitization and photosensitization using an RIPT with UV exposure. Occlusive patches containing 15 ppm were applied for 24 h to the forearms and upper arms of 27 subjects three times per week for a total of 10 induction exposures. Sites on the forearms were irradiated after each patch removal with nonerythrogenic UVA light for 15 minutes at a distance of 10 cm (4400 $\mu W/cm^2$). Two and four weeks after the last induction, challenge patches containing 15 and 50 ppm, respectively, were applied to previously untreated sites; the appropriate sites were irradiated after each patch removal. Dermal responses were recorded after each patch removal during the induction and challenge phases as well as 24 and 48 h after irradiation during the challenge phase. Slight (±) scattered transient reactions were noted during the induction phase. No reactions indicative of sensitization were observed. The investigators concluded that MI/MCI-CG did not induce photosensitization or sensitization under the conditions of this test.[30]

An aqueous solution of MI/MCI-CG was evaluated for phototoxicity using 25 subjects. Single occlusive patches containing 15 ppm were applied for 24 h to the inner aspects of the subjects' forearms. Upon patch removal, one arm was designated as the nonirradiated site while the other arm was irradiated with UVA light for 15 minutes at a distance of 10 cm (4400 $\mu W/cm^2$). Dermal responses were recorded upon patch removal as well as immediately, 24 and 48 h, and one week after irradiation. "Nonspecific" and transient erythema was observed in 4/25 subjects; these were not considered to be phototoxic reactions. It was concluded that MI/MCI-CG was not phototoxic under the conditions of this test.[30]

SUMMARY

Methylisothiazolinone and Methylchloroisothiazolinone are heterocyclic organic compounds also known as 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one, respectively. These compounds are the active ingredients of a family of commercial microbiocides and preservatives under the trade name Kathon. Cosmetic manufacturers are supplied a biocide product, MI/MCI-CG, containing 0.35% Methylisothiazolinone and 1.15% Methylchloroisothiazolinone in aqueous solution [total active ingredients (a.i.) = 1.50%]. Magnesium salts (23%) are also present as stabilizers.

MI/MCI-CG is readily miscible in water, lower alcohols, glycols, and other hydrophilic organic solvents. Although Methylisothiazolinone and Methylchloroisothiazolinone are relatively unstable compounds, their shelf lives may be extended up to one year by the formation of adducts with calcium or magnesium salts.

Methylisothiazolinone and Methylchloroisothiazolinone are prepared by a process using chlorine-induced cyclization of 3,3-dithiodipropionamides. MI/MCI-CG has been determined using thin-layer chromatography with UV, high performance liquid chromatography, and gas chromatography coupled with mass spectrometry.

Low concentrations of dimethylnitrosamine (DMN), a carcinogenic impurity, have been detected in mixtures of Methylisothiazolinone and Methylchloroisothiazolinone; however, subsequent development of a manufacturing process using a specific reactant, methyl-3-mercaptoproprionate, has limited the presence of DMN in a mixture of Methylisothiazolinone and Methylchloroisothiazolinone to concentrations ranging from 0.1 to 0.8 ppm.

MI/MCI-CG is used in cosmetics as a broad spectrum preservative and is effective against both gram-negative and gram-positive bacteria, as well as fungi and yeast. The chemical supplier of MI/MCI-CG has recommended use of its product in cosmetics at concentrations ranging from 0.02 to 0.1% as supplied [3–15 ppm (0.003–0.0015%) a.i.]. According to the data voluntarily submitted to the FDA, MI/MCI-CG, Methylisothiazolinone and Methylchloroisothiazolinone were used in 381 cosmetic products as of 1986. These ingredients (mostly as the commercial biocide product MI/MCI-CG) were used largely in hair and shampoo formulations and skin care preparations at concentrations of $\leq$0.1%. The highest reported concentration range was >0.1 to 1.0%.

Methylisothiazolinone and Methylchloroisothiazolinone are the active ingredients in a variety of commercial and industrial antimicrobial products. They have recently been approved as indirect food additives at a concentration not to exceed 50 ppm.

In aquatic and terrestrial environments, degradation of Methylisothiazolinone and Methylchloroisothiazolinone (as calcium chloride salts) occurred rapidly by hydrolytic,

photochemical, and biological action. The principal degradative pathway involved dissociation of calcium chloride, ring opening, loss of chlorine and sulfur, and formation of *N*-methylmalonamic acid. Subsequent degradation led to carbon dioxide as the end product.

Absorption and metabolism studies have been conducted using various routes of administration. MI/MCI-886 was appreciably absorbed after oral administration to rats; the majority of the administered dose was readily excreted in the urine or feces while storage in the tissues was minimal. After a single i.v. administration of MI/MCI-CG to rats, approximately one-third of the dose persisted in the blood, suggesting that the radioactivity was bound to erythrocyte macromolecules and was eliminated during normal erythrocyte clearance while the remaining two-thirds of the dose was recovered in the feces and urine (one-third each). Only 4% was recovered as exhaled carbon dioxide. Storage in the tissues was minimal.

From 39 to 62% of a single percutaneous dose of [^{14}C]MI/MCI-CG or [^{14}C]MI/MCI-886 was bound to the site of application 24 hours after exposure. The MI/MCI-CG bound to the skin had a 13.1 day half-life. Repeated application at the same site may result in an accumulation of MI/MCI-CG at the site.

Radioactive Methylchloroisothiazolinone and Methylisothiazolinone MI/MCI-886 were similar in the degree of dermal absorption, binding to application sites, and excretion patterns as well as percent excreted following i.v., oral, and dermal administration. However, Methylisothiazolinone-radioactive MI/MCI-886 produced higher blood concentrations after dermal or oral administration and a 45% greater relative absorption after oral administration than Methylchloroisothiazolinone-radioactive MI/MCI-886. Both dose-dependent and saturable processes governed the absorption, distribution, and elimination of [^{14}C]MI/MCI-CG in the rat. Profiles of the urinary metabolites following oral or dermal dosing of [^{14}C]Methylisothiazolinone or [^{14}C]Methylchloroisothiazolinone MI/MCI-886 also were qualitatively similar.

No radioactivity was detected in the blood of rabbits after dermal application of [^{14}C]MI/MCI-CG at a concentration of 100 ppm for three consecutive days.

In acute studies, Methylisothiazolinone and Methylchloroisothiazolinone (as MI/MCI-886) were toxic to both fresh and marine fish as well as avian species.

Results of acute toxicity studies with MI/MCI-CG and MI/MCI-886 indicated that Methylisothiazolinone and Methylchloroisothiazolinone were moderately to highly toxic to rats and highly toxic to rabbits when administered orally. The major signs of toxicity were severe gastric irritation, lethargy, and ataxia. These compounds were moderately toxic when applied dermally to rabbits; the major signs of toxicity included lethargy, severe cutaneous irritation, and eschar formation. The intraperitoneal LD_{50} values for male and female rats were 4.6 and 4.3 mg/kg; major signs of toxicity were decreased motor activity and peritonitis. The inhalation LC_{50} values were variously reported as ranging from 0.2 to >1.4 mg/L air; the major signs of toxicity included pulmonary congestion and edema, marked dyspnea, salivation, hemorrhage, and death.

The ocular irritation produced by Methylisothiazolinone and Methylchloroisothiazolinone was concentration dependent in numerous Draize eye irritation tests. MI/MCI-886 and MI/MCI-CG were corrosive when tested as supplied. Aqueous dilutions of MI/MCI-886 with concentrations of 560 ppm were nonirritating; 2800 ppm was slightly to moderately irritating; 5600 and 17,000 ppm were moderately to severely irritating; and 28,000 and 56,000 ppm were corrosive. An aqueous dilution of 56 ppm MI/MCI-886 was not considered an ocular irritant when tested in the eyes of rabbits 5 days per week for four weeks.

The dermal irritation of Methylisothiazolinone and Methylchloroisothiazolinone was concentration dependent. MI/MCI-CG and MI/MCI-886 were severely irritating to rabbit skin when tested as supplied. Under occlusive patches, aqueous dilutions of MI/MCI-886 containing 560 ppm were nonirritating; 2800 ppm was moderately irritating; 5600 ppm was severely irritating; and 56,000 ppm was corrosive.

In short-term toxicity studies, no treatment-related effects were observed in rats which received MI/MCI-886 orally at doses up to 24.4 mg/kg/day for two weeks. Slight decreases in feed consumption, leukocyte counts and blood glucose were noted in beagle dogs administered MI/MCI-886 orally at a dose of 29 mg/kg/day for two weeks. Doses of MI/MCI-886 up to 2.8 mg/kg/day applied dermally to rabbits five days per week for three weeks produced moderate irritation at the application site, but no systemic toxicity. The no-observable-effect-level (NOEL) was <0.03 mg/L air in rats exposed daily for two weeks to MI/MCI-886.

Results of subchronic toxicity studies indicated no toxicologically significant treatment-related effects in rats and dogs administered MI/MCI-886 in the diet for three months at doses up to 30 and 28 mg/kg/day, respectively. MI/MCI-886 administered in the drinking water to rats for three months produced slight gastric irritation at a dose of 20 mg/kg/day; the NOEL was 8 mg/kg/day. Dermal application of MI/MCI-886 at doses up to 0.4 mg/kg/day for three months produced no systemic toxicity in rabbits.

Sensitization reactions were produced by MI/MCI-886 in four of six sensitization tests using guinea pigs. The potential of MI/MCI-CG to induce sensitization, when assayed using a modified Buehler technique, appears to be dependent on both the induction and challenge concentrations. In one study, the estimated EC_{50} (elicitation concentration of induction for 50% of the test group) in guinea pigs challenged with 2000 ppm was 88 ppm. The EC_{50} in guinea pigs induced with 1000 ppm was 429 ppm. The number of induction doses may also be an important factor in demonstrating the sensitization potential of MI/MCI-886. MI/MCI-886 containing 56 ppm produced no sensitization in guinea pigs tested using the Magnusson-Kligman maximization procedure. MI/MCI-CG, 1500 ppm, produced no sensitization in guinea pigs, although the induction period consisted of only one application per week for three weeks. One of the studies was conducted with UV radiation; MI/MCI-886 (induction at 1400 ppm, challenge at 420 and 1400 ppm) was neither phototoxic nor photosensitizing.

The genotoxic potential of MI/MCI-886 and MI/MCI-CG has been extensively studied. The steep dose–response toxicity curve has made the detection of a mutagenic response difficult. MI/MCI-886 and MI/MCI-CG were mutagenic in two species of bacteria, *S. typhimurium* (strain TA100 only) and *E. coli*, and in a mouse lymphoma cell line *in vitro*. The mutagenicity of the biocide in *S. typhimurium* strain TA100 in some studies has been observed only in the absence of metabolic activation. In other studies, it was mutagenic both with and without metabolic activation, although the addition of S-9 mix reduced the mutagenic effect as well as the toxicity. MI/MCI-886 was mutagenic to *E. coli* and to mouse lymphoma L5178Y cells both with and without activation, although a concentration 10 times higher was needed to produce an effect in the lymphoma cells in the presence of metabolic activation. MI/MCI-886 was not mutagenic in *S. typhimurium* strains TA1535, TA1537, TA1538, and TA98, or to *Saccharomyces cerevisiae* strain D-4 with or without activation. MI/MCI-886 induced no unscheduled DNA synthesis in primary rat hepatocytes, no point mutations in *Drosophilia*, no chromosomal aberrations in mouse or rat bone marrow cells, and no type III transformed foci in mouse embryo fibroblasts. MI/MCI-CG induced no chromosomal aberrations in Chinese hamster lung fibroblasts. Methylisothiazolinone and Methylchloroisothiazolinone were individually evaluated for mutagenicity in the Ames

test with *S. typhimurium* strains TA1535, TA1537, TA98, and TA100; Methylisothiazolinone was not mutagenic in any strain with or without metabolic activation, while Methylchloroisothiazolinone was mutagenic only in strain TA100 without metabolic activation. Neither of the pure compounds had any clastogenic activity when evaluated in a mouse micronucleus test. The Environmental Protection Agency has stated that bacterial systems (for mutagenicity) are not appropriate for assessing the mutagenic potential of microbiocides in mammalian systems.

Dermal application of 400 ppm MI/MCI-CG three times a week for 30 months produced no local or systemic tumorigenic effect in male mice.

MI/MCI-886 administered by gavage to pregnant rabbits at doses of 1.5 to 13.3 mg/kg/day was toxic to the dam, embryo, and fetus; however, it was not teratogenic. Similarly, doses of 1.5 to 15 mg/kg/day MI/MCI-886 administered to pregnant rats were maternally toxic but not teratogenic. No adverse effects on fertility, reproduction, fetal survival, or health were observed in rats administered $\leq$20 mg/kg/day MI/MCI-886 in the drinking water for 15 weeks prior to mating.

The irritation and sensitization potential of MI/MCI-CG and MI/MCI-886 in humans has been studied extensively. The irritation produced by the biocide (MI/MCI-886) was dose dependent: 400 to 800 ppm was strongly irritating; 200 ppm was slightly irritating; and 100 ppm was essentially nonirritating. The available sensitization test data on healthy volunteers at concentrations of 50 ppm and above are not in agreement. In one study, six applications of 150 ppm MI/MCI-CG in petrolatum under occlusive patches followed by 300 ppm in water under occlusive patches sensitized 7 of 196 subjects. In another study, 63 of 216 healthy human volunteers reacted sufficiently to two occlusive patches containing 100 ppm of aqueous MI/MCI-CG to prompt the investigator to reduce the dose to 50 ppm under semiocclusive patches for the remaining seven exposures. Forty of the subjects were considered sensitized to MI/MCI-CG under the conditions of this test. There is general agreement among investigators that MI/MCI-CG is a sensitizer; however, the concentrations of MI/MCI-CG in cosmetic products at which sensitization has occurred have varied. Sensitization occurred in some of the 250 subjects in a study in which 15 ppm MI/MCI-CG in a lotion was tested. Two recent RIPT studies, one at 15 ppm MI/MCI-CG on 189 subjects and 212 water controls and the second at 7.5 ppm on 184 subjects and 184 water controls, did not indicate that the compound was a sensitizer. The lowest concentration of MI/MCI-CG in a cosmetic formulation that produced sensitization in a nonclinical population of over 200 subjects was 7.5 ppm. In patients already sensitized, the lowest concentration of MI/MCI-CG that produced a positive patch test reaction was 1.5 ppm. In clinical studies, the number of patients responding to 100 ppm MI/MCI-CG varied from approximately 1–7%. In some studies, MI/MCG-CG was detected in the cosmetics used by patients who responded positively to the 100 ppm challenges. The concentration of MI/MCI-CG in these cosmetics was 15 ppm or less. Both "leave-on" and "rinse-off" types of cosmetics containing less than 15 ppm were reported. Results of patch tests with various fractions of MI/MCI-CG have indicated that Methylchloroisothiazolinone was the main contact sensitizer in MI/MCI-CG, although Methylisothiazolinone was also a sensitizer.

MI/MCI-CG at a concentration of 15 ppm was neither photosensitizing nor phototoxic in 27 and 25 subjects, respectively.

DISCUSSION

During the CIR Expert Panel's evaluation of the safety of use of Methylisothiazolinone and Methylchloroisothiazolinone in cosmetic products, all of the available data in

each area of testing were extensively reviewed and discussed in a series of open public meetings. During this review, there were two major areas of concern to the Expert Panel. They were: (1) the potential for MI/MCI-CG to produce adverse human genotoxic effects, and (2) the increasing number of reported human contact dermatitis responses in patients who had been previously exposed to low concentrations of MI/MCI-CG in cosmetic products.

In its initial reviews of the genotoxicity data, it was noted that positive data were reported in two out of eight mutagenic assays; also, the Expert Panel challenged the adequacy of the vehicle and the number of mice used in a 30-month carcinogenicity assay. Subsequently, the Expert Panel received and accepted the opinion of the Environmental Protection Agency's Scientific Advisory Committee that neither of the two mutagenic assays (Ames Assay with TA100 and the mouse lymphoma L5178Y cells) which gave positive mutagenic responses should be used to evaluate the mutagenicity of biocides, i.e., MI/MCI-CG. The Expert Panel noted that even though the number of animals used in the 30-month carcinogenesis assay was low, a 30-month study was sufficiently long. The adequacy of the water vehicle used in the carcinogenicity skin painting study was also challenged. This was resolved by evaluating results of dermal absorption studies which showed that significant amounts of MI/MCI-CG were absorbed when water was used as the vehicle. Subsequently, by majority vote, the Expert Panel concurred that the existing 30-month carcinogenic study was valid and that they were no longer concerned about the possible genotoxicity of MI/MCI-CG.

In response to the Expert Panel's concern with the contact dermatitis responses in patients, additional sensitization testing on nonclinical subjects was undertaken by the manufacturer. Three RIPT studies, two at 15 ppm and one at 7.5 ppm, were conducted at three different laboratories and the data were submitted to the Expert Panel. Additional cosmetic product formulation sensitization test data on nonclinical subjects were also submitted. In the first 15 ppm RIPT study using normal subjects, a lotion containing 15 ppm MI/MCI-CG was applied under occlusive patches for the induction and challenge phases of the study. All of the volunteers in the study were prescreened for sensitization to MI/MCI-CG. Of the 244 subjects who completed the induction patch series, 13 responded to the challenge treatment. Using a scoring scale of 0–7, six subjects received a score of 1 and seven subjects received a score of 4+. Subsequent rechallenge of 6 of the subjects who received the score of 4+ was reconfirmed in 5 of the 6 cases. The manufacturer who supported the study concluded that the testing program was flawed and the test results should not be used in evaluating the safety of use of MI/MCI-CG in cosmetic products.

In the second RIPT study at 15 ppm, a significant number of test and control subjects gave a maximum irritation type of reaction during the induction phase of the study, but not during the challenge phase. There were no indications that 15 ppm MI/MCI-CG was a sensitizing agent under the conditions of the test protocol. The positive responses observed for both the control (12/205) and test groups (14/189) during the induction phase of the study could not be explained. The usefulness of these data were limited.

In the third RIPT study which used 184 test subjects and 184 controls, there was no indication that 7.5 ppm MI/MCI-CG was a sensitizer. No significant irritation responses were reported for either the controls or test subjects during the induction phase of the study.

The results from an international multicenter clinical study to determine frequency of sensitization in clinical patients indicated that 2.9% of 3645 patients in Europe and 1.9% of 949 patients in the United States tested at 100 ppm MI/MCI-CG gave a positive reaction. The Expert Panel noted that the percentages of positive clinical

responses to MI/MCI-CG were similar to those reported by the North American Contact Dermatitis Group for other active preservative compounds now being used in cosmetic products.

Essentially all of the safety test data, both from clinical and nonclinical studies, supported the conclusion that MI/MCI-CG could be safely used in "rinse-off" products at a concentration not to exceed 15 ppm. In establishing a safe level of use for "leave-on" products, the Expert Panel noted that the safety tests which indicated that MI/MCI-CG was a human sensitizer at concentrations lower than 15 ppm were mainly from repeat insult patch testing. Data on the increase in use of MI/MCI-CG for both cosmetic and noncosmetic uses have not caused a measurable increase in the frequency of allergic reactions in patients. However, the Expert Panel and other interested groups have noted that there are significant differences in the length and type of exposure an individual can experience when using "leave-on" cosmetic products containing MI/MCI-CG, as compared with that received from "rinse-off" products. The Expert Panel concluded that the difference in exposure conditions and the troublesome inability to explain the positive results from both clinical and nonclinical sensitization safety evaluations justify a more conservative use of MI/MCI-CG in "leave-on" cosmetic products.

As required by the CIR Procedures, a 90-day public comment period must be allowed before a Final Report may be issued. One 90-day public comment period had elapsed, but due to the large amount of new data received during that comment period and a change in the earlier conclusion on the safety of use of MI/MCI-CG in "leave-on" cosmetic products, a second 90-day public comment period was given for this revised report.

During the first 90-day public comment period, one comment disagreed with the Expert Panel's conclusion that MI/MCI-CG was unsafe for use in "leave-on" products, but did not challenge the Expert Panel's conclusion relative to the safe use of MI/MCI-CG in "rinse-off" products at concentrations not to exceed 15 ppm. In a public meeting held on April 16, 1990, this same commentor agreed that 7.5 ppm MI/MCI-CG would provide adequate preservation to "leave-on" cosmetic products and requested that the Expert Panel provide a new definition of a "leave-on" product. A suggested definition was provided. However, the Expert Panel declined to change its existing definition that states that a "rinse-off" product is one that is designed to be removed from the skin by rinsing with water; all other products are considered to be "leave-on." A second comment was received that agreed with the Expert Panel's earlier opinion that MI/MCI-CG was safe for use in "rinse-off" products at a concentration of 15 ppm, but was unsafe for use in "leave-on" cosmetic products.

The Expert Panel now believes that the new RIPT sensitization test data included in this report, at 7.5 ppm, as well as the new nonclinical test data on formulations are sufficient to change its earlier opinion that MI/MCI-CG was unsafe for use in "leave-on" cosmetic products. The Panel concluded that MI/MCI-CG could be safely used in "leave-on" cosmetic products at a concentration not to exceed 7.5 ppm. In reaching this conclusion, the CIR Expert Panel was assured by the ingredient supplier that: (1) 7.5 ppm MI/MCI-CG would provide adequate preservative effect for the majority of "leave-on" type cosmetic products, (2) that the industry supported multicenter clinical study would continue to monitor the dermatologic patient response to MI/MCI-CG, and (3) that the results from the clinical studies would be made available to the CIR Expert Panel.

No comments were received during the second public comment period.

CONCLUSION

Methylisothiazolinone/Methylchloroisothiazolinone may be safely used in "rinse-off" products at a concentration not to exceed 15 ppm and in "leave-on" cosmetic products at a concentration not to exceed 7.5 ppm. The stated safe use concentration refers to a mixture containing 23.3% Methylisothiazolinone and 76.7% Methylchloroisothiazolinone.

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