ADMIN

Memo

Agenda

Minutes

Aluminum Starch

Octenylsuccinate (RRSummary)

MI/MCI (Strategy Memo)

Triacetin

CIR EXPERT PANEL MEETING

SEPTEMBER 24-25, 2018

Commitment & Credibility since 1976

MEMORANDM

To: CIR Expert Panel Members and Liaisons
From: Bart Heldreth, Ph.D., Executive Director, Cosmetic Ingredient Review
Subject: 148th Meeting of the CIR Expert Panel — Monday and Tuesday, September 24 - 25, 2018
Date: August 29, 2018

Welcome to the September 2018 CIR Expert Panel Meeting. Enclosed are the agenda and accompanying materials for the 148th CIR Expert Panel Meeting to be held on September 24 - 25, 2018. The location is the same as the last meeting – the Darcy Hotel, 1515 Rhode Island Avenue, NW, Washington, District of Columbia, 20005-5595. Phone: (202) 232-7000.

The meeting agenda includes the consideration of 14 reports advancing in the review process, including 5 final reports, 3 tentative reports, 5 draft reports, and 1 re-review. Also on the agenda are a re-review summary of Aluminum Starch Octenylsuccinate and a strategy memo regarding the re-review of the combination use of the preservatives MI and MCI.

Schedule and hotel accommodations

We have reserved rooms for the nights of Sunday, September 23rd and Monday, September 24th at the Darcy Hotel. If you encounter travel problems, please contact Monice on her cell phone at 703-801-8156.

Team Meetings

Draft Reports - there are 5 draft reports for review.

1. Brown Algae (agenda and flash drive name – Brown Algae) – This is the first time that the Panel is seeing this report on brown algae-derived ingredients. In July 2018, a Scientific Literature Review (SLR) was issued with an invitation for submission of data on these ingredients. According to the *Dictionary*, these brown algae-derived ingredients are most commonly used as skin conditioning agents.

   Information received from the Personal Care Products Council (Council) include: use concentration data, information regarding hydrolyzed fucoidan extracted from *Laminaria digitata*, information regarding a trade name mixture containing Ascophyllum Nodosum Extract, ocular irritation and sensitization data of products containing Sargassum Muticum Extract, sensitization data of a night cream containing Alaria Esculenta Extract, and comments on the SLR. Council comments have been addressed. Also included in this review package is a copy of the presentation on algal diversity provided to the Panel by Rex L. Lowe.

   According to VCRP survey data received in 2018, Fucus Vesiculosus Extract is reported to be used in 287 formulations (201 in leave-on formulations, 75 in rinse-off formulations, and 11 diluted for the bath; Table 20). Laminaria Digitata Extract is reported to be used in 235 formulations, Macrocystis Pyrifera (Kelp) Extract in 188 formulations, and Laminaria Saccharina Extract is used in 132 formulations. All other in-use ingredients are reported to be used in 77 formulations or fewer.

The results of the concentration of use surveys conducted by the Council in 2015 and 2016 indicate Laminaria Digitata Powder has the highest reported maximum concentration of use; it is used at up to 40% in face and neck formulations. Macrocystis Pyrifera (Kelp) Extract is reported to be used at up to 36.4% in eye lotions. The other ingredients are reported to be used at 6% or less.

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, or unsafe conclusion. If the available data are insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

2. Hydroxyethyl Urea (agenda and flash drive name – Hydroxyethyl Urea). This is the first time that the Panel is seeing this report on Hydroxyethyl Urea. On June 21, 2018, CIR issued the SLR for this ingredient. According to the *Dictionary*, this ingredient is reported to function as a humectant and hair- and skin-conditioning agent in cosmetics.

   The Council provided method of manufacture, impurities, dermal absorption data, concentration of use survey data, and comments. Comments on the SLR from the Council were addressed.

   According to 2018 VCRP data, Hydroxyethyl Urea is used in 432 rinse-off personal cleanliness products, with the majority of the uses (407) reported in bath soaps and detergents. The results of the concentration of use survey conducted in 2017 by the Council indicate that Hydroxyethyl Urea is used in leave-on products at a maximum use concentration range of 0.00046% to 20.6%, with the highest maximum concentration of use reported to be in moisturizing products (not spray).

   If no further data are needed to arrive at a Conclusion of safe, safe with qualifications or unsafe, the Panel should formulate a Discussion and issue a Tentative Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an IDA.

3. Alkoxylated Fatty Amides (agenda and flash drive name – Alkoxylated Fatty Amides). This is the first time the Panel is seeing this safety assessment of 41 structurally-related alkoxylated simple amides. On July 30th, 2018, CIR issued the SLR for these ingredients. According to the *Dictionary*, all but a few of these ingredients are reported to function in cosmetics as a surfactant – emulsifying agent. Although a few of the ingredients in this report (e.g., PEG-3 Cocamide DEA and PEG 2 Tallowamide DEA) are di-*N*,*N*-alkoxyl-substituted amides, most of these alkoxylated fatty amides are mono-*N*-alkoxyl-substituted.

   According to 2018 VCRP survey data, PPG-2 Hydroxyethyl Cocamide is reported to be used in 342 formulations, and PEG-4 Rapeseedamide is reported to be used in 280 formulations. All other in-use ingredients are reported to be used in less than 30 formulations. The results of the concentration of use survey conducted by the Council in 2015 indicate PEG-4 Rapeseedamide has the highest concentration of use, at 9.3% in hair dyes and colors. The ingredient with the next highest reported concentration of use is PPG-2 Hydroxyethyl Cocamide; it is used at 7.5% in “other” non-coloring hair preparations. The alkoxylated fatty amides are primarily used in rinse-off formulations, with few uses reported in leave-on formulations. Most of the reported uses are in some type of hair or cleansing formulation. However, there are some uses that result in leave-on dermal exposure; the highest concentration of use reported for products resulting in leave-on dermal exposure is 3% PPG-2 Hydroxyethyl Cocamide in body and hand products.

   If the data included in this report adequately address the safety of the alkoxylated fatty amides, the Panel should be prepared to formulate a tentative Conclusion, provide the rationale to be described in the Discussion, and issue a Tentative Report for public comment. If the data are not sufficient for making a determination of safety, then an IDA should be issued that provides a listing of the additional data that are needed.

4. Xanthine Alkaloids - (agenda and flash drive name – Xanthine Alkaloids). This is the first time the Panel is seeing this safety assessment of 3 xanthine alkaloid ingredients, comprising Caffeine and 2 structurally related analogs. On August 2nd, 2018, CIR issued the SLR for these

ingredients. According to the *Dictionary*, all three of these ingredients are reported to function as skin-conditioning agents in cosmetic products.

According to 2018 VCRP data, Caffeine is reported to be used in 1033 formulations, 882 of which are leave-on products and 151 are rinse-off. Theobromine and Theophylline are reported to have much smaller frequencies of use at 5 formulations each. The results of the concentration of use survey conducted by the Council indicate Caffeine also has the highest concentration of use in a leave-on formulation; it is used at up to 6% in non-spray body and hand products.

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety the Panel should identify matters to be addressed in the Discussion, and then issue a Tentative Report with a safe as used, safe with qualifications, or unsafe Conclusion. If, however, the available data are insufficient, the Panel should issue an IDA, specifying the data needs therein.

5. Acrylates Copolymers (agenda and flash drive name – Acrylates Copolymers). At the March 2018 meeting, the Panel determined that the Final Report on the Safety Assessment of Acrylates Copolymer and 33 Related Cosmetic Ingredients, published in 2002, should be reopened to add a number of other copolymers. The expanded document includes copolymers prepared from monomers that comprise, in part, acrylic acid and/or methacrylic acid; the methyl, ethyl, propyl, or butyl ester(s) of these acids; or the salts of one or both of these two acids. A complete listing of the 128 ingredients being reviewed is provided.

   According to FDA VCRP data, the frequency of use of Acrylates Copolymer has increased significantly since these ingredients were originally reviewed. In 1998, Acrylates Copolymer was reported to be used in 227 formulations; according to VCRP data received in 2018, it has more than 3100 uses. Several other ingredients included in this safety assessment also have a very high frequency of use.

   It should be noted that when the acrylates copolymers were originally reviewed, concentration of use data were not being reported, but there was an indication that these polymers were being used at a maximum of 25%. Acrylates Copolymer is now being used at up to 98.6% in nail formulations; however, the maximum reported concentration of use for leave-on products with dermal exposure is 25%. However, other ingredients are being used in dermal formulations.

   Council comments were received at the time of the March meeting. Some, but not all, of the comments have been addressed. Please review the outstanding issues discussed in those comments, and provide guidance.

   The Panel should review the Draft Amended Report and determine if the data are sufficient to come to a conclusion of safety. If the data are sufficient, the Panel should be prepared to formulate a Discussion and issue a Tentative Amended Report. If the data are insufficient, the Panel should issue an IDA, with the data needs detailed therein.

Draft Tentative Reports – there are 3 draft tentative reports.

1. Titanium Complexes (agenda and flash drive name – Titanium Complexes). Please note that the title of this safety assessment has been changed from Organo-Titanium Ingredients to Titanium Complexes. At the June 2018 Panel meeting, an IDA with the following data requests was issued:

   Isopropyl Titanium Triisostearate:
   - 28-day dermal toxicity data
     - Depending on the results of this study, additional systemic toxicity data may be needed
   - Mammalian genotoxicity data

   Titanium Citrate, Titanium Ethoxide, Titanium Isostearates, and Titanium Salicylate:
   - Use concentration data
   - Method of manufacture and impurities

- 28-day dermal toxicity data; depending on the results of this study, additional systemic toxicity data may be needed
- Skin irritation and sensitization data at cosmetic use concentrations

The following data on black iron oxide with 2% Isopropyl Titanium Triisostearate were received in response to this announcement: (1) acute oral toxicity (rats), (2) skin irritation (rabbits), and (3) ocular irritation (rabbits). Neither 28-dermal toxicity data nor mammalian genotoxicicty data were submitted for Isopropyl Titanium Triisostearate. No data were submitted with regard to the needs for Titanium Citrate, Titanium Ethoxide, Titanium Isostearates, or Titanium Salicylate.

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, or unsafe Conclusion, and identify issues to be addressed in the Discussion. If the available data are yet insufficient, the Panel should issue a Tentative Report with an insufficient data Conclusion, specifying the data needs in the Discussion section of the report.

2. Vinylpyrrolidone Polymers (agenda and flash drive name – Vinylpyrrolidone Polymers). At the June 4-5, 2018 meeting, the Panel issued an IDA on the 30 vinylpyrrolidone copolymers (21 ingredients reviewed for the first time + 9 ingredients previously reviewed) reviewed in this safety assessment.

   The data requests were as follows:
   - Method of manufacture
   - Impurities

   Therein, the Panel noted that data on a representative ingredient for each type of monomer composition would be sufficient for the group. It was also noted that it would be useful to know whether or not the product of each manufacturing process is an emulsion or solid, pure polymer. Furthermore, it would be useful to know the molecular weight % range for each ingredient.

   To date, the following data were received in response to this request:
   - Method of manufacture and residual monomer data on VP/VA Copolymer
   - Method of manufacture and impurities data on PVP

   After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, or unsafe Conclusion, and identify issues to be addressed in the Discussion. If the available data are still considered insufficient, the Panel should issue a Tentative Report with an insufficient data conclusion, specifying the data needs in the Discussion section of the report. If the data are sufficient to determine safety for some ingredients, but insufficient for others, a split conclusion can be issued.

3. Parabens (agenda and flash drive name – Parabens). At the March 2018 meeting, the Panel reviewed the new data in the category of endocrine activation, developmental and reproductive toxicity (DART), and epidemiology. The Panel discussed the topics and issues related to EU regulations of parabens, bioaccumulation potential, aggregate exposure, and estrogen receptor binding capability of paraben metabolites.

   The Panel reviewed additional studies submitted by various stakeholders or discovered by CIR, as well as interpretation of this data in Dr. George Daston's presentation, titled "Assessing the Developmental and Reproductive Toxicity of Parabens." The Panel requested all relevant new information be included in this Draft Tentative Amended Report.

   Taking Dr. Daston's presentation into account, the Panel considered whether no-observed-adverse-effect-level (NOAEL) data from new DART studies warranted a dose lower than the 1000 mg/kg/day which was used for margin of safety (MOS) calculation in the previous CIR safety assessment of parabens. After careful consideration of all the new data, the Panel determined an adequate NOAEL of 160 mg/kg/day for Butylparaben. The MOS for Butylparaben was re-calculated in this Draft Tentative Amended Report accordingly, and can be inferred to other members of the parabens group.

The Panel should review the available data to either affirm or change the conclusion from the 2008 report for the original seven paraben ingredients. The Panel should also determine if this conclusion can be applied to the newly added ingredients, or if a split conclusion is warranted. Whether the conclusion remains the same (and extends to all of the new ingredients) or is to be changed and/or split, the Panel should develop the basis for the Discussion and Conclusion, and issue a Tentative Amended Report.

Draft Final Reports - there are 5 draft final reports for consideration (including two amended reports). After reviewing these drafts, especially the rationales provided in the Discussion sections, the Panel should issue them as Final Reports, as appropriate.

1. *Ginkgo biloba*-derived Ingredients (agenda and flash drive name – Ginkgo biloba). In June 2018, the Panel reviewed data that had been received from Industry, including updated use concentrations. Based on the new data and the available dermal irritation and sensitization data, the Panel issued a revised Tentative Report with the conclusion that the following 5 ingredients are safe in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing:

   Ginkgo Biloba Leaf Extract
   Ginkgo Biloba Leaf
   Ginkgo Biloba Leaf Cell Extract
   Ginkgo Biloba Leaf Powder
   Ginkgo Biloba Leaf Water

   The Panel also determined that the data are insufficient to determine the safety of the following 5 ingredients:

   Ginkgo Biflavones
   Ginkgo Biloba Meristem Cell
   Ginkgo Biloba Nut Extract
   Ginkgo Biloba Root Extract
   Ginkgo Leaf Terpenoids

   The data needed to determine safety for these cosmetic ingredients are:
   - Method of manufacturing, composition, and impurities data for each of these ingredients, except Ginkgo Biloba Meristem Cell
   - 28-Day dermal toxicity data for each of these ingredients
     - Dependent on the results of these studies, additional data on other toxicological endpoints, such as developmental and reproductive toxicity and carcinogenicity, may be needed
   - Dermal irritation and sensitization data at leave-on use concentrations
   - Ocular irritation data, if available

   No new data has been received since the June Panel meeting. Comments provided by the Council prior to the June meeting and on the revised Tentative Report have been addressed. The Panel should carefully review the Abstract, Discussion, and Conclusion of this safety assessment. If these are satisfactory, the Panel should issue a Final Report.

2. Hydrogen Peroxide (agenda and flash drive name – Hydrogen Peroxide). The Panel reviewed this document for the first time at the June 2018 Panel meeting, and issued a Tentative Report for public comment with the conclusion that Hydrogen Peroxide is safe in cosmetics in the present practices of use and concentration described in the safety assessment.

   The Panel noted the positive genotoxicity studies, but determined these results are not relevant to cosmetic use because Hydrogen Peroxide is hydrolyzed quickly on the skin surface. Also, dermal carcinogenicity and reproductive and developmental toxicity studies were negative with 6% Hydrogen Peroxide (tested in formulation). The Panel found that the irritation and sensitization data given in this report are sufficient.

   The Panel should carefully review the Abstract, Discussion, and Conclusion of this safety assessment. If these are satisfactory, the Panel should issue a Final Report.

3. Dialkyl Dimer Dilinoleates (agenda and flash drive name – Dialkyl Dimer Dilinoleates). In 2003, CIR published the Final Report on the Safety Assessment of Diisopropyl Dimer Dilinoleate, Dicetearyl Dimer Dilinoleate, Diisostearyl Dimer Dilinoleate, Diethylhexyl [previously dioctyl] Dimer Dilinoleate, Dioctyldodecyl Dimer Dilinoleate, and Ditridecyl Dimer Dilinoleate. Based on the available data, the Panel concluded that the dialkyl dimer dilinoleates named in that report are safe for use in cosmetics.

   The report was then brought to the Panel at the June 2018 Panel meeting as a re-review document. The Panel stated that it was appropriate to re-open the document and include the proposed add-ons: Di-C16-18 Alkyl Dimer Dilinoleate and Di-C20-40 Alkyl Dimer Dilinoleate. The Panel reaffirmed the previous conclusion of "safe as used" for the original 6 dialkyl dimer dilinoleates, and deemed that it applies to the two new ingredients.

   The 2018 concentration of use data was given to the Panel as Wave 2 data at the June 2018 Panel meeting, and the current iteration of the report now includes these updated data. According to 2018 VCRP data, Diisopropyl Dimer Dilinoleate is used in 145 formulations with a maximum concentration of use of 29% in lipsticks. Diisostearyl Dimer Dilinoleate is the only other dialkyl dimer dilinoleate reported to be in use (20 formulations; maximum concentration of 16% in lipsticks).

   The Panel should carefully review the Abstract, Discussion, and Conclusion of this safety assessment. If these are satisfactory, the Panel should issue a Final Amended Report.

4. Polyol Phosphates (agenda and flash drive name – Polyol Phosphates). At the June 2018 Panel meeting, the Panel reviewed the safety of 10 polyol phosphates, and issued a Tentative Report with the following split conclusion:

   Sodium Phytate, Phytic Acid, Phytin, and Trisodium Inositol Triphosphate are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

   The data are insufficient to determine the safety of the following 6 ingredients: Disodium Glucose Phosphate, Manganese Fructose Diphosphate, Sodium Mannose Phosphate, Trisodium Fructose Diphosphate, Xylityl Phosphate, and Zinc Fructose Diphosphate. The Panel determined that the following data are needed to assess the safety of these 6 ingredients: (1) Method of manufacture, (2) Impurities data, and (3) Absorption, distribution, metabolism, and excretion (ADME) data. To date, the data requested have not been received.

   After reviewing the data included in the safety assessment, the Panel will need to determine whether or not a Final Report with the conclusions stated above should be issued.

5. Polyfluorinated Polymers (agenda and flash drive name – Polyfluorinated Polymers). At the June 2018 Panel meeting, the Panel issued a Tentative Report with the following conclusions:

   PTFE and Hexafluoropropylene/Tetrafluoroethylene Copolymer are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

   The data are insufficient to determine the safety of the following ten ingredients:

   Acrylates/Perfluorohexylethyl Methacrylate Copolymer
   Behenyl Methacrylate/Perfluorooctylethyl Methacrylate Copolymer
   C6-14 Perfluoroalkylethyl Acrylate/HEMA Copolymer
   Stearyl Methacrylate/Perfluorooctylethyl Methacrylate Copolymer
   Acrylates/Methoxy PEG-23 Methacrylate/Perfluorooctyl Ethyl Acrylate Copolymer
   PEG-10 Acrylate/Perfluorohexylethyl Acrylate Copolymer
   Polyperfluoroethoxymethoxy Difluoroethyl PEG Diisostearate
   Polyperfluoroethoxymethoxy Difluoroethyl PEG Ether
   Polyfluoroethoxymethoxy Difluorohydroxyethyl Ether
   Polyperfluoroethoxymethoxy Difluoromethyl Ether

   The Panel determined that the following data are needed to determine the safety of these ten

ingredients: (1) method of manufacture and impurities data and (2) skin sensitization data at the highest maximum use concentration.

To date, these data have not been received for any of the ingredients. However, the Council provided updated use concentration data on PTFE, and these data are attached for the Panel's review. Therein, the previously reported use of PTFE in the Other Oral Hygiene Products category (at concentrations up to 0.44%) was determined to be erroneous. Accordingly, this data point has been deleted from this safety assessment.

Although the available information now shows no indication that PTFE is used in cosmetic oral hygiene products, 2018 FDA VCRP data indicate that it is used in lipsticks (use concentration data unavailable). Therefore, incidental ingestion remains a possibility under conditions of use. At the June Panel meeting, the Panel determined that a value for the greatest possible amount of incidentally ingested PFOA and PFOS that would result from the use of oral hygiene products at the maximum use concentration of PTFE needed to be calculated and included in the safety assessment on Polyfluorinated Polymers. Since cosmetic oral hygiene use is no longer reported, the use of PTFE in products that are applied to the lips was considered in this calculation and described in the Risk Assessment section of this Draft Final Report.

The Panel should carefully review the Abstract, Discussion, and Conclusion of this safety assessment. If these are satisfactory, the Panel should issue a Final Report.

Re-Review – there is 1 Re-Review Report

1. Triacetin (agenda and flash drive name – Triacetin). In 2003, the Panel published the Final Report on the Safety Assessment of Triacetin. The Panel concluded that Triacetin is "safe as used in cosmetics." According to its Procedures, CIR evaluates the conclusions of previously-issued reports every 15 years. Therefore, this report is being presented for the Panel's consideration for re-review.

   With the exception of a negative DART study and additional negative Ames tests found in the ECHA REACH database, no new relevant data were found in the published literature. Additionally, no ingredients are being proposed for inclusion in this re-review. The frequency of use increased from 13 to 59 uses, however, the maximum use concentrations decreased, from 4% to 0.95%. Therefore, the only reason to re-open this report would be to reconsider the conclusion.

   If there is concern about the existing conclusion, the review should be re-opened. Otherwise, the existing conclusion should be reaffirmed, and the report will not be re-opened.

Re-Review Summary – there is 1 Re-Review Summary

1. Aluminum Starch Octenylsuccinate (agenda and flash drive name). The Panel published the Final Report on the Safety Assessment of Aluminum Starch Octenylsuccinate in 2002. Since then some new data, including methods of manufacture, were identified in the published literature. The Panel reviewed updated information regarding product types and ingredient use frequencies provided by the FDA and maximum use concentrations provided by the Council. The Panel reaffirmed the original conclusion of safe as used in cosmetic formulations, provided that established limitations imposed on heavy metal concentrations are not exceeded.

   The Panel should read this summary carefully and confirm that it is in agreement with their thinking.

Strategy Memo - there is 1 strategy memo

1. MI/MCI (agenda and flash drive name – MI-MCI). The CIR "Final Report on the Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone" was published in 1992. The conclusion stated that "Methylisothiazolinone/Methylchloroisothiazolinone may be safely used in "rinse-off" products at a concentration not to exceed 15 ppm and in "leave-on" cosmetic products at a concentration not to exceed 7.5 ppm. The stated safe use concentration refers to a mixture containing 23.3% Methylisothiazolinone and 76.7% Methylchloroisothiazolinone."

While the safety assessment for Methylisothiazolinone (MI) alone was published in 2010 and re-addressed in 2014, the combination use of MI and Methylchloroisothiazolinone (MCI) is overdue for re-review. (Reminder: CIR was informed that MCI is not used alone in cosmetics without MI.) At the time of the 1992 publication, there were 381 uses of this preservative combination. Current data, obtained from the FDA VCRP in 2018, indicate this preservative is now used in 4595 formulations in the US. Accordingly, a maximum concentration of use survey will be requested and a re-review document will be prepared for the Panel's consideration to re-open (likely for the March 2019 meeting).

However, this strategy memo is being issued in advance to obtain Panel input and direct the CIR staff towards information sought in that re-review document. In contrast to the conclusion issued for the MI/MCI combination use, the conclusion for MI alone is "safe for use in rinse-off cosmetic products at concentrations up to 100 ppm and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a QRA." Consequently, if the Panel is likely to revise their conclusion for the use of the MI/MCI ingredient combination to include the caveat determination by a quantitative risk assessment (QRA), what information / data / calculations (e.g., MOS) would be most useful?

Secondly, a recent article titled, "Risk Assessment of the Skin Sensitization Induction Potential of Kathon CG in Rinse-off and Leave-on Personal Care and Cosmetic Products" is directed at the risk of skin sensitization resulting from use of the MI/MCI combination ("Kathon CG" is a tradename mixture comprising MI/MCI, magnesium salts, and water). Would the Panel please comment on the merits/utility of this assessment (e.g., do you agree with the no effect sensitization induction level (NESIL) the authors chose therein)?

No vote is required for this administrative item; neither does a decision to re-open need to be made at this meeting. We are merely seeking your expert input in advance of document preparation.

Full Panel Meeting

Remember, the breakfast buffet will open at 8:00 am and the meeting starts at 8:30 am on day 1 and on day 2.

The Panel will consider the 5 reports to be issued as final safety assessments, followed by the remaining reports advancing in the process (including the tentative reports, draft reports, and re-review); a re-review summary; and a strategy memo. It is likely that the full Panel session will conclude before lunch on day 2, so plan your travel accordingly.

Have a safe journey!

Agenda
148th Cosmetic Ingredient Review Expert Panel Meeting
September 24th - 25th, 2018

The Darcy Hotel
1515 Rhode Island Avenue, NW,
Washington, District of Columbia, 20005-5595

Monday, September 24th

8:00 am	**CONTINENTAL BREAKFAST**	
8:30 am	**WELCOME TO THE 148th EXPERT PANEL TEAM MEETINGS**	**Drs. Bergfeld/Heldreth**
8:45 am	**TEAM MEETINGS**	**Drs. Marks/Belsito**

Dr. Marks' Team

DAR (MF)	Acrylates Copolymers
DR (MF)	Alkoxylated Fatty Amides
RR (MF)	Triacetin
FR (WJ)	Polyol Phosphates
FR (WJ)	Polyfluorinated Polymers
TR (WJ)	Titanium Complexes
TR (WJ)	Vinylpyrrolidone Polymers
DR (AA)	Hydroxyethyl Urea
RRsum (AA)	Aluminum Starch Octenylsuccinate
FR (CB)	Ginkgo biloba
FAR (PC)	Dialkyl Dimer Dilinoleates
DR (PC)	Xanthine Alkaloids
DR (PC)	Brown Algae
FR (PC)	Hydrogen Peroxide
TAR (PC/JZ)	Parabens
Admin (BH)	MI-MCI

Dr. Belsito's Team*

Admin (BH)	MI-MCI
TAR (PC/JZ)	Parabens
FAR (PC)	Dialkyl Dimer Dilinoleates
DR (PC)	Xanthine Alkaloids
DR (PC)	Brown Algae
FR (PC)	Hydrogen Peroxide
FR (CB)	Ginkgo biloba
FR (WJ)	Polyol Phosphates
FR (WJ)	Polyfluorinated Polymers
TR (WJ)	Titanium Complexes
TR (WJ)	Vinylpyrrolidone Polymers
DR (AA)	Hydroxyethyl Urea
RRsum (AA)	Aluminum Starch Octenylsuccinate
DAR (MF)	Acrylates Copolymers
DR (MF)	Alkoxylated Fatty Amides
RR (MF)	Triacetin

The purpose of the Cosmetic Ingredient Review is to determine those cosmetic ingredients for which there is a reasonable certainty in the judgment of competent scientists that the ingredients are safe under intended conditions of use.

FR: Final Report // FAR: Final Amended Report // TR: Tentative Report // TAR: Tentative Amended Report // DR: Draft Report // DAR: Draft Amended Report // RR: Re-Review // RRsum: Re-Review Summary // Admin: Administrative item

(AA): Alice Akinsulie || (CB): Christina Burnett || (BH): Bart Heldreth || (JZ): Jinqiu Zhu || (MF): Monice Fiume || (PC): Priya Cherian || (WJ): Wilbur Johnson

*Team moves to breakout room.

Tuesday, September 25th

8:00 am	**CONTINENTAL BREAKFAST**		
8:30 am	**WELCOME TO THE 148th FULL CIR EXPERT PANEL MEETING**		**Dr. Bergfeld**
8:45 am	Admin	**MINUTES OF THE JUNE 2018 EXPERT PANEL MEETING**	**Dr. Bergfeld**
9:00 am	**DIRECTOR'S REPORT**		**Dr. Heldreth**
9:10 am	**FINAL REPORTS, REPORTS ADVANCING TO THE NEXT LEVEL, OTHER ITEMS**		

Final Reports

FR (CB)	Ginkgo biloba – *Dr. Marks Reports*
FR (PC)	Hydrogen Peroxide – *Dr. Belsito Reports*
FAR (PC)	Dialkyl Dimer Dilinoleates – *Dr. Marks Reports*
FR (WJ)	Polyol Phosphates – *Dr. Belsito Reports*
FR(WJ)	Polyfluorinated Polymers – *Dr. Marks Reports*

Reports Advancing

TR (WJ)	Titanium Complexes – *Dr. Belsito Reports*
TR (WJ)	Vinylpyrrolidone Polymers – *Dr. Marks Reports*
TAR (PC/JZ)	Parabens – *Dr. Belsito Reports*
DR (PC)	Xanthine Alkaloids – *Dr. Marks Reports*
DR (PC)	Brown Algae – *Dr. Belsito Reports*
DAR (MF)	Acrylates Copolymers – *Dr. Marks Reports*
DR (MF)	Alkoxylated Fatty Amides – *Dr. Belsito Reports*
RR (MF)	Triacetin – *Dr. Marks Reports*
DR (AA)	Hydroxyethyl Urea – *Dr. Belsito Reports*

Other Items

RRsum (AA)	Aluminum Starch Octenylsuccinate – *Dr. Marks Reports*
Admin (BH)	MI-MCI

ADJOURN - Next meeting *Monday and Tuesday, December 3rd - 4th, 2018,* at The Darcy Hotel, 1515 Rhode Island Avenue, NW, Washington, District of Columbia, 20005-5595

On the basis of all data and information submitted, and after following all of the Procedures (https://www.cir-safety.org/supplementaldoc/cir-procedures), the Expert Panel shall determine whether each ingredient, under each relevant condition of use, is safe, safe with qualifications, unsafe, or there are insufficient data or information to make a determination of safety. Upon making such a determination, the Expert Panel shall issue a conclusion.

FR: Final Report // FAR: Final Amended Report // TR: Tentative Report // TAR: Tentative Amended Report // DR: Draft Report // DAR: Draft Amended Report // RR: Re-Review // RRsum: Re-Review Summary // Admin: Administrative item

(AA): Alice Akinsulie || (CB): Christina Burnett || (BH): Bart Heldreth || (JZ): Jinqiu Zhu || (MF): Monice Fiume || (PC): Priya Cherian || (WJ): Wilbur Johnson

Commitment & Credibility since 1976

ONE HUNDRED FORTY-SEVENTH MEETING

OF THE

EXPERT PANEL

June 4-5, 2018

Darcy Hotel

Washington, D.C.

Expert Panel Members

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Don Bjerke	P & G
Roshil Budhram	L-Brands
Christina Burnett	CIR
Priya Cherian	CIR
Carol Eisenmann	PCPC
Monice Fiume	CIR
Eileen Francis	The Rose Sheet
Kevin Fries	CIR
Thomas Gremillion	CFA
Bart Heldreth	CIR
Carla Jackson	CIR
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Alexandra Kowcz	PCPC
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Zemin Wang	FDA
Teresa Washington	FDA
M. Keith Wyatt	FDA
Jinqiu Zhu	CIR
Merle Zimmermann	AHPA

MINUTES FROM THE 147th CIR EXPERT PANEL MEETING

CHAIRMAN'S OPENING REMARKS

Dr. Bergfeld welcomed the attendees to the 147th meeting of the Cosmetic Ingredient Review (CIR) Expert Panel. She announced that CIR will be celebrating the 150th meeting of the Expert Panel in March of 2019, and thanked the Panel for their perserverance. She also thanked the CIR staff for their efforts in preparation for this Panel meeting.

Dr. David Steinberg, who is retiring, was recognized by Dr. Bergfeld for his guidance over a period of many years at meetings of the CIR Expert Panel. She noted his 30 + years of service to the cosmetics industry, and that he once served as a National President of the Society of Cosmetic Chemists. Dr. Bergfeld extended a cordial thank you to Dr. Steinberg for his service to CIR.

APPROVAL OF MINUTES

The minutes of the March 5-6, 2018 CIR Expert Panel meeting were unanimously approved.

DIRECTOR'S REPORT

Dr. Heldreth expressed gratitude for the Panel's and other stakeholders' continued support of the CIR program.

He noted that the CIR Steering Committee had convened on June 4th. The Committee elected one new member, Dr. James Selwyn Taylor, to fill the seat of an independent representative of the American Academy of Dermatology. The Committee is seeking nominees to fill the seat of an independent representative of the Society of Toxicology. The Committee also approved some updates to the CIR Procedures document. The updated document is available on the CIR website: https://www.cir-safety.org/supplementaldoc/cir-procedures.

Dr. Heldreth also noted that on April 1st of 2016, the Panel concluded that Polysilicone-2, Polysilicone-4, and Polysilicone-5 are safe when used to coat metal oxide particles and that the data are insufficient to determine safety if these ingredients are used independently in cosmetics. The requested data have not been submitted and, thus, for uses other than coating metal oxide particles, these ingredients have been moved to the Use Not Supported category.

On that same date, the Panel also concluded that the available data are insufficient for evaluating the safety of Pyrus Malus (Apple) Root Extract, Malus Domestica (Apple) Oil, and Malus Domestica (Apple) Stem Extract. The data needs have not been met and these three ingredients have also been moved to the Use Not Supported category. The Panel also concluded that the data are insufficient for Pyrus Malus (Apple) Stem Extract and Malus Domestica (Apple) Callus Extract, which are not reported to be in use. Thus, these two ingredients are now categorized as No Reported Use.

Dr. Heldreth pointed out that five CIR safety assessment reports have been published in the May/June Supplement of the *International Journal of Toxicology*. These include the assessments of Tromethamine, Polyether Lanolins, Alkyl Betaines, Magnesium Sulfate, and Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten.

Dr. Heldreth also gave an overview of some of the efforts by CIR staff to advance their understanding and involvement in the development of alternative test methods. Since the last Panel meeting, Ms. Fiume attended the annual meeting of the Society of Toxicology, in San Antonio (March 11-15), and learned a great deal on the advances being made in the realm of alternative test methods. Dr. Heldreth also had the opportunity to learn a great deal about such evolving methodologies when he attended and presented at a Risk & Regulatory Symposium of the German Cosmetic, Toiletry, Perfumery, and Detergent Association, in Frankfurt (Industrieverband Körperpflege- und Waschmittel e. V. (IKW)) in April.

Finally, Dr. Heldreth noted that CIR has also been fortunate to collaborate with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICETAM) in an effort to advance alternative methodologies for skin sensitization testing. This project is part of an initiative put forth by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). Nicole Kleinstreuer and her team at NICETAM are doing all of the hard work, of course, but CIR was happy to contribute the wealth of

unpublished HRIPT summary data that have been generated for CIR Reports over the years. This work is very promising and can be tracked at NTP's website for Integrated Chemical Environment tools (https://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/comptox/ct-its/its.html).

Final Safety Assessments

Eucalyptus globulus (Eucalyptus)-Derived Ingredients

The Panel issued a final report for the following 6 *Eucalyptus globulus*-derived ingredients with the conclusion that these ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing.

Eucalyptus Globulus Leaf	Eucalyptus Globulus Leaf Oil	Eucalyptus Globulus Leaf/Twig Oil*
Eucalyptus Globulus Leaf Extract	Eucalyptus Globulus Leaf Powder	Eucalyptus Globulus Leaf Water

**Not reported to be in current use. If the ingredient in this group not in current use was to be used in the future, the expectation is that it would be used in product categories and at concentrations comparable to others in this group.*

The Panel examined the data on oral, dermal and inhalation toxicity, ocular and dermal irritation, sensitization, developmental and reproductive toxicity, genotoxicity, and phototoxicity. The Panel also considered toxicity data on eucalyptol, a high concentration constituent of Eucalyptus Globulus Leaf Oil and Eucalyptus Globulus Leaf/Twig Oil. The Panel noted the lack of toxicity and the lack of irritation and sensitization at relevant concentrations of use of these ingredients. The genotoxicity studies and the carcinogenicity study (on eucalyptol) did not give cause for concern.

The Panel noted that, because botanical ingredients are complex mixtures, there is concern that multiple botanical ingredients in one product formulation may each contribute to the final concentration of a single shared constituent. Therefore, when formulating products, manufacturers should avoid reaching concentrations of botanical constituents that may cause sensitization or other adverse effects.

Triphenyl Phosphate

The Panel issued a final report with the conclusion that Triphenyl Phosphate is safe in cosmetics in the present practices of use and concentration described in the safety assessment.

The Panel found that the systemic toxicity data, including developmental and reproductive toxicity and short-term toxicity studies, and dermal irritation and sensitization data in this report were sufficient. The Panel noted the lack of carcinogenicity data, but this gap was mitigated by multiple genotoxicity studies that were negative. This ingredient is only used in nail products, and the maximum reported use concentration is 14.5%. The Panel also noted that it is not in their purview to perform occupational safety assessments.

The Panel noted that zebra fish studies on Triphenyl Phosphate demonstrated some hormone effects in fish, but did not indicate that cosmetic use would result in endocrine disruption. For further explanation of what qualifies as endocrine activity or disruption, please refer to the CIR resource document: https://www.cir-safety.org/supplementaldoc/cir-precedents-endocrine-activity.

Tentative Safety Assessments

The Panel issued a tentative report for public comment with the conclusions that 1) PTFE and Hexafluoropropylene/Tetrafluoroethylene Copolymer are safe in cosmetics in the present practices of use and concentration described in the safety assessment, and 2) the data are insufficient to determine the safety of the following ten ingredients:

Acrylates/Perfluorohexylethyl Methacrylate Copolymer
Behenyl Methacrylate/Perfluorooctylethyl Methacrylate Copolymer
C6-14 Perfluoroalkylethyl Acrylate/HEMA Copolymer
Stearyl Methacrylate/Perfluorooctylethyl Methacrylate Copolymer
Acrylates/Methoxy PEG-23 Methacrylate/Perfluorooctyl Ethyl Acrylate Copolymer
PEG-10 Acrylate/Perfluorohexylethyl Acrylate Copolymer
Polyperfluoroethoxymethoxy Difluoroethyl PEG Diisostearate
Polyperfluoroethoxymethoxy Difluoroethyl PEG Ether
Polyfluoroethoxymethoxy Difluorohydroxyethyl Ether
Polyperfluoroethoxymethoxy Difluoromethyl Ether

The Panel determined that the following data are needed to determine the safety of these ingredients:

- Method of manufacture and impurities data
- Skin sensitization data at the highest maximum use concentration

The need for skin sensitization data at the highest maximum use concentration of PTFE (13%) was included in the insufficient data announcement (IDA) on this entire group of ingredients that was issued at the March 2018 Panel meeting. However, negative HRIPT data on PTFE at a concentration of 9% were received, and the Panel agreed that these data at a lower concentration of PTFE are sufficient for determining that Hexafluoropropylene/ Tetrafluoroethylene Copolymer and PTFE would not be skin sensitizers at cosmetic use concentrations up to 13%.

Method of manufacture and impurities data on PTFE were also received in response to the IDA. These data indicate that the tetrafluoroethylene monomer is undetectable (75 ppb detection limit), that perfluorooctanoic acid (PFOA) is present at concentrations of < 25 ppb, and that the incidental content of perfluorooctyl sulfonate (PFOS) is detectable in the ppb range. In light of the Environmental Protection Agency's 70 ppt limit on PFOA and PFOS combined in drinking water and the developmental toxicity/carcinogenicity that is associated with these impurities, the Panel determined that a value for the greatest possible amount of incidentally ingested PFOA and PFOS that would result from the use of oral hygiene products at the maximum use concentration of PTFE needs to be calculated and included in this safety assessment.

The Panel also discussed the issue of incidental inhalation exposure from powders, having taken into consideration that PTFE is reported as being used in [fragrance] powders (dusting and talcum, excluding aftershave talcum) and in face powders, which may result in incidental inhalation exposure. Data received from the Personal Care Products Council (Council) indicate that PTFE is being used in face powders at maximum use concentrations ranging from 0.5% to 3%. The Panel noted that conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

***Ginkgo biloba*-Derived Ingredients**

The Panel issued a revised tentative report for public comment with the conclusion that the following 5 ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing:

Ginkgo Biloba Leaf Extract	Ginkgo Biloba Leaf Cell Extract*	Ginkgo Biloba Leaf Powder
Ginkgo Biloba Leaf*		Ginkgo Biloba Leaf Water*

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

The Panel also determined that the data are insufficient to determine the safety of the following 5 ingredients:

Ginkgo Biflavones	Ginkgo Biloba Nut Extract	Ginkgo Leaf Terpenoids
Ginkgo Biloba Meristem Cell	Ginkgo Biloba Root Extract	

The data needed to determine safety for these cosmetic ingredients are:

- Method of manufacturing, composition, and impurities data for each of these ingredients, except Ginkgo Biloba Meristem Cell
- 28-Day dermal toxicity data for each of these ingredients
    - Dependent on the results of these studies, additional data on other toxicological endpoints, such as developmental and reproductive toxicity and carcinogenicity, may be needed
- Dermal irritation and sensitization data at leave-on use concentrations
- Ocular irritation data, if available

The Panel determined that the previous safety test data, methods of manufacturing, and composition and impurities data insufficiencies on Ginkgo Biloba Leaf Extract have been resolved, and reasonable inferences to 4 other leaf-derived ingredients can be made. The Panel noted the positive carcinogenic findings of the rodent studies performed by NTP, but determined that the *Ginkgo biloba* leaf extract used therein contained unusually high concentrations of certain constituents that are markedly different from those found in the leaf extracts used in dietary supplements. Furthermore, the NTP study administered this specific leaf extract at high doses by gavage, allowing for concentrations in the blood that would not be achieved through cosmetic use. The leaf extract that is similar to that used in dietary supplements did not produce increased incidences of cancer in a dietary study. This, combined with a long history of use of *Ginkgo biloba* leaf extracts in folk medicine, indicated that the findings of the NTP study are not relevant to cosmetic use.

The Panel noted that, because botanical ingredients are complex mixtures, there is concern that multiple botanical ingredients in one product formulation may each contribute to the final concentration of a single shared constituent. Therefore, when formulating products, manufacturers should avoid reaching concentrations of botanical constituents that may cause sensitization or other adverse effects.

Hydrogen Peroxide

The Panel issued a tentative report for public comment with the conclusion that Hydrogen Peroxide is safe in cosmetics in the present practices of use and concentration described in the safety assessment.

Hydrogen Peroxide is reported to be used in hair dyes at up to 15% in a professional product (that is diluted for use) and at up to 12.4% in consumer hair dyes and colors formulations; Hydrogen Peroxide is reported to be used in a total of 390 cosmetic ingredients, 250 of which are hair-coloring formulations. Other categories of use include formulations that result in dermal leave-on exposure at up to 3%, and there are reported to be 93 formulations that, under conditions of intended use, come in contact with mucous membranes and may result in incidental ingestion, including at up to 4.6% in dentifrices.

The Panel noted positive genotoxicity study results, but determined these are not relevant to cosmetic use because Hydrogen Peroxide is hydrolyzed quickly on the skin surface. Also, dermal carcinogenicity and reproductive and developmental toxicity studies were negative with 6% Hydrogen Peroxide. The Panel found that the irritation and sensitization data given in this report are sufficient.

Polyol Phosphates

The Panel issued a tentative report for public comment with the following 2 conclusions:

1) Sodium Phytate, Phytic Acid, Phytin*, and Trisodium Inositol Triphosphate* are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

2) The data are insufficient to determine the safety of the following 6 ingredients: Disodium Glucose Phosphate, Manganese Fructose Diphosphate, Sodium Mannose Phosphate, Trisodium Fructose Diphosphate, Xylityl Phosphate, and Zinc Fructose Diphosphate.

The Panel determined that the following data are needed to assess the safety of these 6 ingredients:

- Method of manufacture
- Impurities data
- Absorption, distribution, metabolism, and excretion (ADME) data

These data were also requested in the IDA that was issued at the March 2018 Panel meeting, but were not received.

The Panel also previously requested skin sensitization data (animal or human) on Phytic Acid at the highest maximum use concentration of 2% or on a cosmetic product containing 2% Phytic Acid. Negative human repeated insult patch test (HRIPT) data on products containing Sodium Phytate (up to 0.1%) and on a moisturizer containing 5% Phytic Acid (highest ingredient concentration tested), as well as negative human photosensitization data on a clear liquid containing 1% Sodium Phytate, were among the data that were received in response to the IDA.

Regarding the safe as used conclusion that was issued on the 4 ingredients (all inositol phosphates), the Panel determined that the data received indicate that these ingredients do not have skin sensitization potential at cosmetic use concentrations.

Salicylic Acid and Salicylates

The Panel issued a tentative amended report for public comment with the conclusion that Salicylic Acid and the 18 salicylate ingredients, listed below, are safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be non-irritating.

Butyloctyl Salicylate
Calcium Salicylate*
C12-15 Alkyl Salicylate*
Capryloyl Salicylic Acid
Ethylhexyl Salicylate
Hexyldodecyl Salicylate*
Isocetyl Salicylate*
Isodecyl Salicylate
Magnesium Salicylate
Methyl Salicylate
Myristyl Salicylate*
Potassium Salicylate*
Salicylic Acid
Sodium Salicylate
TEA-Salicylate
Tridecyl Salicylate
Amyl Salicylate
Hexyl Salicylate
Isotridecyl Salicylate*

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

Ingredients identified by blue text were not previously included in the safety assessment, and were added at this meeting.

The Expert Panel originally published a Safety Assessment of Salicylic Acid and 16 salicylates in 2003 with the conclusion: that Salicylic Acid, the salts Calcium Salicylate, Magnesium Salicylate, MEA-Salicylate, Potassium Salicylate, Sodium Salicylate, and TEA-Salicylate; the esters Capryloyl Salicylic Acid, C12-15 Alkyl Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Methyl Salicylate, Myristyl Salicylate, Ethylhexyl Salicylate; and Tridecyl Salicylate, and the compounds Butyloctyl Salicylate and Hexyldodecyl Salicylate are safe as used when formulated to avoid skin irritation and when formulated to avoid increasing the skin's sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection. The complete report is available on the CIR website (http://www.cir-safety.org/ingredients).

In accordance with its Procedures, the CIR evaluates the conclusions of previously-issued reports every 15 years; therefore a re-review was initiated. The Panel determined it was appropriate to re-open the safety assessment to amend the conclusion and to include additional related ingredients (i.e., Amyl Salicylate, Hexyl Salicylate,

Isotridecyl Salicylate). The re-review document consists primarily of safety test data summaries from the original published report, safety test data on the ingredients originally reviewed that entered the published literature since the final report was issued by the Panel, and safety test data on ingredients that have been added to the safety assessment. Also there was one deletion; because MEA-Salicylate was recently reviewed in the CIR safety assessment of Ethanolamine and Ethanolamine Salts, it is not included in this re-review.

According to 2018 VCRP data, the ingredient with the greatest frequency of use is Ethylhexyl Salicylate (3474 uses), followed by Salicylic Acid (1300 uses). The results of a concentration of use survey conducted by the Council in 2018 indicate that Butyloctyl Salicylate is used at concentrations up to 35.9% in leave-on products (lipstick), which is the highest maximum use concentration that is reported for ingredients reviewed in this safety assessment.

The original conclusion (stated above) has been revised to omit the qualification relating to formulating products to avoid increasing the skin's sun sensitivity. This is based on results from an NTP photocarcinogenicity study indicating that Salicylic Acid had some protective effect against photocarcinogenicity, at lower light intensities. In the NTP study, the effects of synthetic solar light on the skin of hairless mice that had been treated with creams containing 2% or 4% Salicylic Acid were evaluated. Creams containing Salicylic Acid decreased the incidence of skin tumors in mice receiving the lower of the two light intensities.

The Panel also expressed concern over the reproductive toxicity of Salicylic Acid, after learning that, in the third trimester, the use of Salicylic Acid can potentially cause early closure of the ductus arteriosus and oligohydramnios. Thus, the Panel requested that CIR calculate a margin of safety for Salicylic Acid exposure, taking into consideration the extent of dermal absorption during cosmetic product use (at highest maximum use concentration of 30% in leave-on products), for inclusion in a future iteration of the report.

Dialkyl Dimer Dilinoleates

The Panel issued a tentative amended report for public comment with the conclusion that the dialkyl dimer dilinoleate ingredients listed below are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

Diisopropyl Dimer Dilinoleate
Dicetearyl Dimer Dilinoleate
Diisostearyl Dimer Dilinoleate
Diethylhexyl Dimer Dilinoleate
Dioctyldodecyl Dimer Dilinoleate*
Ditridecyl Dimer Dilinoleate*
Di-C16-18 Alkyl Dimer Dilinoleate*
Di-C20-40 Alkyl Dimer Dilinoleate*

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

Ingredients identified by blue text were not previously included in the safety assessment, and were added at this meeting.

The Panel published a safety assessment on 6 of these 8 dialkyl dimer dilinoleates in 2003, and concluded that those ingredients are safe as used in cosmetics. At this meeting, the Panel stated that it was appropriate to re-open the document and include the proposed add-ons: Di-C16-18 Alkyl Dimer Dilinoleate and Di-C20-40 Alkyl Dimer Dilinoleate. The Panel reaffirmed the previous conclusion of "safe as used" for the original 6 dialkyl dimer dilinoleates, and concluded that it applies to the two newly added ingredients.

Re-Reviews

In accordance with its Procedures, the Panel considered a re-review of the 2003 safety assessment on the following 17 silicate and clay ingredients:

Aluminum Silicate
Attapulgite
Bentonite
Calcium Silicate
Fuller's Earth
Hectorite
Kaolin
Lithium Magnesium Silicate
Lithium Magnesium Sodium Silicate

Magnesium Aluminum Silicate	Magnesium Trisilicate	Sodium Magnesium Silicate
Magnesium Silicate	Montmorillonite	Zeolite
	Pyrophyllite	Zirconium Silicate

At the time of the report was originally reviewed, the Panel concluded that these ingredients were safe as used in cosmetic products. The Panel decided to re-open this report and add an additional 25 ingredients, which include 9 silica and silicate ingredients that were previously reviewed by the Panel and 16 ingredients that have not been reviewed by the Panel.

Aluminum Calcium Sodium Silicate*	Aluminum Iron Calcium Magnesium Zirconium Silicates
Aluminum Iron Silicates*	Ammonium Silver Zinc Aluminum Silicate
Hydrated Silica*	Ammonium Silver Zeolite
Magnesium Aluminometasilicate*	Calcium Magnesium Silicate
Potassium Silicate*	Gold Zeolite
Silica*	Silver Copper Zeolite
Sodium Metasilicate*	Silver Zinc Zeolite
Sodium Potassium Aluminum Silicate*	Sodium Magnesium Aluminum Silicate
Sodium Silicate*	Sodium Silver Aluminum Silicate
Activated Clay	Titanium Zeolite
Aluminum Calcium Magnesium Potassium Sodium Zinc Silicates	Tromethamine Magnesium Aluminum Silicate
Aluminum Iron Calcium Magnesium Germanium Silicates	Zinc Silicate
	Zinc Zeolite

**Ingredients previously reviewed by the Panel.*

The Panel noted that for many of the previously reviewed ingredients, uses have increased significantly. For example, Silica was previously reported to have 3276 total uses at the time of the original review; in 2018, a total of 8024 uses were reported. A new concentration of use survey for these ingredients will be completed before the Panel reviews this amended safety assessment later in 2018.

Aluminum Starch Octenylsuccinate

In accordance with its procedures, the Panel considered a re-review of the safety assessment of Aluminum Starch Octenylsuccinate, which was published in 2002.

The Panel reviewed updated information regarding product types and ingredient use frequencies provided by the FDA VCRP and maximum use concentrations provided by the Council. Based on the available data, the Panel decided not to re-open the document, reaffirming the previous conclusion of safe as used.

Insufficient Data Announcements

Organo-Titanium Ingredients (New Name: Titanium Complexes)

The Panel reviewed the safety of the following 5 organo-titanium ingredients for the first time at this meeting, and issued an IDA:

Isopropyl Titanium Triisostearate	Titanium Ethoxide	Titanium Salicylate
Titanium Citrate	Titanium Isostearates	

These organo-titanium ingredients are reported to have the following functions in cosmetics: surface modifiers (Isopropyl Titanium Triisostearate); colorants, humectants (Titanium Citrate); binders (Titanium Ethoxide); film formers, opacifying agents (Titanium Isostearates); and preservatives (Titanium Salicylate).

Only one of these ingredients is reported to be in use. According to 2018 VCRP data, Isopropyl Titanium Triisostearate is reported to be used in 580 cosmetic products (573 leave-on and 7 rinse-off products). The results of a concentration of use survey conducted by the Council in 2017 indicate that Isopropyl Titanium Triisostearate is being used at concentrations up to 1.5% in leave-on products (eye shadows) and at concentrations up to 0.3% in rinse-off products (eye make-up removers).

The Panel issued the following data requests:

Isopropyl Titanium Triisostearate

- 28-day dermal toxicity data
    - Depending on the results of this study, additional systemic toxicity data may be needed
- Mammalian genotoxicity data

Titanium Citrate, Titanium Ethoxide, Titanium Isostearates, and Titanium Salicylate

- Use concentration data
- Method of manufacture and impurities
- 28-day dermal toxicity data; depending on the results of this study, additional systemic toxicity data may be needed
- Genotoxicity data
- Skin irritation and sensitization data at cosmetic use concentrations

Vinylpyrrolidone Polymers

The Panel reviewed the safety of the following ingredients for the first time at this meeting, and issued an IDA for the following 30 vinylpyrrolidone polymers evaluated in this safety assessment:

VP/Hexadecene Copolymer
VP/Eicosene Copolymer
Acrylates/Stearyl Methacrylate/VP Copolymer
Acrylic Acid/VP Crosspolymer
Butylated PVP
Ethylhexyl Acrylate/VP/Dimethicone Methacrylate Copolymer
Ethylhexyl Methacrylate/Methyl Methacrylate/VP Copolymer
Hydrolyzed Wheat Protein/PVP Crosspolymer
Maltodextrin/VP Copolymer
PVP/Decene Copolymer
PVP/VA/Itaconic Acid Copolymer
PVP/VA/Vinyl Propionate Copolymer
Triacontanyl PVP
Triacontene/VP Copolymer
Vinyl Caprolactam/VP/Dimethylaminoethyl Methacrylate Copolymer
VP/Acrylates/Lauryl Methacrylate Copolymer
VP/Dimethiconylacrylate/Polycarbamyl/Polyglycol Ester
VP/Dimethylaminoethylmethacrylate/Polycarbamyl Polyglycol Ester
VP/DMAPA Acrylates Copolymer
VP/Polycarbamyl Polyglycol Ester
VP/Vinyl Alcohol Copolymer
VP/Vinyl Caprolactam/DMAPA Acrylates Copolymer
Acrylates/VP Copolymer*
Ammonium Acryloyldimethyltaurate/VP Copolymer*
Methacrylic Acid/Styrene/VP Copolymer*
PVP*
Sodium Acryloyldimethyltaurate/VP Crosspolymer*
Styrene/VP Copolymer*
VP/Dimethylaminoethylmethacrylate Copolymer*
VP/VA Copolymer*

**Previously reviewed by the Panel*

The Panel issued the following data requests on this group of ingredients:

- Method of manufacture
- Impurities data

The Panel noted that a representative ingredient for each type of monomer composition would be sufficient for the group. It was also noted that it would be useful to know whether or not the product of each manufacturing process is an emulsion or solid, pure polymer. Furthermore, it would be useful to know the molecular weight range for each ingredient.

Most of these vinylpyrrolidone polymers have the reported film former function in cosmetics in common, and viscosity increasing agent and binder are two other functions that are frequently reported. According to VCRP data received in 2018, the greatest use frequency is reported for PVP, which is used in 900 cosmetic products (798 leave-on products + 101 rinse-off products + 1 product diluted for bath use). The second highest use frequency (597 cosmetic products: 525 leave-on products + 62 rinse-off products) is reported for Ammonium Acryloyldimethyltaurate/VP Copolymer. The results of a concentration of use survey conducted by the Council in 2017 indicate that the highest maximum ingredient use concentration of vinylpyrrolidone polymers in leave-on products is reported for PVP, which is reported to be used at concentrations up to 35% in leg and body paints. Notably, in 2013, the highest maximum use concentration of PVP in leave-on products was only 12%.

Tabled

Polyaminopropyl Biguanide

The Panel tabled the draft final report, pending receipt of the following data that are needed for completion of this safety assessment:

- Human repeated insult patch test (HRIPT) on Polyaminopropyl Biguanide involving a diverse population (i.e., one with a range of Fitzpatrick skin types) of 100 subjects tested with doses of 500 μg/cm^2 and of 1,000 μg/cm^2
- Consumer use data on pump and propellant hair sprays, for use in determining the extent of exposure to Polyaminopropyl Biguanide during product use

The decision to table the safety assessment was based on an oral commitment from the Council (at this meeting) to provide these data. The expectation is that the data will be made available this winter or early in 2019.

The following rationale for requesting the HRIPT was stated in the announcement of the September 2017 Panel meeting results, and, as determined by the Panel, remains valid: The QRA for contact dermatitis with Polyaminopropyl Biguanide in cosmetics yielded a no expected sensitization induction level (NESIL) of 1,000 μg/cm^2, which supports the use of this ingredient at concentrations of ≤ 0.1%. Among the human data that were used to derive the NESIL was an HRIPT involving 26 subjects tested with 1% Polyaminopropyl Biguanide at a dose of 1,000 μg/cm^2, the highest non-sensitizing dose in relation to all of the HRIPT data that were considered. The Panel noted the small subject population in this HRIPT (≥ 100 subjects usually preferred). Furthermore, in an HRIPT on a neck cream containing 0.2% Polyaminopropyl Biguanide (dose = 100 μg/cm^2) that involved more than 100 subjects, faint, pink reactions were observed at various times during challenge or during induction and the skin types evaluated were not sufficiently diverse. Based on these observations, the Panel suggested that the NESIL of 1,000 μg/cm^2 may not be correct and determined that an HRIPT (100 subjects) on Polyaminopropyl Biguanide at doses of 500 and 1,000 μg/cm^2 is needed.

Similarly, the following rationale for requesting consumer use data was also announced previously and, as determined by the Panel, remains valid: The ConsExpo Web Spray Model (used to estimate the inhalation exposure concentrations of Polyaminopropyl Biguanide) and a no observed adverse effect concentration (NOAEC) (from a 28-day inhalation study in which rats were exposed, nose only, to Polyaminopropyl Biguanide in an aerosolized water solution (6 h/day, 5 days/week)) were used in the margin of safety (MOS) calculations. MOS values for pump hair sprays (MOS = 11) and propellant hair sprays (MOS = 200) were calculated. Exposure concentrations that would yield an MOS of 100 for propellant and pump hair sprays were also calculated. After reviewing this risk assessment, the Panel noted that the exposure scenario in the 28-day inhalation study is not representative of pump and propellant hair spray product use and determined that consumer use data on these product types are needed. Furthermore, at this meeting, the Panel emphasized their concern over multiple exposures (as well as the duration of exposure) to cosmetic products containing Polyaminopropyl Biguanide daily, whereby inhalation is a potential route

of exposure. Also, in the absence of consumer use data, the Panel speculated that brief inhalation exposure to these products would not be a major concern. The Panel agreed that the consumer use data would be needed in order to accurately calculate an inhalation MOS (using the exposure dose).

Other Items:

Hair Dye Epidemiology Resource Document

The Panel reviewed the latest draft of the Hair Dye Epidemiology Resource Document. The previous draft was reviewed by the Panel at the December 2017 meeting. At that meeting, the Panel requested that the services of an expert epidemiologist be retained, with experience specifically relevant to factors associated with breast cancer. Explicitly, this expert would be asked to evaluate all of the currently available epidemiology studies that investigated the potential association between hair dye use and breast cancer, reconcile the disparities in the results of those studies, and provide the Panel with a concise summary for inclusion in this Resource document. Dr. Luigi Naldi, Director of the Department of Dermatology, San Bortolo Hospital in Vicenza, Italy, obliged the Panel's request. Dr. Naldi's analyses, as well as three recently discovered studies, were added to the document.

The Panel noted that further investigations are warranted to determine whether hair dye use increases breast cancer risk, including studies that unravel the mechanism, if any, under which particular chemical components of hair dyes potentially promote carcinogenesis. At this meeting, the Panel requested that Dr. Naldi comment on the two newly discovered studies on the potential breast cancer-hair dye association, and provide a clarifying statement on the types of further investigations that are necessary to examine the association between hair dye use and the incidence of breast cancer.

The Panel also suggested that the Resource Document be reformatted. This document will be brought before the Panel once more before finalization.

Final 2019 Priorities

The CIR Procedures require preparation of the Draft 2019 Priority List for public comment by June 1, 2018. The Draft 2019 Priority List was prepared and issued for public comment earlier in the process (at the March 2018 meeting) to allow more time for the acquisition of data. The list is based on stakeholder requests; frequency of use data (FOU) from FDA's Voluntary Cosmetic Registration Program (VCRP) received from the FDA on February 5, 2018; and on CIR staff and Panel workflow.

Comments were received and incorporated in the Draft Final 2019 Priority List. At this meeting, the Panel assessed the potential twenty-six groupings covering 187 ingredients, including 3 ingredients proposed for cause, and the rest of the ingredients included based on FOU. The list of lead ingredients is below and the full priorities with groupings are available here https://www.cir-safety.org/supplementaldoc/2019-priority-list-ingredient-groups-finalized.

Per interest	*Frequency of Use (FOU)*
BENZISOTHIAZOLINONE – a potential preservative	not reported in 2018 VCRP (6 uses in 2015)
CAPRYLHYDROXAMIC ACID – per Finnish Study	147
BASIC BROWN 17 - Hair Dye	45
Per FOU	
HONEY	949
SACCHARUM OFFICINARIUM (SUGARCANE) EXTRACT	406
EQUISETUM ARVENSE EXTRACT	369
SACCHARIDE ISOMERATE	365
PORTULACA OLERACEA (PURSLANE) EXTRACT	363
UBIQUINONE	343
DIATOMACEOUS EARTH	337
SODIUM LEVULINATE	331
GLUCONOLACTONE	329
ACETYL HEXAPEPTIDE-8	318
CALCIUM SULFATE	317
HONEY EXTRACT	306 (to be grouped w/Honey)
CHONDRUS CRISPUS (CARRAGEENAN) EXTRACT	299
ROSA DAMASCENA (DAMASK ROSE) FLOWER OIL	298
SALVIA OFFICINALIS (SAGE) LEAF EXTRACT	292
ROSA DAMASCENA (DAMASK ROSE) FLOWER WATER	289
DICAPRYLYL ETHER	288
PEG/PPG-8/3 DIISOSTEARATE	277
POLYQUATERNIUM-51	274
DIACETONE ALCOHOL	268
ACETYL GLUCOSAMINE	265
POLYQUATERNIUM-6	265
OLEA EUROPAEA (OLIVE) LEAF EXTRACT	257

ALUMINUM STARCH OCTENYLSUCCINATE

CONCLUSION: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) published the Final Report on the Safety Assessment of Aluminum Starch Octenylsuccinate in 2002.[1] Since then some new data, including methods of manufacture, were identified in the published literature.[2-5] The Panel reviewed updated information regarding product types and ingredient use frequencies provided by the Food and Drug Administration (FDA)[6] and maximum use concentrations provided by the Personal Care Products Council (Table 1).[7] The Panel reaffirmed the original conclusion of safe as used in cosmetic formulations, provided that established limitations imposed on heavy metal concentrations are not exceeded.

DISCUSSION: The reported frequency of use, according to Voluntary Cosmetic Registration Program (VCRP) survey data received from the FDA, of Aluminum Starch Octenylsuccinate in cosmetics has increased since safety was originally reviewed; 172 uses were reported 1998,[1] and 786 uses were reported in 2018.[6] However, the use concentration in leave-on products has remained the same (30%).[1,7] The number of uses in formulations with intentional application near the eye area increased from 11 to 138, but the maximum concentration of use reported for this type of exposure decreased from 30% to 15.3%.

The Panel also noted the use of Aluminum Starch Octenylsuccinate in cosmetic sprays which could possibly be inhaled. The concentration of use in spray formulations has increased since the original review, from 2% to 22.9% in "other" fragrance preparations. 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 < 10 µm compared with pump sprays.[8-11] Also, the Panel acknowledged the use of Aluminum Starch Octenylsuccinate that is reported in powders, which could also result in possible inhalation. Concentration of use in powder products has also increased since the original safety review, from 15% to 30% in face powder formulations. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[12-14]

As in the original assessment, the Panel recognizes the absence of data indicating concentrations of toxic metals that can be found as contaminants in this ingredient.[1] The Panel limited the concentrations of the toxic metals in cosmetic-grade Aluminum Starch Octenylsuccinate to the same concentrations as have been established for food-grade modified starches. The Panel stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulation.

The Panel determined not to re-open this safety assessment and confirmed that Aluminum Starch Octenylsuccinate is safe as used in cosmetic formulations (Table 1), provided that established limitations imposed on heavy metal concentrations are not exceeded.

Table 1. Current and historical frequency and concentration of use according to duration and exposure

	# of Uses		*Max Conc of Use (%)*	
	2018[6]	**1998[1]**	**2018[7]**	**1998/1999[1]**
Totals*	**786**	**172**	**0.087-30**	**30**
Duration of Use				
Leave-On	*744*	*158*	*0.087-30*	*0.5-30*
Rinse-Off	*42*	*12*	0.86-6.7	*1-6*
Diluted for (Bath) Use	*NR*	*2*	NR	*NR*
Exposure Type				
Eye Area	138	11	0.9-15.3	0.5-30
Incidental Ingestion	32	9	7.4-12.9	15
Incidental Inhalation-Spray	14; 122[a]; 115[b]	3; 13[a]; 17[b]	0.1-22.9; 2-2.5[a]; 26[b]	2; 1-5[a]; 0.5-10[b]
Incidental Inhalation-Powder	71;115[b]; 1[c]	55; 17[b]	15-30; 26[b]	1-15; 0.5-10[b];
Dermal Contact	699	159	0.087-30	0.5-30
Deodorant (underarm)	4[a]	NR	NR	4[a]
Hair - Non-Coloring	35	2	1.1-11.4	NR
Hair-Coloring	13	NR	0.1-6.7	NR
Nail	NR	NR	NR	NR
Mucous Membrane	34	18	7.4-12.9	15
Baby Products	1	NR	NR	NR

*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.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays..

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders

NR – no reported use

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Bart Heldreth, Executive Director
Date: August 29, 2018
Subject: Strategy Memo for the Safety Assessment of Methylisothiazolinone/Methylchloroisothiazolinone as Used in Cosmetics

The CIR "Final Report on the Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone" was published in 1992. The conclusion stated that "Methylisothiazolinone/Methylchloroisothiazolinone may be safely used in "rinse-off" products at a concentration not to exceed 15 ppm and in "leave-on" cosmetic products at a concentration not to exceed 7.5 ppm. The stated safe use concentration refers to a mixture containing 23.3% Methylisothiazolinone and 76.7% Methylchloroisothiazolinone."

While the safety assessment for Methylisothiazolinone (MI), alone, was published in 2010, and re-addressed in 2014, the combination use of MI and Methylchloroisothiazolinone (MCI) is overdue for re-review. (Reminder: CIR was informed that MCI is not used alone in cosmetics without MI.) At the time of the 1992 publication, there were 381 uses of this preservative combination. Current data, obtained from the FDA VCRP in 2018, indicate this preservative is now used in 4595 formulations in the US. Accordingly, a maximum concentration of use survey will be requested and a re-review document will be prepared for the Panel's consideration to re-open (likely for the March 2019 meeting).

However, this strategy memo is being issued in advance to obtain Panel input and direct the CIR staff towards information sought in that re-review document. In contrast to the conclusion issued for the MI/MCI combination use, the conclusion for MI, alone, is "safe for use in rinse-off cosmetic products at concentrations up to 100 ppm and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a QRA." ***Consequently, if the Panel is likely to revise their conclusion for the use of the MI/MCI ingredient combination to include the caveat determination by a quantitative risk assessment (QRA), what information / data / calculations (e.g., margin of safety (MOS)) would be most useful?***

Secondly, a recent article, "Risk Assessment of the Skin Sensitization Induction Potential of Kathon CG in Rinse-off and Leave-on Personal Care and Cosmetic Products," Towle et al., *Dermatitis,* May/June 2018 - Volume 29 - Issue 3 – pp 132–138, is directed at the risk of skin sensitization resulting from use of the MI/MCI combination ("Kathon CG" is a tradename mixture comprising MI/MCI, magnesium salts, and water). ***Would the Panel please comment on the merits/utility of this assessment (e.g., do you agree with the no effect sensitization induction level (NESIL) the authors chose therein)?***

No vote is required for this administrative item; neither does a decision to re-open need to be made at this meeting. We are merely seeking your expert input in advance of document preparation.

Included herein, please find reports and article referenced above:
MI-MCI_1992_CIR (1992 CIR publication re: MI/MCI combination use)
MI_2010_CIR (2010 CIR publication re: MI alone)
MI_2014_CIR (2014 CIR Final Report re: MI alone)

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Final Report of the Safety Assessment of Methylisothiazolinone

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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
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[3] Cosmetic Ingredient Review Director

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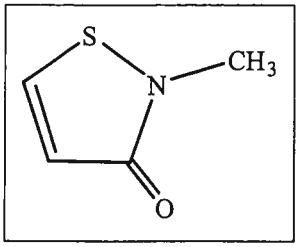

Figure 1. Methylisothiazolinone.

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 $\geq$ 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 $\geq$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.

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Amended Safety Assessment of Methylisothiazolinone as Used in Cosmetics

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ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of methylisothiazolinone (MI), which functions as a preservative. The Panel reviewed relevant animal and human data provided in this safety assessment, and concluded that MI is safe for use in rinse-off cosmetic products at concentrations up to 100 ppm and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a quantitative risk assessment (QRA).

INTRODUCTION

In 2010, the Panel published a 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 were 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 additional data.

In June 2014, the Panel reviewed the results of QRAs performed by Cosmetics Europe and the CIR Science and Support Committee (CIR SSC). The results supported the safety of the use of MI in rinse-off product categories at concentrations up to 100 ppm. However, the QRAs indicated that MI use in many leave-on product categories would be safe only at lower concentrations. The Panel issued a tentative amended safety assessment for public comment with the conclusion that MI is safe for use in rinse-off cosmetic products at concentrations up to 100 ppm and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a QRA.

The Panel previously reviewed the safety of the mixture MCI/MI (sold at a ratio of 3:1; trade names include 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]

Data from the original MI safety assessment report, which was finalized in 2008 and published in 2010, are summarized in *italics* in each appropriate section of this report.

CHEMISTRY

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

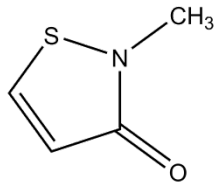

Figure 1. Methylisothiazolinone

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 from the Food and Drug Administration (FDA) 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] It should be noted that the information from the VCRP in 2007 did not clearly distinguish cosmetic products in which MI was used in combination with MCI from products in which MI was used without MCI. This safety assessment addresses the use of MI in cosmetic products that do not also contain MCI. In 2008, 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 2014, the VCRP database indicated that MI is used as an ingredient in 745 cosmetic products that do not also contain MCI, with the majority of the uses reported in leave-on products such as skin moisturizers.[4] A survey of use concentrations conducted by the Personal Care Products Council (Council) in 2014 reported a maximum concentration of use range of 3.5×10^{-8}% to 0.01%, with 0.01% reported in multiple product categories including eye makeup remover, hair shampoos and conditioners, and skin care products (both leave-on and rinse-off).[5]

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) recently released an updated opinion on the use of MI.[10] It states 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 recommended that concentrations up to 0.0015% (15 ppm) MI are safe, in terms of the potential for induction of contact allergy, but stated that there is no information available to evaluate the potential for this ingredient to elicit contact allergy. Furthermore, the SCCS opinion states that MI should not be added to cosmetic products that contain MCI/MI.

Cosmetics Europe, the personal care products industry trade association in Europe, has recommended the discontinuation of MI specifically in leave-on skin products, including wet wipes.[11]

Non-Cosmetic

The non-cosmetic uses of MI include use in water-based paints, which has been noted in a number of case studies of sensitization reactions (e.g., see Table 3). The uses of MI in paints and other non-cosmetic products were 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 0.026 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-hydroxyl-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 on body lotion, shampoo, and sunscreen formulations in rats 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 when 97.5% MI was administered to rats in drinking water for 13 weeks at concentrations of 0, 75, 250, or 1000 ppm. 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 [and malonic acid (MA), up to 220 ppm and 44 ppm in the diet, respectively]* *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* [up to 500 ppm NMMA and 100 ppm MA]* *in their diets for 3 months had no systemic toxicity.*

*Bracketed text presents corrections to the original report

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 MCI/MI tested up to 300 ppm was not a carcinogen.

GENOTOXICITY

MI (up to 1000 µg/plate) and the metabolite NMMA (up to 5000 µg/plate) 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 (up to 300 µM) 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 (up to 3.0 µM) 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 [neat] *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 one local lymph node assay (LLNA) indicated that 99.8% MI produced sensitization at >10,000 ppm. In one local lymph node assay (LLNA), the EC_3 for MI was calculated to be 25,150 ppm. In another LLNA, the calculated EC_3 was 0.86% (8600 ppm). In a study using both the LLNA and cytokine profiling to assess MI, the EC_3 for MI diluted in acetone/olive oil was 0.4% (4,000 ppm), and it was 2.2% (22,000 ppm) when diluted in propylene glycol (a moderate skin allergen); however the cytokine profile of 0.5% MI in acetone/olive oil was not typical for respiratory allergens and the authors concluded that MI was not likely 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.

A letter to the editor reporting the re-evaluation of published LLNA data indicated that MI should be categorized as a strong sensitizer and not a moderate sensitizer, in contrast to previous reports.[12] The earlier reports incorrectly reported 1.9% as the EC_3 for MI; the correct value is 0.4%, which is the lowest EC_3 estimated from multiple LLNAs using, for example, an acetone/oil vehicle.

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), 32% of the subjects with known sensitivity to MCI/MI reacted 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 at concentrations of 200, 300, 400, 500, or 600 ppm concluded that MI up to 600 ppm was not a dermal sensitizer.

MI was named the Allergen of the Year for 2013 by the American Contact Dermatitis Society because of the increasing frequency of use of this preservative in consumer products and the increasing incidences of contact allergy reported to be associated with exposures to MI, especially in the European Union.[13-16] The standard series of patch testing includes exposures to 100 ppm MCI/MI mixture (3:1 ratio). This test may miss up to 40% of subjects with contact allergy to MI, alone, because of the relatively low MI concentration in the MCI/MI mixture tested (approximately 25 ppm MI in a 100 ppm MCI/MI test solution).[17,18] Recommendations have been made to test for contact allergy to MI alone, although there currently is no consensus about the concentration of MI that should be used in such testing.[13,19-24]

The dose-response relationship of contact allergy to MI was investigated in 11 MI-allergic patients.[25] 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 10% ethanol and 90% aqua. (Phenoxyethanol may increase antimicrobial efficacy of MI and was tested to determine if it influenced reactivity to MI). 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 µg MI/cm^2. Controls (n=14) who were not MI-allergic patients 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 of the test solution from 4 different bottles twice a day to four 3 cm^2 areas of the volar forearm. 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 that 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 control subjects. 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.

In a HRIPT of 226 subjects performed in accordance with the International Contact Dermatitis Research Group (ICDRG) criteria for MI, 56 subjects received 100 ppm MI alone and the remaining 170 subjects received 100 ppm MI in combination with various glycols that are used as preservative boosters.[26] No evidence of induced allergic contact dermatitis was observed in any of the subjects, with or without glycols. The study concluded that 100 ppm MI does not cause a risk in cosmetic products when applied on uncompromised skin in the general population.

QUANTITATIVE RISK ASSESSMENT

Both Cosmetics Europe and the CIR SSC conducted QRAs, assuming 100 ppm (0.01%) MI in many categories of cosmetic products, in response to the increased incidences of contact sensitization to MI in Europe.[27,28] Both of these QRAs were conducted using the same no expected sensitization induction level (NESIL = 15 µg/cm^2/day) and sensitization assessment factors (SAFs).

Table 2 summarizes the QRA conducted by the CIR SSC. A conservative NESIL of 15 µg/cm^2/day was derived for MI based on a weight-of-evidence (WoE) evaluation of data from 5 HRIPTs and 4 LLNAs. The NESIL was then used to calculate acceptable exposure levels (AELs) for the potential for the induction of sensitization from dermal exposure to MI in cosmetic products, assuming the maximal use concentration of 100 ppm MI and product-category-specific SAFs. The ratio of the AEL and the consumer exposure level (CEL) was then calculated for each of many cosmetic product categories, ranging from hair conditioners (CEL = 0.02 µg/cm^2/day) to lipsticks (CEL = 1.15 µg/cm^2/day). The concentration of an ingredient is considered to be acceptable in a product when AEL/CEL $\geq$ 1 (i.e., AEL $\geq$ CEL).

According to the Cosmetics Europe calculations the lowest estimated CEL to MI was 0.0011µg/cm^2/day for shower gel, and the highest estimated exposure was 2.27 µg/cm^2/day for a nail varnish. The AEL/CEL ratios indicated that concentrations of MI up to 100 ppm (0.01%) would be acceptable for 20 of the 42 categories assessed by Cosmetics Europe and for 27 of the 60 categories assessed by the CIR SSC.

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 reported 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.

A sampling of case reports and retrospective and multicenter studies reporting MI allergy are summarized in Tables 3 and 4, respectively. Numerous reports of contact allergy, particularly to toilet wipes and water-based

wall paint containing MI, have been reported.[30-38] Incidences of contact allergy to MI, tested separately from MCI/MI, appear to be increasing in Europe in recent years.[39-50]

SUMMARY

In 2010, the Panel published the final report of the safety assessment of 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 newly provided clinical data indicating a higher than expected frequency of individuals who have allergic reactions to the preservative MI. This summary only contains newly identified information on the MI. The original report should be consulted for the information that was previously reviewed by the Panel.

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 4×10^{-6}% to 0.01%, with 0.01% reported in leave-on and rinse-off baby, non-coloring hair, and dermal contact products. The information obtained from the VCRP in 2007 did not clearly distinguish cosmetic products in which MI was used in combination with MCI from cosmetic products in which MI was used without MCI. This safety assessment addresses the use of MI in cosmetic products that do not also contain MCI. In 2014, the VCRP database indicated that MI was used as an ingredient in 745 cosmetic products that do not also contain MCI, with the majority of the uses reported in leave-on products such as skin moisturizers. A survey of use concentrations conducted by the Council in 2014 reported a maximum concentration of use range of 3.5×10^{-8}% to 0.01%, with 0.01% reported in multiple product categories including eye makeup remover, hair shampoos and conditioners, and skin care products (both leave-on and rinse-off).

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 to evaluate the potential for this ingredient to elicit contact allergy. Furthermore, the SCCS states that MI should not be added to cosmetic products that contain MCI/MI.

A re-evaluation of the LLNA results reported in the published literature in an editorial article indicates that MI should be categorized as a strong sensitizer, and not a moderate sensitizer as previously reported.

MI was named 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. A standard series of patch testing includes the mixture MCI/MI, which may miss 40% of contact allergy to MI alone due to the relatively 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 about the concentration of MI that 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. In a HRIPT of 100 ppm MI, with or without various glycols, no evidence of induced allergic contact dermatitis was observed in any of the subjects.

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.

Cosmetics Europe and the CIR SCC conducted QRAs of MI in response to the increased incidences of contact sensitization to MI in Europe. The QRA, which used a conservative NESIL of 15 µg/cm^2/day that was derived based on a WoE evaluation of data from 5 HRIPTs and 4 LLNAs, predicted that consumer exposures to 100 ppm MI in skin leave-on products and cosmetic wet wipes could induce skin sensitization, while exposures to the same concentration in rinse-off products and hair care leave-on products would not induce skin sensitization.

DISCUSSION

The Panel noted the numerous reports of contact allergy to MI in Europe and the increased incidences of contact allergy to MI observed in their own clinical experience. The Panel also noted that MI was named Allergen of the Year for 2013 by the American Contact Dermatitis Society because of the increasing incidence of contact allergy associated with the increasing use of this ingredient as a preservative in cosmetics. The Panel reviewed the results of QRAs performed by Cosmetics Europe and the CIR Science and Support Committee using an appropriate NESIL (i.e., 15 µg/cm^2/day) selected based on a WoE evaluation of EC_3 values from LLNAs and the results of

HRIPTs. The results supported the safety of the use of MI in rinse-off product categories at concentrations up to 100 ppm. However, the QRA indicated that MI use in many leave-on product categories would be safe only at concentrations lower than 100 ppm. As shown in Table 2, for example, the AEL/CEL calculated for 100 ppm (0.01%) MI in baby wipes was 0.13, which the Panel recognizes to be consistent with the reports of increasing incidence of contact allergy associated with the use of MI in wet wipes.

Based on the QRA results, the Panel felt that the current limitation of 100 ppm supported the safety of MI in rinse-off products. Nonetheless, they felt that leave-on products should be formulated to contain MI concentrations below 100 ppm and to be non-sensitizing, as demonstrated, for example, by QRA estimates of safe exposures (typically expressed in $\mu g/cm^2/day$) for the relevant cosmetic product category.

The risk of inducing sensitization depends on the dose of MI per unit area of the skin exposed (e.g., expressed in units of $\mu g/cm^2/day$). One type of cosmetic product will differ from another in the potential to cause sensitization at a given MI concentration if they differ substantially in application rate, which depends on the amount of product applied per day and the total surface area of the skin to which the product is applied. This helps to explain why the risks associated with MI in rinse-off products are less than those associated with leave-on products and, for instance, why the risks associated with exposures to MI in leave-on hair conditioners would likely be substantially lower than those associated with MI in wipes.

It is important to note that appropriate exposure assumptions used in a QRA can vary depending on factors such as differences in regional habits and practices, properties of the formulation, and degree to which conservative default assumptions and exposure scenarios may be refined based on specific exposure data. The Panel stressed the importance of clearly identifying and justifying the exposure assumptions, and the sources of the assumptions, used in any QRA that might be conducted to predict concentrations of MI unlikely to induce sensitization from the use by consumers of a specific cosmetic product or product category.

The Panel determined that the maximum MI concentration should never exceed 100 ppm (0.01%) in any hair product, leave-on product, or rinse-off product, based on the potential for inducing sensitization and concentrations greater than 100 ppm.

The Panel's recommendations for MI in rinse-off and leave-on cosmetic products are intended to prevent the induction of sensitization to MI. The Panel cautioned that following these recommendations may not necessarily prevent the elicitation of allergic reactions in individuals who are already allergic to MI. Individuals sensitized to MI should avoid products that contain MI.

The Panel discussed the issue of incidental inhalation exposure to MI in non-coloring hair sprays and hair tonics or dressings. There were no chronic inhalation toxicity data identified or provided. MI reportedly is used at concentrations up to 0.01% in cosmetic products that may be aerosolized. The Panel noted that 95% – 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. Coupled with the small actual exposures expected in the breathing zone and the absence of significant signs of toxicity in subchronic, chronic, and reproductive and developmental animal studies reviewed previously by the Panel, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

CONCLUSION

The CIR Expert Panel concluded that MI is safe for use in rinse-off cosmetic products at concentrations up to 100 ppm and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a QRA.

TABLES

Table 1. Historical and current use and concentration of use data for methylisothiazolinone.[1,4,5]

	# of Uses		*Max Conc of Use (%)*	
Data Year	**2007***	**2014****	**2007**	**2014**
Totals†	**1125**	**745**	**4×10^{-6}-0.01**	**3.5×10^{-8}-0.01**
Duration of Use				
Leave-On	236	478	0.002-0.01	3.5×10^{-8}-0.01
Rinse-Off	807	260	4.0×10^{-6}-0.01	2.5×10^{-7}-0.01
Diluted for (Bath) Use	82	7	NR	0.0002-0.01
Exposure Type				
Eye Area	6	22	NR	0.00019-0.01
Incidental Ingestion	NR	1	NR	0.0048
Incidental Inhalation-Spray	4; 86[a]; 54[b]	3; 268[a]; 114[,b]	0.005; 0.008-0.009[a]	0.0002-0.01[a]; 0.0002-0.01[c]
Incidental Inhalation-Powder	1; 2[d]	114[b]	NR	NR
Dermal Contact	469	544	0.0008-0.01	3.5×10^{-8}-0.01[e,f]
Deodorant (underarm)	2[a]	NR	NR	0.0095[g]
Hair - Non-Coloring	579	190	4.0×10^{-6}-0.01	4.0×10^{-6}-0.01
Hair-Coloring	76	NR	NR	5.6×10^{-5}-0.0095
Nail	1	5	NR	0.0002-0.006
Mucous Membrane	241	103	0.0015-0.01	9.0×10^{-7}-0.01
Baby Products	14	6	0.002-0.01[h]	0.0002-0.0075

* Data provided are not clear as to whether uses are MI alone or include uses of MI/MCI.
** Data provided are for uses of MI alone.
NR = Not reported
†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.
a. Includes products that can be sprays, but it is not known whether the reported uses are sprays.
b. Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
c. 0.01% 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.
d. Includes products that can be powders, but it is not known whether the reported uses are powders.
e. 0.00023-0.01% in a hand soap; 0.01% in a foot scrub.
f. The Council survey requested that wipe products be identified. One product containing MI was identified as being used as a skin cleansing wipe at a concentration of 0.005%.
g. Not a spray deodorant.
h. 0.01% in baby wipes.

Table 2. Quantitative risk assessment of methylisothiazolinone (MI) at highest maximum use concentration (100 ppm) in cosmetic products.[28]

Product Category*	Product Amount Applied / day (µg/cm^2)	Consumer Exposure Level (CEL; µg/cm^2/day)	Sensitization Assessment Factor (SAF)	Acceptable Exposure Level (AEL; µg/cm^2/day)**	AEL/CEL
Baby shampoo	200	0.02	100	0.15	7.50
Baby lotions, oils, powders, creams	2200	0.22	300	0.05	0.23
Baby wipes	4000	0.40	300	0.05	0.13
Other baby products (powders and talcs)	4200	0.42	100	0.15	0.36
Other baby products (washes)	200	0.02	100	0.15	7.50
Bath oils, tablets and salts	200	0.02	100	0.15	7.50
Bath soaps and detergents	10	<0.01	100	0.15	150
Bubble baths	200	0.02	100	0.15	7.50
Other bath preparations	200	0.02	100	0.15	7.50
Eyebrow pencil	2200	0.22	300	0.05	0.23
Eyeliners	2170	0.22	300	0.05	0.23
Eye shadow	2170	0.22	300	0.05	0.23
Eye lotion	2170	0.22	300	0.05	0.23
Eye makeup remover	900	0.09	100	0.15	1.67
Mascara	2170	0.22	300	0.05	0.23
Other eye makeup	2170	0.22	300	0.05	0.23
Cologne and toilet waters	17700	1.77	100	0.15	0.08
Blushers	1000	0.10	100	0.15	1.50
Other fragrance products	2200	0.22	100	0.15	0.68
Hair conditioners	200	0.02	100	0.15	7.50
Hair sprays (aerosol fixatives)	1390	0.14	100	0.15	1.08
Hair sprays (pump)	2200	0.22	100	0.15	0.68
Hair straighteners	4200	0.42	100	0.15	0.36
Permanent waves	4200	0.42	100	0.15	0.36
Rinses (noncoloring)	170	0.02	100	0.15	8.82
Shampoos (noncoloring)	170	0.02	100	0.15	8.82
Tonics, dressings and other hair grooming aids	990	0.10	100	0.15	1.52

Table 2. Quantitative risk assessment of methylisothiazolinone (MI) at highest maximum use concentration (100 ppm) in cosmetic products.[28]

Product Category*	Product Amount Applied / day (µg/cm^2)	Consumer Exposure Level (CEL; µg/cm^2/day)	Sensitization Assessment Factor (SAF)	Acceptable Exposure Level (AEL; µg/cm^2/day)**	AEL/CEL
Wave sets	4200	0.42	100	0.15	0.36
Other noncoloring hair products	1000	0.10	100	0.15	1.50
***Hair dyes and colors	1000	0.10	100	0.15	1.50
***Hair tints	990	0.10	100	0.15	1.52
Hair rinses (coloring)	200	0.02	100	0.15	7.50
***Hair bleaches	1000	0.10	100	0.15	1.50
Other hair coloring preparations	1000	0.10	100	0.15	1.50
Face powders	1000	0.10	100	0.15	1.50
Foundations	3170	0.32	100	0.15	0.47
Lipsticks	11460	1.15	300	0.05	0.04
Other makeup preparations	4200	0.42	100	0.15	0.36
Other manicuring preparations	1000	0.10	100	0.15	1.50
Other personal cleanliness products	4400	0.44	300	0.05	0.11
Aftershave lotions	2210	0.22	100	0.15	0.68
Preshave lotions (all types)	2200	0.22	100	0.15	0.68
Shaving cream (aerosol, brushless and lather)	70	0.01	300	0.05	7.14
Shaving soaps (cakes, sticks, etc.)	70	0.01	300	0.05	7.14
Other shaving preparations	2200	0.22	100	0.15	0.68
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	900	0.09	100	0.15	1.67
Depilatories	200	0.02	100	0.15	7.50
Face and neck creams, lotions, powders and sprays	2700	0.27	100	0.15	0.56
Body and hand creams, lotions and powders	1120	0.11	300	0.05	0.45
Moisturizers	2700	0.27	100	0.15	0.56
Nail care creams and lotions	970	0.10	100	0.15	1.55
Deodorants (underarm)	8500	0.85	300	0.05	0.06
Night creams, lotions, powders, and sprays	3170	0.32	100	0.15	0.47
Paste masks (mud packs)	4200	0.42	100	0.15	0.36

Table 2. Quantitative risk assessment of methylisothiazolinone (MI) at highest maximum use concentration (100 ppm) in cosmetic products.[28]

Product Category*	Product Amount Applied / day (µg/cm^2)	Consumer Exposure Level (CEL; µg/cm^2/day)	Sensitization Assessment Factor (SAF)	Acceptable Exposure Level (AEL; µg/cm^2/day)**	AEL/CEL
Skin fresheners	150	0.02	100	0.15	10
Other skin care products	2200	0.22	100	0.15	0.68
Suntan gels, creams, liquids and sprays	2200	0.22	100	0.15	0.68
Indoor tanning preparations	2200	0.22	100	0.15	0.68
Other tanning preparations	2200	0.22	100	0.15	0.68
Foot powders and sprays	2200	0.22	100	0.15	0.68

Shaded rows indicate the ratio of AEL x CEL^{-1} is less than 1.
*Exposure values assumed for each product category were from the IFRA RIFM QRA Information Booklet (2011)[51] and Api et al. (2008)[52]
**Based on No Expected Sensitizing Induction Level (NESIL) of 15 µg/cm^2/day
***Note that this product category may be diluted prior to application

Table 3. 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.	[30]
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.	[30]
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.	[30]
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.	[30]
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.	[30]
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.	[30]

Table 3. 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.	[30]
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.	[32]
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.	[31]
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.	[33]
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.	[34]

Table 3. 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 (workplace) had been painted with a paint that contained MI.	[35]
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.	[35]
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.	[36]
"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.	[37]
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.	[38]

Table 4. 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	[39]
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	[40]
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.	[41]
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.	[42]
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.	[43]
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 (BIT).	[44]

Table 4. 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.	[45]
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.	[46]
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.	[47]
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.	[48]
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.	[49]
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.	[50]

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6

Final Report on the Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone

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

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

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

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

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

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

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

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

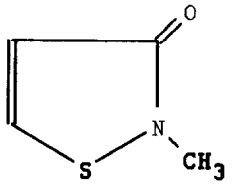
INTRODUCTION

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

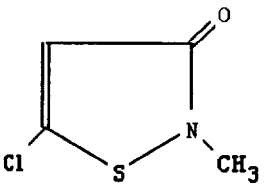
CHEMISTRY

Definition and Structure

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



Methylisothiazolinone



Methylchloroisothiazolinone

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

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

Composition for Cosmetic Use

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

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

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

Properties

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

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

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

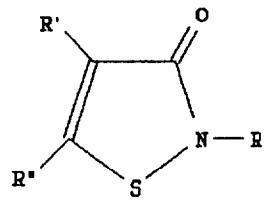
TABLE 1. COMPOSITION, CHEMICAL, AND PHYSICAL PROPERTIES OF MI/MCI-CG[a]

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

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

Source: Ref. 3.

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



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

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

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

Source: Ref. 7.

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

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

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

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

Method of Manufacture/Analytical Methods

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

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

Impurities

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

USE

Cosmetic

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

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

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

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

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

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

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

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

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

Source: Ref. (21).

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

Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators must conform to the format of concentration ranges and product categories as described in Title 21 Part 720.4 of the Code of Federal Regulations.[22] Since certain cosmetic ingredients are supplied to the formulator at less than 100% concentration (in this case a concentration of 1.5%), the concentration reported by the formulator may not necessarily reflect the actual concentration found in the finished cosmetic product; the actual concentration would be a fraction of that reported to the FDA. Data submitted within the framework of a "concentration range" provides opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing a two- to ten-fold error in the assumed ingredient concentration.

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

Noncosmetic

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

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

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

BIOLOGY

Fate in the Environment

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

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

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

Antimicrobial Activity

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

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

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

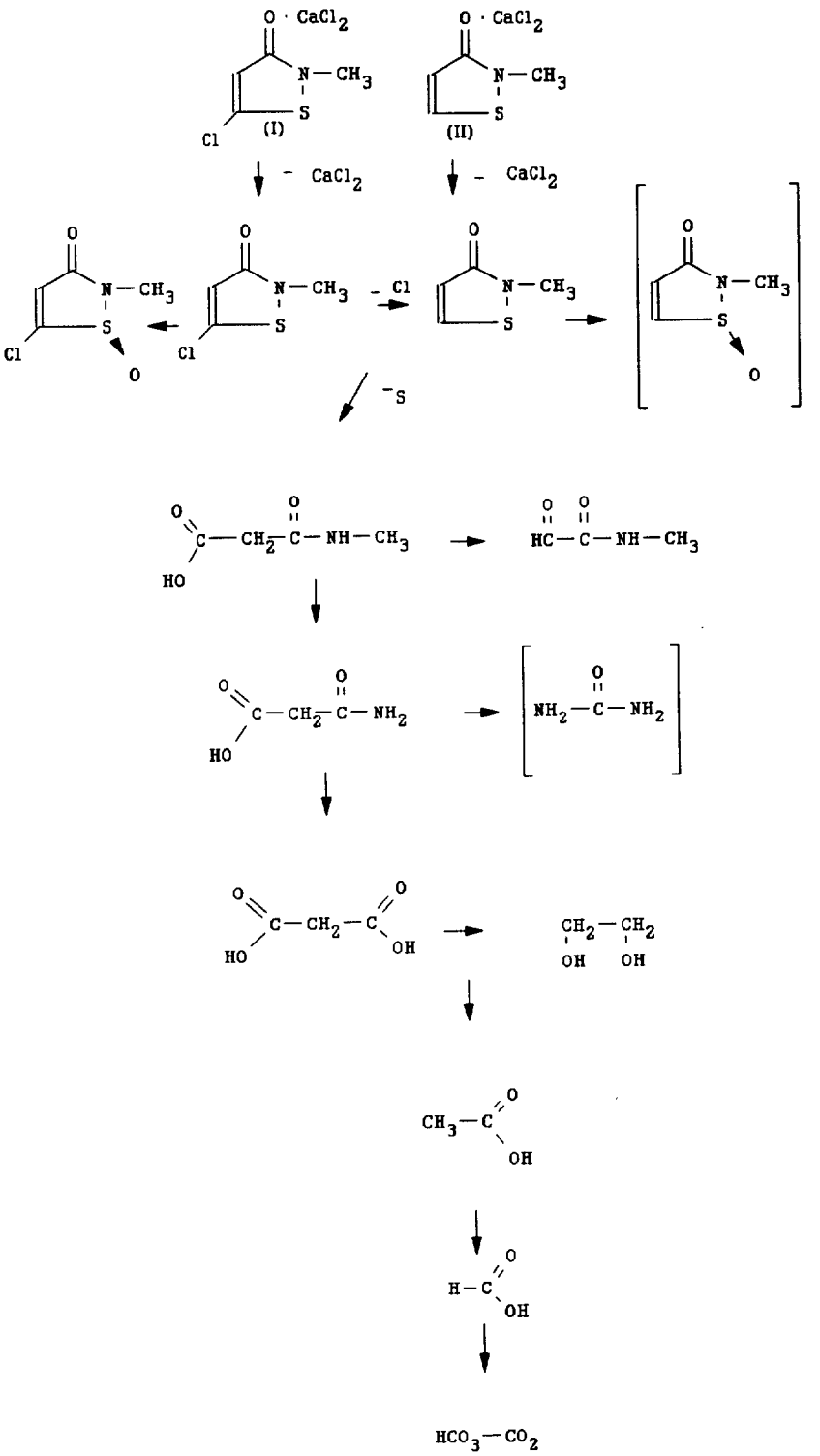

FIG. 1. Major degradative pathway of the calcium chloride salts of Methylchloroisothiazolinone (I) and Methylisothiazolinone (II) (bracketed structures are postulated) Ref. 12.

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

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

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

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

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

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

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

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

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

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

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

TABLE 4. RESULTS OF ABSORPTION STUDY WITH MI/MCI-CG AND MI/MCI-886 IN RATS

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

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

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

[c]ND = Not determined.

Source: Ref. 32.

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

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

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

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

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

TOXICOLOGY

Aquatic and Avian Toxicity

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

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

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

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

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

Acute Toxicity

Oral

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

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

Dermal

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

Intraperitoneal

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

Inhalation

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

Irritation

Chorioallantoic Membrane

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

Ocular

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

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

Dermal

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

Short-Term Toxicity

Oral

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

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

Dermal

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

Inhalation

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

Subchronic Toxicity

Oral

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

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

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

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

Dermal

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

Sensitization, Photosensitization, and Phototoxicity

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

TABLE 5. INCIDENCE OF DELAYED CONTACT DERMATITIS IN GUINEA PIGS INDUCED AND CHALLENGED BY VARIOUS CONCENTRATIONS OF MI/MCI BIOCIDE

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

[a]Dosage volume = 0.4 ml/patch.

[b]a.i. = active ingredients.

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

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

Source: Ref. 5.

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

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

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

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

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

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

GENOTOXICITY

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

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

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

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

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

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

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

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

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

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

TABLE 6. GENOTOXICITY OF METHYLISOTHIAZOLINONE AND METHYLCHLOROISOTHIAZOLINONE

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

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

[a]a.i = active ingredients

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CARCINOGENICITY

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

Teratogenicity and Reproductive Toxicity

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

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

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

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

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation and Sensitization

Predictive Tests

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[a]Study conditions

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Provocative Tests

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

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

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

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

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

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

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

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

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

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

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

TABLE 10. PROVOCATIVE PATCH TEST RESULTS WITH 100 PPM OF MI/MCI-CG[90]

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Photosensitization and Phototoxicity

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

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

SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

No comments were received during the second public comment period.

CONCLUSION

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

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Monice M. Fiume MMF
Senior Director, CIR
Date: August 29, 2018
Subject: Re-Review of the Final Report on the Safety Assessment of Triacetin

In 2003, the CIR Expert Panel published the Final Report on the Safety Assessment of Triacetin. The Panel concluded that Triacetin is "safe as used in cosmetics." According to its Procedures, CIR evaluates the conclusions of previously-issued reports every 15 years. Therefore, this report is being presented for consideration for re-review.

No new ingredients are being proposed for inclusion in this re-review. With the exception of the following data that were found in the ECHA REACH database, no new relevant data were found in the published literature:

- DART study: Combined Repeated Dose Toxicity Study with the Reproduction /Developmental Toxicity Screening Test
  - in accord with OECD Guideline 422, 12 rats/sex received doses of 0 (vehicle; distilled water), 40, 200, and 1000 mg/kg bw/day Triacetin by gavage; males were exposed for 44 days from 2 weeks prior to mating and females were exposed for 41-48 days from 14 days before mating to day 3 postpartum; no maternal toxicity was observed, and there were not fetotoxic or developmental effects; both the maternal and developmental NOAEL was established as 1000 mg/kg bw/day
- Genotoxicity studies:
  - Triacetin was not genotoxic in a mouse lymphoma L5178Y assay, with or without metabolic activation, at doses up to 5000µg/ml
  - In a chromosomal aberration test (OECD TG 473) that was conducted in cultured Chinese hamster lung (CHL/IU) cells, induction of chromosome aberrations was observed in the cultured cells only at the highest concentration (2.2 mg/mL, 10 mM) with metabolic activation; however, the results were negative without activation; due to the high cytotoxicity (74.5 %) under acidic conditions at the highest test substance concentration, the observed chromosomal aberrations were judged to be not biologically relevant.

Updated frequency of use data were obtained from the FDA VCRP database, and concentration of use data were received in response to the Council's survey. These data are attached for your consideration, as is an updated use table (*triace092018FDA, triace092018data,* and *triace092018use tbl,* respectively). The frequency of use of Triacetin in cosmetics increased from 13 to 59 uses, however, the maximum use concentrations decreased, from 4% to 0.95%. Therefore, the only reason to re-open this report would be to reconsider the conclusion.

Because there are no new data, other than the updated use table and the three studies briefly described above, the original report is included for your review (*triace092018orig*), as opposed to a complete re-review document.

Other supporting documents include:
- search strategy (*triace092018strat*)
- minutes from the original deliberations (*triace092018prev min*)

If there is concern about the existing conclusion, the review should be re-opened. Otherwise, the existing conclusion should be reaffirmed, and the report will not be re-opened.

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Current and historical frequency and concentration of use of Triacetin, according to duration and exposure

	# of Uses		*Max Conc of Use (%)*	
	2018[1]	**1998[2]**	**2018[3]**	**1999[2]**
Totals*	**59**	**13**	**0.0000013 – 0.95**	**0.8 - 4**
Duration of Use				
Leave-On	*57*	*13*	*0.0000013 – 0.95*	*0.8 – 2*
Rinse-Off	*2*	*NR*	*0.00012 – 0.08*	*4*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	4	4	0.000025 – 0.008	1-2
Incidental Ingestion	NR	NR	0.00047 – 0.054	NR
Incidental Inhalation-Spray	1; 21[a]; 7[b]	NR	0.0000013 – 0.95; 0.00047 – 0.021[a]	0.8
Incidental Inhalation-Powder	7[b]	NR	0.008 – 0.015[c]	NR
Dermal Contact	34	11	0.000025 – 0.14	1
Deodorant (underarm)	NR	NR	0.13 (not spray) 0.14 (spray)	NR
Hair - Non-Coloring	4	NR	0.0000013 – 0.95	0.8
Hair-Coloring	NR	NR	NR	NR
Nail	20	NR	0.2	1-4
Mucous Membrane	NR	NR	0.00047 – 0.08	NR
Baby Products	NR	NR	NR	NR

*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.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.

[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, there fore the information is captured in both categories

NR – no reported use

1. US Food and Drug Administration (FDA) Center for Food Safety & Applied Nutrition (CFSAN). Voluntary Cosmetic Registration Program (VCRP) - Frequency of Use of Cosmetic Ingredients. College Park, MD: 2018. Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 3 2018; received February 5 2018).

2. Andersen FA (ed). Final Report on the Safety Assessment of Triacetin. *Int J Toxicol*. 2003;22(Suppl 2):1-10.

3. Personal Care Products Council. 6-1-2018. Concentration of Use by FDA Product Category: Triacetin. Unpublished data submitted by Personal Care Products Council on June 1, 2018.

Final Report on the Safety Assessment of Triacetin[1]

Triacetin, also known as Glyceryl Triacetate, is reported to function as a cosmetic biocide, plasticizer, and solvent in cosmetic formulations, at concentrations ranging from 0.8% to 4.0%. It is a commonly used carrier for flavors and fragrances. Triacetin was affirmed as a generally recognized as safe (GRAS) human food ingredient by the Food and Drug Administration (FDA). Triacetin was not toxic to animals in acute oral or dermal exposures, nor was it toxic in short-term inhalation or parenteral studies, and subchronic feeding and inhalation studies. Triacetin was, at most, slightly irritating to guinea pig skin. However, in one study, it caused erythema, slight edema, alopecia, and desquamation, and did cause some irritation in rabbit eyes. Triacetin was not sensitizing in guinea pigs. Triacetin was not an irritant or a sensitizer in a clinical maximization study, and only very mild reactions were seen in a Duhring-chamber test using a 50% dilution. In humans, Triacetin reportedly has caused ocular irritation but no injury. Triacetin was not mutagenic. Although there were no available reproductive and developmental toxicity data, Triacetin was quickly metabolized to glycerol and acetic acid and these chemicals were not developmental toxins. Reports of 1,2-glyceryl diesters, which may be present in Triacetin, affecting cell growth and proliferation raised the possibility of hyperplasia and/or tumor promotion. The Cosmetic Ingredient Review (CIR) Expert Panel concluded, however, that the effects of 1,2-glyceryl diesters on cell growth and proliferation require longer ester chains on the glycerin backbone than are present when acetic acid is esterified with glycerin, as in Triacetin. On the basis of the available information, the CIR Expert Panel concluded that Triacetin is safe as used in cosmetic formulations.

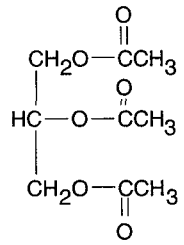
INTRODUCTION

This report reviews the safety of Triacetin, an ingredient that is reported to function as a cosmetic biocide, plasticizer, and solvent in cosmetic formulations (Pepe, Wenninger, and McEwen 2002).

CHEMISTRY

Definition and Structure

Triacetin (CAS no. 102-76-1) is the triester of glycerin and acetic acid that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



Triacetin is also known as Glyceryl Triacetate (Pepe, Wenninger, and McEwen 2002; National Academy of Science [NAS] 1996; United States Pharmacopeial Convention, Inc. [USP] 1995; Unichema Chemie B.V. 1994; Lewis 1993a; Budavari 1989); Glycerol Triacetate (Unichema Chemie B.V. 1994; Lide 1993; Lewis 1993a); Glycerin Triacetate (Unichema Chemie B.V. 1994); Glycerine Triacetate (Lewis 1993a); Triacetyl Glycerine (Unichema Chemie, B.V. 1994; Budavari 1989); Acetin, Tri-; 1,2,3-Triacetoxypropane (Unichema Chemie B.V. 1994); 1,2,3-Propanetriol Triacetate (Pepe, Wenninger, and McEwen 2002; USP 1995; Unichema Chemie B.V. 1994; Lewis 1993a; Budavari 1989); 1,2,3-Propanetriyl Triacetate (ChemID 1998); and Acetic, 1,2,3-Propanetriyl Ester (Pepe, Wenninger, and McEwen 2002; Unichema Chemie B.V. 1994).

Physical and Chemical Properties

Physical and chemical properties are described in Table 1. Published data on the ultraviolet radiation absorbance of Triacetin were not found.

Manufacture and Production

Triacetin is derived by the action of acetic acid on glycerol, with vacuum distillation used as the method of purification (Lewis 1993b). It can also be prepared by esterification of glycerin with acetic anhydride (Gennaro 1990), by the acetylation of glycerol, by the reaction of oxygen with a liquid-phase mixture of allyl acetate and acetic acid using bromide as a catalyst (Budavari 1989), and from glycerin and acetic anhydride

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Monice Zondlo Fiume, former Scientific Analyst/Report Management Coordinator, Address correspondence to F. Alan Andersen, Cosmetic Ingredient Review Director, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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TABLE 1
Chemical and physical properties of Triacetin

		Reference
Physical Characterization	Colorless, oily liquid with a slight fatty odor and taste	Lewis 1993a
	Colorless, somewhat oily liquid having a slight, fatty odor and a bitter taste	NAS 1996; Budavari 1989
Empirical formula	$C_9H_{14}O_6$	Pepe, Wenninger, and McEwen 2002
Molecular weight	218.21	NAS 1996
	218.20	Budavari 1989; Grant 1972
Melting point	−78°C	Lewis 1993a; Budavari 1989
Boiling point	258°C	Lewis 1993a; Grant 1972
	258°C–260°C	Lewis 1993b
	260°C	Gennaro 1990
	258°C–260°C; 172°C (bp_{40})	Budavari 1989
Solubility	Soluble in water; miscible with alcohol, ether, and chloroform	NAS 1996; Lewis 1993a
	Soluble in acetone, ethanol, benzene, and chloroform	Unichema Chemie B.V. 1994
	Soluble in alcohol, ether, acetone, benzene, and chloroform	Lide 1993
	Slightly soluble in water; very soluble in alcohol, ether, and other organic solvents	Lewis 1993b
	Soluble in 14 parts water and in alcohol, chloroform, and ether	Gennaro 1990
	Soluble in 14 parts water; miscible with alcohol, ether, chloroform; slightly soluble in carbon disulfide	Budavari 1989
log $P_{o/w}$	0.1, 0.368	Unichema Chemie B.V. 1994
Specific gravity	1.154–1.158 (food-grade)	NAS 1996
	1.152–1.158 (USP-grade)	USP 1995
	1.1596 (20/4)	Lide 1993
	1.161	Lewis 1993a; Grant 1972
	1.160	Lewis 1993b
	1.1562 (d_4^{25}); 1.1596 (d_4^{20}); 1.163 (d_{20}^{20})	Budavari 1989
Index of refraction	1.429–1.431 (25°C) (food-grade)	NAS 1996
	1.429–1.430 (USP-grade)	USP 1995
	1.4301 (20°C)	Lide 1993
	1.4312 (20°C)	Lewis 1993b
	1.4307 (n_D^{20})	Budavari 1989
Stability	Combustible	Lewis 1993b
Reactivity	A polar substance that is easily hydrolyzed with the liberation of acetic acid	Unichema International 1996
Flash point	280°F (COC)	Lewis 1993a; Sax 1979
	300°F	Lewis 1993b
Autoignition temperature	812°F	Lewis 1993a; Sax 1979

using phosphoric acid or zinc chloride as the condensing agent (Opdyke 1978).

Triacetin occurs naturally as an oil from cod-liver oil, butter, and other fats (Grant 1972).

Analytical Methods

Triacetin has been determined using gas chromatography (Ogawa et al. 1988, 1992; Uematsu et al. 1997), thin layer chromatography, and infrared spectroscopy (Ogawa et al. 1992).

Impurities

Triacetin contains trace moisture and acetic acid (Unichema Chemie B.V. 1994). Food-grade Triacetin must be at least 98.5% $C_9H_{14}O_6$, and it must not contain >5 mg/kg heavy metals (as Pb) or >0.2% water (NAS 1996). USP-grade Triacetin must contain not less than 97.0% and not greater than 100.5% $C_9H_{14}O_6$, calculated on the anhydrous basis (USP 1994).

USE

Cosmetic

Triacetin is reported to function as a cosmetic biocide, plasticizer, and solvent in cosmetic formulations (Pepe, Wenninger, and McEwen 2002). It is a commonly used carrier for flavors and fragrances (Unichema International 1996).

As shown in Table 2, the product formulation data reported by the cosmetics industry to the Food and Drug Administration (FDA) in 1998 indicate that Triacetin was used in a total of 13 cosmetic formulations. Concentration of use data submitted by the Cosmetic, Toiletry, and Fragrance Association (CTFA) indicated that Triacetin was used in a number of product types at concentrations of 0.8–4% (CTFA 1999).

According to the Ministry of Health, Labor and Welfare (MHLW) in Japan, Triacetin is not restricted in any manner in cosmetic formulations (MHLW 2001).

Triacetin does not appear in Annex II (list of substances that must not form part of the composition of cosmetic products) or Annex III (list of substances that cosmetic products must not contain except subject to the restrictions and conditions laid down) of the *Cosmetics Directive of the European Union* (European Commission 2003).

TABLE 2
Triacetin use in cosmetic formulations

Product category (total no. formulations in category) (FDA 1998)	Total no. containing ingredient (FDA 1998)	Concentration of use (%) (CTFA 1999)
Eyeliner (514)	2	1
Mascara (167)	2	2
Tonics, dressings, and other hair-grooming aids (549)	—	0.8
Makeup bases (132)	6	1
Other makeup preparations (135)	3	—
Basecoats and undercoats (manicuring preps) (48)	—	1
Nail polish and enamel (80)	—	1
Nail polish and enamel removers (34)	—	4
1998 Triacetin use	13	0.8–4

Noncosmetic

Triacetin is a generally recognized as safe (GRAS) ingredient (21 CFR 184.1901) by the FDA. It is used in foods as a flavoring agent and adjuvant, formulation aid, humectant, and solvent and vehicle; it has no limitations other than good manufacturing practice. In its determination that Triacetin was a GRAS ingredient (FDA 1983, 1989), FDA relied upon an evaluation on glycerin and glycerides prepared by the Federation of American Societies for Experimental Biology (FASEB) that included safety-test data on Triacetin. This FASEB evaluation found that Triacetin was without toxic effects in long-term feeding studies in rats that used doses that were higher than those to which consumers would be exposed (FASEB 1975). In addition, FDA contracted with a laborartory to perform a mutagenic evaluation of Triacetin, the results of which demonstrated no mutagenic activity (Litton Bionetics, Inc. 1976).

Triacetin is used in pharmaceuticals as a hydrophilic plasticizer in polymeric coatings of capsules, tablets, beads, and granules, with typical concentrations of 10% to 35% *w/w* (Unichema International 1996). Triacetin is used as an antifungal drug for treatment of superficial fungus infections of the skin (Gennaro 1990). However, data are inadequate to establish general recognition of the safety or effectiveness of Triacetin as an over-the-counter topical antifungal drug (21 CFR 310.545). Triacetin has been used with prostaglandin E_2 (PGE_2) to form a gel used for preinduction cervical softening (Graves et al. 1985; Noah et al. 1985). According to Yalkowsky and Roseman (1979), Triacetin stabilized PGE.

Triacetin had wide use in foundry applications as a curing agent for phenol resins used in the manufacture of sand molds (Unichema International 1996). It is used as a cellulose acetate plasticizer in the manufacture of cigarette filters, a tobacco humidifier, a plasticizer for cellulose nitrate, and a solvent for basic dyes and in the manufacture of celluloid and photographic films, as well as in the paint, lacquer, and varnish industries (Unichema Chemie B.V. 1994). Triacetin is also used in removal of carbon dioxide from natural gas (Lewis 1993b). According to Budavari (1989), technical Triacetin (a mixture of mono-, di-, and small quantities of Triacetin) is used as a solvent for basic dyes, particularly induline dyes, and tannin in dyeing.

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

In an in vitro study by Stoughton (1970), the penetration of Triacetin into the corium of human skin was determined by topical application of Triacetin to the epidermis (which was later separated from the corium), and then measuring the antimicrobial activity. Using leg skin, a plastic cylinder was attached to the epidermis, and 0.01 cc Triacetin was applied for 20 to 24 h. The skin was then washed and the epidermis removed. Six-millimeter punches of the corium were taken and implanted on the culture medium, with the epidermal side in contact with the medium. When inhibition of growth around the corium

occurred, the radius of inhibition was measured. There was no average inhibition of positive responses with Triacetin cream. The researchers speculated that an antimicrobial agent may penetrate into the corium and not diffuse into the surrounding medium when assayed, and that this might give a false-negative report of its ability to penetrate the epidermis.

von Oettingen (1960) stated that Triacetin is absorbed from the gastrointestinal tract, but no experimental details were provided.

Pharmacological Effects

In a series of studies, the use of Triacetin in total parenteral nutrition was examined.

Six female mongrel dogs were used to determine the effect of Triacetin on serum phosphorus, calcium, and magnesium metabolism (Bailey, Heath, and Miles 1989). A 5% *v/v* aqueous solution of Triacetin was infused intravenously at a rate of 47 μmol/kg/min for 3 h. Arterial blood was sampled at 15 to 30-min intervals, and urine was collected during infusion. No significant changes in total serum calcium or phosphorus concentrations were observed; however, serum magnesium concentrations were statistically significantly decreased 90 min after the initiation of dosing and remained decreased until the end of the study. During Triacetin infusion, the plasma acetate concentration increased from 0.13 to 1.32 mmol/L at 30 min; the concentration gradually declined to ~1 mmol/L during the last hour of the study. No change was observed in the fractional excretion of calcium, magnesium, or phosphorus. The authors speculated that the decrease in serum magnesium was probably because of cellular uptake rather than accelerated excretion. Baseline blood pH was not significantly altered.

Bailey, Haymond, and Miles (1991) also used groups of female mongrel dogs to study the metabolic effects of isocaloric and hypercaloric infusions of 5% *v/v* aqueous Triacetin. A primed, continuous infusion of ~5 μmol/kg (0.3 μCi/kg/min) [^{13}C]-acetoacetate and ~1.0 μCi/kg (0.01 μCi/kg/min) [^{3}H]-glucose was continued for 6 h. Three hours after the start of the isotope infusion, dosing with Triacetin was started. Six animals were infused at a rate of 47 μmol/kg/min and seven were infused at a rate of 70 μmol/kg/min Triacetin for 3 h. Blood and breath samples were taken at 15 to 30-min intervals. A group of four animals was infused with 70 μmol/kg/min glycerol and used as the control for the hypercaloric infusion.

During isocaloric infusion of Triacetin, plasma acetate and free fatty acid concentrations were significantly increased at 30 and 60 min, respectively, and remained elevated. During hypercaloric infusion, plasma acetate concentration increased progressively throughout the study, whereas the plasma free fatty acid concentration did not change. Plasma pyruvate and lactate concentrations were significantly decreased after 30 and 90 min, respectively, and throughout the study with both isocaloric and hypercaloric infusion. The plasma insulin concentrations were modestly increased during both infusions. Plasma glucose concentration was significantly decreased during isocaloric Triacetin infusion; a slight but significant increase was observed with hypercaloric infusion. Glucose clearance decreased significantly in both groups during the last hour of Triacetin infusion. Plasma ketone body concentrations increased significantly by 60 min, and they remained elevated with isocaloric infusion and increased progressively with hypercaloric infusion of Triacetin; the increased concentrations were due to increased ketone body production. During the last hour of infusion, resting energy expenditure was significantly increased with isocaloric Triacetin (Bailey, Haymond, and Miles 1991).

In a study examining the effect of Triacetin on nitrogen balance, whole-body kinetics, and muscle and liver fractional protein synthetic rates, Bailey, Barker, and Karlstad (1992) infused male Sprague-Dawley rats with isovolemic, isocaloric, and isonitrogenous parenteral diets. Thirty percent of the nonprotein energy of the diets was lipid energy, and for groups of 6, 10, and 6 animals, the lipid energy was composed of 0%, 50%, and 90% Triacetin, respectively. No difference was observed in the plasma acetate concentration of animals that received Triacetin compared to those that did not. The liver weight was significantly decreased in the animals given Triacetin. Protein concentration in the liver and rectus muscle was similar for all three groups. Cumulative nitrogen balance was positive for all animals, and no significant difference in nitrogen balance was observed on days 6 and 7. Lipid composition had very little effect on plasma leucine kinetics. Also, fractional protein synthetic rates in the rectus muscle and liver were similar for all animals.

Further work by Bailey, Miles, and Haymond (1993) reported the effect of a 5% *v/v* aqueous solution of Triacetin on leucine metabolism using female mongrel dogs. A primed, continuous infusion of L-[1-^{14}C]-leucine was continued for 6 h. Three hours after the start of the isotope infusion, administration of Triacetin was started. Six animals were infused at a rate of 47 μmol/kg/min and seven were infused at a rate of 70 μmol/kg/min Triacetin for 3 h. Blood and breath samples were taken at 15 to 30-min intervals. A group of four animals was infused with 70 μmol/kg/min glycerol and a group of five animals was infused with saline; both were used as control groups. During the last hour of dosing, plasma acetate concentrations increased from 0.13 to 0.99 and from 0.10 to 11.8 for the animals infused with 47 and 70 μmol/kg/min Triacetin, respectively. Plasma glucose concentration decreased slightly but significantly for the animals given 47 μmol/kg/min Triacetin. Plasma leucine concentration decreased significantly in the animals given 70 μmol/kg/min Triacetin. Plasma α-ketoisocaproate concentration and specific activity increased with both doses. $^{14}CO_2$ excretion was increased with the low dose and decreased with the high dose of Triacetin.

The conclusion of this series of studies was that due to the water solubility, minimal effect on mineral metabolism, improved nitrogen balance, lack of toxicity, and favorable effects

on protein metabolism, Triacetin warranted further study as a parenteral nutrient.

Bleiberg et al. (1993) used mongrel dogs to determine the systemic, hindlimb, gut, hepatic, and renal uptake of acetate during infusion of a 5% *v/v* aqueous solution of Triacetin. A primed, continuous infusion of [1-^{14}C]-acetate was continued for 7 h with 10 animals. Three hours after the start of the tracer infusion, the animals were infused with Triacetin at a rate of 47 μmol/kg/min for 4 h. Blood and breath samples were taken at 15-min intervals for the last 30 min. Steady-state conditions were achieved in plasma acetate concentrations and specific activity and in expired $^{14}CO_2$. Plasma acetate concentrations were ≈1180, ≈935, ≈817, ≈752, and ≈473 μmol/L in the aorta, renal vein, portal vein, femoral vein, and hepatic vein, respectively. The acetate turnover rate during Triacetin infusion was 2214 μmol/min; systemic acetate turnover accounted for 68% of Triacetin-derived acetate. The researchers concluded that the majority of triacetin undergoes intravascular hydrolysis, and the majority of the resulting acetate is oxidized.

Lynch, Miles, and Bailey (1994) determined the metabolic effect of Triacetin on intestinal mucosa cells and plasma substrates in a 30-day feeding study in which male Sprague-Dawley rats were given a diet that contained Triacetin. Groups of eight animals were fed a diet in which 30% of the energy was supplied as lipids, and Triacetin composed either 0 or 95% of the lipids (long-chain triglyceride [LCT] or Triacetin group, respectively); the remainder of the lipid was a long-chain triglyceride. (In the Triacetin-containing feed, Triacetin composed 19% of the diet by weight.) A control group of eight animals was fed chow that supplied 5% of the energy as LCTs. Body weights and feed consumption were measured throughout the study.

At study termination, animals of the LCT group weighed more than the animals of the Triacetin or control groups. Feed consumption was not statistically significantly different between the Triacetin and LCT groups; however, during week 2 for the Triacetin group and weeks 2 to 4 for the LCT group, feed consumption was significantly greater than that of controls. No significant differences in lactate, ketone body, or glucose concentration were observed among the groups. Plasma pyruvate concentration in the Triacetin group was significantly decreased compared to the LCT group, and plasma free fatty acids were significantly decreased and the plasma triglyceride concentration was significantly increased in the Triacetin group compared to animals of the control and LCT groups. Total intestinal DNA, RNA, protein, and protein: DNA ratio were measured. No significant difference in mucosal protein concentration was observed in the jejunum and colon. Jejunal and colonic DNA content was significantly increased (and therefore protein: DNA ratio decreased), whereas jejunal RNA was significantly decreased in animals fed Triacetin. No significant differences in crypt depth or mean villus height were observed (Lynch, Miles, and Bailey 1994).

Lynch and Bailey (1995) fed groups of eight male Sprague-Dawley rats the diets described in the preceding paragraph for 30 days, and the effect on total adiposity, fat distribution, and body composition was determined. At study termination, animals of the LCT group weighed more than the animals in the Triacetin or control groups. Feed consumption was not significantly different between the Triacetin and LCT groups; however, the controls ate less than animals of the test groups. Also, energy intake was not significantly different between animals of the Triacetin and LCT groups, but it was significantly less in animals of the control group during weeks 2 to 4. Animals of the Triacetin group had the least adipose tissue mass (measured in three depots) compared to the other groups; animals in the LCT group had the greatest adipose tissue mass. Triacetin decreased adipocyte size, but total fat cell number did not differ among the groups.

ANIMAL TOXICOLOGY

Table 3 summarizes the acute toxicity findings as a function of the route of administration and animals used.

Acute Oral Toxicity

Gast (1963) stated that the oral LD_{50} of Triacetin for male and female mice was 1.8 and 1.1 g/kg, respectively, although study details were not provided.

The acute oral toxicity of Triacetin was determined using a group of 10 mice (Lawrence, Malik, and Autian 1974). The animals were given a single dose and observed for 7 days. The calculated oral LD_{50} for mice was 8.0 ml/kg.

Groups of five mice and five rats were used to determine the acute oral toxicity of Triacetin (Anstadt 1976). The dose ranges tested were 1.6 to 25.6 and 0.8 to 12.8 g/kg for mice and rats, respectively. The approximate LD_{50} was 3.2 to 6.4 g/kg for mice and 6.4 to 12.8 g/kg for rats.

Additional oral LD_{50} values were 3.2 to 6.1 g/kg for mice, 3.0 and >2.0 g/kg for rats, and >2.0 g/kg for rabbits (Unichema Chemie B.V. 1994).

Groups of eight Long-Evans rats were given as a continuous nasogastric infusion 462 kJ/kg of a diet containing either 16% or 32% Triacetin (Robertson et al. 1992). No adverse effects, such as diarrhea or change in normal activity, were observed.

Acute Dermal Toxicity

The acute dermal LD_{50} of Triacetin was determined using groups of five albino rabbits (Food and Drug Research Laboratories, Inc. 1976). A dose of 5 g/kg was applied to intact and abraded skin. The animals were observed for 7 days after dosing. None of the animals died. The dermal LD_{50} of Triacetin for rabbits was >5 g/kg. Additional dermal LD_{50} values were >20 ml/kg for guinea pigs and >2 g/kg for rabbits (Unichema Chemie B.V. 1994).

Acute Inhalation Toxicity

Unichema Chemie B.V. (1994) stated that, using five male and five female rats and a 4-h exposure, the LC_{50} of Triacetin was >1.721 mg/L. The particle size was not specified.

TABLE 3
Triacetin acute toxicity studies

Route of administration	Animal	LD_{50}	Reference
Oral	Mice (male)	1.8 g/kg	Gast 1963
Oral	Mice (female)	1.1 g/kg	Gast 1963
Oral	Mice (sex not given)	8 ml/kg	Lawrence, Malik, and Autian 1974
Oral	Mice (sex not given)	3.2–6.4 g/kg	Anstadt 1976
Oral	Mice (sex not given)	3.2–6.1 g/kg	Unichema Chemie B.V. 1994
Oral	Rats (sex not given)	6.4–12.8 g/kg	Anstadt 1976
Oral	Rats (sex not given)	3.0 and >2.0 g/kg	Unichema Chemie B.V. 1994
Oral	Rabbits (sex not given)	>2.0 g/kg	Unichema Chemie B.V. 1994
Dermal	Rabbits (sex not given)	>5 g/kg	Unichema Chemie B.V. 1994
Dermal	Rabbits (sex not given)	>2 g/kg	Unichema Chemie B.V. 1994
Dermal	Guinea pigs (sex not given)	>20 ml/kg	Unichema Chemie B.V. 1994
Parenteral	Mice (sex not given)	2.3 cc/kg	Li, Sah, and Anderson 1941
Parenteral	Mice (sex not given)	1.6 g/kg	Wretlind 1957
Parenteral	Mice (male)	1.7 g/kg	Gast 1963
Parenteral	Mice (female)	1.4 g/kg	Gast 1963
Parenteral	Mice (sex not given)	1.52 ml/kg	Lawrence, Malik, and Autian 1974
Parenteral	Mice (sex not given)	~0.8–1.6 g/kg	Anstadt 1976
Parenteral	Mice (sex not given)	1.2 ml/kg	Tarr, Sambandan, and Yalkowsky 1987
Parenteral	Rats (sex not given)	2.8 cc/kg	Li, Sah, and Anderson 1941
Parenteral	Rats (sex not given)	~0.8–1.6 g/kg	Anstadt 1976
Parenteral	Rabbits (sex not given)	0.75 ml/kg	Opdyke 1978
Parenteral	Guinea pigs (sex not given)	1.5 cc/kg	Lipschitz et al. 1942
Parenteral	Dogs (sex not given)	1.5–2.0 ml/kg	Opdyke 1978
Parenteral	Dogs (sex not given)	>70 μmol/kg/min	Bailey, Haymond, and Miles 1991
Inhalation	Rats (male and female)	>1.721 mg/L (LC_{50})	Unichema Chemie B.V. 1994

Acute Parenteral Toxicity

Li, Sah, and Anderson (1941) determined the subcutaneous (SC) LD_{50} of Triacetin for inbred white mice and inbred albino rats. Groups of 10 mice were dosed with 1.0 to 3.0 cc/kg and groups of 10 rats were dosed with 2.0 to 10.0 cc/kg aqueous Triacetin. The mice and rats were observed for 5 and 15 days after dosing, respectively. Two, 2, 7, and 10 mice of the 1.0-, 2.0-, 3.0-, and 3.5-cc/kg groups and 1, 6, and 10 rats of the 2.0-, 3.0-, and 4–10-cc/kg groups died, respectively. The animals usually died within 20 min to 3 or 4 h after injection. The authors noted marked depression, weakness, prostration, and in some animals, labored respiration just before death. Hemorrhagic areas in the lungs and some swelling of the convoluted tubules of the kidney were observed, along with hydropic degeneration and necrosis of the tubules in some areas. Also, the authors stated that the liver appeared to be congested. The SC LD_{50} was calculated to be 2.3 and 2.8 cc/kg for mice and rats, respectively. Diacetin was slightly less toxic than Triacetin, and the researchers concluded that the order of toxicity of the acetins appears to increase with the degree of acetylation.

Lipschitz et al. (1942) determined the intramuscular (IM) toxicity of Triacetin using guinea pigs. The lethal dose was 1.5 cc/kg, and dyspnea followed by death was observed. The researchers stated that the behavior of the animals suggested to them that the lethal effect might be attributable to the large amount of the acids liberated by relatively rapid hydrolysis.

Wretlind (1957) determined the intravenous (IV) LD_{50} of a 25% emulsion of Triacetin. Six groups of 10 animals were dosed with 1 to ~2.5 g/kg Triacetin. The IV LD_{50} of Triacetin was 1.6 g/kg for mice. Injection with Triacetin produced almost immediate convulsions, failure of the righting reflexes, and respiratory arrest.

von Oettingen (1960) reported a study using rabbits in which a SC injection of 0.8 g/kg of Triacetin given in three fractional doses caused a temporary stimulation of respiration with no narcosis. This author also reported that a slow IV injection of 1.48 g/kg Triacetin given in three fractional doses of 2.4 g/kg given in two fractional doses, and of 1.46 g/kg given as a single dose caused deep sleep, reduction of the tendon reflexes, and reduction of sensitivity to pain in rabbits. Respiration became slow and labored, and the authors stated that animals died from respiratory arrest.

Gast (1963) reported that the intraperitoneal (IP) LD_{50} of Triacetin for male and female mice was 1.7 and 1.4 g/kg, respectively. Based on a study using a limited number of adult rats,

this author also stated that the IP LD_{50} was 2.1 g/kg, although no study details were provided.

Lawrence, Malik, and Autian (1974) determined the acute IP toxicity of Triacetin using a group of 10 mice. The animals were given a single injection and observed for 7 days. The calculated IP LD_{50} for mice was 1.52 ml/kg.

Anstadt (1976) used groups of five mice and five rats to determine the acute IP toxicity of Triacetin. The dose ranges tested were 0.4 to 6.4 and 0.8 to 6.4 for mice and rats, respectively. The approximate LD_{50} was 0.8 to 1.6 g/kg for both mice and rats.

The IV LD_{50} of Triacetin for rabbits and dogs has been reported as 0.75 and 1.5 to 2.0 ml/kg, respectively (Opdyke 1978).

Tarr, Sambandan, and Yalkowsky (1987) determined the IV LD_{50} of an emulsion consisting of 50% Triacetin, 1.5% soy lecithin, 1.5% pluronic F68, and 2.0% ethyl oleate for Swiss-Webster mice. Groups of eight animals were dosed intravenously with 0.8 to 2.0 ml/kg of the emulsion. The IV LD_{50} for mice of the Triacetin emulsion was 1.2 ml/kg.

Bailey, Barker, and Karlstad (1991), in a study described earlier, infused female mongrel dogs with 47 or 70 μmol/kg/min Triacetin for 3 h, reported no evidence of acute toxicity.

Short-Term Oral Toxicity

According to Opdyke (1978), rats fed diets containing 55% Triacetin instead of fat gained weight.

Shapira et al. (1969) stated that, in 3-month feeding studies, rats tolerated up to 20% Triacetin without weight loss as compared to control values; but that diets containing 60% Triacetin were associated with a large loss in weight and considerable mortality. The authors stated that the type and quantity of protein present influenced weight gain.

Shapira, Vann, and Furst (1975) stated that, in groups of eight male Sprague-Dawley rats that were fed diets containing 30% Triacetin (and 30% glycerin or propylene glycol) as a starch substitute for 3 to 4 or 12 to 13 weeks, growth was relatively poor. Liver enlargement was observed in all animals.

In a study by Lynch, Miles, and Bailey (1994) described previously, the metabolic effect of Triacetin on intestinal mucosal cells and plasma substrates in male Sprague-Dawley rats was assessed. Rats were fed for 30 days a diet in which 28.5% of the total calories were supplied by Triacetin. No overt signs of toxicity were observed.

Short-Term Inhalation Toxicity

Anstadt (1976) stated that no toxicity was observed when rats were exposed to 8200 ppm Triacetin (saturated vapor) for 6 h per day for 5 days.

Unichema Chemie B.V. (1994) exposed a group of three rats to 250 ppm Triacetin for 6 h per day, 5 days per week, for 64 days. The no-observed-effect level (NOEL) was 250 ppm. Three rats were also exposed to 8271 ppm Triacetin for 6 h per day for 64 days. The NOEL was 8271 ppm.

Short-Term Parenteral Toxicity

Bailey, Barker, and Karlstad (1992) infused rats intravenously with 0%, 50%, or 90% (15.2 and 27.2 g/L) Triacetin for 7 days. No overt signs of toxicity were observed.

Subchronic Oral Toxicity

Blumenthal (1964) stated that Triacetin was not toxic to groups of five male rats fed a diet containing 1%, 2%, 4%, or 8% Triacetin for 16 weeks. A control group was fed basal diet.

Subchronic Inhalation Toxicity

Unichema International (1996) exposed rats to concentrations of 250 ppm Triacetin, which was produced by using heated vapor, 5 days per week for 13 weeks. No changes in liver and kidney weight, blood counts, or urinalysis were observed.

Chronic Toxicity

Published data on the chronic toxicity of Triacetin were not found.

Dermal Irritation

Anstadt (1976) determined the dermal irritation potential of Triacetin using two guinea pigs. Doses of 5 and 10 cc/kg were applied under an occlusive patch for 24 h. Slight erythema was observed. Occlusive patches with 5 to 20 cc/kg were tested using three animals. Slight edema and "1-3 erythema" were observed. Slight skin irritation was observed in the high dose animals.

Unichema Chemie B.V. (1994) stated that groups of three, three, and two guinea pigs were used to determine the irritation potential of Triacetin, but no study details were provided. At the end of a 14-day observation period, erythema, slight edema, alopecia, and desquamation were observed. They also stated that another study found no skin irritation in guinea pigs, but no details were available.

Other reports stated that Triacetin was not a skin irritant in guinea pigs (Unichema International 1996; Opdyke 1978) or rabbits (Unichema Chemie B.V. 1994).

Dermal Sensitization

Anstadt (1976) reported that no sensitization was observed using the "drop-on method" in a test using five guinea pigs.

Unichema Chemie B.V. (1994) evaluated the sensitization potential of Triacetin in acetone, dioxane, and guinea pig fat (7:2:1) using guinea pigs. The animals were initially dosed three times over 5 days and challenged after 1, 2, or 3 weeks. A vehicle and positive control were used. Triacetin was nonsensitizing.

Other reports found that Triacetin was not a sensitizer in guinea pigs (Unichema International 1996; Unichema Chemie B.V. 1994; Opdyke 1978).

Ocular Irritation

Li, Sah, and Anderson (1941) stated that application of 50% Triacetin (and diacetin) into the conjunctival sac of the eyes of rabbits resulted in "marked congestion and moderate edema."

In the original work of Draize, Woodard, and Calvery (1944), undiluted Triacetin, 0.1 ml, instilled into the conjunctival sac of the eyes of rabbits yielded average scores of 2.1 and 1.5/110 after 1 and 24 h, respectively.

Conquet et al. (1977) determined the ocular irritation potential of Triacetin using albino rabbits. In the first study, 0.1 ml undiluted Triacetin was instilled into the conjunctival sac of the eyes of six rabbits, and the eyes were scored according to the methods of Draize; corneal thickness was also measured. In a second study using groups of four rabbits, in which ocular tissue sampling was done, 0.1 ml was instilled into both eyes. A control group of four rabbits was not treated. Animals were killed after 2 or 24 h. In the Draize test, Triacetin had a total Draize score of 1 after 2 h (0 for the cornea and 0.7 for the conjunctiva); corneal thickness did not change. After 2 h, the corneal percentage of dry weight/wet weight was significantly decreased, but not after 24 h, compared to controls. No difference was observed for conjunctival percentage of dry weight/wet weight or Evan's blue concentration/dry weight at 2 or 24 h.

Unichema Chemie B.V. (1994) reported a study in which one drop of Triacetin was placed into the eyes of two rabbits; the eye of one rabbit was washed. The eyes were evaluated after 1, 24, and 48 h. Triacetin was slightly irritating. The authors stated that Triacetin, as tested using rabbits according to Organization for Economic Cooperation and Development (OECD) guidelines, was reported to be nonirritating.

In other reports, Triacetin, 100 μl, was a low-to-moderate irritant in rabbit eyes (Unichema International 1996). No damage was observed when a rabbit eye was irrigated continuously for 6 min (Unichema International 1996; Opdyke 1978).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Published data on the reproductive and developmental toxicity of Triacetin were not found.

GENOTOXICITY

In Vitro

Several laboratories have reported results from different versions of the Ames test for bacterial genotoxicity.

Litton Bionetics, Inc. (1976) evaluated the mutagenic potential of Triacetin in a plate test using *Salmonella typhimurium* strains TA1535, TA1537, and TA1538 with and without metabolic activation. Test concentrations were 0.0013%, 0.00065%, and 0.000325% and the solvent was dimethyl sulfoxide (DMSO). A negative control (solvent) and appropriate positive controls were used and gave expected results. Triacetin was not mutagenic with or without metabolic activation.

The mutagenic potential of Triacetin was also evaluated in a suspension test with and without metabolic activation. Test concentrations were 0.0013%, 0.00065%, and 0.000325% with *S. typhimurium* strains TA1535, TA1537, and TA1538 and 1.25%, 2.5%, and 5.0% with *Saccharomyces cerevisiae* strain D4. DMSO was used as the solvent. Appropriate negative and positive controls were used and gave expected results. Triacetin was not mutagenic in the suspension tests with or without metabolic activation (Litton Bionetics, Inc. 1976).

Unichema Chemie B.V. (1994) reported that Triacetin was not mutagenic at 50 to 5000 μg/plate in an Ames test using *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 with and without metabolic activation.

In Vivo

The mutagenic potential of Triacetin was determined using adult *Drosophila melanogaster* (Efremova 1962). A dose of 0.2 to 0.3 mg Triacetin had a spontaneous mutation rate of approximately one mutation per 750 chromosomes.

CARCINOGENICITY

Published data on the carcinogenicity of Triacetin were not found.

CLINICAL ASSESSMENT OF SAFETY

Irritation and Sensitization

A maximization test for undiluted Triacetin was completed using 33 subjects (Epstein 1976). Triacetin was applied under an occlusive patch to the volar aspect of the forearm for 48 h on 5 alternate days. Because a pretest indicated the Triacetin was not an irritant, the test site was pretreated for 24 h with 2% sodium lauryl sulfate (SLS) under an occlusive patch prior to application of the initial test patch. After a 10 to 14-day nontreatment period, challenge patches were applied to a previously unexposed site on the right side of the back. Prior to challenge, 2% SLS was applied for 30 min under an occlusive patch to the left side of the back. Additional SLS control patches and petrolatum patches were placed on the left and right sides, respectively, and used as controls. Undiluted Triacetin did not produce an irritant or sensitization reaction.

A Duhring-chamber test was performed using 20 subjects (Unichema Chemie B.V. 1994). Triacetin was applied as a 50% dilution for 24 h. No further details were available. The authors stated that only very mild skin reactions were observed.

Ocular Irritation

According to Unichema Chemie B.V. (1994), commercial Triacetin, which can contain diacetin and monoacetin, caused severe burning, pain, and much redness of the conjunctiva, but no injury. The authors claimed that diacetin causes considerably more discomfort than pure Triacetin.

Toxicity

The safety and effectiveness of a mucosa-adhesive polymer film used to alleviate acute radiation-induced oral mucositis and prevent infections was analyzed using 25 patients (Oguchi et al. 1998). The film contained 24 mg Triacetin, 600 mg hydroxypropylcellulose, 5 mg each of tetracaine hydrochloride, ofloxacine, and miconazole nitrate, 0.6 mg guaiazulene, and 100 ml ethyl alcohol. No acute or chronic adverse effects on the oral mucosa or gastrointestinal tracts were observed, and no allergic dermal reactions were reported. No changes in liver or renal function and no hematological toxicity were noted.

Unichema Chemie B.V. (1994) stated that Triacetin "appears to be innocuous when swallowed, inhaled or in contact with the skin, but may cause slight irritation to sensitive individuals."

SUMMARY

Triacetin, also known as Glyceryl Triacetate, is reported to function as a cosmetic biocide, plasticizer, and solvent in cosmetic formulations. In 1998, it was reported to FDA that Triacetin was used in a total of 13 cosmetic formulations. Industry reported that it was used at concentrations of 0.8% to 4%. Triacetin was affirmed as a GRAS human food ingredient by FDA.

The acute dermal LD_{50} of Triacetin was >5 g/kg for rabbits and >20 ml/kg for guinea pigs. The oral LD_{50} for mice has been reported as 1.8 and 1.1 g/kg for males and females, respectively, and as 3.2 to 6.4 g/kg; it has been calculated as 8.0 ml/kg. For rats, the oral LD_{50} was 6.4 to 12.8 g/kg. The inhalation LC_{50} was >1.721 mg/L for rats. The SC LD_{50} was 2.3 cc/kg for mice and 2.8 cc/kg for rats. For mice, the IV LD_{50} values of 25% and 50% emulsions of Triacetin were 1.6 g/kg and 1.2 ml/kg, respectively. For rabbits, the IV LD_{50} of Triacetin was 0.75, and for dogs, it was 1.5 to 2.0 ml/kg. The IP LD_{50} for Triacetin in mice was determined to be 1.7 and 1.4 g/kg for males and females, respectively, and 0.8 to 1.6 g/kg; it was also calculated as 1.52 ml/kg for mice. The IP LD_{50} for rats was 2.1 g/kg; in another study, it ranged from 0.8 to 1.6 g/kg. The IM lethal dose was 1.5 cc/kg for guinea pigs.

In short-term feeding studies, Triacetin affected weight gain. Triacetin was not toxic in short-term studies when administered via inhalation or parenterally or in subchronic studies when administered via feed or inhalation.

Triacetin was, at most, slightly irritating to guinea pig skin. However, in one study, it caused erythema, slight edema, alopecia, and desquamation. Triacetin was not sensitizing in guinea pigs; however, test concentrations were not stated. Triacetin caused some irritation in rabbit eyes.

Triacetin, with and without metabolic activation, was not mutagenic in the Ames assay or a suspension test. It was also not mutagenic in an in vivo assay using *Drosophila*.

Triacetin (test concentrations not provided) was not an irritant or a sensitizer in a clinical maximization study, and only very mild reactions were seen in a Duhring-chamber test using a 50% dilution. In humans, commercial Triacetin has caused ocular irritation but no injury.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel reviewed the safety of Triacetin for use as a cosmetic ingredient. The Expert Panel considered the FDA affirmation of glycerides, including Triacetin, as a GRAS human food ingredients to be supportive of the overall safety of this ingredient.

The Expert Panel did recognize that reproductive and developmental toxicity data on Triacetin are absent from the report. Because Triacetin is thought to be hydrolyzed to glycerol and acetic acid and these chemicals are not developmental toxins, the Expert Panel concluded that the use of Triacetin in cosmetics does not present a risk of reproductive or developmental toxicity.

The Expert Panel also noted that there are reports indicating that 1,2-glyceryl diesters (also known as 1,2-diacylglycerols) can affect cell growth and proliferation, raising the possibility of hyperplasia and/or tumor promotion. This was an issue for discussion because, although Triacetin is a glyceryl triester, it is recognized that there would be some small amounts of glyceryl diesters present, some of which could be 1,2-glyceryl diesters. The Panel concluded, however, that the effects of 1,2-glyceryl diesters on cell growth and proliferation require ester chains longer than two carbon atoms on the glycerin backbone. Thus, any glyceryl 1,2-diacetyl esters present in Triacetin would be inactive in hyperplasia and tumor promotion.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that Triacetin is safe as used in cosmetic formulations.

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TRIACETIN – MINUTES FROM ORIGINAL DELIBERATIONS

February 14-15, 2000

Dr. Schroeter stated that the Panel issued a Tentative Report with the following conclusion at the September 9-10, 1999 Panel meeting: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that Triacetin is safe for use in cosmetic formulations.

Dr. Belsito requested that wording in the Tentative conclusion be changed from "safe for use" to "safe as used".

The Panel voted unanimously in favor of issuing a Final Report on Triacetin with the following conclusion: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that Triacetin is safe as used in cosmetic formulations.

Dr. Belsito confirmed Dr. Slaga's agreement with the following statement (in report discussion) relating to the presence of 1,2-glyceryl diesters in Triacetin: The Panel concluded, however, that the effects of 1,2-glyceryl diesters on cell growth and proliferation require longer ester chains on the glycerin backbone than are presented when acetic acid is esterified with glycerin. Thus, any glyceryl 1,2-diesters present in Triacetin would be inactive in hyperplasia and tumor promotion.

September 9-10, 1999

Dr. Belsito stated that Triacetin is a triester and that the Panel concluded earlier this year that other triesters are safe as used in cosmetics. He also noted that the fact that Triacetin is an indirect food additive that is generally recognized as safe (GRAS), but not a direct food additive, somewhat changes the level of his Team's concern about the presence of 1,2-diesters and the safety of Triacetin. Additionally, Dr. Belsito noted that his Team discussed the need for some type of risk assessment and data on the concentration of 1,2-diesters in Triacetin. The latter concern was based on the observation that Triacetin contains diacetylated glycerol.

Dr. Slaga stated that the acetates of the 1,2-diesters are inactive.

Dr. Belsito said that his Team could conclude that Triacetin is safe as used based on Dr. Slaga's comment.

Dr. Klaassen said that he was hoping that the diacetates would not be a problem. He also recommended that "good" references clarifying this potential concern be incorporated into the current report.

Concerning the regulatory status of Triacetin, Dr. Bailey noted that Triacetin is listed as a prior sanctioned GRAS ingredient for direct food use. Dr. Bailey said that being prior sanctioned means that the amount of data in the record could be somewhat limited. He also noted that the citation in the CIR report is wrong, and that it should be 21CFR 184.1901. He said that Triacetin is used as a flavoring agent (direct food additive use), in addition to a number of other uses, and that it also has several indirect food additive uses. The citation included in the CIR report refers to these indirect food additive uses.

The Panel unanimously concluded that Triacetin is safe as used in cosmetics.

June 14-15, 1999 Panel Meeting

Dr. Schroeter noted that the following informal data request was issued at the March 3-4, 1999 Panel meeting.

(1) UV absorption data; if significant absorption occurs in the UVA or UVB range, photosensitization data may be needed
(2) Irritation and sensitization data at concentration of use
(3) Genotoxicity testing using a mammalian system; if positive, a two-year dermal carcinogenicity assay performed according to NTP methods may be needed
(4) Octanol/water partition coefficient
(5) Gross pathology and histopathology in skin and other major organ systems associated with repeated dermal exposures (if these data cannot be obtained from other sources)
(6) Ocular toxicity data, if available

Concerning the request for genotoxicity data (item 3), he noted that it had been brought to his attention that Triacetin is a GRAS ingredient (food additive). With this in mind, he said that if FDA has genotoxicity data on Triacetin, then item 3 could be deleted. Dr. Schroeter also said that if Triacetin is accepted as a food additive, with data from FDA, then the need for gross pathology and histopathology data in other major organ systems (item 5) could also be deleted (Note: Skin gross pathology and histopathology data are still needed, and this request should not be deleted). Additionally, Dr. Schroeter noted that ocular toxicity data (item 6) have been received, but that items 1, 2, and 3 have not been received and are still needed.

Dr. Schroeter said that his Team agrees that an insufficient data announcement based on the preceding observations should be issued.

Dr. Shank wanted to know if Dr. Schroeter meant that the Panel should expect to receive genotoxicity data from FDA.

Dr. Schroeter said that genotoxicity data will be received from FDA if they are available.

Dr. Bailey indicated that he had made an effort to obtain the Federal Register announcement on the preceding day, but was unable to do so because of the short notice that he had been given.

Dr. Belsito noted that Triacetin is a glyceryl triester, and that the Panel has already arrived at a conclusion on the safety of glyceryl triesters. He also recalled that Triacetin is a triester in which the fatty acid is acetic acid, a GRAS compound. Additionally, Dr. Belsito noted that the available human (33 subjects) and animal (several studies) sensitization data on Triacetin are completely negative, that other ingredient families in this class are also completely negative for sensitization, and that impurities data on both USP and food grade Triacetin are available. Thus, Dr. Belsito's Team determined that no additional data are needed and also proposed that Triacein be incorporated into the existing CIR Final Report on Glyceryl Triesters.

Dr. McEwen said that because Triacetin is a GRAS compound, he assumes that FDA has data on this ingredient.

Dr. Bailey said that his assumption is the same, but that he needs to confirm that FDA has these data.

Dr. McEwen said that Triacetin was not originally included in the CIR report on Glyceryl Triesters because its function in cosmetics (biocide) is different from that of the other Glyceryl Triesters.

Dr. Andersen said that it would be problematic for Triacetin to now be incorporated into the existing Final Report on Glyceryl Triesters, and that it would be easier for the report on Triacetin to exist as a separate report.

On the issue of the need for sensitization data, Dr. McEwen noted that maximization test data are included in the present report. He also noted that the CIR report contains negative mutagenicity data and subchronic toxicity tests, and that the current concentration of use data indicate fairly low use concentrations. The higher use concentrations are in nail polish and mascara, to which there is very little skin contact.

Dr. Shank said that if Triacetin is used as a biocide, then he does not think that the Panel is being excessive by asking for mammalian mutagenicity data and more skin sensitization data.

Dr. Schroeter stated that few sensitization studies (animal and human) are included in the present report and that the human data indicate that Triacetin is not a sensitizer. However, he requested that sensitization data remain in the list of the Panel's data needs because of the possibility that some company may have data to submit.

Dr. Andersen said that if there is substantial comfort that the data are likely to be negative, the Panel could vote to issue a Tentative report with a safe as used conclusion. Thus, any data (genotoxicity included) that will be submitted by FDA could be considered a comment to the Tentative report.

Dr. Shank said that if cosmetics manufacturers are adding Triacetin to cosmetics for its biocidal properties, then biological activity must be associated with this ingredient. He also noted that data in the current CIR report do not support any Triacetin-induced biological activity. Specifically, there was no evidence of toxicity in Ames tests, meaning that it isn't a very good bacteriocide, and no antifungal activity was associated with Triacetin.

Dr. Bailey said that the report on Triacetin contains very little data He also said that in terms of the rigor of the process, if the Panel is going to accept data such as these, this would lead one to question the CIR process. Furthermore, Dr Bailey indicated that if Triacetin is supposed to be used as a biocide and is not effective, then the public should be allerted concerning this.

Dr. McEwen stated that based upon the existing knowledge of the chemistry of Triacetin, the CIR report contains a sufficient amount of information.

Dr. Belsito said that some of the data from the report on Glyceryl Triesters could be incorporated into the report on Triacetin.

Dr. Andersen said that he was unsure of the basis for adding data on fatty acid esters to support the safety of a glyceryl

ester that is defined as glycerine esterified with acetic acid.

Dr. McEwen that the introduction in the Triacetin report should contain a statement indicating that the Panel has evaluated the safety of other Glyceryl Triesters.

Dr. Bergfeld questioned any decision by the Panel to issue a Tentative report at this point, considering that additional information needs to be added and reviewed.

Dr. Andersen expressed the view that if the Panel really wants to review the FDA data first, then the report on Triacetin should be tabled.

The Panel voted in favor of tabling the report on Triacetin. Dr. Belsito voted against the motion.

Dr. Bergfeld noted that the report on Triacetin is being tabled to allow the submission of data that were used by FDA to approve the use of Triacetin as a direct food additive. She also stated that, in the meantime, an effort can be made to obtain the study details that are identified in text as missing from the Triacetin report

March 3-4, 1999 – Team Meeting Minutes

The ingredients that were discussed only in Team meetings are included in this section. As described earlier in the full minutes, these represent CIR staff notes.

Triacetin

Just prior to the meeting, current concentration of use data were received. These data will be incorporated following the meeting.

The Belsito and Schroeter Teams informally requested the following information:

1. UV absorption data; if significant absorption occurs in the UVA or UVB range, photosensitization data may be needed
2. Irritation and sensitization data at concentration of use
3. Genotoxicity testing using a mammalian system; if positive, a 2-yr dermal carcinogenicity assay performed according to NTP methods may be needed;
4. Octanol/water partition coefficient
5. Gross pathology and and histopathology in skin and other major organ systems associated with repeated dermal exposures (if these data cannot be obtained from other sources)

The Schroeter Team also requested:

1. Ocular toxicity data, if available

PubMed – 8/1/18

((((triacetin) OR "glyceryl triacetate") OR "glycerol triacetate") OR 102-76-1[EC/RN Number]) AND ("1997"[Date - Publication] : "3000"[Date - Publication]) – 254 hits/4 obtained/0 useful

REACH – dossier available

TRIACETIN	03D - Eye Lotion	2
TRIACETIN	03F - Mascara	1
TRIACETIN	03G - Other Eye Makeup Preparations	1
TRIACETIN	04E - Other Fragrance Preparation	1
TRIACETIN	05E - Rinses (non-coloring)	1
TRIACETIN	05I - Other Hair Preparations	3
TRIACETIN	07H - Makeup Fixatives	1
TRIACETIN	08A - Basecoats and Undercoats	1
TRIACETIN	08E - Nail Polish and Enamel	15
TRIACETIN	08G - Other Manicuring Preparations	4
TRIACETIN	12C - Face and Neck (exc shave)	4
TRIACETIN	12D - Body and Hand (exc shave)	3
TRIACETIN	12F - Moisturizing	15
TRIACETIN	12G - Night	5
TRIACETIN	12H - Paste Masks (mud packs)	1
TRIACETIN	12I - Skin Fresheners	1

Concentration of Use by FDA Product Category – Triacetin

Product Category	Maximum Concentration of Use
Eye shadows	0.008%
Eye lotions	0.000025-0.008%
Hair conditioners	0.00012-0.028%
Hair sprays	
Aerosol	0.00031-0.95%
Pump spray	0.0000013%
Shampoos (noncoloring)	0.031%
Tonics, dressings and other hair grooming aids	0.012-0.021%
Foundations	0.005-0.008%
Makeup bases	0.004%
Makeup fixatives	0.00005%
Other manicuring preparations	0.2%
Dentifrices	0.054%
Mouth washes and breath fresheners	0.00047%
Bath soaps and detergents	0.08%
Deodorants	
Not spray	0.13%
Aerosol	0.14%
Aftershave lotions	0.00008%
Preshave lotions	0.0002%
Shaving cream	0.00018-0.004%
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0022-0.008%
Face and neck products	
Not spray	0.012-0.015%
Body and hand products	
Not spray	0.008-0.011%
Moisturizing products	
Not spray	0.012%
Night products	
Not spray	0.0003%
Paste masks and mud packs	0.00018-0.006%
Other skin care preparations	0.008%
Suntan products	
Not spray	0.005%
Other suntan preparations	0.004%

Information collected in 2018

Table prepared June 1, 2018