Amended Safety Assessment of Methylisothiazolinone as Used in Cosmetics

Status:	Draft Amended Report for Panel Review
Release Date:	February 21, 2014
Panel Meeting Date:	March 17-18, 2014

The 2014 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, DPA. This safety assessment was prepared by Christina L. Burnett, Scientific Analyst/Writer.

1620 L St NW, Suite 1200 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊fax 202.331.0088
◊ cirinfo@cir-safety.org

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett
Scientific Writer/Analyst
Date: February 21, 2014
Subject: Draft Tentative Amended Report on Methylisothiazolinone (MI)

At the March 2013 CIR Expert Panel meeting, the Panel reviewed newly provided clinical data indicating a higher than expected frequency of individuals who have allergic reactions to the preservative MI. The Panel reopened this safety assessment to gather and evaluate further clinical data. Interested parties were encouraged to provide all available data relevant to this concern about allergic reactions.

The Panel issued a final safety assessment of MI in 2008, which was published in 2010, with the conclusion that this ingredient is safe for use in cosmetic formulations at concentrations up to 100 ppm. The available relevant data in the published literature since this review, which mainly consisted of case reports and retrospective and multicenter studies, have been incorporated into tables in this report. Excerpts from the 2008 report summary are included in each appropriate report section, and are indicated by italicized text. The Discussion section of the original safety assessment is presented as a reminder of the deliberations from the original review.

Concentration of use data submitted by the Council has been incorporated into the report. No other data have been received. The data are available for your review in this report's package.

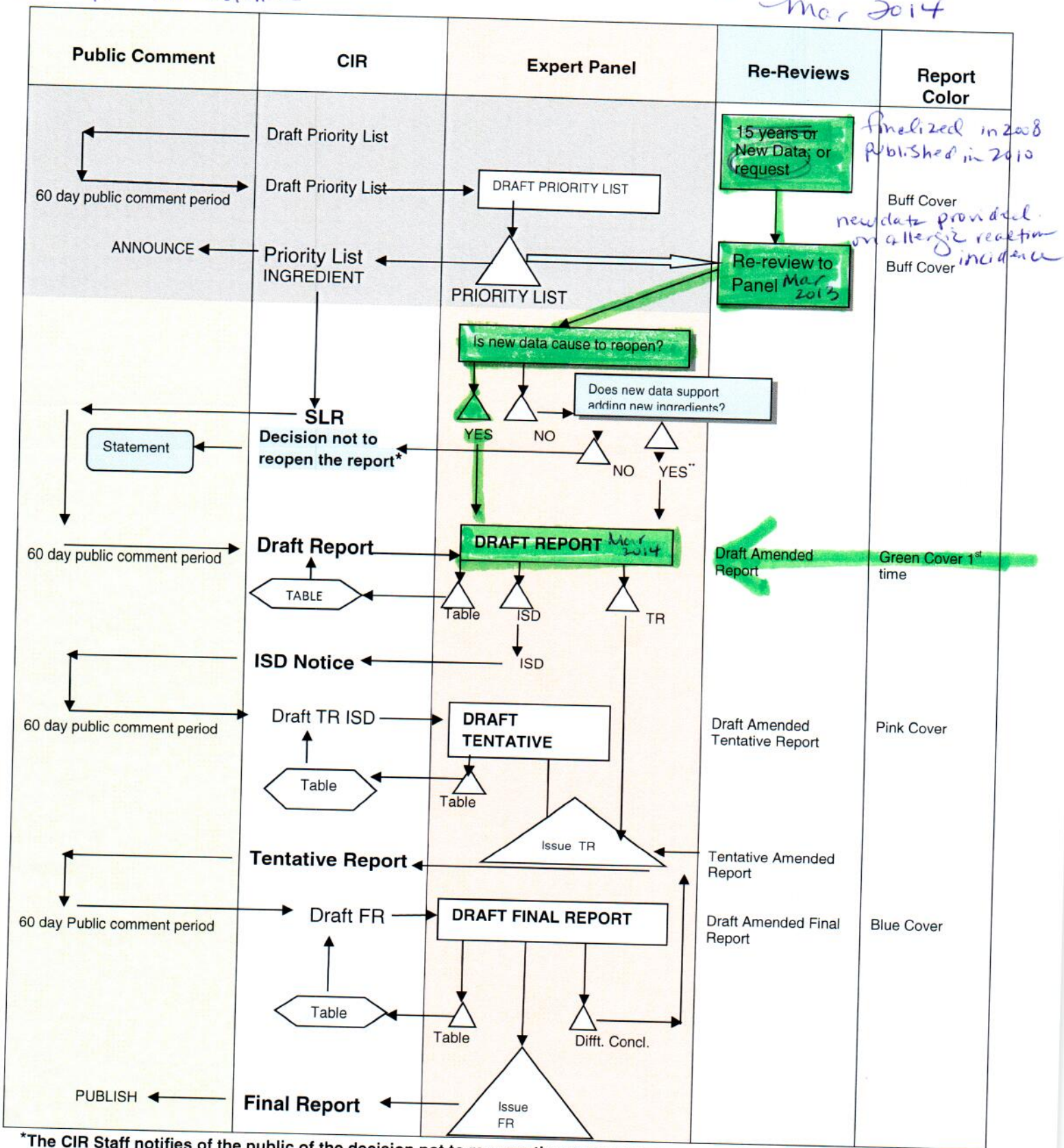
If no further data are needed, the Panel should issue a Tentative Amended Report with the appropriate conclusion.

1620 L Street NW, Suite 1200, Washington, DC 20036
(Main) 202-331-0651 (Fax) 202-331-0088
(Email) cirinfo@cir-safety.org (Website) www.cir-safety.org

Methylisothiazolone

SAFETY ASSESSMENT FLOW CHART

Mar 2014



*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

**If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

Expert Panel Decision

Document for Panel Review

Option for Re-review

Methylisothiazolinone History

2008 – The CIR Expert Panel issued a final safety assessment of MI with the conclusion that this ingredient is safe for use in cosmetic formulations at concentrations up to 100 ppm.

2010 – The safety assessment was published in the International Journal of Toxicology.

March 2013 - The Expert Panel reviewed newly provided clinical data indicating a higher than expected frequency of individuals who have allergic reactions to the preservative MI. The Panel reopened this safety assessment to gather and evaluate further clinical data. Interested parties were encouraged to provide all available data relevant to this concern about allergic reactions.

Methylisothiazolinone Data Profile – March 2014 – Writer, Christina Burnett																				
	In-Use	Composition /Impurities	Method of Mfg	Toxicokinetics	Acute Tox - Derm	Acute Tox - Oral	Acute Tox - Inhalation	Repeated Dose - Dermal	Repeated Dose - Oral	Repeated Dose - Inhalation	Neurotoxicity	Repro/Dev Tox	Genotoxicity	Carcinogenicity/ Tumor Promoton	Dermal Irritation – Non-Human	Dermal Irritation- Human	Dermal Sens – Non-Human	Dermal Sens – Human	Ocular /Mucosal Irritation	Case Studies and Retro-Multicenter	Phototoxicity/ Photosensitization
Data in Original Assessment	X	X	X	X	X	X	X		X		X	X	X	*	X	X	X	X	X	X	X
New Data in Re-Review Assessment	X																	X		X	

"X" indicates that data were available in the category for that ingredient.
* Data on mixture, MCI/MI, not on solo ingredient.

Search Strategy for Methylisothiazolinone

December 2013 and January 2014: SCIFINDER, TOXLINE, and PUBMED search for methylisothiazolinone from 2007 to 1/10/2014

Searches were then further filtered for adverse effects and document type.

	TOXLINE, minus PUBMED	SCIFINDER	PUBMED
Methylisothiazolinone	57	103	85

Reviewed references individually to remove those reporting exclusively on MCI/MI mixture.

Total references ordered: 38

March 18-19, 2013 CIR Panel Meeting

Marks' Team

DR. MARKS: Okay, the next ingredient on my agenda is in the Admin Buff Book. It's methylisothiazolinone, or MIT. And the last time we discussed this, the MIT alumni took over the discussion. I must recuse myself, since

DR. SHANK: I object to that acronym.

DR. MARKS: Yes.

DR. SHANK: The Europeans use MI, but CIR staff insists on this MIT. I just register that I object to that acronym.

DR. MARKS: Well, the three of you can have a powwow as who is going to lead it, but I must recuse myself since for a number of years I ran a meeting in Hershey which Rohm and Hass who is now a part of Dow and I'm not exactly sure if it's a subsidiary or whatever but any rate, got financial support to run that meeting. And actually, when the original MCI/MI was brought up to the panel I testified, came down and testified on that. So, I am going to recuse myself at this point and, Ron, as I remember, you led the discussion before.

It's pretty easy today. Do you re open or not re-open?

DR. SHANK: I think we have to re-open it in order to consider the sensitization issue.

DR. SLAGA: I agree with that.

DR. BERGFIELD: I do, too.

DR. HILL: Yes.

DR. SHANK: Okay. So, I guess that's it, right?

(Laughter)

SPEAKER: For today, that's all you have to do.

DR. ANSELL: Yeah, we agree.

DR. SHANK: Next?

DR. SLAGA: Next? You can't escape.

Belsito's Team

DR. BELSITO: Okay, next one. Are we further talking about methylisothiazolinone?

DR. LIEBLER: I think so.

DR. BELSITO: Okay. So, you know, we approved the use of methylisothiazolinone up to 100 parts per million in both leave on and wash off products, and since that time there have been increasing reports coming out of Europe about sensitization as a result of this. In the U.S., there hasn't been because the major group, the North American group that I'm a member of, hasn't really started testing it as a group until January of this year. So, if we wait for the North American group data, it's 3 years away unless we somehow get them to end their cycle or look at it mid cycle.

I'm concerned because in my practice I've seen about 9 percent of the patients that I've tested positive to MI. Most of them were either weakly positive to the methylchloroiso combination or were negative so that reports out of the U.S. are going to miss it because the standard allergen, the methyliso, is not picking them up.

A lot of them have been in baby wipes in my experience. It may be bias because I get a large pediatric referral population because we have a very strong pediatric Derm Department at Columbia. So I asked several other colleagues who have been testing it what their experience was.

And Joe Fowler in Louisville is getting about 6 percent rate on his tests. Now he wasn't able to tell me how these people broke down with the methylchloro combination versus just the MI.

Denis Sasseville from Canada was much more detailed. He has tested 590 patients. He's in Montreal. And he had 28 that reacted to MI or MCI/MI, 18 of them were atopic, eight reacted to MI alone, and 2 reacted to MCI MI alone. And of the 26 that reacted to both, a good proportion of them were more strongly reactive to the methyliso than the combination, suggesting that it was the methyliso. So his data are somewhere around 4 percent lower than what we're seeing.

I think there are regional variations. There certainly are referral biases. But this just came out 2 years ago.

I mean, these are presumably newly sensitized cases, and we're not dealing with a backlog of cases like when we test for MCI/MI where that combination has been out for years and years and you get a group of people who are sensitized from past exposure plus newly sensitized represented in your patch test numbers. These, presumably, are people who have been sensitized in the last couple of years since we let it out.

So I think the issue from my standpoint is really, you know, qualitative risk assessment. I think it's being used too high in some products, in my case, baby wipes, perhaps in other products. So I think we need to look at it. The biggest issue is how much data we're going to have from the U.S., it's going to be very limited for at least 3 years, but I think the European data will be very robust. I mean, they're going to, I think they're going to move very quickly to lower the limits in the E.U.

That's all I have to say. I think I sent you all the papers that have come out of Europe.

DR. BRESLAWEC: We would support reopening this.

COURT REPORTER: Speak up, please.

DR. BRESLAWEC: We would support reopening this as well.

DR. SNYDER: Yeah, I mean I think that's the information we're looking for and we need. I mean, it's highly pertinent.

And I think also the fact that this is used as a preservative, there are lots of other preservatives they could use that are not sensitizing.

So we've kind of used that in some instances before, to say: Come on, this is a preservative. There are lots better preservatives. You don't need to use one that's sensitizing.

So I think that all plays into it. Reopen it.

MS. SHAW: Can I just make a comment? I'm Dolores Shaw from the Dow Company. We brought this product to market. I just wanted to make a statement that we have been quite aware of what's been happening in Europe. We're concerned. We do support reopening this because we'd like to really understand more detail into what is bringing these folks to the clinic and what's the relevance of these folks coming into the clinic.

So we do support reopening that I don't know who the gentleman is at the end of-- just to comment. In fact, there really aren't a lot of preservatives to pick from anymore. So to say that there's less sensitizing may not be completely accurate.

In fact, we think because the tool box has shrunk, and they're really looking at this in Europe. Because the tool box has shrunk, we believe the MIT has ramped up much faster than we would have ever imagined, and as you have more people using you're going to have more people present.

So, you know, as a company, Dow is supporting that we take a look at this more closely. We do understand, and if we need to make some modifications, we will.

DR. BELSITO: All right.

DR. LIEBLER: So it make sense to reopen.

DR. KLAASSEN: Sure.

DR. BELSITO: Okay.

DR. ANDERSEN: Don, a question, just a big picture kind of question. What experience in the clinical setting would have led you to say, well, we made the right decision?

DR. BELSITO: With methyliso?

DR. ANDERSEN: Yeah.

DR. BELSITO: Well, you know, I guess if the reports hadn't come out of Europe, because I was just testing with a combination, which is 100 ppm, so it's 25 ppm of methylisothiazolinone I would have missing a lot of these cases. But when I started seeing these reports come out from Europe, I added just methyliso alone to the tray, and that's when I started, you know, picking up a good number of cases that were just MI positive.

Again, a lot of mine have been in baby wipes, used obviously on babies but also used by women to remove eye makeup and facial makeup. It's amazing what people use baby wipes for. So, you know, very sensitive areas.

If I had seen these reports from Europe, as has happened in some other ingredients, and started testing for it and I wasn't seeing it in the U.S., then I wouldn't have brought it up.

DR. ANDERSEN: But it's another example of you don't find what you're not looking for.

DR. BELSITO: Right.

DR. ANDERSEN: Once you started looking, you found some cases. Okay. Thank you.

MS. SHAW: May I ask one more question? What was the level for the patch testing that you used?

DR. BELSITO: I'm using 1,000 ppm. That's the other major debate as to what the appropriate level is. The Danes use 2,000 ppm. The Germans use 500 ppm. You know, the Swedes, I think, and most of Scandinavia uses 1,000 ppm. So I decided to go halfway in between and look at that number. But it's not active sensitization. I mean, these are coming up at 48 hours. Okay. Anything else on MIT? Okay. So re review summaries.

DR. LIEBLER: Can I ask one question?

DR. BELSITO: Sure.

DR. LIEBLER: Have we surveyed for new use?

DR. BELSITO: Yeah, it's gone way up. Oh, not new use, but volume of use.

DR. LIEBLER: Yeah. So we have that?

DR. BELSITO: It's gone like close to 3,000 now, right?

DR. LIEBLER: From 1,000 to 3,000?

DR. BELSITO: With methylisos?

DR. BRESLAWEC: We haven't looked at it recently, but we will.

DR. ANDERSEN: Let me see whether I can access the VCR go ahead on, Don.

Full Panel Meeting

DR. BERGFELD: All right, moving on to the next issue, are you going to take that up, the methylisothiazolinone? Why don't you do that?

DR. ANDERSEN: Well, I can

DR. BERGFELD: Introduce it anyway.

DR. ANDERSEN: Yes, yesterday, each team considered new information to summarize a short version. In Europe, testing has been done over the past several years of methylisothiazolinone alone and the findings have been a higher rate of positive responses than at least as Don Belsito looked at it were expected. That may be reflective of the fact that when the safety assessment of methylisothiazolinone was done, there were about 1,300 uses and the number of uses reported to FDA in 2013 VCRP data are up to 3 times that number. So, it's clearly uses have gone up.

Don also provided in addition to studies from Europe the identification of methylisothiazolinone. You notice I'm carefully avoiding calling it MIT so that Dr. Shank doesn't jump up and say that's a university in Massachusetts. So, we'll stick with methylisothiazolinone.

DR. SNYDER: Thank you for that. (Laughter)

DR. ANDERSEN: The methylisothiazolinone has been named "Allergen of the Year" by the American Contact Dermatitis Society, which suggests that in the United States, testing is going to start happening with a vengeance. Don in response to seeing the information developing in Europe had begun himself testing to methylisothiazolinone alone and found a lot more positive responses than he had expected, but he did note that the responses seemed to be very product specific, which was an interesting piece of information. Don provided data from two other patch testing machines, locations that had no dissimilar data.

So, the question of whether the blanket panel's conclusion that methylisothiazolinone was safe for use in cosmetics up to 100 parts per million is suggested needs to be reexamined with all of the available new data with I think a particular focus on quantitative risk assessment that factors in specific product usage and concentrations is a function of product type. So, that was the information provided and it says "Belsito." I think, Paul, I would appreciate a motion to reopen it so we can get this resolved.

DR. SHANK: Thank you for not making me pronounce MIT. (Laughter)

SPEAKER: Thank you.

SPEAKER: MI.

DR. SHANK: We had a lengthy discussion about this. It was nice to have Don reflect some of his personal experiences and things and some other issues that were brought up were there appears to be disagreement among dermatologists actually what concentration you test and he expressed that some results may be negative because some people are only testing at 200 parts per million, some are testing

at 2,000 parts per million. He himself is testing at 1,000 parts per million and they get a positive result relatively quickly, within 48 hours.

A representative from DOW also made a comment to us and they indicated that they would be in favor of reopening and trying to better understand what the issues related to this ingredient. And, so, our team would like to make the motion that we would reopen and re review this ingredient.

DR. BERGFELD: Is there a second?

SPEAKER: Second.

DR. BERGFELD: Second. Any further discussion? Oh, that's right, you're

DR. MARKS: Just for the record, I reclused myself from evaluating this ingredient since in the past Rhom and Haas supported a number of meetings which were conducted in Hershey.

DR. SNYDER: But one last comment was it was shared with us that this ingredient functions as a preservative and as a number of preservatives have decreased over time, that we're probably going to see increased usage of preservatives, particularly this one. And, so, this will only become probably a greater issue as it's used more and more.

DR. BERGFELD: Ron, did you have a comment?

DR. HILL: Who me?

DR. BERGFELD: No, no, Shank, sorry.

DR. SHANK: No, just that I agree. It needs to be reopened for the sensitization issue.

SPEAKER: We'll need a vote.

DR. BERGFELD: Okay. All right, we'll call for a vote to reopen. All those in favor please indicate by raising your hands.

(Hands raised.)

Amended Safety Assessment of Methylisothiazolinone as Used in Cosmetics

Status:	Draft Amended Report for Panel Review
Release Date:	February 21, 2014
Panel Meeting Date:	March 17-18, 2014

The 2014 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, DPA. This safety assessment was prepared by Christina L. Burnett, Scientific Analyst/Writer.

1620 L St NW, Suite 1200 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊fax 202.331.0088
◊ cirinfo@cir-safety.org

INTRODUCTION

In 2010, the Cosmetic Ingredient Review (CIR) Expert Panel published the final report of the safety assessment of methylisothiazolinone (MI) with the conclusion that "MI is safe for use in cosmetic formulations at concentrations up to 100 ppm (0.01%)."[1] At the March 2013 CIR Expert Panel meeting, the Panel reviewed newly provided clinical data indicating a higher than expected frequency of individuals who have allergic reactions to the preservative MI. In some cases, comparative data are available indicating a higher frequency of positive reactions than currently seen with the combination preservative, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI). The Panel reopened this safety assessment to gather and evaluate further clinical data. Interested parties were encouraged to provide all available data relevant to this concern about allergic reactions.

The Panel previously has reviewed the safety on the mixture MCI/MI (commercially known as Kathon™ microbiocides) with the conclusion that the mixture "may be safely used in 'rinse-off' products at a concentration not to exceed 15 ppm and in 'leave-on' products at a concentration not to exceed 7.5 ppm".[2]

Excerpts from the 2010 report summary are included in each appropriate report section, and are indicated by italicized text. The Discussion section of the original 2010 safety assessment is presented here as a reminder of the deliberations from the original review.

CHEMISTRY

The definition, physical and chemical properties, method of manufacturing, and impurities of MI are described in the original safety assessment.[1]

USE

Cosmetic

Table 1 presents the historical and current product formulation data for MI. MI functions as a preservative in cosmetic products.[3] According to information supplied to the Food and Drug Administration (FDA)'s Voluntary Cosmetic Registration Program (VCRP) database in 2007, MI had 1125 reported uses, with the majority of the uses reported in non-coloring hair conditioners and shampoos.[1] Industry reported the maximum use concentration range to be 4×10^{-6}% to 0.01%, with 0.01% reported in both leave-on and rinse-off baby, non-coloring hair, and dermal contact products.[1] In 2013, the VCRP database indicated that uses have increased for MI, which now has 3339 reported uses, with the majority of the uses reported in rinse-off products such as bath soaps and detergents.[4] A survey of use concentrations conducted by the Personal Care Products Council (Council) reported a maximum concentration of use range of 3.5×10^{-8}% to 0.011%, with 0.011% reported in an aerosol hair spray.[5] It should be noted that the information provided under the VCRP in 2007 and in 2013 did not clearly indicate whether MI is used alone in products or is used in combination with MCI.

MI was reported to be used in non-coloring hair sprays and hair tonics or dressings that may be aerosolized or become airborne and could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm compared with pump sprays.[6-9] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[7,8]

The European Union's Scientific Committee on Consumer Safety (SCCS) has a recently updated opinion on the use of MI.[10] It has found that in leave-on cosmetic products (including "wet wipes"), no safe concentration has been adequately demonstrated for induction or elicitation of contact allergy. In rinse-off cosmetic products, the SCCS has concluded that concentrations up to 0.0015% (15 ppm) MI are safe in terms of induction of contact allergy but recognized that there is no information available on the elicitation of contact allergy with this ingredient. Furthermore, the SCCS states that MI should not be used as an addition to cosmetic products already containing MCI/MI.

Non-Cosmetic

The non-cosmetic uses of MI are described in the original safety assessment.[1]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

The percutaneous absorption of radiolabeled MI (99.88% radiochemical purity) was determined using rat skin mounted on diffusion cells. Over a 24-hour period, the rate of absorption was 0.0059, 0.0277, and 0.0841 µg equivalents/cm^2/h for 25, 75, and 150 ppm dose groups, respectively, and the mean amount of total applied

radioactivity absorbed was 21.4%, 33.7%, and 51.2% for 25, 75, and 150 ppm dose groups, respectively. The total dose absorbed of aqueous solutions containing radiolabeled MI (96.90% radiochemical purity) in human epidermis was 29.8%, 38.0%, and 54.7% for 52.2, 104.3, and 313 μg MI/ml dose groups. The rate of absorption was 0.037 μg/cm^2/h over a 24-hour exposure. In the same study, the total dose absorbed from shampoo, body lotion, and facial cream formulations containing 100 μg MI/ml was 29.5%, 8.98%, and 19.6%, respectively. The rates for absorption of MI in the formulations over a 24-hour exposure ranged from 0.007 to 0.0026 μg/cm^2/h. After oral dosing of 100 mg/kg radiolabeled MI (96.70% radio purity) in mice, total radioactive residues (TRR) were highest in the liver and lowest in the bone 1 h post-dosing. At 24 h post-dosing, TRR declined significantly in all tissues and the tissue-to-plasma ratio showed that the radiolabel partitioned preferentially from plasma to tissues. Blood had the highest tissue-to-plasma ratio at 48 h. TRR was higher in male tissues than female tissues overall. Most radiolabeled metabolites of MI (99.08% radio purity) were excreted in urine and feces by rats within 24 h of oral dosing. Tissue sampling at 96 h post-dosing found 1.9-3.6% of the radiolabel, mainly in blood. Total mean recovery of the radiolabel was 92-96%. Major metabolites in urine were N-methyl malonamic acid (NMMA), 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propionamide, and N-methyl-3-hyrdoxyl-propamide. Another metabolism study of radiolabeled MI (96.90% radio purity) conducted on bile duct-cannulated rats had an 88% recovery of the dose at 24 h post oral dosing. The majority of the radiolabel was found in bile, urine, and feces. No intact MI was recovered and the main metabolites were NMMA and 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propionamide.

TOXICOLOGICAL STUDIES

Acute Toxicity

In acute oral toxicity studies, MI was slightly toxic in rats in concentrations ranging from 9.69% to 99.7%. At 9.69%, the LD_{50} for male and female rats was 274.6 and 105.7 mg/kg body weight, respectively. Rats that died during these studies had reddened intestines and/or stomach mucosa, clear or red/yellow fluid in the intestines and/or stomach; blackened intestines and distended stomachs. Studies in rats in body lotion, shampoo, and sunscreen formulations containing 100 ppm MI found no treatment related effects and an LD_{50} greater than 2000 mg formulation/kg body weight. Slight toxicity, including gastrointestinal changes, was observed in mice that orally received 97.5% MI. The LD_{50} was 167 mg/kg body weight. An acute oral toxicity study of the metabolite NMMA found the substance slightly toxic. The calculated oral LD_{50} for NMMA in males and females was 3550 and 4100 mg/kg body weight, respectively. MI at 97.5% was slightly toxic in rats in an acute dermal toxicity study. The substance was corrosive to the skin. The LD_{50} was calculated to be 242 mg/kg body weight. In another acute dermal toxicity study, 9.69% MI was corrosive to rat skin, but no deaths occurred during the study. The LD_{50} was greater than 484.5 mg/kg body weight. Acute inhalation toxicity studies in rats found that 53.52% and 97.8% MI were slightly toxic after 4 h exposures. The LC_{50} were 0.35 and 0.11 mg/L. Rats that died during these studies had reddened lungs and distended gastrointestinal tracts. Mice exposed to 10 minutes of atomized 98.6% MI had up to 47% decrease in respiratory rates that equated to moderate responses for sensory irritation.

Repeated Dose Toxicity

No toxic effects were observed in a rat study where 97.5% MI was administered to drinking water for 13 weeks. Dogs that were fed diets prepared with 51.4% MI for 3 months had a NOAEL of 1500 ppm. In a subchronic study, rats fed the metabolites NMMA or malonamic acid for 3 months had no effects observed in body weight, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, or gross pathologic changes. Beagle dogs that received these metabolites in their diets for 3 months had no systemic toxicity.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a teratogenicity study, MI was administered by daily single oral doses to pregnant rats at doses of 5, 20, or 60 (reduced to 40) mg/kg body weight/day on gestation days 6-19. Females in the high dose group had clinical signs of rales, gasping, and labored breathing and at necropsy had red areas in the glandular portion of the stomach and lungs. No treatment-related effects were observed in the fetuses. The maternal and developmental NOAEL were 20 mg/kg/day and 40 mg/kg/day, respectively. In a teratogenicity study of MI in rabbits, pregnant females received daily single oral doses of 3, 10, or 30 mg/kg/day MI on gestation days 6-28. Maternal effects in the 30 mg/kg/day group included decreased defecation and dark red areas in the stomach. The maternal NOAEL was 10 mg/kg/day. No treatment-related effects were observed in the fetuses and the developmental NOAEL was determined to be 30 mg/kg/day. A two-generation reproduction toxicity test found that MI in drinking water at concentrations up to 1000 ppm was not a reproductive toxicant.

CARCINOGENICITY

Studies of the carcinogenicity of the sole ingredient MI were not available; however, a 2 year drinking water study in rats concluded that the mixture MI/MCI was not a carcinogen.

GENOTOXICITY

MI and the metabolite NMMA were not mutagenic in the Ames test when tested with and without metabolic activation. In a Chinese hamster ovary cell assay, 97.5% pure MI was non-mutagenic when tested with and without metabolic activation (0.5 - 40.0 μg/ml). However, another CHO assay that studied MI at 97.5% a.i. (0.0785 - 5000 μg/ml) found significant increases in cells with chromosome aberrations, with and without metabolic activation. The aberrations were accompanied by significant cytotoxicity, which may have caused a false positive in this assay. MI was non-mutagenic in an unscheduled DNA synthesis assay and in a micronucleus test.

NEUROTOXICITY

An acute in vitro neurotoxicity study of MI in embryonic rat cortical neurons and glia observed widespread neuronal cell death within 24 h in the cortical cultures. Gliotoxicity was low. A 14-hour in vitro neurotoxicity study of MI from the same laboratory concluded that prolonged exposure to MI and related isothiazolones may damage developing nervous systems. However, no evidence of neurotoxicity has been observed in vivo.

IRRITATION AND SENSITIZATION

Irritation

Non-Human

A bovine cornea study classified MI as mildly irritating. Ocular irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm MI found the formulations non-irritating in rabbit eyes. Undiluted 97.8% MI was corrosive to intact rabbit skin after an exposure period of 1 h. Rabbit dermal irritation studies of MI at 9.69% and 10% concluded the chemical was non-irritating. In EpiDerm skin constructs, 1.7% MI applied for 3 or 60 minutes were non-corrosive. In the same study, 51.5% MI was non-corrosive in the 3 minute exposure but corrosive at the 60 minute exposure.

Human

A single 24-hour application of 100 ppm MI in 40 volunteer subjects did not produce skin irritation. Respective skin irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm MI also found MI to be nonirritating.

Sensitization

Non-Human

In a guinea pig maximization test, 0.076% w/v MI was a weak sensitizer and a follow-up study found that 0.015% MI produced no sensitization. An investigation using the Buehler method found that 99.8% MI was a sensitizer at concentrations $\geq$ 1000 ppm. Another maximization test that evaluated the sensitization potential of 99.7% MI concluded that the chemical was not a sensitizer at concentrations up to 800 ppm. MI was a sensitizer at concentrations $\geq$ 1.5% in an open epicutaneous test. Results from local lymph node assays indicated that 99.8% MI and 10.37% MI produced sensitization at >10,000 ppm and >0.76%, respectively. In a joint study, a local lymph node assay testing MI at concentrations up to 0.85% in acetone/olive oil and up to 9.85% in propylene glycol found MI was a skin allergen with moderate strength, but the cytokine profile of 0.5% MI was not typical of chemical respiratory allergens and concluded that MI was not likely to have a significant potential to cause sensitization of the respiratory tract. The metabolite NMMA did not induce hypersensitivity in a local lymph node assay up to and including 30% concentration.

Human

In a clinical study of 22 patients tested with fractions isolated from Kathon CG that included MI and MCI, only 2 patients had positive reactions to MI. Sensitization may have been due to cross-reactions to MCI. MI was determined to be a weak sensitizer in a study of 12 patients. In a cumulative irritation/sensitization study of MI in 80 subjects, the sensitization threshold was determined to be at or around 1000 ppm. Eighty-five patients with pre-determined sensitization to MI/MCI were tested epicutaneously to 500 or 1000 ppm MI. The results show that at high concentrations of MI (500 to 1000 ppm), a proportion of the subjects with known sensitivity to MCI/MI may

also react to MI.A human RIPT in 98 subjects tested with 100 ppm MI concluded that MI did not induce skin sensitization in humans. A series of RIPT evaluating the sensitization of 50% MI in 200, 300, 400, 500, or 600 ppm doses concluded that MI up to 600 ppm was not a dermal sensitizer.

Dermal – Human

MI was named Society Contact Allergen of the Year for 2013 by the American Contact Dermatitis Society due to the rise of use of the preservative and the increased incidences of contact allergy being reported, especially in the European Union.[11] Standard series of patch testing includes the mixture MCI/MI, which may miss 40% of contact allergy to MI alone due to the low concentration of MI (approximately 3.75 ppm in rinse-off products or 1.8 ppm in leave-on products) in a 100 ppm mixture. Recommendations have been made to test for MI contact allergy separate from the MCI/MI, although there currently is no consensus of what concentration of MI should be tested.[11-14]

The dose-response relationship of contact allergy to MI was investigated in 11 MI-allergic patients.[15] The patients were patch tested with 2 dilution series of 12 doses of MI (Neolone 950™ 9.7% active ingredient) in 10% ethanol and 90% aqua and 12 doses of MI with 9.26 µg phenoxyethanol/cm^2 in the same vehicle. The MI doses with and without phenoxyethanol were 0.0105, 0.105, 0.147, 0.21, 0.441, 1.47, 2.94, 4.41, 8.82, 15, 30, and 60 µgMI/cm^2. Controls (n=14) were patch tested with 60 µg MI/cm^2 and 9.26 µg phenoxyethanol/cm^2. Each test site received 15 µl of each dilution applied by filter disc in a Finn Chamber and were occluded for 2 days. Readings were performed on days 2, 3 or 4, and 7. The subjects also underwent a repeated open application test (ROAT) with a cream that contained 0, 0.0105, 0.105, or 0.21 µg MI/cm^2 (0, 5, 50, or 100 ppm MI) with phenoxyethanol in 10% ethanol and 90% water. The patients applied 20 µl from 4 different bottles twice a day on 4 areas of the volar forearm that were 3 cm^2 each. Sites were read on days 2, 3 or 4, 7, 14, and 21, with additional reading if a reaction occurred between visits. In the patch test, results showed the phenoxyethanol had no influence on reactions to MI. The lowest eliciting dose in the patch test was 1.47 µg MI/cm^2 (49 ppm). No reactions were observed at 0.441 µg MI/cm^2 (15 ppm) or lower, nor were there any reactions in the controls. In the ROAT, 7 patients (64%) reacted to 0.105 and 0.21 µg MI/cm^2 and 2 patients (18%) reacted to 0.0105 µg MI/cm^2. The authors of this study recommended that the permitted amount of MI in cosmetics be reduced from 100 ppm.

PHOTOTOXICITY

MI at 100 ppm was not phototoxic or photosensitizing in guinea pig studies. No phototoxic effects were observed in a study of 200 ppm MI in 12 female subjects. A photosensitization study of 200 ppm MI in 32 subjects did not produce photoallergic reactions.

CLINICAL USE

Case Reports

Three cases of allergic contact dermatitis were reported in patients that had come into contact with coolant solutions containing biocides. Patch testing in 2 of the patients revealed 2+ and 3+ reactions to MI, respectively. An investigator in this study developed eczematous dermatitis while isolating coolant components and had a 2+ reaction to MI during patch testing. Another case study reported hand eczema in a diesel mechanic that was exacerbated with the use of moist toilet paper. The diesel oil and the toilet paper the man came in contact with both contained Kathon biocides. Positive reactions to MI were observed with patch testing. Two cases of occupational contact allergy and dermatitis were reports in patients exposed to compounds containing the biocide MI. Patch testing revealed +++ reactions to MI and Neolone 950. Four out of 14 workers at a Danish paint factory were observed with contact dermatitis after exposure to paint additives containing 7-10% MI. Positive reactions were observed in all 4 patients during patch testing.

Case reports and retrospective and multicenter studies reporting MI allergy are summarized in Tables 2 and 3, respectively. Numerous reports of contact allergy, particularly to toilet wipes and water-based wall paint containing MI, have been reported.[16-24] Incidences of contact allergy to MI, tested separately from MCI/MI, appear to be increasing in Europe in recent years.[25-36]

SUMMARY

In 2010, the Cosmetic Ingredient Review (CIR) Expert Panel published the final report of the safety assessment of methylisothiazolinone (MI) with the conclusion that “MI is safe for use in cosmetic formulations at concentrations up to 100 ppm (0.01%)”. At the March 2013 CIR Expert Panel meeting, the Panel reopened this

safety assessment to gather and evaluate further clinical data based on newly provided clinical data indicating a higher than expected frequency of individuals who have allergic reactions to the preservative MI.

According to the FDA's VCRP database in 2007, MI had 1125 reported uses, with the majority of the uses reported in non-coloring hair conditioners and shampoos. Industry reported the maximum use concentration range to be 4 x 10^{-6}% to 0.01%, with 0.01% reported in both leave-on and rinse-off baby, non-coloring hair, and dermal contact products. In 2013, the VCRP database indicated that uses have increased for MI to 3339 reported uses, with the majority of the uses reported in rinse-off products such as bath soaps and detergents. A survey of use concentrations conducted by the Council reported a maximum concentration of use range of 3.5 x 10^{-8}% to 0.011%, with 0.011% reported in an aerosol hair spray. It should be noted that the information provided under the VCRP in 2007 and in 2013 did not clearly indicate whether MI is used alone in products or is used in combination with MCI.

The European Union's SCCS has a recently updated opinion on the use of MI and has found that in leave-on cosmetic products (including "wet wipes"), no safe concentration has been adequately demonstrated for induction or elicitation of contact allergy. In rinse-off cosmetic products, the SCCS has concluded that concentrations up to 0.0015% (15 ppm) MI are safe in terms of induction of contact allergy but recognized that there is no information available on the elicitation of contact allergy with this ingredient. Furthermore, the SCCS states that MI should not be used as an addition to cosmetic products already containing MCI/MI.

MI was named Society Contact Allergen of the Year for 2013 by the American Contact Dermatitis Society due to the rise of use of the preservative and the increased incidences of contact allergy being reported, especially in the European Union. Standard series of patch testing includes the mixture MCI/MI, which may miss 40% of contact allergy to MI alone due to the low concentration of MI in the mixture. Recommendations have been made to test for MI contact allergy separate from the MCI/MI, although there currently is no consensus of what concentration of MI should be tested.

In sensitization studies conducted in 11 MI-allergic patients, the lowest eliciting dose in a patch test was 1.47 µg MI/cm^2 (49 ppm). No reactions were observed at 0.441 µg MI/cm^2 (15 ppm) or lower, nor were there any reactions in the controls. In a ROAT, 7 patients (64%) reacted to 0.105 and 0.21 µg MI/cm^2 and 2 patients (18%) reacted to 0.0105 µg MI/cm^2.

Numerous reports of contact allergy, particularly to toilet wipes and water-based wall paint containing MI, have been reported. Incidences of contact allergy to MI, tested separately from MCI/MI, appear to be increasing in Europe in recent years.

ORIGINAL DISCUSSION

In 1992, the CIR Expert Panel concluded that the mixture MI/MCI (23.3% MI and 76.7% MCI) 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. Currently, MI is used as a stand-alone biocide. Accordingly, it was considered necessary to evaluate the safety of MI alone.

The CIR Expert Panel noted that in vitro studies on MI and related isothiazolinone compounds were positive for neurotoxicity. However, in vivo studies described in this report, including subchronic, chronic, and reproductive and developmental animal studies did not report significant signs of toxicity, including neurotoxicity. The Expert Panel does not consider MI as used in cosmetics to be neurotoxic.

The Expert Panel observed that MI of undetermined particle size had adverse effects in acute inhalation studies in animals. However, the Expert Panel determined that MI can be used safely in hair sprays and other spray products, because cosmetic product sprays contain particles of sizes that are not respirable. The available data demonstrated that the particle size of aerosol hair sprays (~38 µm) and pump hair sprays (>80 µm) is large compared to respirable particulate sizes (≤10 µm).

The Expert Panel noted that MI was a sensitizer in both animal and human studies. A threshold dose response was observed in these studies. Cosmetic products formulated to contain concentrations of MI at 100 ppm (0.01%) or less are not expected to pose a sensitization risk. The Expert Panel also recognizes that cross-sensitization to MCI may occur in individuals sensitized with MI. Most individuals sensitized with MCI, however, do not cross-react with MI. These animal and clinical data support that MCI is a strong sensitizer and MI is a weak sensitizer.

TABLES

Table 1. Historical and current use and concentration of use data for methylisothiazolinone.

	*# of Uses**		*Max Conc of Use (%)*	
Data Year	**2007**	**2013**	**2007**	**2013**
Totals[1]	**1125**	**3339**	**4×10^{-6}-0.01**	**3.5×10^{-8}-0.011**
Duration of Use				
Leave-On	236	726	0.002-0.01	3.5×10^{-8}-0.011
Rinse-Off	807	2477	4.0×10^{-6}-0.01	2.5×10^{-7}-0.01
Diluted for (Bath) Use	82	136	NR	0.0002-0.01
Exposure Type				
Eye Area	6	39	NR	0.00019-0.01
Incidental Ingestion	NR	2	NR	NR
Incidental Inhalation-Spray?[2,5]	144	550	0.005-0.009	0.00018-0.01
Confirmed Spray[3]	NS	NS	NR	0.0002-0.011[a]
Incidental Inhalation-Powder?[4,5]	101	347	NR	0.01
Confirmed Powder[3]	NR	NR	NR	NR
Dermal Contact	469	2008	0.0008-0.01	3.5×10^{-8}-0.01[b,c]
Deodorant (underarm)-Spray?[2]	2	NR	NR	NR
Confirmed Spray[3]	NR	NR	NR	NR
Not Spray[3]	NR	NR	NR	0.0095
Hair - Non-Coloring	579	1292	4.0×10^{-6}-0.01	4.0×10^{-6}-0.011
Hair-Coloring	76	29	NR	5.6×10^{-5}-0.0095
Nail	1	3	NR	0.0002-0.0006
Mucous Membrane	241	1280	0.0015-0.01	9.0×10^{-7}-0.01[b]
Baby Products	14	17	0.002-0.01[d]	0.0002

* Data provided are not clear as to whether uses are MI alone or include uses of MI/MCI.
NR = Not reported
1. Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
2. It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
3. Use has been confirmed by the Council.
4. It is possible these products may be powders, but it is not specified whether the reported uses are powders.
5. Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
a. 0.01-0.011% in an aerosol hair spray; 0.0002-0.01% in a pump hair spray; 0.006-0.0095% in a pump hair tonic or dressing.
b. 0.00023-0.01% in a hand soap; 0.01% in a foot scrub.
c. The Council survey requested that wipe products be identified. One product containing MI was identified as being used as a skin cleansing wipe.
d. 0.01% in baby wipes.

Table 2. Case studies

Mode of Contact	Patient(s)	Indication	Reference
MI in toilet wipes, carpet glue (100 ppm), and water-based paint (100 ppm and also 100 ppm MCI/MI)	55-year-old non-atopic male employed as a bank clerk	-eczematous eruptions on the face, neck, retroauricular area, and forearms that appeared after exposure to fresh paint at his place of employment; -earlier in the year, suffered from pruritus ani and occasional eczema in the perineal area after use with a toilet wipe, facial dermatitis following first uses of a perfume after shaving, and dermatitis following use of deodorant; -previous patch tests with a baseline and cosmetic series were negative; -further testing performed with wipes, perfume, the individual ingredients of these products, and fragrance mix II and its components yielded positive reactions to the wipes, perfume, MI, and fragrance mix II on day 2; -day 2 results from additional testing with repeated baseline series and aqueous dilutions of MI and MCI/MI found +? reaction to 100 ppm MCI/MI, ++ reaction to 1000 ppm MI, and + reaction to a brand of wipes; -on day 4, + or +? reactions to 10, 50, and 100 ppm MCI/MI, + reaction to 10 ppm MI, ++ reactions to 100 and 500 ppm MI, +++ reactions to 1000 ppm MI, and ++ reaction to the wipes.	[16]
toilet wipes that contain 90 ppm MI and water-based paint that contained 0.01% MI and 0.01% MCI/MI	62-year-old non-atopic female	-eczematous eruptions affecting face, trunk, arms, and legs that had started 1 month earlier as acute eczema in the perineal area that the patient attempted to treat with feminine hygiene products; -symptoms occurred 2 months following the initial use of a toilet wipe; -patch testing with European baseline, cosmetic series, the toilet wipe, and a feminine hygiene product yielded positive reactions to the wipe (++ days 2 and 4) and the feminine hygiene product (+ day 4) as well as to 100 ppm MCI/MI (++ days 2 and 4); -patient returned 4 months later with 1-week history of swollen eyelids and face with severe itching and burning following exposure to water-based wall paint in her home; -patch testing with paint produced a ++ reaction.	[16]
toilet wipes that contain 90 ppm MI	50-year-old non-atopic female	-patient presented with a 1-year history of perianal dermatitis following the use of moist toilet paper to control anal pruritus; -patch testing with European baseline, 1000 ppm MI, and 200 ppm MCI/MI yielded a + reaction to 200 ppm MCI/MI (day 4) and a + (day 2) and ++ (day 4) reaction to 1000 ppm MI.	[16]
toilet wipes that contain 90 ppm MI	43-year-old non-atopic female	-patient presented with a 3-month history of eczematous lesions on the genital and perianal area; -patch testing with European baseline, 1000 ppm MI, and toilet wipe yielded a + (day 2) and ++ (day 4) reaction to 1000 ppm MI.	[16]
toilet wipes that contain 90 ppm MI	20-year-old non-atopic female	-perianal itch and genital lesions that had lasted 4 years that the patient treated under physician's guidance with toilet wipes and then worsened into oozing dermatitis; -patch testing with European baseline and toilet wipe yielded a ++ reaction (day 4) to 100 MCI/MI, a ++ reaction (day 4) to 1000 ppm MI, and ++ reactions (day 2 and 4) to the wipes.	[16]
eye cleansing lotion that contained MI	57-year-old atopic female	-patient presented eczematous lesions to the eyelids, mainly localized in corners of eyes, with 6 months duration; -patch testing with European baseline, cosmetic series, and 1000 ppm MI yielded + reactions (days 2 and 4) to 1000 ppm MI.	[16]

Table 2. Case studies

Mode of Contact	Patient(s)	Indication	Reference
toilet wipes that contain 90 ppm MI	44-year-old atopic female	-patient presented pruritus and perianal eczema with 1-year duration following use of toilet wipes that were initially used 2 years prior; -patient also had reactions previously to perfumed bath salts and has experienced severe scalp itch; -patch testing with European baseline, cosmetic series, 10 and 1000 ppm MI, 10 ppm MCI/MI, fragrance mix II ingredients, lavender oil, and the toilet wipe yielded a +++ reactions (days 2 and 4) to 100 ppm MCI/MI, +++ (day 2) and ++ (day 4) reactions to 1000 ppm MI, a + (day 4) reaction to 10 ppm MI, and ++ reactions (days 2 and 4) to the toilet wipes.	[16]
deodorant containing MI used for 2 weeks	37-year-old atopic woman with past history of jewelry intolerance and no history for previous skin reactions to perfumes and deodorants	-eczematous lesions affecting both axillae that cleared after treatment with topical corticosteroids; -patch testing with Portuguese baseline series, a fragrance series, and to patient's own product yielded ++ reactions to nickel, 100 ppm MCI/MI, and to the deodorant; -repeated open allocation test on the volar forearm with the deodorant was strongly positive on day 2; -patch testing with 200 ppm MI yielded at ++ reaction on day 2.	[18]
water-based wall paint containing 0.0053% (53 ppm) MI that had been applied to bedroom walls	4-year-old girl with mild atopic dermatitis since birth	-papular dermatitis affecting face, including nasolabial folds and lower eyelids, followed by generalized skin lesions accentuated at the knee and elbow folds; - rash "waxed and waned" for about 4 weeks with corticosteroid treatment while patient continued to sleep in painted bedroom and then started to clear; -patch testing with adapted European baseline series for children had a + reaction on D4 for MCI/MI at 0.01% or 100 ppm; -child had history of extensive dermatitis following use of a moist toilet paper that contained MI but not MCI.	[17]
toilet cleaner containing 10 ppm MI with additional occupational exposures	32-year-old man	-severe widespread dermatitis caused by heavy exposure to MCI/MI and MI while working at a glue factory; -patch testing revealed + reaction to MCI/MI and ++ reaction to MI; -during treatment, patient also developed a 5-cm eczematous reaction on left inner thigh extending to the buttock; -patient had a new toilet cleaner in home toilet that contained both MCI and MI at 11 ppm and 10 ppm, respectively; -eczema improved after removal of toilet cleaner from home.	[19]
wall paint containing MI	23-year-old non-atopic woman	-initial symptoms of facial dermatitis including periorbital edema that progressed to vesicular dermatitis began 2 months prior to examination after the patient started working at a restaurant that had just been freshly painted; -patient also experienced burning sensation of the cheeks, malaise, and dizziness that worsened the more consecutive days she worked and improved during days off; -patch testing with European baseline series, an extended series with the patient's own cosmetic products, and an extended series with fragrance ingredients yielded ++ reactions to 0.01% MCI/MI and to 0.2% MI; -after initial airborne exposure, patch testing and onset of dermatitis, patient was re-exposed to MI in a cleansing product to which she had never been exposed and immediately experience marked aggravation of facial dermatitis.	[20]

Table 2. Case studies

Mode of Contact	Patient(s)	Indication	Reference
wall paint containing MI	36-year-old non-atopic male	-dermatitis on the legs that spread to the face, shoulders, back, abdomen, and arms as well as intense headache that worsened while the patient was at work, but improved on days off; -initial patch testing showed ++ reaction to 2% formaldehyde and +? Reactions to fragrance and 0.2% MI; -symptoms disappeared after 2.5 months of sick leave, but reappeared after patient moved to a newly refurbished apartment; -both the apartment and casino had been painted with a paint that contained MI.	[21]
wall paints containing 1.2-187 ppm MI, 0.3-10 ppm MCI/MI, and 8.5 - 187ppm benzisothiazolinone (BIT)	57-year-old non-atopic male with a long history of hand eczema and contact allergy	-patient developed facial erythema, cough, and difficulty breathing a few days after using paint containing isothiazolinones; -during the same time period, the patient was participating in a clinical investigation of the dose-response relationship of MI in MI-allergic patients; -patient previously had positive patch tests to formaldehyde, quaternium-15, DMDM hydantoin, *p*-phenylenediamine, melamine formaldehyde, urea formaldehyde, MCI/MI, and MI; -treatment with prednisolone, cetirizine, and corticosteroids helped alleviate the symptoms while at the hospital but all symptoms reoccurred when the patient returned home and even worsened to include dermatitis reactions at the MI test sites from the dose-response study.	[21]
wall paint containing MI	53-year-old non-atopic female	-patient presented with severe respiratory symptoms, erythema in the face, and edema around the eyes that occurred after the patient moved into a freshly painted apartment; - patch testing with the European baseline series, an extended standard, and a paint series yielded + reactions to 2000 ppm MI and 5% farnesol; -symptoms resolved after the patient moved out of her apartment.	[22]
"waist reduction belt" contact gel containing MI	68-year-old male with longstanding perianal dermatitis and recurrent hand eczema	-patient presented with pruritic, erythematous patches on abdomen corresponding to contact areas for the gel of a waist reduction belt; -patient used the device 3x/day for 10 min each for a few days before developing progressive skin changes; -patch testing with baseline series, preservative series, 5% propylene glycol, and 3 ultrasonic contact gels, including the one used by the patient, yielded doubtful reactions to fragrance mix I and MCI/MI and ++ reaction to 0.05% MI; -labeling of the contact gel used by patient indicated the presence of both MCI and MI.	[23]
household wipes and skin cleansing products containing MI	39-year-old non-atopic female employed as a neonate nurse	-patient presented with eczematous skin lesions on the arms, neck and trunk of 7-month duration; -patient also developed palmar hand dermatitis 2-months later, after receiving treatment for the initial symptoms; -patient had previously developed a severe eczematous reaction on the hands to water-soluble paint and eyelid dermatitis while her house was being painted; -patient had daily contact to nitrile gloves, hospital soap, skin cleansing products, baby wipes, household wipes, and rubber; -patch testing with the European baseline series, cosmetic and rubber series, and patient's products and the known allergens in them yielded + reactions to 500 ppm MI, 5% Compositae mix, a cosmetic body milk tested "as is" and a household wipe tested "as is"; -household wipes were analyzed by a lab that determined they contained 60 ppm MCI/MI, however, the patient tested negative to 100 ppm MCI/MI.	[24]

Table 3. Retrospective and multicenter studies

Number of dermatitis patients tested, location	Concentration of MI tested	Years analyzed	Results	Reference
2536; Gentofte, Denmark	2000 ppm in supplemented European baseline series	May 2006 – Feb 2010	-1.5% (37/2536) of the patients patch-tested with MI had contact allergy; -MI contact allergy more often associated with occupational exposure, hand eczema, and age above 40 years. -12/37 cases (32%) were cosmetics exposure and 11/37 cases (30%) were occupational exposure, with half of these occurring in painters	[25]
10,821; Finland	0.1% (1000 ppm) and 0.03% (300 ppm) in addition to being tested with MCI/MI	2006-2008	-1.4% and 0.6% had positive patch test reactions to 0.1% and 0.03% MI, respectively. -66% of those who were MI-positive were also positive to 100 ppm MCI/MI -Of 33 patients that submitted to a use test, 10 had positive results	[26]
653; Australia	200 ppm in the Australian baseline series; testing with100 and 200 ppm MCI/MI also performed	January 1, 2011 to June 30, 2012	-43 (7%) reactions were observed, 23 (4%) of which were deemed relevant; -7 of the patients were parents of young children with hand dermatitis caused by allergic contact dermatitis to MI in baby wipes; -remaining patients reacted to MI in shampoos, conditioners, deodorants, moisturizers, a skin cleanser, and a facial wipe; -3 patients had occupational exposure to hand cleansers; -34/43 patients (79%) had concomitant reactions with MCI/MI.	[27]
2766 to MI, 2802 to MCI/MI, and 2413 to BIT; Gentofte, Denmark	2000 ppm MI, 100 ppm MCI/MI, and 1000 ppm BIT	2010-2012	-contact allergy to MI increased from 2.0% in 2010 to 3.7% in 2012; -contact allergy to MCI/MI increased from 1.0% in 2010 to 2.4% in 2012; -MI-allergic patients tended to have occupational exposure, hand and face dermatitis, and were > 40-years-old; -cosmetic products were the most common substances causing relevant exposure in both MCI/MI- and MI-allergic patients.	[28]
1289; London	500 ppm MI in a cosmetics/ face patch test series	July 2010 to September 2012	-in 2010, 1/85 patients (0.5%) had a positive reaction to MI; -in 2011, 18/521 patients (3.5%) had a positive reaction to MI; -in 2012, 33/584 patients (5.7% had a positive reaction to MI; -reactions appeared to be more prevalent in patients ≥ 40-years-old.	[29]
219 painters and 1095 controls; Gentofte, Denmark	0.01% MCI/MI in European baseline series with testing with MI and other isothiazolinones of unreported concentrations performed as dictated by patient's exposure history	2001 to 2010	-22/219 (10%) of painters had positive reactions to MCI/MI ($p<0.0001$); -11/41 (27%) of painters had positive reactions to MI; -5/21 (25%) of painters had positive reactions to octylisothiazolinone; -7/37 (19%) of painters had positive reactions to benzisothiazolinone.	[30]

Table 3. Retrospective and multicenter studies

Number of dermatitis patients tested, location	Concentration of MI tested	Years analyzed	Results	Reference
~120,000 with baseline series and ~13,000 with preservative series; Germany, Switzerland, Austria (IVDK network)	0.05% MI in pet. and 0.01% MCI/MI in pet.	January 1996 to December 2009	-2.22% of patients had positive reactions to MCI/MI in baseline series; -1.54% of patients had positive reactions to MI in preservative series; -67% (134/199) of MI positive patients also reacted to MCI/MI; -MI sensitization observed more often with occupational dermatitis.	[31]
563 and 2056 for 2 different concentrations of MI, 2489 for MCI/MI; Leeds, UK	0.002% MI (2009-2012); 0.2% (2011-2012); and 0.02% MCI/MI (2008-2012)	January 2008 to June 2012	-3.8% and 4.6% of patients had positive reactions to 0.2% MI in 2011 and 2012, respectively; -percentage of patients positive to 0.02% MI increased from 0.6% in 2009 to 2.5% in 2012; -percentage of patients positive to 0.02% MCI/MI increased from 0.9% in 2008 to 4.9% in 2012.	[32]
245 for MI and ~25,000 for MCI/MI; European Surveillance System on Contact Allergy Network	0.05% MI and 0.01% for MCI/MI	2007 to 2008	-2.6% of patients (n=245 in the Netherlands) had positive reactions to MI; -additional results reported were 1.1% and 1.7% positive reactions in 281 Finnish patients to 0.03% MI and 0.1% MI, respectively, and 1.4% positive reactions in 1280 Danish patients to 0.2% MI; -for MCI/MI, an average of 2.5% of the patients across 11 countries had positive reactions.	[33]
28,922; IVDK network	0.05% MI (500 ppm) in water	2009 to 2012	-an average of 3.83% of patients tested had positive reactions to MI; -prevalence of MI sensitization reported to have increased from 1.94% in 2009 to 6.02% in 2012; -increases observed in female patients $\geq$ 40 years-old, patients with face dermatitis, and use of cosmetics.	[34]
477; France	0.02% and 0.05% (200 and 500 ppm) MI	2 year period, years not reported	-out of 477 patients tested with European baseline and two concentrations of MI, 10 patients had relevant reactions; -all 10 patients reaction to 0.05% MI, while only 5 reacted to 0.02% MI; -only 1 patient of the 10 reacted to 100 ppm MCI/MI -all 5 patients that had been tested with personal care products containing MI reacted.	[35]
12,427 in 2009, 12,802 in 2010, and 12,575 in 2011; IVDK network	500 ppm MI and 100 ppm MCI/MI	2009-2011	-1.9%, 3.4%, and 4.4% positive reactions in 2009, 2010, and 2011, respectively; -proportion of MI-positive patients in those reacting to MCI/MI increased from 43% to 59% between 2009 and 2011.	[36]

References

1. Burnett CL, Bergfeld WF, Belsito DV, Klaassen CD, Marks JG, Shank RC, Slaga TJ, Snyder PW, and Andersen FA. Final Report on the Safety Assessment of Methylisothiazolinone. *IJT*. 2010;29(Suppl 3):187-213.

2. Elder RL (ed). Final report on the safety assessment of methylisothiazolinone and methylchloroisothiazolinone. *JACT*. 1992;11:75-128.

3. Gottschalck TE and Breslawec HP. International Cosmetic Ingredient Dictionary and Handbook. 14 *ed*. Washington, DC: Personal Care Products Council, 2012.

4. Food and Drug Administration (FDA). Frequency of use of cosmetic ingredients. *FDA Database.* 2013. Dated Jan 15.

5. Personal Care Products Council. 8-29-2013. Concentration of Use by FDA Product Category: Methylisothiazolinone. Unpublished data submitted by Personal Care Products Council. 3 pages.

6. Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, and Gronewold C. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.

7. Rothe H. Special Aspects of Cosmetic Spray Evalulation. 9-26-2011. Unpublished data presented at the 26 September CIR Expert Panel meeting. Washington, D.C.

8. Bremmer HJ, Prud'homme de Lodder LCH, and Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 2006. Report No. RIVM 320104001/2006. pp. 1-77.

9. Johnsen MA. The Influence of Particle Size. *Spray Technology and Marketing*. 2004;14(11):24-27.

10. European Commission. Scientific Committee on Consumer Safety (SCCS) Opinion on Methylisothiazolinone (P94) Submission II (Sensitization Only). http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_145.pdf. Date Accessed 1-23-2014.

11. Castanedo-Tardana MP and Zug KA. Methylisothiazolinone. *Dermatitis*. 2013;24(1):2-6.

12. Bruze M, Engfeldt M, Gonçalo M, and Goossens A. Recommendation to include methylisothiazolinone in the European baseline patch test series - on behalf of the European Society of Contact Dermatitis and the European Environmental and Contact Dermatitis Research Group. *Contact Dermatitis*. 2014;69:263-270.

13. Gonçalo M and Goossens A. Whilst Rome burns: The epidemic of contact allergy to methylisothiazolinone. *Contact Dermatitis*. 2013;68:257-258.

14. Lundov MD, Krongaard T, Menné T, and Johansen JD. Methylisothiazolinone contact allergy: A review. *Brit J Dermatol*. 2011;165:1178-1182.

15. Lundov MD, Zachariae C, and Johansen JD. Methylisothiazolinone contact allergy and dose-response relationships. *Contact Dermatitis*. 2011;64:330-336.

16. García-Gavín J, Vansina S, Kerre S, Naert A, and Goossens A. Methylisothiazolinone, an emerging allergen in cosmetics? *Contact Dermatitis*. 2010;63:96-101.

17. Aerts, O, Cattaert N, Lambert J, and Goossens A. Airborne and systemic dermatitis, mimicking atopic dermatitis, caused by methylisothiazolinone in a young child. *Contact Dermatitis.* 2013;68:250-256.

18. Amaro C, Santos R, and Cardoso J. Contact allergy to methylisothiazolinone in a deodorant. *Contact Dermatitis*. 2011;64:289-302.

19. Lundov MD and Menné T. Airborne exposure to methylchloroisothiazolinone and methylisothiazolinone from a toilet cleaner. *Contact Dermatitis.* 2013;68:250-256.

20. Kaae J, Menné T, and Thyssen JP. Presumed primary contact sensitizatin to methylisothiazolinone from paint: a chemical that became airborne. *Contact Dermatitis*. 2012;66:340-355.

21. Lundov MD, Mosbech H, Thyssen JP, Menné T, and Zachariae C. Two cases of airborne allergic contact dermatitis caused by methylisothiazolinone in paint. *Contact Dermatitis*. 2011;65:175-185.

22. Lundov MD, Friis UF, Menné T, and Johansen JD. Methylisothiazolinone in paint forces a patient out of her apartment. *Contact Dermatitis*. 2013;69:251-259.

23. Uter W, Uter M, Steen-Schuberth B, and Schnuch A. Allergic contact dermatitis caused by methylisothiazolinone from a 'waist reduction belt'. *Contact Dermatitis*. 2012;66:347-348.

24. Vanneste L, Persson L, Zimerson E, Bruze M, Luyckx R, and Goossens A. Allergic contact dermatitis caused by methylisothiazolinone from different sources, including 'mislabelled' household wet wipes. *Contact Dermatitis*. 2013;69:311-312.

25. Lundov MD, Thyssen JP, Zachariae C, and Johansen JD. Prevalence and cause of methylisothiazolinone contact allergy. *Contact Dermatitis*. 2010;63:164-167.

26. Ackermann L, Aalto-Korte K, Alanko K, Hasan T, Jolanki R, Lammintausta K, Lauerma A, Laukkanen A, Liippo J, Riekki R, Vuorela AM, and Rantanen T. Contact sensitization to methylisothiazolinone in Finland - a multicentre study. *Contact Dermatitis.* 2010;64:49-53.

27. Boyapati A, Tam M, Tate B, Lee A, Palmer A, and Nixon R. Allergic contact dermatitis to methylisothiazolinone: Exposure from baby wipes causing hand dermatitis. *Australas J Dermatol*. 2013;54:264-267.

28. Lundov MD, Opstrup MS, and Johansen JD. Methylisothiazolinone contact allergy - a growing epidemic. *Contact Dermatitis.* 2013;69:271-275.

29. McFadden JP, Mann J, White JML, Banerjee P, and White IR. Outbreak of methylisothiazolinone allergy targeting those aged $\geq$ 40 years. *Contact Dermatitis*. 2013;69:53-55.

30. Mose AP, Lundov MD, Zachariae C, Menné T, Veien NK, Laurberg G, Kaaber K, Avnstorp C, Andersen KE, Paulsen E, Mørtz CG, Sommerlund M, Danielsen A, ThormannJ, Kristensen O, Kristensen B, Andersen BL, Vissing S, Nielsen NH, and Johansen JD. Occupational contact dermatitis in painters - an analysis of patch test data from the Danish Contact Dermatitis Group. *Contact Dermatitis.* 2012;67:293-297.

31. Schnuch A, Lessmann H, Geier J, and Uter W. Contact allergy to preservatives. Analysis of IVDK data 1996-2009. *Brit J Dermatol*. 2011;164:1316-1325.

32. Urwin R and Wilkinson M. Methylchloroisothiazolinone and methylisothiazolinone contact allergy: A new 'epidemic'. *Contact Dermatitis*. 2013;68:250-256.

33. Uter W, Aberer W, Armario-Hita JC, Fernandez-Vozmediano JM, Ayala F, Balato A, Bauer A, Ballmer-Weber B, and et al. Current patch test results with the European baseline series and extensions to it from the 'European Surveillance System on Contact Allergy' network, 2007-2008. *Contact Dermatitis*. 2012;67:9-19.

34. Uter W, Geier J, Bauer A, and Schnuch A. Risk factors assocaited with methylisothiazolinone contact sensitization. *Contact Dermatitis.* 2013;69:231-238.

35. Waton J, Poreaux C, Schmutz JL, and Barbaud A. Is 500 ppm a better concentration than 200 ppm for diagnosing contact allergy to methylisothiazolinone? *Contact Dermatitis.* 2013;69:251-252.

36. Geier J, Lessmann H, Schnuch A, and Uter W. Recent increase in allergic reactions to methylisothiazolinone/methylisothiazolinone: Is methylisothiazolinone the culprit? *Contact Dermatitis*. 2012;67:334-341.

2013 Raw FDA VCRP Data

01A - Baby Shampoos	2682204	METHYLISOTHIAZOLINONE	7
01B - Baby Lotions, Oils, Powders, and Creams	2682204	METHYLISOTHIAZOLINONE	3
01C - Other Baby Products	2682204	METHYLISOTHIAZOLINONE	7
02A - Bath Oils, Tablets, and Salts	2682204	METHYLISOTHIAZOLINONE	7
02B - Bubble Baths	2682204	METHYLISOTHIAZOLINONE	100
02D - Other Bath Preparations	2682204	METHYLISOTHIAZOLINONE	29
03A - Eyebrow Pencil	2682204	METHYLISOTHIAZOLINONE	2
03B - Eyeliner	2682204	METHYLISOTHIAZOLINONE	2
03C - Eye Shadow	2682204	METHYLISOTHIAZOLINONE	2
03D - Eye Lotion	2682204	METHYLISOTHIAZOLINONE	8
03E - Eye Makeup Remover	2682204	METHYLISOTHIAZOLINONE	5
03F - Mascara	2682204	METHYLISOTHIAZOLINONE	4
03G - Other Eye Makeup Preparations	2682204	METHYLISOTHIAZOLINONE	16
04A - Cologne and Toilet waters	2682204	METHYLISOTHIAZOLINONE	1
04E - Other Fragrance Preparation	2682204	METHYLISOTHIAZOLINONE	3
05A - Hair Conditioner	2682204	METHYLISOTHIAZOLINONE	392
05B - Hair Spray (aerosol fixatives)	2682204	METHYLISOTHIAZOLINONE	6
05C - Hair Straighteners	2682204	METHYLISOTHIAZOLINONE	5
05D - Permanent Waves	2682204	METHYLISOTHIAZOLINONE	1
05E - Rinses (non-coloring)	2682204	METHYLISOTHIAZOLINONE	3
05F - Shampoos (non-coloring)	2682204	METHYLISOTHIAZOLINONE	643
05G - Tonics, Dressings, and Other Hair Grooming Aids	2682204	METHYLISOTHIAZOLINONE	167
05H - Wave Sets	2682204	METHYLISOTHIAZOLINONE	4
05I - Other Hair Preparations	2682204	METHYLISOTHIAZOLINONE	64
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	2682204	METHYLISOTHIAZOLINONE	8
06B - Hair Tints	2682204	METHYLISOTHIAZOLINONE	2
06D - Hair Shampoos (coloring)	2682204	METHYLISOTHIAZOLINONE	16
06H - Other Hair Coloring Preparation	2682204	METHYLISOTHIAZOLINONE	3
07B - Face Powders	2682204	METHYLISOTHIAZOLINONE	1
07C - Foundations	2682204	METHYLISOTHIAZOLINONE	3
07E - Lipstick	2682204	METHYLISOTHIAZOLINONE	2
07I - Other Makeup Preparations	2682204	METHYLISOTHIAZOLINONE	3
08G - Other Manicuring Preparations	2682204	METHYLISOTHIAZOLINONE	3
10A - Bath Soaps and Detergents	2682204	METHYLISOTHIAZOLINONE	569
10C - Douches	2682204	METHYLISOTHIAZOLINONE	1
10E - Other Personal Cleanliness Products	2682204	METHYLISOTHIAZOLINONE	572
11A - Aftershave Lotion	2682204	METHYLISOTHIAZOLINONE	5
11D - Preshave Lotions (all types)	2682204	METHYLISOTHIAZOLINONE	1
11E - Shaving Cream	2682204	METHYLISOTHIAZOLINONE	8
11F - Shaving Soap	2682204	METHYLISOTHIAZOLINONE	1
11G - Other Shaving Preparation Products	2682204	METHYLISOTHIAZOLINONE	7
12A - Cleansing	2682204	METHYLISOTHIAZOLINONE	198
12B - Depilatories	2682204	METHYLISOTHIAZOLINONE	1
12C - Face and Neck (exc shave)	2682204	METHYLISOTHIAZOLINONE	113
12D - Body and Hand (exc shave)	2682204	METHYLISOTHIAZOLINONE	54
12F - Moisturizing	2682204	METHYLISOTHIAZOLINONE	146

12G - Night	2682204	METHYLISOTHIAZOLINONE	16
12H - Paste Masks (mud packs)	2682204	METHYLISOTHIAZOLINONE	30
12I - Skin Fresheners	2682204	METHYLISOTHIAZOLINONE	14
12J - Other Skin Care Preps	2682204	METHYLISOTHIAZOLINONE	51
13A - Suntan Gels, Creams, and Liquids	2682204	METHYLISOTHIAZOLINONE	3
13B - Indoor Tanning Preparations	2682204	METHYLISOTHIAZOLINONE	26
13C - Other Suntan Preparations	2682204	METHYLISOTHIAZOLINONE	1

International Journal of Toxicology
29(Supplement 3) 187S-213S

DOI: 10.1177/1091581810374651
http://ijt.sagepub.com
SAGE

Final Report of the Safety Assessment of Methylisothiazolinone

Christina L. Burnett[1], Wilma F. Bergfeld, MD, FACP[2], Donald V. Belsito, MD[2], Curtis D. Klaassen, PhD[2], James G. Marks Jr, MD[2], Ronald C. Shank, PhD[2], Thomas J. Slaga, PhD[2], Paul W. Snyder, DVM, PhD[2], and F. Alan Andersen, PhD[3]

Abstract

Methylisothiazolinone (MIT) is a heterocyclic organic compound used as a preservative in cosmetics and personal care products in concentrations up to 0.01%. MIT is a colorless, clear liquid with a mild odor that is completely soluble in water; mostly soluble in acetonitrile, methanol, and hexane; and slightly soluble in xylene. Consistent with its solubility, dermal penetration is low. The Cosmetic Ingredient Review Expert Panel noted the in vitro evidence of neurotoxicity but concluded that the absence of any neurotoxicity findings in the many in vivo studies, including subchronic, chronic, and reproductive and developmental animal studies, suggests that MIT would not be neurotoxic as used in cosmetics. Although recognizing that MIT was a sensitizer in both animal and human studies, the panel concluded that there is a threshold dose response and that cosmetic products formulated to contain concentrations of MIT at 100 ppm (0.01%) or less would not be expected to pose a sensitization risk. Accordingly, MIT may be safely used as a preservative in cosmetics up to that concentration.

Keywords

methylisothiazolinone, safety, cosmetics

In 1992, the Cosmetic Ingredient Review (CIR) Expert Panel issued a final report on the mixture methylisothiazolinone/methylchloroisothiazolinone (commercially known as Kathon microbiocides) with the conclusion that the mixture "may be safely used in 'rinse-off' products at a concentration not to exceed 15 ppm and in 'leave-on' products at a concentration not to exceed 7.5 ppm."[1,p75] This report reviews the safety of the ingredient methylisothiazolinone alone, because it now has reported cosmetic applications as a biocide without methylchloroisothiazolinone.

In the 1992 report, methylisothiazolinone and methylchloroisothiazolinone were abbreviated as MI and MCI, respectively. In recognition of the global use currently, the abbreviations MIT and CMIT, respectively, have been used throughout this new report.

Chemistry

Definition and Structure

According to the *International Cosmetic Ingredient Dictionary and Handbook*,[2] methylisothiazolinone (CAS No. 2682-20-4) is the heterocyclic organic compound that conforms to the formula shown in Figure 1.

Synonyms and trade names for MIT as used in cosmetic products are listed in Table 1.

Physical and Chemical Properties

Table 2 lists the physical and chemical properties of MIT as they were provided by Rohm & Haas, LLC.[4] The ultraviolet (UV)/visible spectrum for the MIT product Kordek 573T microbicide, an industrial biocide, had peak wavelengths at 274 nm for a neutral solution, 266 nm for an acidic solution, and 274 nm for a basic solution.[4]

Method of Manufacture

MIT is produced by the controlled chlorination of dimethyldithiodipropionamide (DPAM) in solvent. MIT is then neutralized and extracted into water followed by a solvent strip.[3]

[1] Cosmetic Ingredient Review Scientific Analyst/Writer
[2] Cosmetic Ingredient Review Expert Panel Member
[3] Cosmetic Ingredient Review Director

Corresponding Author:
Christina L. Burnett, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036
Email: cirinfo@cir-safety.org

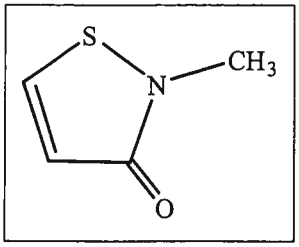


Figure 1. Methylisothiazolinone.

Table 1. Technical and Trade Names for Methylisothiazolinone[2,3]

Synonyms	3(2H)-Isothiazolone, 2-methyl- 2-Methyl-3(2H)-isothiazolone 2-Methyl-4-isothiazolin-3-one
Trade names	Microcare MT Neolone 950 preservative OriStar MIT

Table 2. Chemical and Physical Properties of Neolone 950 Preservative[4]

Property	Description
Physical description	Colorless, clear with a mild odor, liquid at 20°C
Molecular weight	115.2
Empirical formula	C_4H_5NOS
Melting point	No data
Boiling point	100°C
Flash point	Not applicable
Density	1.02 g/mL at 25°C
Viscosity	3.95 cP at 25°C
Solubility	Completely soluble in water Mostly soluble in acetonitrile, methanol, hexane Slightly soluble in xylene
pH at 25°C	3.87
Vapor pressure	2 × 10–2 torr at 25°C
Octanol/water partition coefficient	log P = –0.486

Analytical Methods

In studies by Bruze et al,[5,6] MIT was isolated from Kathon CG and identified by high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance spectrometry (NMR).

In a study by Connor et al,[7] MIT was isolated from Kathon 886 by thin-layer chromatography (TLC) and identified by gas chromatography/mass spectrometry (GC/MS).

According to Rohm & Haas,[3] MIT is identified and quantified using reverse-phase HPLC.

Impurities

The composition of technical grade MIT is described in Table 3.[4] Most toxicity testing performed by Rohm & Haas, which is described in this safety assessment, used this material. Table 4 describes the impurities profile for Neolone 950 preservative (9.5% active ingredient).

Table 3. Composition of MIT Technical Grade[4]

Component	% by Weight
MIT	96.8
5-chloro-2-methyl-4-isothiazolin-3-one	0.1
4,5-dichloro-2-methyl-4-isothiazoline-3-one	0.1
N, N′-dimethyl-3,3′-dithiodipropionamide	0.2
N,N′-dimethyl-3,3′-trithiodipropionamide	0.5
N-methyl-3-chloropropionamide	0.1
Ammonium chloride	0.3
Water	0.2
Ethyl acetate	0.1
Acetic acid	0.1
Unknown compounds[a]	1.5

[a] Fraction of 9 minor components that have been tentatively identified by liquid chromatography/mass spectrometry as chlorination products of monosulfide by-products produced during amidation of methyl-3-mercaptopropionate.

Table 4. Impurities Profile of Neolone 950 Preservative[3]

Component	ppm
4,5-dichloro-2-methyl-4-isothiazoline-3-one	0
N-methyl-3-chloropropionamide	0
N, N′-dimethyl-3,3′-dithiodipropionamide	490
5-chloro-2-methyl-4-isothiazolin-3-one	44-79
N,N′-dimethyl-3,3′-trithiodipropionamide	79-103

Reactions

According to Collier et al,[8] MIT oxidatively reacts with thiols, such as glutathione, to form disulfides. Reaction rates are dependent on pH. Cystine is released and mercaptoacrylamide is formed when MIT further interacts with thiols.

Use

Cosmetic

Table 5 represents the current uses and concentrations for MIT as a function of product category. According to information supplied to the US Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Ingredient Registration Program (VCRP), MIT is used in a total of 1125 cosmetic products.[9] The information provided under the VCRP, however, does not clearly indicate whether MIT is used alone in products or is used with CMIT.[3]

Based on an industry survey of use concentrations of MIT alone, current concentrations of use are shown in Table 5 and range from 0.000004% to 0.01%.[10] According to Gottschalck and Bailey,[2] MIT functions as a preservative.

Use data from the industry database Mintel show that many (83) products in the United States contain MIT without the

Table 5. Current Cosmetic Product Uses and Concentrations for Methylisothiazolinone

Product Category (total no. of products in each category)	Ingredient Uses in Each Product Category[a] (FDA)[9]	Use Concentrations, %[10]
Baby products		
Shampoos (38)	5	—
Lotions, oils, powders, and creams (67)	2	—
Other (64)	7	0.002–0.01[b]
Bath products		
Soaps and detergents (594)	117	0.008
Bubble baths (256)	37	—
Other (276)	45	—
Eye makeup		
Eyeliners (639)	1	—
Eye makeup remover (114)	4	—
Other (229)	1	—
Makeup		
Blushers (459)	1	—
Face powders (447)	1	—
Fragrance products		
Other (187)	2	—
Noncoloring hair care products		
Conditioners (715)	206	0.000 004–0.01
Sprays/aerosol fixatives (294)	2	0.005
Straighteners (61)	1	—
Rinses (46)	3	—
Shampoos (1022)	275	0.004–0.01
Tonics, dressings, etc (623)	34	0.008–0.009
Wave sets (59)	3	—
Other (464)	50	—
Hair coloring products		
Dyes and colors (1600)	13	—
Tints (56)	38	—
Shampoos (27)	18	—
Bleaches (103)	1	—
Other (73)	6	—
Nail care products		
Creams and lotions (13)	1	—
Personal hygiene products		
Underarm deodorants (281)	2	—
Other (390)	42	0.0015– 0.01
Shaving products		
Aftershave lotions (260)	3	—
Shaving cream (135)	3	0.005
Shaving soap (2)	1	—
Other (64)	4	—
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1009)	62	0.0008–0.008
Depilatories (49)	1	—
Face and neck creams, lotions, powder and sprays (546)	23	0.006[c]
Body and hand creams, lotions, powder and sprays (992)	31	—
Moisturizers (1200)	30	—
Night creams, lotions, powder and sprays (229)	4	—
Paste masks/mud packs (312)	4	—
Skin fresheners (212)	10	—
Other (915)	23	—
Suntan Products		
Suntan gels, creams, liquids and sprays (138)	5	—
Indoor tanning preparations (74)	1	—
Other (41)	2	—
Total uses/ranges for methylisothiazolinone	1125	0.000 004–0.01

[a] Data provided are not clear as to whether uses are methylisothiazolinone alone or include uses of methylisothiazolinone/methylchloroisothiazolinone.
[b] 0.01% in baby wipes.
[c] 0.006% does not represent a spray product.

chlorinated counterpart, CMIT. This information is represented in Table 5.

According to Rohm & Haas,[4] MIT is a broad-spectrum preservative that is used in cosmetic formulations. Neolone 950 contains 9.5% of the active ingredient (a.i.) MIT and is used at a maximum concentration of 100 ppm a.i.

Neolone 950 is reported to be safe and suitable for over-the-counter (OTC) products used for rinse-off and leave-on applications on unbroken skin at this maximum concentration.[11] OTC applications include antidandruff shampoos and sunscreens but would not include anti-acne creams, because open sores may be present in acne cases.

MIT is used in hair sprays and possibly other spray products, and effects on the lungs that may be induced by aerosolized products containing this ingredient are of concern.

The potential adverse effects of inhaled aerosols depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system.[12] In general, the smaller the particle, the farther into the respiratory tree the particle will deposit and the greater the impact on the respiratory system.[13]

Anhydrous hair spray particle diameters of 60 to 80 μm have been reported, and pump hair sprays have particle diameters of 80 μm or larger.[14] The mean particle diameter is around 38 μm in a typical aerosol spray.[15] In practice, aerosols should have at least 99% of particle diameters in the 10- to 110-μm range. This means that most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

In Japan, MIT is restricted to a maximum level of 0.01 g/100 g (100 ppm) in both wash-off and leave-on cosmetics.[16] MIT has not been evaluated for use on mucous membranes to date. MIT (listed as 2-methyl-4-isothiazolin-3-one) is also considered to be a quasi-drug that may be used directly on the body.[17] Quasi-drugs are defined as having a mild effect on the body but are not intended for the diagnosis, prevention, or treatment of disease or to affect the structure or function of the body.

The European Union[18] has approved the use of MIT in preservatives at a maximum concentration of 0.01%.[19]

MIT has been reviewed and approved for use up to 0.01% (100 ppm) in both leave-on and rinse-off products by the following nations: the Association of Southeast Asian Nations (Brunei Darussalam, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, Vietnam), Argentina, Australia, Brazil, Canada, China, Iceland, Israel, Korea, Mexico, Norway, Russia, Switzerland, and Turkey.[3]

Noncosmetic

MIT is used as a preservative in cleaning products such as carpet cleaners, dishwashing liquids, fabric softeners, floor polishes, general cleaners, and sprinkler liquids.[20]

MIT is registered by the US Environmental Protection Agency (EPA) as an antimicrobial agent. MIT is used to control slime-forming bacteria, fungi, and algae in pulp/paper mills, cooling water systems, oil field operations, industrial process waters, and air washer systems. MIT is used to control mold, mildew, and sap stain on wood. It also is used as a preservative in adhesives, coatings, fuels, metalworking fluids, resin emulsions, paints, and other specialty products.[21]

Rohm & Haas[4] reported that MIT is approved by the FDA as a preservative in regulated diagnostic reagents.

General Biology

Absorption, Distribution, Metabolism, Excretion

Absorption. The in vitro percutaneous absorption of MIT was determined using Charles River Crl:CD hairless rat skin.[22] MIT was radiolabeled on the fourth and fifth carbon of the isothiazolone ring (99.88% radiochemical purity with specific activity of 39.05 mCi/g). The [^{14}C]-MIT was applied to the epidermal surface of the rat skin that was mounted on Bronaugh flow-through diffusion cells at the following concentrations: 25 ppm, 75 ppm, or 150 ppm in water. The receptor fluid was evaluated for radiolabel over a 24-hour period. Radioactivity was measured in all fractions.

Most of the radiolabel was in the epidermal sections of the skin (29.2%-46.4% of applied radioactivity), and smaller amounts were in the stratum corneum (3.8%-10.4% of applied radioactivity) and dermis (0.2%-0.9% of applied radioactivity). The rate of absorption over the 24-hour period was 0.0059 ± 0.0024, 0.0277 ± 0.0079, and 0.0841 ± 0.0265 μg equivalents per square centimeter for hour for 25-, 75-, and 150-ppm dose groups, respectively. During the 24-hour exposure period, the mean amount of total applied radioactivity absorbed was 21.4% ± 8.8%, 33.7% ± 9.6%, and 51.2% ± 16.1% for 25-, 75-, and 150-ppm dose groups, respectively.[22]

In another in vitro percutaneous absorption study by Rohm & Haas,[23] [^{14}C]-MIT (96.90% radiochemical purity, specific activity 48.50 mCi/g) was applied to human epidermis in 3 aqueous solutions (52.2, 104.3, and 313.0 μg of MIT per milliliter) and 3 formulations (shampoo, body lotion, and facial cream at a concentration of 100 μg of MIT per milliliter). The aqueous solutions were applied to the membranes at a rate of 20 μL/cm^2 and the formulations were applied at a rate of 20 mg/cm^2. The applications were occluded for 24 hours, after which the distribution of the radiolabel was measured.

In the aqueous solutions, 11% to 13% of applied radioactivity was found in the donor chamber and 7% to 15% of applied radioactivity was washed from the skin. The percentage of applied radioactivity recovered ranged from 2% to 4% in the stratum corneum and from 11% to 36% in the remaining epidermis. The amount of total dose absorbed in the aqueous solutions was 29.8% ± 10.1%, 38.0% ± 12.1%, and 54.7% ± 12.0% for the groups receiving 52.2, 104.3, and 313.0 μg of MIT per milliliter, respectively. In the formulations, 4% to 9% of applied radioactivity was found in the donor chamber, and 30% to 69% of dose was washed from the skin. The percentage of applied radioactivity recovered ranged from 2% to 4% in the stratum corneum and from 17% to 20% in the remaining epidermis.

The amount of total dose absorbed was 29.5% ± 13.4%, 8.98% ± 3.10%, and 19.6% ± 10.0% in the shampoo, body

lotion, and facial cream formulations, respectively. The authors suggested that the ^{14}C recovered in the receptor fluid may represent MIT metabolites. The rates of absorption for MIT (100 μg/mL concentration) across human epidermis over a 24-hour exposure ranged from 0.007 to 0.026 μg/cm^2/h in the formulations. The rate of absorption for the aqueous MIT solutions (104 μg/mL concentration) was 0.037 μg/cm^2/h over the same exposure time.[23]

Distribution. Rohm & Haas[24] evaluated the distribution of [^{14}C]-MIT (96.70% radio purity, 51.4% nonradiolabeled purity, and specific activity 13.72 mCi/g) using CD-1 mice (average body weights 27 g in males and 23 g in females). Fifteen mice of each sex were dosed with 100 mg/kg radiolabeled MIT by oral gavage. One mouse served as a control. At 1, 3, 6, 24, and 48 hours post dosing, 3 mice per sex were killed, and blood, plasma, bone marrow, femurs, and livers were collected and measured for radiolabel content.

At early time points, total radioactive residues (TRRs) derived from the radiolabeled MIT were high in all tissues, with the highest levels in the liver and lowest in the bone. At 24 hours post dosing, the TRR declined significantly in the tissues. A tissue to plasma ratio showed that the radiolabel partitioned preferentially from plasma to tissues. At 48 hours post dosing, blood had the highest tissue to plasma ratio. For the 48-hour period, the mean concentrations of TRR in the bone marrow ranged from 1.2 to 39.4 ppm in males and 1.1 to 30.4 ppm in females. TRR appeared to be higher in male tissues than female tissues overall.[24]

Metabolism. The metabolism of 4,5-[^{14}C]-MIT (99.08% radio purity, specific activity 25.20 mCI/g) was evaluated in 36 Sprague-Dawley rats by Rohm & Haas.[25] The test substance was administered by oral gavage at either 5 or 50 mg/kg. The study was 96 hours in duration. At 24-hour intervals, urine, cage rinse, and feces were collected from rats. A group of 4 rats of each sex that received 5 mg/kg were killed 1 hour post dosing for tissue sampling. All rats were killed at the end of study, and the tissues were sampled for radiolabel.

Most of the radiolabel was excreted within 24 hours (80%-87%) and was mainly recovered in the urine and cage rinse (53%-70%) and in the feces (21%-37%). At the 96-hour tissue sampling, only 1.9% to 3.6% of the radiolabel was measured, and this was mainly in the blood. The total mean recovery of the radiolabel was 92% to 96%. The half-life of elimination ($T_{1/2}$ initial) of radiolabel derived from MIT from plasma was 3 to 6 hours and was not dose dependent. No difference between the genders was observed. All radiolabel that was recovered was in 23 different metabolite components of the test substance as measured by HPLC radioprofiling. The test substance itself was not detected in either the urine or feces.

The metabolites were identified with liquid chromatography/mass spectroscopy (LC/MS), liquid chromatography/tandem mass spectroscopy (LC/MS/MS), and 1-dimensional (1D) and 2D NMR. The major metabolites in urine were *N*-methyl malonamic acid (NMMA), 3-mercapturic acid conjugate of 3-thiomethyl-*N*-methyl-propionamide, and *N*-methyl-3-hydroxyl-propionamide at 21% to 23%, 10% to 23%, and 4% to 5% of the dose, respectively.[25]

Rohm & Haas[26] conducted another study on the metabolism of radiolabeled MIT (96.90% radio purity, 51.4% nonradiolabeled purity, and specific activity 48.50 mCi/g) using bile duct–cannulated female Sprague-Dawley rats (body weight range, 251-276 g). Four rats received a single oral dose of 50 mg/kg. Bile, urine, cage wash, and feces were collected from the rats for 24 hours post dosing. At the end of the 24-hour period, the rats were killed.

More than 88% of the dose was recovered in the 24-hour period, with most of the radiolabel found in the bile (29.09%), urine and cage rinse (52.92%), and feces (6.14%). The radiolabel was recovered in 31 metabolite forms of MIT; no intact MIT was recovered. The main metabolites recovered were *N*-methyl malonamic acid and 3-mercapturic acid conjugate of 3-thiomethyl-*N*-methyl-propionamide. The metabolites were identified with LC/MS and LC/MS/MS.[26]

Animal Toxicology

Acute Toxicity

Acute toxicity studies for MIT are summarized in Table 6 and described below for oral, dermal, and inhalation routes of exposure in studies using rats and mice.

Acute Oral Toxicity

MIT—rats. An acute oral toxicity study of MIT (99.7%) was performed using 60 Crl:CD BR rats (36 males and 24 females).[27] MIT was diluted with distilled water, and the solutions were administered to the rats at 75, 150, 180, and 225 mg/kg body weight. Males were also dosed at 300 mg/kg body weight. The animals received a single dose by gavage at a volume of 10 mL/kg body weight. The rats were observed for 14 days thereafter, during which they were allowed feed and water ad libitum.

In the male rats, 4 of 12 and 6 of 6 in the 225- and 300-mg/kg dose groups, respectively, died. No deaths were reported in the remaining male dose groups. In the female rats, 4 of 6 and 5 of 6 in the 180- and 225-mg/kg dose groups, respectively, died. Again, no deaths were reported in the remaining female dose groups.

Females at all doses and males in the 150-mg/kg dose groups and higher exhibited signs of intoxication beginning at 1 hour post dosing. Intoxication was resolved by day 6 in surviving rats.

At necropsy, rats that died during the observation period had reddened intestines, red-tinged fluid or red/red-tinged material in the intestines, reddened glandular portion of the stomach, red-tinged fluid or mucus in the stomach, and stomach distended by air. No gross changes were observed in survivors.

The median lethal dose (LD_{50}) for MIT in male rats was 235 mg/kg body weight (95% confidence interval [CI], 216-336

Table 6. Acute Toxicity of MIT in Rats and Mice

Concentration of MIT	Dose Range	No. of Animals and Type	Results	Reference No.
Oral—rats				
99.7%	75-300 mg a.i./kg	36 male and 24 female Crl:CD BR rats	LD_{50} = 235 mg a.i./kg males; 183 mg a.i./kg females	27
9.69% in formulation	1000-5000 mg/kg of formulation	24 male and 18 female Crl:CD BR rats	LD_{50} = 274.6 mg a.i./kg males; 105.7 mg a.i./kg females	28
100 ppm tested in a lotion at a 1:9 dilution	0 (vehicle control) and 2000 mg/kg of formulation	10 male and 10 female Crj:CD(SD)IGS rats	LD_{50} >2000 mg formulation/kg for both sexes	29
100 ppm tested in a shampoo at a 1:9 dilution	0 (vehicle control) and 2000 mg/kg of formulation	10 male and 10 female Crj:CD(SD)IGS rats	LD_{50} >2000 mg formulation/kg for both sexes	30
51.4%	180-300 mg a.i./kg	18 male and 18 female Crl:CD BR rats	LD_{50} = 232-249 mg a.i./kg males; 120 mg a.i./kg females	32
Oral—mice				
97.5%	150-250 mg/kg	18 male and 18 female Crl:CD-1(ICR) BR mice	LD_{50} = 167 mg/kg for both sexes	33
Dermal—rats				
97.5%	100-400 mg a.i./kg	24 male and 18 female Crl:CD BR rats	LD_{50} = 242 mg a.i./kg for both sexes	35
9.69%	193.8-484.5 mg a.i./kg	18 male and 18 female Crl:CD BR rats	LD_{50} >484.5 mg/kg for both sexes	36
Inhalation—rats				
97.8%	0.046-2.09 mg a.i./L	30 male and 30 female Crl:CD BR rats	LC_{50} = 0.11 mg a.i./L combined	37
53.52%	0.15-0.68 mg a.i./L	20 male and 20 female Crl:CD BR rats	LC_{50} = 0.35 mg a.i./L	38,39
Inhalation—mice				
98.6%	3.12-157 μg/L	36 male Crl:CFW(SW)BR mice	RD_{50} > 157 μg/L	40

a.i., active ingredient; LC_{50}, mean lethal concentration; LD_{50}, mean lethal dose; RD_{50}, 50% respiratory rate decrease.

mg/kg). In female rats, the LD_{50} was 183 mg/kg body weight (95% CI, 154-214 mg/kg).[27]

Rohm & Haas[28] performed an acute oral toxicity study in Crl:CD BR rats using Neolone 950 (MIT 9.69%). The test substance was administered undiluted via a single oral gavage dose. A total of 24 male and 18 female rats were used in the experiment. The rats were observed for clinical signs of toxicity beginning 1 hour post dosing through day 4.

In the males, 1 of 5, 3 of 6, 2 of 6, and 6 of 6 of the 2000-, 2500-, 3000-, and 5000-mg/kg dose groups, respectively, died before the end of the study period. In the females, 1 of 6, 6 of 6, and 5 of 6 of the 1000-, 1500-, and 2000-mg/kg dose groups, respectively, died before the end of the study period.

Clinical signs of toxicity were observed. No effects on body weight were observed in rats surviving until the end of the study compared with historical control data. Rats that died during the study had reddened intestines and/or stomach mucosa, clear or red/yellow fluid in the intestines and/or stomach, blackened intestines, and distended stomachs.

The acute oral LD_{50} for Neolone 950 preservative in male rats was 2834 mg of product per kilogram of body weight (95% confidence limits of 2047 and 4377 mg/kg body weight) and in females was 1091 mg of product per kilogram of body weight (95% confidence limits of 891 and 1334 mg/kg body weight). The calculated corresponding LD_{50} values for the active ingredient, MIT, were provided without further explanation: 274.6 mg/kg body weight (95% CI, 198.4-424.1 mg/kg body weight) in male rats and 105.7 mg/kg body weight (95% CI, 86.3-129.3 mg/kg body weight) in female rats.[28]

An anionic body lotion containing 100 ppm MIT was tested on Crj:CD(SD)IGS rats.[29] The anionic body lotion was mixed with distilled water at a ratio of 1:9 while another emulsion of an anionic body lotion without the active ingredient was also prepared. The rats (5 per sex per dose group) were dosed at a volume of 20 mL of solution per kilogram of body weight via a single oral gavage dose. The rats were allowed food and water ad libitum and were observed for 14 days.

No mortalities or treatment-related effects were observed. The acute oral LD_{50} was greater than 2000 mg of lotion per kilogram of body weight for both lotions in rats.[29]

The acute oral toxicity of a generic shampoo containing 100 ppm MIT was tested on Crj:CD(SD)IGS rats using the same protocol as described in the previous study.[30] No mortalities were observed in either test group. Half of the animals in both dose groups had loose, muddy, or jelly-like stools from 2 hours after dosing. The changes in the stools were attributed to the generic shampoo and not to MIT. No other treatment-related effects were observed. The acute oral LD_{50} was greater than 2000 mg of shampoo per kilogram of body weight for both shampoos in rats.

The acute oral toxicity of a high-SPF sunscreen containing 100 ppm MIT was tested on Crj:CD(SD)IGS rats using the same protocols as described in the previous 2 studies.[31] No mortalities or treatment-related effects were observed in either test group. The acute oral LD_{50} was greater than 2000 mg of sunscreen per kilogram of body weight for both sunscreens in rats.

An acute oral toxicity study using Crl:CD BR rats tested MIT at 51.4%.[32] The MIT was diluted in distilled water and the solution was administered to the rats at a volume of 10 mL of solution per kilogram of body weight via a single oral gavage dose in dose groups receiving 150 to 300 mg of a.i. per kilogram of body weight. Following dosing, the rats were allowed food and water ad libitum and were observed for 14 days.

In male rats, 4 of 6, 1 of 6, and 6 of 6 of the 180-, 225-, and 300-mg/kg dose groups, respectively, died by day 6 of the study. In the females, 4 of 6, 5 of 6, and 5 of 6 of the 150-, 180-, and 225-mg/kg dose groups, respectively, also died by day 6.

Clinical signs of toxicity were observed but surviving animals recovered by day 7 and had normal body weight changes. At necropsy, animals that died during the study had gastrointestinal (GI) changes (no details were available) and surviving animals had no gross changes.

The LD_{50} was 232 to 249 mg of a.i. per kilogram of body weight (95% CI, 176-306 mg of a.i. per kilogram of body weight) and 120 mg of a.i. per kilogram of body weight (95% CI, 79-182) in male and female rats, respectively.[32]

MIT—mice. An acute oral toxicity study in Crl:CD-1(ICR) BR mice tested MIT at 97.5%.[33] The MIT was diluted in distilled water, and the solution was administered to the mice at a volume of 10 mL of solution per kilogram of body weight via a single oral gavage dose. The dose groups were 150, 200, and 250 mg/kg body weight. There were 6 of each sex in each dose group (body weight range, 29-34 g males, 23-29 g females). The mice were observed for 14 days and were allowed food and water ad libitum.

All mice in the 250-mg/kg dose group died before the end of the observation period, and 2 of 6 of each sex in the 150-mg/kg dose group and 4 of 6 males and 5 of 6 females in the 200-mg/kg dose group died before the end of the study.

Clinical signs of toxicity were observed in both sexes in all dose groups started at 1 hour after dosing but resolved in surviving animals by day 2. No effects on body weight were observed. At necropsy, animals that had died during the study had GI changes (no details were available) and surviving animals had no gross changes.

The LD_{50} for male and female mice was 167 mg/kg body weight (95% CI, 137-187 mg/kg).[33]

N-methyl-malonamic acid—rats. The effects of the MIT metabolite NMMA (100%) were studied in an acute oral study using rats (strain not specified).[34] The rats were divided into 3 dose groups with 6 of each sex in the 1000-, 2500-, and 5000-mg/kg dose groups. NMMA was diluted in 0.5% methylcellulose and administered by a single oral gavage. The rats were allowed food and water ad libitum and were observed for 14 days.

In the 5000-mg/kg dose group, 5 of 6 males and 4 of 6 females died before the end of the observation period. One male and 1 female died in the 2500-mg/kg dose group.

Clinical signs of toxicity were observed. At necropsy of the decedents, mucosal congestion, petechial hemorrhage, and GI tract irritation were observed. No clinical signs of toxicity or gross changes at necropsy were observed in rats in the 1000- or 2500-mg/kg dose group.

The calculated LD_{50} in males was 3550 mg/kg body weight (95% CI, 2649-4787 mg/kg), and the calculated LD_{50} in females was 4100 mg/kg body weight (95% CI, 2808-5986 mg/kg).[34]

Acute Dermal Toxicity

MIT—rats. The acute dermal toxicity of 97.5% MIT was studied in Crl:CD BR rats.[35] The rats were divided into 4 dose groups with 6 of each sex in the 100-, 200-, and 400-mg/kg dose groups and 6 males in the 300-mg/kg dose group. MIT was administered undiluted in a single 24-hour occluded topical application on shaved intact skin of the trunk, and the rats were observed for 14 days before necropsy.

In the male rats, 5 of 6 of both the 300- and 400-mg/kg dose groups died during the observation period. In females, 3 of 6 of the 200-mg/kg dose group and 6 of 6 of the 400-mg/kg dose group died during the observation period.

Clinical signs of toxicity were noted in all dose levels and both sexes beginning on day 1. Surviving rats recovered by day 5. Body weight gains decreased in surviving rats of both sexes in the 200-mg/kg and higher dose groups compared with historical controls. Blanching, edema, darkened areas, eschar, sloughing, scabbed areas, and desiccation were observed in both sexes in all dose groups throughout the observation period.. Rats that died during the study had GI changes at necropsy, whereas surviving rats had no gross changes.

The acute dermal LD_{50} for 97.5% MIT was calculated to be 242 mg/kg body weight (95% CI, 192-294 mg/kg) in male and female rats.[35]

In another acute dermal toxicity study by Rohm & Haas,[36] MIT at 9.69% in Neolone 950 was tested on Crl:CD BR rats. The dose groups were 193.8, 339.2, and 484.5 mg of a.i. per kilogram of body weight (6 of each sex in each dose group). The test substance was administered undiluted by a single 24-hour occluded topical application on shaved intact skin of the trunk (area = 6 cm × 6-7 cm) and the rats were observed for 14 days.

There was no mortality during the observation period. Scant feces were observed in females of the 339.2-mg/kg and 484.5-mg/kg dose groups on days 2 and 3 and in 1 male in the 484.5-mg/kg dose group on day 3. Skin effects noted through the observation period included pocketing edema/edema, erythema, blanching, desiccation, darkened or reddened area,

scabs, eschar, and/or sloughing. No changes in body weight or gross changes at necropsy were observed in any of the rats.

The acute dermal LD_{50} for 9.69% MIT was determined to be greater than 484.5 mg/kg body weight in male and female rats.[36]

Acute Inhalation Toxicity

MIT—rats. An acute inhalation toxicity study of 97.8% MIT was performed on 60 Crl:CD BR rats (30 of each sex) by Rohm & Haas.[37] The test material was diluted 1:1 wt/wt with tap water and the rats were exposed (groups of 6 males and 6 females) for 4 hours, nose-only in exposure chambers, to concentrations of 0.046, 0.012, 0.15, 1.07, and 2.09 mg/L.

In the 1.07- and 2.09-mg/L dose groups, all males died and half of the females died. In the 0.150-mg/L dose group, half of the males died and 5/6 females died. No deaths were observed in the 0.012-mg/L dose group and 1 male died in the 0.046-mg/L dose group. Most of the deaths occurred during the exposure.

Clinical signs of toxicity were observed. No exposure-related effects on body weight gain were noted in surviving rats. Necropsies of all rats showed signs of slight to severe redness in all lobes of the lung, scattered incidences of red pinpoint foci on the lungs, and gas-filled stomachs.

The combined LC_{50} was 0.11 mg MIT/L (95% CI, 0.07-0.25 mg/L).[37]

In another acute inhalation toxicity study reported by Rohm & Haas,[38,39] 40 Crl:CD BR rats were exposed to 53.52% MIT. There were 10 animals (5 of each sex) in each of the following dose groups: 0.15, 0.25, 0.47, and 0.68 mg of a.i. per liter. The rats were exposed for 4 hours by nose only using a glass nebulizer in an exposure chamber.

No deaths were observed in the 0.15-mg/L dose group. In the male rats, 2 of 5, 1 of 5, and 5 of 5 died in the 0.25-, 0.47-, and 0.68-mg/L dose groups, respectively. In the female rats, 3 of 5, 3 of 5, and 4 of 5 died in the 0.25-, 0.47-, and 0.68-mg/L dose groups, respectively.

Rats were observed for clinical signs of toxicity after removal from the exposure chamber through day 6. Clinical signs of toxicity were observed.

Necropsies of rats that died during the exposure and observation periods revealed pale and/or reddened lungs, distended intestines, and/or wet muzzle. No gross changes were observed in rats that survived the exposure and observation periods. Body weight gain was decreased 25% to 39% in females exposed to 0.25 mg/L and above during the 14-day observation period; there was no effect on body weight in males during the same observation period.

The combined LC_{50} for MIT was 0.35 mg/L (95% CI, 0.27-0.45 mg/L).[38,39]

MIT—mice. The irritation effects of 98.6% MIT on the upper respiratory tract were studied in 36 male Crl:CFW(SW)BR mice. There were 4 males in each of the following dose groups: 3.12, 6.76, 10.5, 27.8, 64.6, 74.9, 90.7, 92.2, and 157 μg/L. The mice were exposed for 10 minutes to the atomized test material (particle diameter not reported) in 3.5-L exposure chambers. Respiratory rates were monitored before, during, and after the exposure, and the average respiratory rates and percentage depression of the rates were calculated. The percentage decrease in respiratory rate was 25% in the 3.12-μg/L group and 44% in the 157-μg/L group, with the greatest depression of 47% occurring in the 74.9-μg/L group. The RD_{50} was greater than 157 μg/L. The decreases in respiratory rates equated to moderate responses for sensory irritation according to the American Standard Test Method (ASTM) E981-84.[40]

Subchronic Oral Toxicity

MIT—rats. In a 3-month study reported by Rohm & Haas,[41] 97.5% MIT was administered diluted in the drinking water of Crl:CD BR rats. MIT was administered at the concentrations of 0, 75, 250, or 1000 ppm, which was equivalent to 0, 6.5 to 9.8, 19 to 25, and 66 to 94 mg of MIT per kilogram of body weight per day, respectively. The dose groups consisted of 10 males and 10 females each. The rats were observed daily, and body weights and water and feed consumption were recorded weekly. Detailed clinical observations were performed weekly. During the 13th week of dosing, a Functional Observational Battery (FOB) was performed on all animals at all dose levels. During the last week of dosing, the motor activity of all animals was assessed using an infrared motion activity cage system. All rats received an ophthalmoscopic examination at the end of the treatment period. The rats were killed and necropsied at the end of the study after samples for hematologic and clinical chemistry measurements were collected.

There was no mortality. Likewise, there were neither systemic nor neurological effects in any of the rats during the treatment period. No treatment-related gross lesions, ocular disease, or changes in hematology and clinical chemistry were observed. There were no treatment-related effects on any organ weights and no microscopic pathological effects on any tissues or organs were observed at any dose level. No treatment-related effects on body weight in male and female rats were observed at doses up to and including 250 ppm.

Treatment-related decreases in cumulative body weight gains were observed in males and females at 1000 ppm for the entire treatment period. Treatment-related decreases in feed consumption in males were also observed in this dose group, and decreases in water consumption were observed in females of the 250- and 1000-ppm dose groups and in males of all dose groups.

The authors suggested that the decreases in body weight, feed, and water consumption were likely due to unpalatability of the drinking water and the refusal of the rats to drink it. The no observed adverse effect level (NOAEL) for the study was considered to be 1000 ppm (66-94 mg of a.i. per kilogram of body weight per day).[41]

MIT—dogs. In a study by Rohm & Haas,[42] groups of 4 male and 4 female Beagle dogs were fed diets containing 0, 100/130, 400, or 1500 ppm MIT (51.4% a.i.) for 3 months. These doses

equated to 3, 10, and 41 mg of a.i. per kilogram of body weight per day, respectively. Lower than acceptable recovery in the 100-ppm dose group caused the researchers to increase the dose level to 130 ppm starting week 4. The dogs were observed at least twice daily, and clinical examinations were conducted weekly on all dogs. Body weight and feed consumption were measured throughout the course of the study. Prior to treatment and at study conclusion, ophthalmoscopic and physical exams were conducted. Hematologic and clinical chemistry measurements were collected prior to treatment, at week 7, and at study termination. At study termination, all dogs were killed and necropsied. Tissues and select organs underwent histopathological evaluation.

There was no mortality, and there were no treatment-related clinical effects or histopathological findings in any of the dogs.

Treatment-related decreases in body weight and cumulative body weight gain were observed in dogs of both sexes exposed to 1500 ppm MIT in week 1 compared with controls, but weight gain was comparable to controls from week 3 (males) and week 4 until treatment conclusion. Feed consumption was also decreased in this dose group in both sexes for the entire treatment period but not always in a statistically significant manner.

In the 1500-ppm group, non–statistically significant changes were observed in some hematology parameters in both sexes. There were no treatment-related effects on organ weights. No treatment-related effects were observed in microscopic pathology.

The authors concluded that the no observed effect level (NOEL) was 400 ppm MIT (10 mg of a.i. per kilogram of body weight per day), and the NOAEL was 1500 ppm MIT (41 mg/kg/d).[42]

NMMA. —rats. In a subchronic oral toxicity study,[43] 45 male and 45 female Charles River CD rats were divided into 3 dose groups that received control vehicle, 33 to 66 ppm NMMA and 6.7 to 13.4 ppm malonic acid (MA), or 110 to 220 ppm NMMA and 22 to 44 ppm MA. The rats received the treatment in their diets for 3 months.

One control rat had slight alopecia. A few rats in each treated dose group showed slight alopecia or reddened raw or scabbed skin. No other clinical signs were observed. No effects on body weight, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, or gross pathologic changes were observed.

There was 1 death in a low-dose female and 1 death in a high-dose male (no further details provided).[43]

NMMA. —dogs. In a subchronic oral toxicity study,[44] 24 male and 24 female Beagle dogs were divided into 3 dose groups that received control vehicle, 150 ppm NMMA and 30 ppm MA, or 500 ppm NMMA and 100 ppm MA. The dogs received the treatment in their diets for 3 months. No systemic toxicity was observed at doses up to 16 to 17 mg/kg/d NMMA when in combination with 3.2 to 3.4 mg/kg/d MA.

Ocular Irritation

Smith and Alexander[45] presented a study in which the ocular irritancy potential of CMIT/MIT, MIT, and CMIT/1,2-benzisothiazolin-3-one (BIT) was tested using bovine corneas at in-use concentrations, 100× in-use concentrations, and neat concentrations. The corneal anterior surface was then treated for 10 minutes with either 0.9% NaCl (control solution), absolute ethanol, or the test compound (3 or 4 per treatment). The corneal permeability was measured using a fluorescein dye solution. The in vitro score (IVS) was then calculated from the opacity and absorbance measurements and assessed according to the prediction model created by Gautheron et al.[46]

The neat concentrations of the isothiazolinones had mean IVS greater than 3, which is the threshold score for irritation. The neat formulations of MIT/BIT and CMIT/MIT had greater eye irritation potentials than MIT (21.8 $\pm$ 3.2, 16.8 $\pm$ 7.3, and 9.3 $\pm$ 5.3, respectively). All the formulations were mild eye irritants according to the model.[45]

Rohm & Haas[47] predicted that MIT at 50% in water would be corrosive to the eyes of rabbits, based on findings in an earlier dermal toxicity study.[48]

In an ocular irritation study[49] in 6 male New Zealand White rabbits, 9.69% MIT in Neolone 950 preservative was instilled into the conjunctival sac of 1 eye of each rabbit. The test substance was diluted in distilled water as a 100-ppm solution of the active ingredient prior to instillation. Both rabbit eyes were rinsed with saline for 1 minute at 24 hours after application. The cornea, iris, and conjunctiva were observed at 1, 24, 48, and 72 hours after application.

No adverse effects were observed, and the authors concluded that 100 ppm MIT in Neolone 950 preservative is nonirritating to rabbit eyes.[49]

Rohm & Haas,[50] formulated Neolone 950 in a generic shampoo to have a final concentration of 100 ppm (0.01%) a.i. The shampoo was studied for eye irritation in Kbl:JW male rabbits. Six of the rabbits were dosed with the shampoo containing MIT in a single instillation of 0.1 mL into the conjunctival sac of 1 eye of each rabbit (the other eye of each rabbit served as an untreated control), whereas 7 rabbits were dosed with a generic shampoo that did not contain MIT (1 treated eye and 1 untreated eye per rabbit). Twenty to 30 seconds following the instillation of the test substances, the eyes of half the animals in each group were rinsed with lukewarm water; the remaining eyes were unwashed. The cornea, iris, and conjunctiva were observed at 1, 24, 48, 72 hours, and once daily for 21 days post application.

Mild to moderate primary irritant effects were observed in the eyes of rabbits treated with both shampoo formulations, and primary ocular mucosal irritation was lower in the rabbits with washed eyes. It was concluded that a shampoo containing 100 ppm MIT is not an eye irritant.[50]

In a similar study,[51] Neolone 950 was formulated in an anionic body lotion to have a final concentration of 100 ppm (0.01%) a.i. The lotion was studied for eye irritation in Kbl:JW male rabbits. Six rabbits were dosed with 0.1 mL of the lotion

containing MIT, whereas another 6 rabbits were dosed with lotion that did not contain MIT. Application and eye-washing protocol were the same as in the previous study.

No adverse effects were observed in the cornea, iris, conjunctivae, or other ocular structures in either lotion formulation in washed and unwashed eyes. The authors considered an anionic lotion containing 100 ppm MIT to be nonirritating.[51]

Rohm & Haas[52] used same protocols as the previous 2 studies to study the effects of a high-SPF sunscreen formulated from Neolone 950 to have a final concentration of 100 ppm (0.01%) a.i. Again, 6 male Kbl:JW rabbits were dosed with 0.1 mL of a formulation containing MIT, whereas another 6 were dosed with a formulation that did not contain MIT.

No adverse effects were observed in the cornea, iris, conjunctivae, or other ocular structures in either sunscreen formulation in washed and unwashed eyes. It was concluded that a high-SPF sunscreen containing 100 ppm MIT is not an eye irritant.[52]

Dermal Irritation

Dermal irritation studies for MIT are summarized in Table 7. All percentages and dose levels are in terms of a.i.

Rohm & Haas[48] performed a dermal irritation study in 7 male New Zealand White rabbits using 97.8% MIT. To the shaved intact skin of the rabbits' trunks, 0.5 mL of the test substance was applied using a 1-inch-square gauze-lined adhesive bandage. The patch site was semi-occluded for 1- and 4-hour exposures and uncuffed for a 3-minute exposure. One rabbit was tested for the 4-hour exposure and another was tested on 2 separate sites for a 1-hour exposure (on right side) and a 3-minute exposure (on left side). An additional 5 rabbits were tested for 3-minute exposures. The skin was evaluated for irritation at 1, 24, 48, and 72 hours after the patch was removed and again at 7 and/or 14 days after patch removal.

During the study, no mortality or signs of systemic toxicity were observed. On the sites exposed to the test substance for 1 and 4 hours, concave eschar was observed on days 7 and 14, respectively. The 3-minute exposure on the rabbit with dual site applications resulted in very slight to well-defined erythema through day 7 and slight edema at the 1-hour observation. The rabbits with just the 3-minute exposure sites had very slight to well-defined erythema through the 48-hour observation. Very slight to moderate edema was observed at 1 and 24 hours. One rabbit had very slight to slight edema at the 48- and 72-hour observations. It was concluded that undiluted MIT is corrosive to the skin after a 1-hour exposure.[48]

In another dermal irritation study, 6 male New Zealand White rabbits were exposed to MIT at 9.69% in Neolone 950. The test substance was diluted in distilled water as a 100-ppm solution of a.i. The solution was applied by a single application of 0.5 mL on a 1-inch-square gauze-lined adhesive bandage to shaved intact skin of the rabbits' trunks. The patch sites were semi-occluded for an exposure duration of 4 hours. After patch removal, the sites were observed for signs of irritation 1, 24, 48, and 72 hours after patch removal. No mortality or clinical signs of systemic toxicity were observed. No erythema or edema was observed, and the Primary Irritation Index was 0.0. The authors concluded that 100 ppm MIT (from 9.69% in Neolone 950) is nonirritating to rabbit skin.[53]

Another dermal irritation study using New Zealand White rabbits used 10% MIT in Neolone 950.[54] Six male rabbits received 0.5 mL of the test substance diluted in water and applied at concentrations of 100, 300, and 1000 ppm a.i. The dilutions were applied for 14 consecutive days on 3 shaved areas of the backs of the rabbits (2.5 × 2.5 cm per area). Sites were not occluded and were observed for erythema, eschar, and edema formation according to the Draize criteria. The rabbits were observed for clinical signs daily through the completion of the study. No dermal abnormalities or abnormal clinical signs were observed in the rabbits at any time during the study, and it was concluded that 100, 300, and 1000 ppm a.i. did not possess any cumulative skin irritant effects.

In an in vitro study by Rohm & Haas,[55] EpiDerm skin constructs were exposed to MIT at either 51.5% or 1.7%. Positive and negative controls were also used. Fifty microliters were applied to 4 skin constructs in a manner so that the upper surface was covered. Tissue viability was determined using MTT (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). It was concluded that 51.5% MIT was noncorrosive after the 3-minute exposure but corrosive at the 60-minute exposure; 1.7% MIT was noncorrosive in both exposures.

Dermal Sensitization

Dermal sensitization studies for MIT are summarized in Table 7. All percentages and dose levels are in terms of a.i.

MIT and CMIT—in vitro. Alvarez-Sánchez et al[56] studied the reactivity of CMIT and MIT with a model peptide derived from the N-terminal chain of globine (without cystine) and glutathione.

Both CMIT and MIT (concentrations not reported) were found to be highly reactive toward glutathione used as a thiol nucleophile model and a mimic of the detoxication process. In the model peptide reaction, MIT did not react with histidine and lysine to form stable adducts.

MIT and CMIT—in vivo. Bruze et al[57] assessed the active ingredients of Kathon CG, CMIT, and MIT for sensitization potential and cross-reactivity patterns in a modified Buehler guinea pig maximization test using female Dunkin-Hartley guinea pigs. The dose groups were composed of the following: 6 positive controls (2-methylol phenol), 12 negative controls (vehicle only), and 24 test animals in each series (1 series for CMIT and 2 series for MIT). Of each group of 24 animals, 12 were challenged on both patches with test chemical and 12 were challenged with 1 patch of test chemical and the other of vehicle.

The guinea pigs were induced with CMIT and MIT with intradermal injections of equimolar concentrations (6.7×10^{-3} mole $\times$ 1^{-1}; CMIT 0.100% wt/vol and MIT

Concentration	No. of Animals Per Model	Procedure	Results	Reference No.
Dermal irritation				
97.8%	12 male New Zealand White rabbits	1- and 4-h application (semi-occluded); 3-min application (uncuffed); all to intact skin	Corrosive to skin after a 1-h exposure	48
9.69% in Neolone 950 diluted to 100 ppm a.i.	6 male New Zealand White rabbits	4-h application to intact skin (semi-occluded)	Nonirritating	53
10% in Neolone 950 diluted to 100, 300, and 1000 ppm a.i.	6 male New Zealand White rabbits	14 consecutive daily applications to intact skin (nonoccluded)	Nonirritating	54
1.7% and 51.5%	4 EpiDerm skin constructs	3- and 60-min exposures followed by rinse; tissue viability measured with MTT	1.7% MIT noncorrosive after 3- and 60-min exposures; 51.5% MIT noncorrosive after 3-min exposure; 51.5% MIT corrosive after 60-min exposure	55
Dermal sensitization				
MIT and CMIT concentration not reported	Model peptide and glutathione	Covalent binding of ^{13}C isothiazolinones to a model peptide and glutathione with NMR spectroscopy analysis	CMIT reacted with histidine and lysine to form stable adducts; MIT was nonreactive under same conditions.	56
CMIT at 0.1% and MIT at 0.076% wt/vol in intradermal induction phase; 0.05% for CMIT and 0.038% wt/vol MIT in topical sensitization induction; 0.02% CMIT and 0.015% wt/vol MIT in challenge and rechallenge phases	48 female Dunkin-Hartley guinea pigs (additional 6 as positive controls and 12 as negative controls)	Modified Buehler maximization test	CMIT was a potent sensitizer and MIT was a weak sensitizer.	57
0.015% MIT	24 female Dunkin-Hartley guinea pigs (additional 12 as controls)	Maximization test	No sensitization	5
99.8% MIT; 1000-30 000 ppm in induction phases, 1000-15 000 ppm in challenge phase	25 male and 25 female Hartley guinea pigs	Buehler method	Sensitization at ≥ 1000 ppm	58
99.7% diluted to 550 or 800 ppm in induction and challenge phases, 1000 ppm in rechallenge phase	60 female Hartley guinea pigs	Maximization test	Not sensitizing up to 800 ppm	59
19.7% MIT; dose concentrations = 0.15%-18%	64 female Hsd Poc: DH [SPF] guinea pigs	Open epicutaneous test	Sensitization at ≥1.5%	60
Local lymph node assay				
99.8% MIT and 99.9% CMIT; dose concentrations = 1000-30 000 ppm	24 CBA/J mice (sex not reported)	LLNA	CMIT sensitization at 100 ppm; MIT sensitization at >10 000 ppm. EC_3 = 25 150 ppm	61
10.37% MIT in Neolone 950; dose concentrations = 0.15%-1.80%	40 female CBA/J mice	LLNA	MIT sensitization at >0.76%. EC_3 = 0.86%	62
19.7% MIT; dose concentrations = 0.049%-0.0985% in acetone/olive oil or 0.99%-9.85% in propylene glycol	44 female CBA/J mice	LLNA	Skin allergen with moderate strength. EC_3 = 0.4% in MIT with acetone: olive oil and EC_3 = 2.2% in MIT with propylene glycol	63
Cytokine profile study				
0.5% MIT in acetone/olive oil	Female Balb/c mice (number not reported)	Cytokine profile study	Cytokine profile not typical of a chemical respiratory allergen	63

CMIT, methylchloroisothiazolinone; EC_3; LLNA, local lymph node assay; MIT, methylisothiazolinone; NMR, nuclear magnetic resonance.

[a] All percentages and dose levels are in terms of active ingredient.

0.076% wt/vol). Twenty-four hours prior to topical sensitization, animals were treated with sodium lauryl sulfate (SLS) solution (200 μL). For the topical sensitization, 200 μL of the suspected sensitizing test chemical in 99.5% ethanol (0.050% wt/vol for CMIT and 0.038% wt/vol for MIT) was placed on a 2 × 4-cm patch at equimolar concentrations (3.3×10^{-3} mole $\times 1^{-1}$) and applied under occlusion for 48 hours.

The challenge procedure occurred 2 weeks after the second sensitization. Thirty microliters of test solution was placed on one or both patches that were applied to the right flank of the animals and occluded for 24 hours. The test chemicals were at equimolar concentrations (1.3×10^{-3} mole $\times 1^{-1}$; 0.020% wt/vol for CMIT and 0.015% for MIT). Test sites were evaluated after the removal of the patches. Animals received an intradermal injection of 0.1 mL of the solution used in the induction 2 days after the first challenge. Five days later, the animals were rechallenged with CMIT or MIT at the same concentrations and procedures as used in the challenge. The first MIT series was not rechallenged.

In the first and second MIT series, 4 of 24 (nonsignificant) and 11 of 24 (significant) guinea pigs had a positive reactions to MIT. In the CMIT series, 19 of 24 animals had positive reactions. No controls reacted in either MIT series and 1 reacted in the CMIT series. In the rechallenge, 8 of 24 MIT-sensitized animals were positive to MIT and 3 of 24 were positive to CMIT. In the CMIT-sensitized rechallenge, 1 of 24 was positive to MIT and 12 of 24 were positive to CMIT. Positive reactions were observed in 4 of 12 controls in the CMIT-sensitized rechallenge with CMIT. No reactions were observed in the MIT-sensitized controls. No cross-reactivity was observed with MIT after sensitization with CMIT; however, cross-reactivity occurred with CMIT following sensitization with MIT.

The authors determined that CMIT is a potent sensitizer but MIT is a weak sensitizer.[57]

In a follow-up guinea pig maximization study of the Kathon CG preservative contaminant 4,5-dichloro-2-methyl-4-isothiazolin-3-one, female Dunkin-Hartley guinea pigs were rechallenged with 0.015% MIT along with other constituents of Kathon CG in the manner described in the previous study. No positive reactions to MIT were observed in the test animals (n = 24) or in the control animals (n = 12).[5]

The sensitization potential of MIT (99.8% a.i.) was evaluated using the Buehler method.[58] Ten 6-hour induction doses of 0, 1000, 5000, 15 000, or 30 000 ppm in distilled water were applied (0.4 mL) on the shaved intact flank skin of Hartley guinea pigs (5 per sex in each dose group). Three doses per week were given for 3.5 weeks and the patches were occluded. After the last induction patch, the animals were allowed to rest for 2 weeks before the challenge application.

At challenge, the guinea pigs were patched with 1000, 5000, or 15 000 ppm in distilled water. The sites were evaluated for erythema 24 and 48 hours after the challenge application.

No incidences of erythema were observed in the controls during challenge. One guinea pig that was induced with 15 000 ppm MIT was observed with erythema at the 1000-ppm MIT challenge. The other induction dose groups had no observable erythema incidences with this challenge. In the 5000-ppm challenge, 2 of 10, 1 of 10, and 2 of 10 guinea pigs had observable erythema in the 5000-, 15 000-, and 30 000-ppm dose induction groups, respectively. No erythema was observed in the 1000-ppm MIT dose group for this challenge group. For the 15 000-ppm challenge, 1 of 10, 6 of 10, 3 of 10, and 5 of 10 guinea pigs had observable erythema in the 1000, 5000-, 15 000-, and 30 000-ppm MIT dose induction groups, respectively.

It was concluded that MIT is a sensitizer at concentrations greater than or equal to 1000 ppm MIT.[58]

Rohm & Haas[59] used a maximization test to evaluate the sensitization potential of MIT (99.7% pure). Sixty female Hartley guinea pigs were used in the study with 20 in each induction dose of 550 or 800 ppm MIT and 10 in a positive control group (25% hexylcinnamaldehyde [HCA] in mineral oil) and 10 in a negative control (water) group. During the induction phase, the guinea pigs received 6 intradermal injections followed 1 week later by a single (0.1 mL) 24-hour topical (occluded) dose. Following a 2-week resting phase, the guinea pigs were challenged with 550 or 800 ppm MIT and rechallenged with 1000 ppm MIT. The sites were evaluated for erythema reactions 24 and 48 hours after the challenge patch.

No dermal reactions were observed in the 550-ppm dose challenge group and only 1 reaction was observed in the 800-ppm dose challenge group after 48 hours. During the rechallenge, less than 30% of the animals exhibited a grade 1 erythema at either observation period.

The authors concluded that MIT is not a sensitizer at concentrations up to 800 ppm.[59]

The sensitization potential of MIT was evaluated using the open epicutaneous test.[60] Groups of 8 female Hsd Poc:DH [SPF] guinea pigs received topical doses of 0.1 mL of 0.15%, 0.25%, 0.4%, 0.6%, 1.5%, or 18% (wt/vol) MIT. Another 2 groups of 8 guinea pigs received positive control (1-chloro-2,4-dintrobenzene) or negative control (ethanol/water). The guinea pigs received a total of 20 doses over 4 consecutive weeks.

Three days after the last induction application, the guinea pigs were challenged with 0.15%, 0.25%, 0.4%, 0.6%, 1.5%, or 18% MIT at a volume of 0.025 mL. A rechallenge occurred 14 days after the challenge, with 0.4%, 0.6%, 1.5%, and 18% MIT applied to groups 3 to 6 in parallel; 0.25%, 0.6%, 1.5%, and 18% applied to group 7; 0.15%, 0.6%, 1.5%, and 18% applied to group 8; and 0.15%, 0.4%, 1.5%, and 18% applied to both control groups in parallel. After an exposure period of 6 hours, the application sites were washed with water. The skin was evaluated for irritation effects at 24, 48, and 72 hours after the first and second challenge applications.

In the first challenge, 1 of 8, 3 of 8, 1 of 8, 1 of 8, and 4 of 8 guinea pigs had signs of allergic reaction during the observation periods in the 0.25%, 0.4%, 0.6%, 1.5%, and 18% MIT dose induction and challenge groups, respectively. In the rechallenge, 2 of 8 guinea pigs in the 1.5% dose induction group had signs of allergic reaction to the 18% rechallenge application and 1 of 8 and 6 of 8 guinea pigs in the 18% dose

induction group had signs of allergic reaction to the 1.5% and 18% rechallenge applications, respectively. Two reactions in the 0.4% induction group to the 0.4% rechallenge application were considered isolated occurrences.

The study concluded that MIT is a sensitizer at concentrations greater than or equal to 1.5%.[60]

Local Lymph Node Assay

Local lymph node assay (LLNA) studies are summarized in Table 7 and described below. All percentages and dose levels are in terms of a.i.

MIT and CMIT. Potter and Hazelton[61] reported the sensitization potentials of 99.8% MIT and greater than 99.9% CMIT using CBA/J mice (sex not reported) in an LLNA. There were 6 mice in each of the MIT dose groups, the CMIT dose groups, an acetone vehicle control group, and a water-vehicle control group. The mice received 25 μL of topical solution consisting of 0, 1000, 10 000, or 30 000 ppm MIT in acetone or 50, 100, 500, or 1000 ppm CMIT in acetone on each ear for 5 consecutive days. Mice treated with the respective isothiazolinone in water received 3 μL on each ear also for 5 consecutive days. On day 5 of the study, the mice were injected with 20 μCi of ^{3}H-thymidine in the tail vein and were killed 5 hours later. The auricular lymph nodes were removed and the lymph node cells were precipitated with 5% trichloroacetic acid (TCA). Quantification of the [^{3}H]DNA was performed by liquid scintillation.

The stimulation indexes (SIs) were determined to be less than 1.0, 2.3, and 3.2 for the 1000-, 10 000-, and 30 000-ppm MIT dose groups, respectively. The SIs for 50-, 100-, 500-, and 1000-ppm CMIT dose groups were 1.7, 3.8, 19.8, and 28.2, respectively. The control groups had SI of 1.0 each. The authors concluded that MIT is a sensitizer at concentrations greater than 10 000 ppm (>250-750 μg of a.i. per square centimeter). The EC_3 was calculated to be 25 150 ppm a.i. (628 μg of a.i. per square centimeter).[61]

Rohm & Haas[62] investigated the sensitization potential of 10.37% MIT in Neolone 950 using female CBA/J mice in an LLNA. There were 5 mice in each of the 6 dose groups and the positive and negative (acetone/olive oil 4:1) control groups. The mice received 25 μL of topical solution consisting of 0%, 0.15%, 0.45%, 0.76%, 1.35%, 1.57%, or 1.80% MIT or positive control on each ear for 3 consecutive days. On day 6 of the study, the mice were injected with 20 μCi of ^{3}H-thymidine and killed 5 hours later.

The SIs were determined to be 2.08, 2.40, 2.23, 6.64, 4.73, and 6.62 for the 0.15%, 0.45%, 0.76%, 1.35%, 1.57%, and 1.80% MIT dose groups, respectively. It was concluded that MIT is a sensitizer at concentrations greater than 0.76%. The EC_3 was calculated to be 0.86%.[62]

In an LLNA and cytokine profiling study performed by Basketter et al,[63] 19.7% MIT was tested for allergenic hazard along with formaldehyde, glutaraldehyde, and CMIT/MIT. In the LLNA portion of the study, female CBA/J mice (aged 6-12 weeks) were divided into groups of 4 mice for each MIT dose group and the vehicle control groups. The mice received 25 μL of topical solution consisting of 0%, 0.049%, 0.099%, 0.197%, 0.493%, or 0.985% MIT in acetone/olive oil (4:1 ratio) or 0%, 0.99%, 1.97%, 4.93%, or 9.85% MIT in propylene glycol on each ear for 3 consecutive days. Five days after the first treatment, the mice were injected with 20 μCi of [^{3}H] methyl thymidine and killed 5 hours later.

The SIs were determined to be 1.0, 1.5, 1.5, 1.8, 3.8, and 2.5 for the 0%, 0.049%, 0.099%, 0.197%, 0.493%, or 0.985% in acetone/olive oil MIT dose groups, respectively. The SIs were 1.0, 1.9, 2.6, 7.0, and 7.6 for 0%, 0.99%, 1.97%, 4.93%, or 9.85% for propylene glycol MIT dose groups, respectively. The authors noted that in the 0.985% MIT acetone/olive oil dose group, the SI value was reduced and likely reflects the skin irritation observed at this concentration. No systemic toxicity was observed. The EC_3 was calculated to be 0.4% in the MIT solutions with acetone/olive oil and 2.2% in the MIT solutions with propylene glycol. It was concluded that MIT is a moderate skin allergen.

The results of this LLNA were used to determine the concentrations used in the cytokine profiling study. In this portion of the study, female Balb/c mice (number not reported) received 50 μL of either 0.5% MIT (prepared in acetone/olive oil), vehicle, 10% trimellitic anhydride (TMA; positive control for respiratory allergen), or 1% 2,4-dinitrochlorobenzene (DNCB; positive control for contact allergen) on shaved flanks on days 0 and 5. Three further applications of 25 μL were made to the dorsum of each ear on days 11, 12, and 13. The auricular lymph nodes were removed aseptically (study day not reported), and the lymph node cells were cultured with 20 μCi of [^{3}H] methyl thymidine to measure in vitro proliferation of lymph node cells with or without T-cell mitogen.

The SI determined in the in vitro lymph node cell proliferation was 2.6. In the enzyme-linked immunosorbent assay (ELISA), the level of cytokine production peaked between 96 and 120 hours for interferon (IFN)-γ, interleukin (IL)-10, IL-5, and IL-13 and at 24 hours for mitogen-induced IL-4. Positive controls yielded anticipated results. The amounts of cytokine produced at 96 hours in the 0.5% MIT dose groups were 2.5, 0.6, 0.9, 0.2, and 0.0 ng/mL for IFN-γ, IL-10, IL-13, IL-5, and IL-4, respectively. The authors concluded that MIT does not have the cytokine profile typical of chemical respiratory allergens and is not likely to have a significant potential to cause sensitization of the respiratory tract.[63]

NMMA. The sensitization potential of NMMA, an MIT metabolite, was studied in 25 female CBA/J mice (body weight range, 18-23 g) in an LLNA.[64] Five mice in each dose group plus a positive control (HCA) received a 25-μL topical application of vehicle (acetone/olive oil, 4:1); 3%, 10%, or 30% NMMA; or 50% HCA to the dorsal surface of both ears once daily for 3 days. After 2 days of rest, the mice were injected with ^{3}H-thymidine and killed 5 hours later.

The SI values were determined to be 0.81, 0.66, and 0.60 for 3%, 10%, and 30% NMMA, respectively. Results of the positive control were not provided. The authors concluded that

NMMA does not induce hypersensitivity in mice in an LLNA up to and including 30% concentration.[64]

Phototoxicity

Rohm & Haas[65] used 10 female Hartley guinea pigs to evaluate the phototoxicity potential of a preservative containing 9.5% to 9.9% MIT. Each guinea pig received 200 ppm MIT, distilled water (vehicle control), and 1% 8-methoxypsoralen (8-MOP; positive control) on 2 separate skin sites at a dose volume of 0.02 mL per site. Thirty minutes after application, the right sides of the animals' backs were covered with aluminum foil, and the animals were irradiated with 10.0 to 11.9 J/cm^2 long-wavelength UVA from 6 fluorescent lamps (300-400 nm). The skin sites were examined 4, 24, and 48 hours after the UV irradiation.

No skin reactions to the UV irradiation were observed at the sites treated with MIT or distilled water. The positive control provided expected results. MIT was not phototoxic in this study.[65]

Rohm & Haas[66] conducted a photosensitization study of a preservative containing 9.5% to 9.9% MIT using female Hartley guinea pigs (body weight range, 322-377 g). The skin on the back of the animals' necks was first treated with 0.1 mL of Freund's complete adjuvant in distilled water (FCA-DW) per site intradermally on the first day of induction. The skin was then stripped with adhesive tape to produce slight erythema, and the test area was treated with 0.1 mL each of 200 ppm MIT, distilled water (vehicle control), and 5.0% wt/vol 6-methylcoumarin (positive control).

Thirty minutes post application, the animals were irradiated with 9.9 to 11.2 J/cm^2 long wavelength UV from 6 fluorescent lamps (300-400 nm). This procedure occurred once daily for 5 consecutive days.

Sixteen days after the first treatment, challenge applications were made to the same sites with 0.02 mL each of 200 ppm MIT, distilled water, and 1.0% wt/vol 6-methylcoumarin per site. Thirty minutes after application, the right side of each animal's back was covered with aluminum foil and the animals were irradiated with 10.0 to 10.2 J/cm^2 long wavelength UV. The skin sites were examined 24 and 48 hours after the challenge irradiation.

No skin reactions were observed in the UV-irradiated and nonirradiated sites treated with MIT and distilled water. Skin reactions were observed at the sites treated with the positive controls. It was concluded that 9.5% to 9.9% MIT is not a photosensitizer at 200 ppm.[66]

Reproductive and Developmental Toxicity

The teratogenicity of MIT (51.4% a.i.) was evaluated by Rohm & Haas[67] using 100 Crl:CD(SD)IGS BR rats. Dose groups were 0, 5, 20, or 60 mg (later reduced to 40 mg) per kilogram of body weight per day and consisted of 25 mated female rats in each dose group. The control was tap water. MIT was administered by a daily single oral (intubation) dose on days 6 to 19 of gestation, and the rats were killed and necropsied on gestation day 20. Because of excessive toxicity in the 60-mg/kg/d dose group, the dosage level of the high-dose group was lowered to 40 mg/kg/d beginning sometime between gestation days 6 and 9.

Mortality occurred in 3 females of the 60/40-mg/kg/d dose group between gestation days 8 and 15. Another 2 females of this dose group were killed in extremis between gestation days 8 and 9.

Clinical signs of toxicity in these 5 rats were greater than those observed in the surviving rats of the 60/40-mg/kg/d dose group. At necropsy, this dose group had red areas in the glandular portion of the stomach and lungs.

Treatment-related net body weight gain and food consumption were noted in the 60-mg/kg/d dose group during gestation days 6 to 9. No effects on body weight gain or food consumption were observed in this group when the dose level was reduced to 40 mg/kg/d, compared with controls. No treatment-related effects on body weight parameters, gravid uterine weight, and food consumption were noted in the 5- and 20-mg/kg/d dose groups.

No treatment-related effects on internal findings, numbers of early or late resorptions, live fetuses per litter, fetal body weight, or sex ratio were observed at any dose level. Intrauterine growth and survival and viable litters were comparable with the control group in all dose groups. Fetal external, visceral, or skeletal malformations were observed in the control group (3 fetuses) and in the 60/40-mg/kg/d dose group (1 fetus) and were considered spontaneous in origin. No treatment-related external, soft tissue, or head malformations, variation, or developmental retardations were observed at any dose level.

The NOAEL for maternal toxicity was determined to be 20 mg/kg/d, and the NOAEL for developmental toxicity was determined to be 40 mg/kg/d.[67]

In another teratogenicity study by Rohm & Haas,[68] MIT (51.4% a.i.) was tested using 100 New Zealand White rabbits. There were 25 mated females in each dose group. The dose groups were 0, 3, 10, and 30 mg/kg/d MIT, and the MIT was administered as a daily single oral dose (intubation) during days 6 through 28 of gestation. Tap water was used as the control. On day 29 of gestation, the rabbits were killed and Caesarean sections were performed.

No treatment-related maternal effects were observed in the 3- and 10-mg/kg/d dose groups. One female in the 10-mg/kg/d dose group was found dead on gestation day 19 from a possible intubation error. In the 30-mg/kg/d dose groups, maternal effects included decreased defecation and dark red areas in the stomach. One female in the 30-mg/kg/d dose group aborted on gestation day 25.

No treatment-related external, visceral, or skeletal malformations or developmental variations were noted at any dose level. External malformations were observed in 2 fetuses in the 3-mg/kg/d dose group and 1 fetus in the 10-mg/kg/d dose group, soft tissue malformations were noted in 1 fetus in the control group and in 2 fetuses in each of the 3- and 10-mg/kg/d dose groups, and skeletal malformations were observed in 3 and

4 fetuses in the 3- and 10-mg/kg/d dose groups, respectively. These malformations were considered to be spontaneous in origin. Malformations were not observed in the 30-mg/kg/d dose group.

The NOAEL for maternal toxicity was determined to be 10 mg/kg/d, and the NOAEL for developmental toxicity was determined to be 30 mg/kg/d.[68]

A 2-generation reproduction toxicity test was used to evaluate the effects of MIT (51.4% a.i.) on Crl:CD IGS BR rats.[69] There were 30 males and 30 females in each dose group. Doses were 0, 50, 200, or 1000 ppm and equated to 0, 4 to 7, 15 to 19, and 69 to 86 mg/kg/d in males and 0, 6 to 13, 22 to 26, and 93 to 115 mg/kg/d in females. The rats were administered the test substance in drinking water, and F_0 and F_1 males and females received the aqueous MIT solution ad libitum for at least 70 days prior to mating and through the mating, gestation, and lactation cycles of the animals until the day they were killed. All animals were observed twice daily for appearance and behavior, and clinical observations, body weights, and water and food consumption were recorded at regular intervals prior to mating and during gestation and lactation. Offspring (30 per sex per group) of the F_0 animals were selected to make up the F_1 generation.

Females of the F_0 and F_1 generations were allowed to deliver and rear their pups until lactation day 21. Litters were observed daily for survival and any changes in appearance or behavior. All pups received physical examinations on postnatal days 1, 4, 7, 14, and 21. In both the F_1 and F_2 generations, 8 pups per litter (4 of each sex if possible) were selected on postnatal day 4 to reduce variability among the litters. F_1 animals began to receive the test substance on postnatal day 22. Developmental landmarks were measured in the selected F_1 rats, and the anogenital distance was measured in F_2 pups. Pups not selected in the F_1 generation and all F_2 pups were necropsied on postnatal day 21, and select organs were weighed. Parental F_0 and F_1 rats received a complete gross necropsy upon the completion of weaning of the F_1 and F_2 pups, and select organs were weighed.

Sperm motility, morphology, and counts were evaluated in all F_0 and F_1 males, and ovarian primordial follicle counts were recorded for F_1 females in the control group and in the high-dose group. Microscopic examinations of select tissues from all parental F_0 and F_1 rats and from parental rats that died or were killed in extremis were conducted. Reproductive organs of females that did not deliver in the low- and mid-dose groups and their paired males were also examined microscopically.

There were no treatment-related deaths in any animals at any dose level. Decreased water consumption was observed in all males in the F_0 generation and in F_0 and F_1 females of the 200- and 1000-ppm dose groups during gestation and lactation. The authors speculated that the decrease in consumption was likely attributable to an aversion to the taste or smell of the water by the rats.

Decreased body weights and food consumption were noted in the 1000-ppm dose group males and females and were likely a result of the decreased water consumption. No clinical signs or physical signs of toxicity were observed in any dose groups. There were no treatment-related effects observed in the tissues or reproductive organs of the F_0 and F_1 generation males and females. No treatment-related effects were observed in F_1 and F_2 pups.

It was concluded that MIT is not a reproductive toxicant at the doses tested (up to 69-86 mg/kg/d in males and 93-115 mg/kg/d in females).[69]

Genotoxicity

Bacterial Assays

MIT. The mutagenicity of MIT (99.9% pure) was tested in Ames assays using *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100. The assays were performed with and without metabolic activation using Arochlor 1254 rat liver extract (S9). The concentration ranges were 0.0001 to 0.25 μg per plate for strains TA1535 and TA1537, 0.0001 to 1 μg per plate for strain TA98, and 0.0001 to 100 μg per plate for strain TA100. Positive controls were 2-anthramine for TA1535, TA1537, and TA100 and 2-acetamidofluorene for TA98; negative control was distilled water. The positive controls gave expected results. Inhibition of growth was observed in TA100 at concentrations of 25 μg per plate or higher. MIT was not mutagenic in this assay.[70]

In another gene mutation assay, MIT (97.5% a.i.) was tested using *S typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102. The assays were performed with and without S9. The test material was tested at the concentration range of 5 to 1000 μg per plate (diluted in distilled water). The positive control in the presence of metabolic activation was 2-anthramine in all strains and 2-nitrofluorene (TA98), sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and mitomycin-C (TA102) in the absence of metabolic activation. The negative control was distilled water. The positive controls gave expected results. Toxicity was observed in all strains at 1000 μg per plate with metabolic activation and at 500 μg per plate in strains TA98, TA100, and TA1535 without metabolic activation. MIT was not mutagenic in this assay.[71]

In a mutagenicity study by Connor et al,[7] MIT was isolated from Kathon 886 via GC/MS, diluted with dimethyl sulfoxide (DMSO), and tested with *S typhimurium* strain TA100 without S-9 metabolic activation in an Ames assay. The authors determined that MIT was nonmutagenic in this assay.

NMMA. In an Ames test, 99.22% NMMA was tested using *S typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2 *uvr*A with or without the presence of S9 metabolic activation. The concentration ranges were 1.5 to 5000 μg per plate and NMMA was diluted in DMSO. Positive controls were 2-anthramine (for all strains) in the presence of S9 and 2-nitroflurorene (for TA98), sodium azide (for TA100 and TA1535), 9-aminoacridine (for TA1537), and methyl methanesulfonate (for WP2 *uvr*A) in the absence of S9. The negative control was DMSO. Precipitation or appreciable toxicity was not observed. There were no increases in the

number of revertants compared with solvent controls. NMMA was not mutagenic in this Ames study.[72]

Mammalian Cell Assays

MIT. The mutagenic potential of MIT (97.5% pure) was assessed using Chinese hamster ovary (CHO) cells, with and without S-9 metabolic activation, in a 2-phase study.[73] In the first definitive phase, the concentrations tested were 0.5, 1.0, 5.0, 10.0, 15.0, and 25.0 µg/mL of culture medium. The cells were exposed for 4 hours and the expression period was 9 days. In the second confirmatory phase, the concentrations tested were 5.0, 10.0, 15.0, 25.0, and 40.0 µg/mL of culture medium, with a 4-hour exposure period and an 8-day expression period. Upon conclusion of the expression period, the cultures were cloned in the presence of 6-thioguanine for HGPRT enzyme-deficient mutant selection. The test material was diluted in deionized water in both phases. The positive controls were ethyl methanesulfonate in the absence of S-9 and 7,12-dimethylbenzanthracene in the presence of S-9. The negative controls were deionized water, DMSO, and acetone.

Relative cloning efficiencies for the definitive phase ranged from 29% to 79% in the presence of S-9 and from 42% to 80% in the absence of S-9. In the confirmatory phase, relative cloning efficiencies ranged from 91% to 5% in cultures exposed to 5.0 to 25 µg/mL without S-9. No surviving colonies occurred in the 40.0 µg/mL concentration. Cloning efficiencies for the cultures exposed to 5.0 to 40.0 µg/mL with S-9 ranged from 104% to 20%.

The mutation frequency at the HGPRT locus was not significantly increased at any dose level, with and without S-9 activation, and it was concluded that MIT was nonmutagenic in this assay.[73]

In another CHO cell assay, MIT (97.5% a.i.) was assessed for mutagenicity in 3 phases.[74] The initial phases tested MIT (diluted in deionized water) at concentrations ranging from 33.9 to 5000 µg/mL of culture medium, but toxicity was excessive. In the definitive phase, concentrations ranged from 0.0785 to 40.0 µg/mL, with and without S-9 metabolic activation. The treatment period lasted 3 hours and cells were harvested 20 hours after the initiation of the treatment. In the confirmatory phases, concentrations ranged from 0.157 to 20.0 µg/mL without S-9 activation and from 1.25 to 20.0 µg/mL with S-9 activation. The treatment period was 17.8 hours without S-9 activation and 3.0 hours with S-9 activation. The positive controls were mitomycin-C (without S-9) and cyclophosphamide (with S-9), and the negative controls were deionized water and growth medium.

Significant increases in the number of cells with chromosome aberrations were observed in cells treated with 9.53 and 12.7 µg/mL without S-9 and in cells treated with 12.7 and 16.9 with S-9 during the initial phase. Higher concentrations were not examined. The increases in the number of aberrations were observed only at concentrations inducing greater than 40% cytotoxicity. Significant increases in the number of cells with chromosome aberrations were also observed in the confirmatory phase in cultures treated with 3.73 and 7.50 µg/mL without S-9 activation and in cultures treated with 7.50 µg/mL with S-9 activation. Chromosomal aberrations were also accompanied by significant cytotoxicity (29%-48% reductions).

The authors cited a study by Hilliard et al[75] that stated chromosomal aberrations may occur as a secondary mechanism of cytotoxicity in some compounds, which can lead to a false positive response in a chromosomal aberration assay and may explain the results seen in this study.[74]

Animal Assays

Rohm & Haas[76] assessed the mutagenicity of MIT (51.1% a.i.) in an unscheduled DNA synthesis assay using male Crl:CD(SD)IGS rats. A range-finding study was used to determine the concentrations for the study. Dose groups consisted of 4 males at 0, 100, and 200 ppm MIT and 6 males at 300 ppm MIT. The dose volume was 10 mL/kg. Rats were killed at either 2 to 4 hours or 14 to 16 hours after dosing, and rat hepatocytes were subsequently harvested. The study also included a negative control group and 2 positive control groups. Following harvest, the hepatocytes were cultured in the presence of 10 µCi/mL ^{3}H-thymidine for 4 hours, washed, and analyzed for radiolabel incorporation with autoradiography.

There was no significant difference in mean net nuclear grain count or the percentage of nuclei between the treated cells at any dose and the negative controls. It was concluded that MIT was not mutagenic in this assay.[76]

A micronucleus test was used to evaluate the mutagenic potential of MIT (97.5% pure) using CD-1 mice.[77] The mice received MIT, diluted with distilled water and administered in a single oral dose of 10 mL/kg, at dose levels of 10, 50, or 100 mg/kg body weight. Groups consisted of 5 males and 5 females except in the 100-mg/kg dose group, which had 2 additional animals per time point. Positive (intraperitoneal injection of 2 mg/kg mitomycin-C) and negative (single oral dose of distilled water) controls were also included in the study. Twenty-four or 48 hours post treatment, the mice were killed and bone marrow smears were prepared.

No increases in the number of micronucleated polychromatic erythrocytes were observed in the mice. The authors concluded that MIT was nonmutagenic in this assay.[77]

Carcinogenicity

No studies examining the carcinogenicity of MIT alone were available. A newly available study of the mixture MIT/CMIT was provided as unpublished data and is included here. Previously available carcinogenicity data on MIT/CMIT were detailed in the earlier safety assessment of MIT/CMIT.[1]

Rohm & Haas[78] evaluated the carcinogenicity of MIT/CMIT (as Kathon 886 microbicide, 14.2% a.i.) using 850 CRL:CD BR rats. There were 90 males and 80 females in each dose group, and the dose groups consisted of 30, 100, and 300 ppm MIT/CMIT (the ratio of MIT:CMIT was 1:3) in addition to 2 control

groups of 1 water and 1 $MgCl_2/Mg(NO_3)_2$ salt. The test material was administered to the rats in drinking water for 2 years. During the treatment period, the rats were observed daily for signs of toxicity, given physical exams, and monitored for body weight and water and food consumption.

Ophthalmoscopic examinations were performed on all rats prior to the start of treatment and on all surviving rats at 24 months. Ultrasound examinations, clinical chemistry, and hematology analysis were conducted. At the 12th and 18th months of treatment, 10 rats per sex per dose group were killed, necropsied, and examined for histopathologic changes, as were rats that died during the treatment period. All surviving rats at the completion of the treatment period were killed, necropsied, and examined for histopathologic changes.

Survival rates of both male and female rats in all dose groups were similar to those of the control groups. There were no treatment-related clinical effects or physical, hematology, clinical chemistry, ophthalmoscopic, or organ weight changes in any dose groups throughout the treatment period.

No treatment-related effects on body weight or body weight gain were observed in the 30- or 100-ppm dose groups. Decreases in body weight and body weight gains were observed in the 300-ppm dose group throughout the study but were thought to be a secondary effect to decreased water consumption.

Treatment-related and dose-dependent decreases in water consumption were seen in all dose groups throughout the treatment period. The authors speculated that the decreases were likely due to the unpalatability of the MIT/CMIT and not to the substance's stabilizer salts because the water consumption of the $MgCl_2/Mg(NO_3)_2$ salt control group was comparable to that of the water control group. There were sporadic increases in urinary specific gravity in the 100- and 300-ppm dose groups, which were likely due to the decreased water consumption as well.

No treatment-related effects were observed in the ultrasounds of the rats at any dose level. No treatment-related neoplasms or evidence of systemic toxicity were observed in any dose group during the study.

There were treatment-related morphological changes in the stomachs of rats of both sexes in the 100- and 300-ppm dose groups. Gastric irritation was marked by thickening of the forestomach mucosa from hyperplasia and hyperkeratosis of the squamous mucosa. In the 300-ppm males, focal necrosis of the superficial glandular mucosa and edema and inflammatory cell infiltration in the forestomach submucosa were observed.

It was concluded that MIT/CMIT was not a carcinogen in this 2-year drinking water study in rats.[78]

Neurotoxicity

In Vitro

Du et al[79] studied the acute neurotoxicity of MIT in mixed 4-week-old cultures of rat cortical neurons and glia from embryonic day-16 Sprague-Dawley rat fetuses. The cells were exposed to 0, 10, 30, 100, or 300 μM MIT for 10 minutes in memantine. The cells were also exposed to neuroprotective compounds 10 minutes before, during, and 18 to 20 hours after MIT exposure. Cell viability was determined 18 to 20 hours after MIT exposure using a lactate dehydrogenase (LDH)–based in vitro toxicity assay. Mitogen-activated protein kinase (MAPK) activation was assessed using the Western blot technique. The cultures also were immunostained and stained with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling. A glutathione assay was performed and electrophysiological techniques were used to measure K^+ currents.

The rat cortical cultures exposed to 100 and 300 μM MIT experienced widespread neuronal cell death within 24 hours. The underlying glial cell layer was spared from MIT toxicity. Exposure to increasing concentrations of MIT increased the number of injured neurons based on release of LDH.

In a neurotoxicity study by He et al,[80] cerebral cortex cultures from embryonic day-17 Sprague-Dawley rat fetuses were plated at a density of 5.21×10^4 cells per square centimeter and treated with 0.1, 0.3, 1.0, and 3.0 μM MIT for 14 hours in serum-containing media. Cell viability was determined after the incubation with MIT using an LDH-based in vitro toxicity assay. The cells were analyzed for morphological changes, and immunoprecipitation, electrophoresis, and immunoblotting were performed. A cell-free tyrosine kinase assay was also performed.

A modest (~35%) level of cell death was observed in the cultures treated with 3.0 μM MIT. No significant cell loss was detected at the remaining concentrations; however, inhibition of process outgrowth was observed. The immunoprecipitation and immunoblotting reactions found that focal adhesion kinase (FAK) phosphorylation was primarily affected by MIT with the phosphorylation level at tyrosines 576 and 861 of FAK significantly decreased. The researchers also found that MIT inhibited Src family kinases (SFKs) in cell-free assays and caused the physical dissociation of FAK from the signaling complexes normally formed with c-Src and Fyn in developing neurons. Increasing the cell density (and thus cell-to-cell contact) of the neuronal cultures increased the kinase activity of SFKs and the tyrosine phosphorylation of FAK, overcoming the toxicity of MIT in the cultures.

The authors suggested that prolonged exposure to MIT and related isothiazolones may damage developing nervous systems.[80]

In Vivo

Based on data provided by Rohm & Haas,[81] recounting studies that have been conducted in various laboratory animal models with several isothiazolone molecules (ie, biocidal actives), including MIT, there was no evidence in vivo of neurotoxicity with any actives within the isothiazolone family. In rodent and nonrodent subchronic studies, for example, there was no clinical or pathological evidence that MIT produces neurotoxicity. These studies included evaluation of detailed clinical observations, functional observation battery tests, motor

activity measurements, and histopathological examination of representative tissues of the central nervous system and peripheral nerves. When MIT was tested in developmental and reproductive studies, there was no evidence of neurotoxicity. No clinical signs of neurotoxicity were evident in developing animals (rat and rabbit) and no evidence of neurotoxicity was observed in parental animals or their offspring across 2 generations (rat). No gross or microscopic changes were observed in the brain of any pups examined in high dose of either generation following exposure to MIT in utero, through nursing, during lactation, or in drinking water following weaning. In chronic studies conducted with MIT, in combination with the structurally related analog CMIT, there was no clinical evidence of neurotoxicity and there were no effects on tissues of the central or peripheral nervous system when examined histopathologically. The authors suggested that the rapid metabolism and excretion of MIT, shown in toxicokinetic studies in the rat and mouse, support the lack of systemic toxicity (including neurotoxicity).

Clinical Assessment of Safety

Dermal Irritation

The irritation potential of MIT was evaluated in 40 volunteer subjects. The test substance (dose volume 15 μL) was applied to the dorsal skin at MIT concentrations of 100, 300, and 600 ppm for a period of 24 hours. The negative control was water. The subjects were observed for skin reactions 1 and 24 hours after application. The skin irritation indices for the test substance were 6.3, 1.3, and 6.3 for 100, 300, and 600 ppm MIT, respectively, and were compared with the irritation index for water, which was 5.0. It was concluded that under the conditions of this study, MIT was not an irritant.[82]

The skin irritation potential of a shampoo containing MIT was evaluated using 40 subjects. The test substance (dose volume 15 μL) and a shampoo without MIT were applied to the dorsal skin at a concentration of 100 ppm for a period of 24 hours. Reactions were scored 1 and 24 hours after application. The skin irritation indices for the shampoo with MIT, for the shampoo without MIT, and for water were 21.3, 15.0, and 5.0, respectively. The authors concluded that a shampoo containing MIT (100 ppm a.i.) was not an irritant in this study.[83]

In another evaluation of irritation potential, 40 subjects were patched with a body lotion containing 100 ppm MIT (9.5%-9.9% a.i.) and a body lotion without MIT. The test substances (dose volume 15 μL) were applied to the dorsal skin of the subjects with Finn chambers and Scanpor tape for 24 hours. Skin reactions were evaluated 1 and 24 hours after application. The skin irritation indices for both test substances were 1.3 and both were considered nonirritating.[84]

Rohm & Haas[85] also studied the irritation potential of a sunscreen containing 100 ppm MIT in 40 subjects. The subjects received single patch applications (15 μL dose volume) of the test substance and of sunscreen without MIT on the dorsal skin for 24 hours. Reactions were scored 1 and 24 hours after application. The skin irritation indices for the sunscreen with and without MIT were 1.3 and 6.3, respectively. The sunscreen containing MIT was not an irritant.

Dermal Sensitization

In a study by Bruze et al,[6] 22 patients who were positive for sensitization to Kathon CG microbicide were patch tested with 5 fractions isolated from Kathon CG via chromatography. Fraction II was determined to be MIT and fraction IV was determined to be CMIT. All fractions were diluted in water/methanol to 10, 30, 100, and 300 ppm. Eighteen of the 22 patients were patch tested with all concentrations of all the fractions, and the remaining 4 were patch tested with only all concentrations of fractions II and IV.

Another 6 patients who had been actively sensitized through patch testing were patch tested with all concentrations of all fractions, and 18 patients (4 patch test sensitized, 14 identified through routine testing) were tested with fraction II at 300 ppm Kathon CG.

All 22 patients had positive reactions to fraction IV (CMIT) and Kathon CG at 300 ppm, whereas only 2 were positive to fraction II (MIT) at this same concentration. Eleven patients had positive reactions to fraction IV, 9 were positive to Kathon CG, and 1 was positive to fraction II at 100 ppm. In the 6 patients who had been actively sensitized, none experienced positive reactions to fraction II at any concentration, whereas all 6 reacted positively toward fraction IV and Kathon CG at 300 ppm. The patch testing of fraction II in the 18 patients at 3 times the concentration found in the test solution of Kathon CG resulted in 4 positive reactions.

The authors concluded that MIT is a sensitizer but is not as potent as CMIT and that sensitization may be due to cross-reactions to CMIT.[6]

Bruze et al[86] studied 12 patients who tested positive for Kathon CG sensitivity. These patients were patch tested with equimolar concentrations of the 2 active ingredients of Kathon CG, CMIT, and MIT, along with 4,5-dichloro-2-methyl-4-isothiazolin-3-one in ethanolic solutions. Although all 12 patients reacted to the chlorinated isothiazolinones, only 3 patients had a doubtful reaction to MIT at 115 ppm and 1 of these patients had another doubtful reaction to MIT at 57.5 ppm. The authors determined that MIT is a weak sensitizer.

Schnuch[87] investigated the sensitization potential of MIT in 85 individuals with predetermined sensitization to CMIT/MIT (Kathon CG). MIT was tested epicutaneously at 500 and 1000 ppm in water for 24 or 48 hours (1000 ppm was determined to be the irritation threshold). CMIT/MIT was also tested in 73 of the individuals to determine sensitization intensity. Readings of test sites were performed daily up to 96 hours post application.

Of the 85 patients, 27 reacted to 1 of the 2 MIT concentrations (32% reacted; CI between 22% and 40%) at intensities ranging from + to ++. Eleven of 18 patients with a strong reaction (++/+++) to CMIT/MIT had a positive reaction to MIT, whereas 12 of 55 with a weak reaction (+) to the mixture had a positive reaction to MIT (at either test concentration).

The authors concluded that at high concentrations of MIT (500 to 1000 ppm), a proportion of the subjects with known sensitivity to CMIT/MIT may also react to MIT.[87]

Isaksson et al[88] studied the potential for cross-reactivity between MIT and CMIT in 4 former or current chemical plant workers. The subjects previously reported occupational sensitization to CMIT/MIT. In this study, the subjects were patch tested with Kathon CG (CMIT/MIT), Neolone 950 (containing 950 ppm MIT), 2-*n*-octyl-4-isothiazolin-3-one (OIT), CMIT and MIT isolated from Kathon CG, and 4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one (dichlorinated OIT). The test was performed according to the International Contact Dermatitis Research Group procedures. The patches were removed after 2 days and the patch sites were scored on day 3.

All 4 of the subjects reacted to CMIT/MIT and 3 subjects reacted to CMIT alone. One subject reacted to a high dose of MIT (1000 pm) but not to Neolone 950. None of the subjects reacted to OIT or dichlorinated OIT. The authors concluded that sensitization to CMIT/MIT leads to sensitization to CMIT. Individuals with high reactivity to CMIT may react to high concentrations of MIT.[88]

Repeated Insult Patch Tests. The cumulative irritation/sensitization potential of 98% MIT was evaluated in a repeated-insult patch test (RIPT) using 80 subjects, with the subjects tested with 50, 100, 250, 500, or 1000 ppm.[89] The test substance (0.1 mL) was applied for 23 hours daily for 21 consecutive days. Following a 10- to 14-day rest period, the subjects were challenged for 23 hours with the same respective concentrations of test substance in the 50-, 100-, or 250-ppm dose groups. The 500-ppm dose group was challenged with 100, 250, and 500 ppm MIT, and the 1000 ppm dose group was challenged with 250, 500, and/or 1000 ppm MIT. The subjects were then evaluated for erythema reactions 48 and 96 hours post challenge.

During the induction phase, irritation reactions were observed in all dose groups. The reactions were grade 1 and considered transient. One cumulative irritation reaction was observed in the 1000-ppm induction group. At challenge, 1 subject in the 500-ppm dose group was observed with a reaction, but this subject also reacted to the marker pen and several consumer products. Two subjects in the 1000-ppm dose group had mild reactions upon challenge and were considered sensitized. The authors concluded that the sensitization threshold for 98% MIT was at or around 1000 ppm.[89]

In an RIPT,[90] 98 subjects who had patch tested negative for 100 ppm Kathon CG were enrolled in the study to evaluate the sensitization potential of MIT. During the induction phase, 100 ppm MIT (dose volume 0.15 mL) was applied for 23 hours 4 times a week for 3 weeks to the subjects' backs using occlusive Webril patches. After the final induction patch, the subjects were allowed a week to rest before the challenge phase began. During the challenge phase, virgin sites were patched with 100 ppm MIT (0.15 mL dose volume) for approximately 24 hours. The skin was observed for erythema or edema reactions 48, 72, and 96 hours after the challenge patch.

One subject had a grade 4 reaction on the fifth day of the induction phase. It was determined that this subject was presensitized to the test material. None of the remaining subjects had reactions to MIT during the induction or challenge phases, and the authors concluded that 100 ppm MIT does not induce skin sensitization in human subjects.[90]

In a series of RIPTs performed by Rohm & Haas,[91-95] 50% MIT was evaluated for sensitization potential at 200, 300, 400, 500, and 600 ppm. The total number of subjects who completed the study in each dose group was 100, 98, 116, 210, and 214, respectively. During the induction phase, the test substance was applied 3 times a week for 3 weeks on the subjects' backs with occlusive Webril patches for 24 hours at a time at a dose volume of 0.2 mL. Following the induction phase, the 200- and 300-ppm dose groups were allowed to rest for a week, and the 400-, 500-, and 600-ppm dose groups were allowed to rest for 10 to 15 days. After the rest periods, the subjects were challenged on a virgin site for 24 hours with the same concentration of MIT that was applied in the induction phase. The subjects were observed for signs of erythema or edema 48 and 72 hours after the application of the challenge patch.

No signs of skin irritation were observed in any of the dose groups during the induction phase, and only 1 subject in each of the 400-ppm and 500-ppm dose groups had a incidence of erythema response. It was concluded that MIT up to 600 ppm is not a dermal sensitizer.[91-95]

Phototoxicity

The phototoxicity of 50% MIT was evaluated in 12 female subjects. The subjects received occluded patches with 200 ppm MIT (50 μL dose volume) on duplicate sites on the lower back. An additional site was treated with an occlusive patch without test substance and was the irradiated control. The patches were removed after 24 hours and the sites were evaluated. Another 50 μL of test substance was reapplied to the test sites and allowed to air dry for 15 minutes, and then 1 of the 2 test sites on each subject and the irradiated control site were exposed to 20 J/cm^2 of UVA (320-400 nm) using a filtered light source and 0.5 minimal erythema dose (MED) of UVB (290-320 nm). The other treated site was the nonirradiated control. The test sites were evaluated 24 and 48 hours after irradiation. No phototoxic effects were observed in this study.[96]

In a study evaluating the photosensitization effects of MIT (raw material concentration 50%), 32 subjects were induced with 200 ppm MIT (20 μL for the first application and 6 μL for the remaining applications) using occluded dermal patches. The patches were applied to irradiated and nonirradiated sites (2× MED UVA/UVB) on the subjects' lower or mid-backs for 24 hours. After the 24-hour application, the patches were removed and the sites were graded for reactions prior to the application of a new patch. This process was repeated 6 times over a 3-week period. A rest period of 9 to 14 days followed the induction phase. During the challenge phase, a 24-hour occluded patch containing 5 μL/cm^2 test material was applied to duplicate virgin sites adjacent to the induction sites. The

following day, the patches were removed, the sites were graded for reactions, a new patch containing 2 μL/cm^2 was applied, and the site was irradiated with 10 J/cm^2 of UVA and 0.5 MED of UVA/UVB. The sites were evaluated 24 and 48 hours after irradiation for skin reactions. No reactions indicating photoallergy to MIT were observed.[97]

Case Reports

Three cases of allergic contact dermatitis to coolant solutions containing biocides were reported by Pilger et al.[98] The 3 patients (26, 39, and 30 years old) were males who had developed eczematous eruptions on the forearms and dorsal hands while working with the coolant solutions. The eruptions cleared when use was discontinued by the patients. The patients were subsequently patch tested with the coolant solution (diluted to 0.1% in petrolatum), components of the coolant solution (including the 0.1% biocide mixture, which was separated into MIT and CMIT at 300 ppm in petrolatum), and the European standard series. One patient had a 2+ reaction (edematous or vesicular reaction) and another had a 3+ reaction (spreading, bullous, or ulcerative reaction) to MIT at both observations. These patients had similar reactions to CMIT. The third patient had no response to any of the components of the coolant solution or the solution itself. While isolating the components of the coolant solution, one of the investigators developed eczematous dermatitis on the forearms and dorsal hands. Patch testing of the investigator revealed a 2+ reaction to both MIT and CMIT.

Bruynzeel and Verburgh[99] reported a case of a 43-year-old man employed as a diesel mechanic with hand eczema of 15 months' duration. The man was unable to work with gloves and had continuous contact with diesel oil. The eczema was exacerbated after using moist toilet paper. A patch test was positive for thimerosal, and subsequent patch tests with additional standard series and series for materials in oils, grease, and metalworking fluids were given. Positive (++) reactions were observed on day 3 and day 7 to CMIT (0.01% aq) and MIT (0.02% aq). Further investigation found that the moist toilet paper contained Kathon CG and the diesel oil at the patient's place of employment contained Kathon FP 1.5 (MIT content 1.5%). The patient's condition improved when he was away from work.

Isaksson et al[100] reported 2 cases of occupational contact allergy and dermatitis in 2 male patients exposed to compounds containing the biocide MIT. In the first case, a 48-year-old male was exposed to wallpaper glues and developed eczematous lesions on his forehead, hands, and dorsal surfaces of his forearms. In the second case, a 58-year-old male was exposed to paper mill preservatives in an accidental spill that led to chemical burns on his feet and vesicular dermatitis on his hands. The glues and preservatives contained the biocide Acticide MBS, which contains less than 0.01% MIT. Both patients were patch tested with the Swedish standard series (containing CMIT/MIT as Kathon CG at a concentration of 200 ppm); a paint series; a standard series that contained a 0.5% aq. test preparation of Neolone 950 (with MIT at a concentration of 475 ppm); serial aqueous dilutions of laboratory isolated CMIT/MIT, Neolone 950, MIT, and CMIT; and serial dilutions of Skane M-8 (active ingredient is 2-n-octyl-4-isothiazolin-3-one). The patient in the second case was also patch tested with propylene glycol. A third case, in which a 50-year-old woman had suspected contact allergy to inhaled corticosteroids, was patch tested with the Swedish standard series, some select allergens, and the serial aqueous dilutions of the laboratory isolated compounds listed above.

The patient in the first case tested positively to CMIT/MIT, Skane M-8, Neolone 950, Acticide MBS, CMIT, and MIT, with +++ reactions to Neolone 950 (475 ppm), CMIT/MIT (100 and 200 ppm), MIT (62-500 ppm), and CMIT (150 ppm). The second patient also tested positively to the above compounds and had +++ reactions to CMIT/MIT (100 and 200 ppm), Neolone 950 (59-475 ppm), MIT (250 ppm), and CMIT (75 ppm). This patient also had +++ reactions to Skane M-8 (62.5-1000 ppm). In both of these patients, the lowest patch test reactivity to a concentration of MIT was about half the concentration of CMIT. The third patient had +++ reactions to CMIT/MIT (100 and 200 ppm) and to CMIT alone (75 and 150 ppm). No reactions to MIT were observed in this patient.

The authors concluded that primary sensitization to MIT differs from primary sensitization to CMIT/MIT, where the sensitization is due to CMIT, and that cross-reactions of these 2 differ.[100]

Four of 14 workers at a Danish paint factory were observed with contact dermatitis after exposure to paint additives that contained the biocide MIT.[101] The 4 workers, all males and ranging in age from 34 to 55 years old, had dermatitis on their hands, neck, chest, armpits, abdomen, leg, and/or feet following contact with the additive that had 7% to 10% MIT. The patients were patch tested with an extended European standard test series supplemented with a paint test series that contained various preservatives. MIT was tested in aqueous solution at 1050 ppm. The patches were removed after day 2 and scoring was made on day 3 and day 7. Positive reactions (+ and ++) were observed in all 4 patients. Reactions to the mixture MIT/CMIT were not as strong (+ and +?). Previous sensitization to MIT/CMIT could not be excluded in the workers.

Margin of Safety

A margin of safety (MOS) was calculated by Rohm & Haas[4] using the following assumptions in a worst case scenario:

- Global (includes use of multiple cosmetics and personal care products) daily exposure is 17.79 g/d
- Maximum permitted concentration is 100 ppm or 0.1 mg/g
- Exposure is to a 60-kg individual
- 100% dermal absorption

Based on these assumptions, the total exposure to a 60-kg person from all products was

$$0.1\text{mg/g} \times 17.79\text{g/d} \times 1 \div 60\text{ kg} = 0.0296\text{ mg/kg/d}.$$

MOS also were calculated in worst case scenarios for specific studies and described earlier in this report. The results were as follows:

- Rat 3-month oral toxicity—NOAEL of 66 to 94 mg/kg/d ÷ maximum cosmetics exposure 0.0296 mg/kg/d = 2230 to 3176 MOS[41]
- Dog 3-month oral toxicity—NOAEL of 41 mg/kg/d ÷ maximum cosmetics exposure 0.0296 mg/kg/d = 1385 MOS[42]
- Rat developmental toxicity—NOAEL of 40 mg/kg/d ÷ maximum cosmetics exposure 0.0296 mg/kg/d = 1351 MOS[67]
- Rabbit developmental toxicity—NOAEL of 30 mg/kg/d ÷ maximum cosmetics exposure 0.0296 mg/kg/d = 1014 MOS[68]
- Rat 2-generation reproduction toxicity—NOEL (F_0) of 69 to 86 mg/kg/d ÷ maximum cosmetics exposure 0.0296 mg/kg/d = 2331 to 2905 MOS (F_0) and NOEL (F_1) of 93 to 115 mg/kg/d ÷ maximum cosmetics exposure 0.0296 mg/kg/d = 3142 to 3885 MOS (F_1)[69]

These authors determined that overall consumer exposures were well below levels that are of concern for sensitization in both rinse-off and leave-on products in deterministic approaches. As an example, rinse-off products, such as a shampoo with 100 ppm MIT, had a point estimate of exposure to the scalp of 0.008 μg of MIT per square centimeter of skin, and leave-on products, such as a body lotion with the same MIT concentration, had a point estimate of exposure to skin of 0.05 μg of MIT per square centimeter of skin. Under probabilistic methods (Monte Carlo simulations), the distribution of exposures to the scalp and skin under rinse-off and leave-on conditions at the 100th percentile was 0.0103 μg of MIT per square centimeter of skin and 0.044 μg of MIT per square centimeter of skin, respectively.[4]

Summary

MIT is a heterocyclic organic compound used in cosmetics and personal care products. A trade name is Neolone 950. MIT is a colorless, clear liquid with a mild odor. MIT is completely soluble in water; mostly soluble in acetonitrile, methanol, and hexane; and slightly soluble in xylene.

MIT functions as a preservative in cosmetic products. It is used in concentrations up to 0.01%. MIT is also used as a preservative and biocide in numerous noncosmetic applications.

The percutaneous absorption of radiolabeled MIT (99.88% radiochemical purity) was determined using rat skin mounted on diffusion cells. Over a 24-hour period, the rate of absorption was 0.0059, 0.0277, and 0.0841 μg equivalents per square centimeter per hour for 25-, 75-, and 150-ppm dose groups, respectively, and the mean amount of total applied radioactivity absorbed was 21.4%, 33.7%, and 51.2% for 25-, 75-, and 150-ppm dose groups, respectively.

The total dose absorbed of aqueous solutions containing radiolabeled MIT (96.90% radiochemical purity) in human epidermis was 29.8%, 38.0%, and 54.7% for groups receiving 52.2, 104.3, and 313.0 μg of MIT per milliliter. The rate of absorption was 0.037 $\mu g/cm^2/h$ over a 24-hour exposure. In the same study, the total dose absorbed from shampoo, body lotion, and facial cream formulations containing 100 μg of MIT per milliliter was 29.5%, 8.98%, and 19.6%, respectively. The rates for absorption of MIT in the formulations over a 24-hour exposure ranged from 0.007 to 0.0026 $\mu g/cm^2/h$.

After oral dosing of 100 mg of radiolabeled MIT (96.70% radio purity) per kilogram of body weight in mice, total radioactive residues (TRRs) were highest in the liver and lowest in the bone 1 hour post dosing. At 24 hours post dosing, TRR declined significantly in all tissues and the tissue-to-plasma ratio showed that the radiolabel partitioned preferentially from plasma to tissues. Blood had the highest tissue-to-plasma ratio at 48 hours. TRR was higher in male tissues than female tissues overall.

Most radiolabeled metabolites of MIT (99.08% radio purity) were excreted in urine and feces by rats within 24 hours of oral dosing. Tissue sampling at 96 hours post dosing found 1.9% to 3.6% of the radiolabel, mainly in blood. Total mean recovery of the radiolabel was 92% to 96%. Major metabolites in urine were *N*-methyl malonamic acid, 3-mercapturic acid conjugate of 3-thiomethyl-*N*-methyl-propionamide, and *N*-methyl-3-hyrdoxyl-propamide. Another metabolism study of radiolabeled MIT (96.90% radio purity) conducted on bile duct–cannulated rats had an 88% recovery of the dose at 24 hours after oral dosing. Most of the radiolabel was found in bile, urine, and feces. No intact MIT was recovered, and the main metabolites were *N*-methyl malonamic acid and 3-mercapturic acid conjugate of 3-thiomethyl-*N*-methyl-propionamide.

In acute oral toxicity studies, MIT was slightly toxic in rats in concentrations ranging from 9.69% to 99.7%. At 9.69%, the LD_{50} for male and female rats was 274.6 and 105.7 mg of a.i. per kilogram of body weight, respectively. Studies in rats in body lotion, shampoo, and sunscreen formulations containing 100 ppm MIT found no treatment-related effects and an LD_{50} greater than 2000 mg of formulation per kilogram of body weight. Slight toxicity, including GI changes, was observed in mice that orally received 97.5% MIT. The LD_{50} was 167 mg of a.i. per kilogram of body weight. An acute oral toxicity study of the metabolite NMMA found the substance slightly toxic. The calculated oral LD_{50} for NMMA in males and females was 3550 and 4100 mg of NMMA per kilogram of body weight, respectively.

MIT at 97.5% was slightly toxic in rats in an acute dermal toxicity study. The substance was corrosive to the skin. The LD_{50} was calculated to be 242 mg of a.i. per kilogram of body weight. In another acute dermal toxicity study, 9.69% MIT was corrosive to rat skin, but no deaths occurred during the study. The LD_{50} was greater than 484.5 mg of a.i. per kilogram of body weight.

Acute inhalation toxicity studies in rats found that 53.52% and 97.80% MIT were slightly toxic after 4-hour exposures.

The LC_{50} values were 0.35 and 0.11 mg of a.i. per liter. Rats that died during these studies had reddened lungs and distended GI tracts. Mice exposed to 10 minutes of atomized 98.6% MIT had up to 47% decrease in respiratory rates that equated to moderate responses for sensory irritation.

No toxic effects were observed in a rat study where 97.5% MIT was administered to drinking water for 13 weeks. Dogs that were fed diets prepared with 51.4% MIT for 3 months had an NOAEL of 1500 ppm.

In a subchronic study of rats fed the metabolites NMMA or malonamic acid for 3 months, no effects were observed in body weight, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, or gross pathologic changes. Beagle dogs that received these metabolites in their diets for 3 months had no systemic toxicity.

A bovine cornea study classified MIT as mildly irritating. Ocular irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm MIT found the formulations nonirritating in rabbit eyes.

Undiluted 97.8% MIT was corrosive to intact rabbit skin after an exposure period of 1 hour. Rabbit dermal irritation studies of MIT at 9.69% and 10% concluded that the chemical was nonirritating. In EpiDerm skin constructs, 1.7% MIT applied for 3 or 60 minutes was noncorrosive. In the same study, 51.5% MIT was noncorrosive in the 3-minute exposure but corrosive at the 60-minute exposure.

In a guinea pig maximization test, 0.076% wt/vol MIT was a weak sensitizer, and a follow-up study found that 0.015% MIT produced no sensitization. An investigation using the Buehler method found that 99.8% MIT was a sensitizer at concentrations of 1000 ppm or higher. Another maximization test that evaluated the sensitization potential of 99.7% MIT concluded that the chemical was not a sensitizer at concentrations up to 800 ppm. MIT was a sensitizer at concentrations of 1.5% or higher in an open epicutaneous test.

Results from local lymph node assays indicated that 99.8% MIT and 10.37% MIT produced sensitization at greater than 10 000 ppm and greater than 0.76%, respectively. A local lymph node assay testing MIT at concentrations up to 0.85% in acetone/olive oil and up to 9.85% in propylene glycol found that MIT was a skin allergen with moderate strength, but that the cytokine profile of 0.5% MIT was not typical of chemical respiratory allergens, and concluded that MIT was not likely to have a significant potential to cause sensitization of the respiratory tract. The metabolite NMMA did not induce hypersensitivity in a local lymph node assay up to and including 30% concentration.

MIT at 100 ppm was not phototoxic or photosensitizing in guinea pig studies.

In a teratogenicity study, MIT up to 40 mg per kilogram of body weight per day resulted in no treatment-related effects in the fetuses. The maternal and developmental NOAELs were 20 mg/kg/d and 40 mg/kg/d, respectively. In a teratogenicity study of MIT in rabbits receiving up to 30 mg/kg/d MIT, the maternal NOAEL was 10 mg/kg/d. No treatment-related effects were observed in the fetuses, and the developmental NOAEL was determined to be 30 mg/kg/d.

A 2-generation reproduction toxicity test found that MIT in drinking water at concentrations up to 1000 ppm was not a reproductive toxicant.

MIT and the metabolite NMMA were not mutagenic in the Ames test when tested with and without metabolic activation. In a CHO cell assay, 97.5% pure MIT was nonmutagenic when tested with and without metabolic activation (0.5-40.0 μg/mL). However, another CHO assay that studied MIT at 97.5% a.i. (0.0785-5000 μg/mL) found significant increases in cells with chromosome aberrations, with and without metabolic activation. The aberrations were accompanied by significant cytotoxicity, which may have caused a false positive in this assay. MIT was nonmutagenic in an unscheduled DNA synthesis assay and in a micronucleus test.

Studies of the carcinogenicity of the sole ingredient MIT were not available; however, a 2-year drinking water study in rats concluded that the mixture MIT/CMIT was not a carcinogen.

An acute in vitro neurotoxicity study of MIT in embryonic rat cortical neurons and glia observed widespread neuronal cell death within 24 hours in the cortical cultures. Gliotoxicity was low. A 14-hour in vitro neurotoxicity study of MIT from the same laboratory concluded that prolonged exposure to MIT and related isothiazolones may damage developing nervous systems. However, no evidence of neurotoxicity has been observed in vivo.

A single 24-hour application of 100 ppm MIT in 40 volunteer subjects did not produce skin irritation. Respective skin irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm MIT also found MIT to be nonirritating.

In a clinical study of 22 patients tested with fractions isolated from Kathon CG that included MIT and CMIT, only 2 patients had positive reactions to MIT. Sensitization may have been due to cross-reactions to CMIT. MIT was determined to be a weak sensitizer in a study of 12 patients. In a cumulative irritation/sensitization study of MIT in 80 subjects, the sensitization threshold was determined to be at or around 1000 ppm. The results show that at high concentrations of MIT (500 to 1000 ppm), a proportion of the subjects with known sensitivity to CMIT/MIT may also react to MIT.

A human RIPT in 98 subjects tested with 100 ppm MIT concluded that MIT did not induce skin sensitization in humans. A series of RIPTs evaluating the sensitization of 50% MIT in up to 600 ppm doses concluded that MIT up to 600 ppm was not a dermal sensitizer.

No phototoxic effects were observed in a study of 200 ppm MIT in 12 female subjects. A photosensitization study of 200 ppm MIT in 32 subjects did not produce photoallergic reactions.

Three cases of allergic contact dermatitis were reported in patients who had come into contact with coolant solutions containing biocides. Patch testing in 2 of the patients revealed 2+ and 3+ reactions to MIT, respectively. An investigator in this study developed eczematous dermatitis while isolating coolant components and had a 2+ reaction to MIT during patch testing. Another case study reported hand eczema in a diesel mechanic

that was exacerbated with the use of moist toilet paper. The diesel oil and the toilet paper that the man came into contact with both contained Kathon biocides. Positive reactions to MIT were observed with patch testing. Two cases of occupational contact allergy and dermatitis were reported in patients exposed to compounds containing the biocide MIT. Patch testing revealed +++ reactions to MIT and Neolone 950. Four of 14 workers at a Danish paint factory were observed with contact dermatitis after exposure to paint additives containing 7% to 10% MIT. Positive reactions were observed in all 4 patients during patch testing.

Margins of safety were calculated for MIT using the concentration of 100 ppm in several worst-case exposure scenarios. It was determined that consumer exposure would be well below levels that are of concern for sensitization in both rinse-off and leave-on products.

Discussion

In 1992, the CIR Expert Panel concluded that the mixture MIT/CMIT (23.3% MIT and 76.7% CMIT) 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. Currently, MIT is used as a standalone biocide. Accordingly, it was considered necessary to evaluate the safety of MIT alone.

The CIR Expert Panel noted that in vitro studies on MIT and related isothiazolinone compounds were positive for neurotoxicity. However, in vivo studies described in this report, including subchronic, chronic, and reproductive and developmental animal studies, did not report significant signs of toxicity, including neurotoxicity. The Expert Panel does not consider MIT as used in cosmetics to be neurotoxic.

The Expert Panel observed that MIT of undetermined particle size had adverse effects in acute inhalation studies in animals. However, the Expert Panel determined that MIT can be used safely in hair sprays and other spray products because cosmetic product sprays contain particles of sizes that are not respirable. The available data demonstrated that the particle size of aerosol hair sprays ($\sim$38 μm) and pump hair sprays (>80 μm) is large compared with respirable particulate sizes ($\leq$10 μm).

The Expert Panel noted that MIT was a sensitizer in both animal and human studies. A threshold dose response was observed in these studies. Cosmetic products formulated to contain concentrations of MIT at 100 ppm (0.01%) or less are not expected to pose a sensitization risk. The Expert Panel also recognizes that cross-sensitization to CMIT may occur in individuals sensitized with MIT. Most individuals sensitized with CMIT, however, do not cross-react with MIT. These animal and clinical data supported that CMIT is a strong sensitizer and MIT is a weak sensitizer.

Conclusion

Based on the available data, the CIR Expert Panel concluded that methylisothiazolinone is safe for use in cosmetic formulations at concentrations up to 100 ppm (0.01%).

Authors' Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1101 17th Street, Suite 412, Washington, DC 20036, USA.

Declaration of Conflicting Interests

No potential conflict of interest relevant to this article was reported. F. Alan Andersen, PhD, and Christina L. Burnett are employed by Cosmetic Ingredient Review.

Funding

The articles in this supplement were sponsored by the Cosmetic Ingredient Review. The Cosmetic Ingredient Review Program is financially supported by the Personal Care Products Council.

References

1. Elder RL. Final report on the safety assessment of methylisothiazolinone and methylchloroisothiazolinone. *JACT.* 1992;11: 75-128.
2. Gottschalck TE, Bailey JE. *International Cosmetic Ingredient Dictionary and Handbook.* Vol 12, No 2, pp 1571-1572. Washington, DC: CTFA; 2008.
3. Rohm & Haas, LLC. 2008. Rohm and Haas Comments on the Scientific Literature Review Methylisothiazolinone, 12 December 2007. 18 pages.
4. Rohm & Haas, LLC. 2006. Methylisothiazolinone Toxicology and Risk Summary, Report No. 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC. 197 pages.
5. Bruze M, Gruvberger B, Persson K. Contact allergy to a contaminant in Kathon CG in the guinea pig. *Dermatosen.* 1987;35: 165-168.
6. Bruze M, Dahlquist I, Fregert S, Gruvberger B, Persson K. Contact allergy to the active ingredients of Kathon CG. *Contact Dermatitis.* 1987;16:183-188.
7. Connor TH, Tee PG, Afshar M, Connor KM. Mutagenicity of cosmetic products containing Kathon. *Environ Mol Mutagen.* 1996; 38:127-132.
8. Collier PJ, Ramsey A, Waigh RD, Douglas KT, Austin P, Gilbert P. Chemical reactivity of some isothiazolone biocides. *J Appl Bacteriol.* 1990;69:578-584.
9. Food and Drug Administration. Frequency of use of cosmetic ingredients. FDA database. Submitted by FDA in response to FOI request F06-18753. Washington, DC: FDA; 2007.
10. Personal Care Products Council (Council). Current use concentration—methylisothiazolinone. 2008. Unpublished data submitted by CTFA.
11. Randazzo DJ. 2008. OTC statement: Neolone 950 preservative. Unpublished data submitted by CTFA. 1 page.
12. Willeke K, Baron PA. *Industrial Hygiene: Aerosol Measurement. Principles Techniques and Applications.* New York, NY: John Wiley; 1993.
13. Klassen CD, ed. *Absorption of Toxicants by the Lungs. Casarret and Doull's Toxicology: The Basic Science of Poisons.* New York, NY: McGraw-Hill; 2008.
14. Bower D. Unpublished information on hair spray particle sizes provided at CIR Expert Panel meeting. September 9, 1999.

15. Johnsen MA. The influence of particle size. *Spray Technology and Marketing*. 2004;November:24-27.
16. Ministry of Health, Labor and Welfare (MHLW). *MHW Ordinance No. 331, Appendix 1. List of Ingredients That Cosmetics Shall Not Contain*. Tokyo, Japan: Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division; 2005.
17. Ministry of Health, Labor and Welfare (MHLW). *MHW Ordinance No. 332. Ingredients of Quasi-Drugs. Products to Be Used Directly on the Body*. Tokyo, Japan: Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division; 2000.
18. European Union. Council Directive 1976/768/EEC of 27 July 1976 on the Approximation of the Laws of the Member States Relating to Cosmetic Products, as amended through Commission Directive 2003/83/ED. http://eur-lex.europa.eu/LexUriServ/site/en/counsleg/1976/L/01976L078-20060809-en.pdf. 2006. Accessed September 12, 2007.
19. Scientific Committee on Cosmetic Products and Non-Food Products (SCCNP).Opinion concerning methylisothiazolinone, COLIPA no. P94. http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out270_en.pdf. Accessed December 12, 2007.
20. Flyvholm M-A. Contact allergens in registered cleaning agents for industrial and household use. *Br J Ind Med*. 1993;50: 1043-1050.
21. Environmental Protection Agency. R.E.D Facts—methylisothiazolinone, prevention, pesticides and toxic substances. 1998. Report No. EPA-738-F98-008. http://www.epa.gov/oppsrrd1/REDs/factsheets/3092fact.pdf. Accessed 2-25-2008.
22. Rohm & Haas, LLC. 2-methyl-4-isothiazolin-3-one: in vitro percutaneous absorption through rat skin. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2003. 3 pages.
23. Rohm & Haas, LLC. 2-Methyl-4-isothiazolin-3-one (MI): in vitro absorption from water and three formulations through human epidermis. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2005. 2 pages.
24. Rohm & Haas, LLC. Tissue distribution of 14C-RH-473 in the mouse. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2003. 2 pages.
25. Rohm & Haas, LLC. Metabolism and pharmacokinetics of ^{14}C-RH-573 in the rat. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2005. 4 pages.
26. Rohm & Haas, LLC. Metabolism of ^{14}C-RH-573 in the biliary cannulated rat. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2005. 4 pages.
27. Rohm & Haas, LLC. RH-573 technical acute oral toxicity in male and female rats (methylisothiazolinone 99.7% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1999. 2 pages.
28. Rohm & Haas, LLC. Neolone 950 preservative: acute oral toxicity study in male and female rats (methylisothiazolinone 9.69% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 2 pages.
29. Rohm & Haas, LLC. A single oral dose toxicity study of anionic body lotion with Neolone 950 (100 ppm AI) in rats (methylisothiazolinone 9.5-9.9% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 2 pages.
30. Rohm & Haas, LLC. A single oral dose toxicity study of generic shampoo with Neolone 950 (100 ppm AI) in rats (methylisothiazolinone 9.5-9.9% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 2 pages.
31. Rohm & Haas, LLC. A single oral dose toxicity study of high SPF sunscreen with Neolone 950 (100 ppm AI) in rats (methylisothiazolinone 9.5-9.9% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 2 pages.
32. Rohm & Haas, LLC. Single dose oral toxicity/LD_{50} in rats with 2-methyl-4-isothiazolin-3-one (methylisothiazolinone 51.4% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2002. 2 pages.
33. Rohm & Haas, LLC. Kordek 573T acute oral toxicity study in male and female mice (methylisothiazolinone 97.5%). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 2 pages.
34. Rohm & Haas, LLC. RH-35375 acute oral LD_{50} report. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1972. 2 pages.
35. Rohm & Haas, LLC. Kordek 573T acute dermal toxicity in male and female rats (methylisothiazolinone 97.5% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1999. 2 pages.
36. Rohm & Haas, LLC. Neolone 950 preservative acute dermal toxicity in male and female rats (methylisothiazolinone 9.69% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 2 pages.
37. Rohm & Haas, LLC. RH-573 technical: acute inhalation toxicity study in rats (methylisothiazolinone 97.8% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1995. 2 pages.
38. Rohm & Haas, LLC. Kordek 573F: acute inhalation toxicity study in rats (methylisothiazolinone 53.52% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001.
39. Rohm & Haas, LLC. Kordek 573F: acute inhalation toxicity study in rats, supplemental report (methylisothiazolinone 53.52% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2002. 3 pages.
40. Rohm & Haas, LLC. RH-573 upper airway irritation RD_{50} evaluation in mice (methylisothiazolinone 98.6% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished

data submitted by Rohm & Haas Chemicals, LLC; 1994. 3 pages.
41. Rohm & Haas, LLC. RH-573 technical: three-month drinking water toxicity study in rats (methylisothiazolinone 97.5% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 5 pages.
42. Rohm & Haas, LLC. 2-Methyl-4-isothiazolin-3-one: a 13 week dietary toxicity study in dogs (methylisothiazolinone 51.4% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2004. 6 pages.
43. Rohm & Haas, LLC. RH-886T, RH-35, 375, and RH-00,345: three month subchronic oral safety evaluation study in rats. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals LLC; 1975. 2 pages.
44. Rohm & Haas, LLC. RH-886T, RH-35, 375, and RH-00,345: three month subchronic oral safety evaluation study in beagle dogs. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1975. 3 pages.
45. Smith C, Alexander B. In vitro assessment of the ocular irritancy potential of isothiazolinone-based preservatives using the BCOP assay [abstract]. *Toxicology*. 2004;194:263-264.
46. Gautheron P, Dukic M, Alix D, Sina JF. Bovine corneal opacity and permeability test: an in vitry assay of ocular irritancy. *Fundam Appd Toxicol*. 1992;18:442-449.
47. Rohm & Haas, LLC. Eye irritation potential of 2-methyl-4-isothiazolin-3-one industrial microbiocide: rationale to use surrogate skin irritation data. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2004. 1 pages.
48. Rohm & Haas, LLC. RH-573 technical skin irritation study in rabbits (methylisothiazolinone 97.8% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1997. 2 pages.
49. Rohm & Haas, LLC. Neolone 950 preservative (100 ppm aqueous solution) eye irritation study in rabbits (methylisothiazolinone 9.69% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 2 pages.
50. Rohm & Haas, LLC. A primary eye irritation study of generic shampoo with Neolone 950 (100 ppm AI) in rabbits. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 2 pages.
51. Rohm & Haas, LLC. A primary eye irritation study of anionic body lotion with Neolone 950 (100 ppm AI) in rabbits. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 2 pages.
52. Rohm & Haas, LLC. A primary eye irritation study of high SPF sunscreen with Neolone 950 (100 ppm AI) in rabbits. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 2 pages.
53. Rohm & Haas Chemicals, LLC. Neolone 950 preservative (100 ppm aqueous solution) skin irritation study in rabbits (methylisothiazolinone 9.69% active ingredient). In: Rohm & Haas Chemicals, LLC Report No. 06R-1002; 2000. 3 pages.
54. Rohm & Haas, LLC. A 14-day cumulative skin irritation study of RH-573 in rabbits. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 1 pages.
55. Rohm & Haas, LLC. 2-Methyl-4-isothiazolin-3-one corrosivity in vitro skin assay using Epiderm™ (EPI-200): 3 and 60-minute exposure protocol. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2005. 2 pages.
56. Alvarez-Sánchez R, Basketter D, Pease C, Lepoittevin J-P. Covalent binding of the 13C-labeled skin sensitizers 5-chloro-methylisothiazol-3-one (MCI) and 2-methylisothiazol-3-one (MI) to a model peptide and glutathione. *Bioorg Med Chem Lett*. 2004;14:365-368.
57. Bruze M, Fregert S, Gruvberger B, Persson K. Contact allergy to the active ingredients of Kathon CG in the guinea pig. *Acta Derm Venereol*. 1987;67:315-320.
58. Rohm & Haas, LLC. Delayed contact hypersensitivity study in guinea (methylisothiazolinone 99.8% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1989. 3 pages.
59. Rohm & Haas, LLC. Methylisothiazolinone: dermal sensitization study in guinea pigs—maximization test (methylisothiazolinone 99.7% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 3 pages.
60. Rohm & Haas, LLC. Methylisothiazolinone 20%—open epicutaneous test in guinea pigs. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 3 pages.
61. Potter DW, Hazelton GA. Evaluation of auricular lymph node cell proliferation in isothiazolone-treated mice. *Fundam Appl Toxicol*. 1995;24:165-172.
62. Rohm & Haas, LLC. Methylisothiazolinone: local lymph node assay (methylisothiazolinone 10.37% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2003. 3 pages.
63. Basketter DA, Gilmour NJ, Wright ZM, Walters T, Boman A, Liden C. Biocides: characterization of the allergenic hazard of methylisothiazolinone. *J Toxicol Cutaneous Ocular Toxicol*. 2003;22:187-199.
64. Rohm & Haas, LLC. N-(methyl) malonamic acid local lymph node assay. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC;2003. 3 pages.
65. Rohm & Haas, LLC. A phototoxicity study of RH-573 in guinea pigs (methylisothiazolinone 9.5-9.9% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 3 pages.
66. Rohm & Haas, LLC. A skin photosensitization study of RH-573 in guinea pigs (adjuvant and strip method) (methylisothiazolinone 9.5-9.9% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 3 pages.

67. Rohm & Haas, LLC. An oral (gavage) developmental toxicity study of 2-Methyl-4-isothiazolin-3-one in rats (methylisothiazolinone 51.4% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2003. 5 pages.
68. Rohm & Haas, LLC. An oral (gavage) developmental toxicity study of 2-Methyl-4-isothiazolin-3-one in rabbits (methylisothiazolinone 51.4% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2003. 6 pages.
69. Rohm & Haas, LLC. A two-generation reproductive toxicity study of 2-Methyl-4-isothiazolin-3-one administered via drinking water in rats (methylisothiazolinone 51.4% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2003. 7 pages.
70. Rohm & Haas, LLC. 2-Methyl-4-isothiazolin-3-one: microbial mutagen test (methylisothiazolinone 99.9% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1982. 2 pages.
71. Rohm & Haas, LLC. Kordek 573T Salmonella typhimurium gene mutation assay (methylisothiazolinone 97.5% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1999. 3 pages.
72. Rohm & Haas, LLC. N-methyl malonamic acid: bacterial reverse mutation (Ames) assay. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2005. 2 pages.
73. Rohm & Haas, LLC. Kordek 573T: test for chemical induction of gene mutation at the HPGRT locus in cultured Chinese hamster ovary (CHO) cells with and without metabolic activation with a confirmatory assay (methylisothiazolinone 97.5% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 3 pages.
74. Rohm & Haas, LLC. Mutagenicity study on Kordek 573T measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells (methylisothiazolinone 97.5% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 4 pages.
75. Hilliard CA, Armstrong MJ, Brandt CI, Hill RB, Greenwood SK, Galloway SM. Chromosome aberrations in vitro related to cytotoxicity of nonmutagenic chemicals and metabolic poisons. *Environ Mol Mutagen*. 1998;31:316-32618.
76. Rohm & Haas, LLC. 2-Methyl-4-isothiazolin-3-one (RH-573): in vivo/in vitro unscheduled DNA synthesis in rat primary hepatocyte cultures at two time points with a dose range-finding assay (methylisothiazolinone 51.1% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2003. 3 pages.
77. Rohm & Haas, LLC. Kordek 573T: micronucleus assay in CD-1 mouse bone marrow cells (methylisothiazolinone 97.5% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 3 pages.
78. Rohm & Haas, LLC. KATHON™ biocide: 24-month drinking water chronic/oncogenic study in rats. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1994. 5 pages.
79. Du S, McLaughlin B, Pal S, Aizenman E. In vitro neurotoxicity of methylisothiazolinone, a commonly used industrial and household biocide, proceeds via a zinc and extracellular signal-regulated kinase mitogen-activated protein kinase-dependent pathway. *J Neurosci*. 2002;22:7408-7416.
80. He K, Huang J, Lagenaur CF, Aizenman E. Methylisothiazolinone, a neurotoxic biocide, disrupts the association of Src family tyrosine kinases with focal adhesion kinase in developing cortical neurons. *J Pharmacol Exp Ther*. 2006;317:1320-1329.
81. Rohm & Haas, LLC. Methylisothiazolinone: no evidence of neurotoxicity in-vivo when tested in a battery of pre-clinical studies, Report No. 07R-1043. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2007. 11 pages.
82. Rohm & Haas, LLC. Human patch test (raw materials) (Neolone 950 [100, 300, and 600 ppm AI aqueous solution]). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 2 pages.
83. Rohm & Haas, LLC. Human patch test (cosmetic product) (shampoo cosmetic product containing Neolone 950 [100 ppm AI]). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 2 pages.
84. Rohm & Haas, LLC. Human patch test (cosmetic product) (body lotion cosmetic containing Neolone 950 [100 ppm AI]). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 2 pages.
85. Rohm & Haas, LLC. Human patch test (cosmetic product) (sunscreen cosmetic product containing Neolone 950 [100 ppm AI]). In: Rohm & Haas Chemicals, LLC Report 06R-1002; 2001. 2 pages.
86. Bruze M, Dahlquist I, Gruvberger B. Short communications: contact allergy to dichlorinated methylisothiazolinone. *Contact Dermatitis*. 1989;20:219-220.
87. Schnuch, A. Testing the frequency of sensitization to MI in MCI/MI (Kathon CG)-sensitized subjects (translation). Unpublished study conducted by Rohm & Haas; 1999. 12 pages.
88. Isaksson M, Bruze M, Gruvberger B. Cross-reactivity between methylchloroisothiazolinone/methylisothiazolinone, methylisothiazolinone, and other isothiazolinones in workers at a plant producing binders for paints and glues. *Dermatitis*. 2008;58:60-62.
89. Rohm & Haas, LLC. RH-573—evaluation of 21-day cumulative irritation potential in humans (methylisothiazolinone 98% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 1994. 2 pages.
90. Rohm & Haas, LLC. A patch test procedure to determine the skin irritation and sensitization propensities of Kordek 50C (methylisothiazolinone 50.61% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 2 pages.
91. Rohm & Haas, LLC. Repeated insult patch study with 2-methylisothiazolin-3-one at an aqueous concentration of

200 ppm active ingredient. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 4 pages.

92. Rohm & Haas, LLC. Repeated insult patch study with 2-methylisothiazolin-3-one at an aqueous concentration of 300 ppm active ingredient. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 4 pages.
93. Rohm & Haas, LLC. Repeated insult patch study with 2-methylisothiazolin-3-one at an aqueous concentration of 400 ppm active ingredient. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 4 pages.
94. Rohm & Haas, LLC. Repeated insult patch study with 2-methylisothiazolin-3-one at an aqueous concentration of 500 ppm active ingredient. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2001. 4 pages.
95. Rohm & Haas, LLC. Repeated insult patch study with 2-methylisothiazolin-3-one at an aqueous concentration of 600 ppm active ingredient. In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2002. 4 pages.
96. Rohm & Haas, LLC. Photoallergy study with 2-methylisothiazolin-3-one at an aqueous concentration of 200 ppm active ingredient (methylisothiazolinone 50% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 2 pages.
97. Rohm & Haas, LLC. Phototoxicity study with 2-methylisothiazolin-3-one at an aqueous concentration of 200 ppm active ingredient (methylisothiazolinone 50% active ingredient). In: Rohm & Haas Chemicals, LLC Report 06R-1002. Unpublished data submitted by Rohm & Haas Chemicals, LLC; 2000. 3 pages.
98. Pilger C, Nethercott JR, Weksberg F. Allergic contact dermatitis due to a biocide containing 5-chloro-2-methyl-4-isothiazolin-3-one. *Contact Dermatitis*. 1986;14:201-204.
99. Bruynzeel DP, Verbugh CA. Occupational dermatitis from isothiazolinones in diesel oil. *Contact Dermatitis*. 1996;34:64-65. 4.
100. Isaksson M, Gruvberger B, Bruze M. Occupational contact allergy and dermatitis from methylisothiazolinone after contact with wallcovering glue and after a chemical burn from a biocide. *Dermatitis*. 2004;15:201-205.
101. Thyssen JP, Sederberg-Olsen N, Thomsen JF, Menne T. Contact dermatitis from methylisothiazolinone in a paint factory. *Contact Dermatitis*. 2006;54:322-324.

Personal Care Products Council
Committed to Safety, Quality & Innovation

TO: Lillian Gill, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: August 29, 2013

SUBJECT: Concentration of Use by FDA Product Category: Methylisothiazolinone

Concentration of Use by FDA Product Category
Methylisothiazolinione

FDA Code†	Product Category*	Maximum Concentration of Use
01A	Baby shampoos	0.0002%
02B	Bubble baths	0.0002-0.00037%
02D	Other bath preparations	0.0002-0.01%
03D	Eye lotion	0.0038%
03E	Eye makeup remover	0.00019-0.01%
03G	Other eye makeup preparations	0.0095%
04A	Colognes and toilet waters	0.0004%
04B	Perfumes	0.0004-0.008%
04E	Other fragrance preparations	0.00018-0.0076%
05A	Hair conditioners	0.000004-0.01%
05B	Hair sprays aerosol pump spray	 0.01-0.011% 0.0002-0.01%
05E	Rinses (noncoloring)	0.00018%
05F	Shampoos (noncoloring)	0.0001-0.01%
05G	Tonics, dressings and other hair grooming aids pump spray	0.0002-0.01% 0.006-0.0095%
05I	Other hair preparations (noncoloring)	0.0095%
06A	Hair dyes and colors (all types requiring caution statement and patch test)	0.000059-0.0082%
06C	Hair rinses (coloring)	0.00027%
06G	Hair bleaches	0.0095%
06H	Other hair coloring preparations	0.000056%
07A	Blushers (all types)	0.000000035%
07C	Foundations	0.0095-0.0097%
07I	Other makeup preparations	0.00037%

08B	Cuticle softeners	0.0002%
08C	Nail creams and lotions	0.0006%
10A	Bath soaps and detergents	0.0000009-0.01%
10B	Deodorants (underarm) not spray	 0.0095%
10E	Other personal cleanliness products hand soap foot scrub	0.0012-0.0079% 0.00023-0.01% 0.01%
11D	Preshave lotions (all types)	0.0074%
11E	Shaving cream (aerosol, brushless and lather)	0.00011-0.01%
12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads) wipe (not baby)	0.000013-0.01% 0.005%
12B	Depilatories	0.00000025%
12C	Face and neck products not spray	 0.0072-0.01%
12D	Body and hand products not spray	 0.00011-0.01%
12E	Foot powders and sprays	0.01%
12F	Moisturizing products not spray	 0.0032-0.0072%
12G	Night products not spray	 0.0066-0.01%
12H	Paste masks and mud packs	0.0066-0.01%
12J	Other skin care preparations	0.00003-0.00026%
13A	Suntan products not spray	 0.0095%

†Product category codes used by FDA

*This survey requested that wipe products be identified. In the response, one product containing Methylisothiazolinone was identified as being in the form of wipes.

Information collected in 2013

Table prepared: August 29, 2013