# Amended Safety Assessment of Methylisothiazolinone as Used in Cosmetics

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| Status: | Draft Amended Report for Panel Review |
| Release Date: | May 15, 2020 |
| Panel Meeting Date: | June 8-9, 2020 |

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer, CIR.



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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, Senior Scientific Writer/Analyst, CIR
Date: May 15, 2020
Subject: Draft Amended Report on the Safety Assessment on Methylisothiazolinone

Enclosed is the draft amended report of the safety assessment of Methylisothiazolinone (MI) as used in cosmetics. (It is identified as *MI062020rep* in the pdf document.) This ingredient functions as a preservative in cosmetics. In 2019, the Panel published an amended safety assessment of Methylisothiazolinone 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 quantitative risk assessment (QRA)." This conclusion superseded the Panel's original conclusion that was published in 2010.

At the September 2019 Expert Panel meeting, based upon the adverse events described in the published literature on the inhalation of humidifier disinfectants containing Methylchloroisothiazolinone/Methylisothiazolinone (MCI/MI), the Panel moved to reopen the safety assessment of MI. The Panel wanted to further investigate the causes of respiratory issues reported in Korea with these disinfectants. A search of inhalation toxicity to Methylisothiazolinone (separate from the combination of MCI/MI) did not yield any new published literature aside from the papers already detailed in the MCI/MI report, but additional relevant data for other toxicological endpoints have been discovered and are included in this draft amended report, along with summary information from the original report (indicated by *italics*).

According to 2019 VCRP survey data, Methylisothiazolinone (when not used with Methylchloroisothiazolinone) is used in a total of 915 formulations; the majority of the uses are in bath soaps and detergents. These uses have increased since the last review where 745 uses were reported; the majority of the uses reported then were in non-coloring hair conditioners and shampoos. In the amended safety assessment published in 2019, the maximum concentration of use range was reported to be 3.5 x $10^{-8}$ % 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). A survey of the present concentration of use is currently being conducted by the Council; these data are expected in time for the Wave 2 submission.

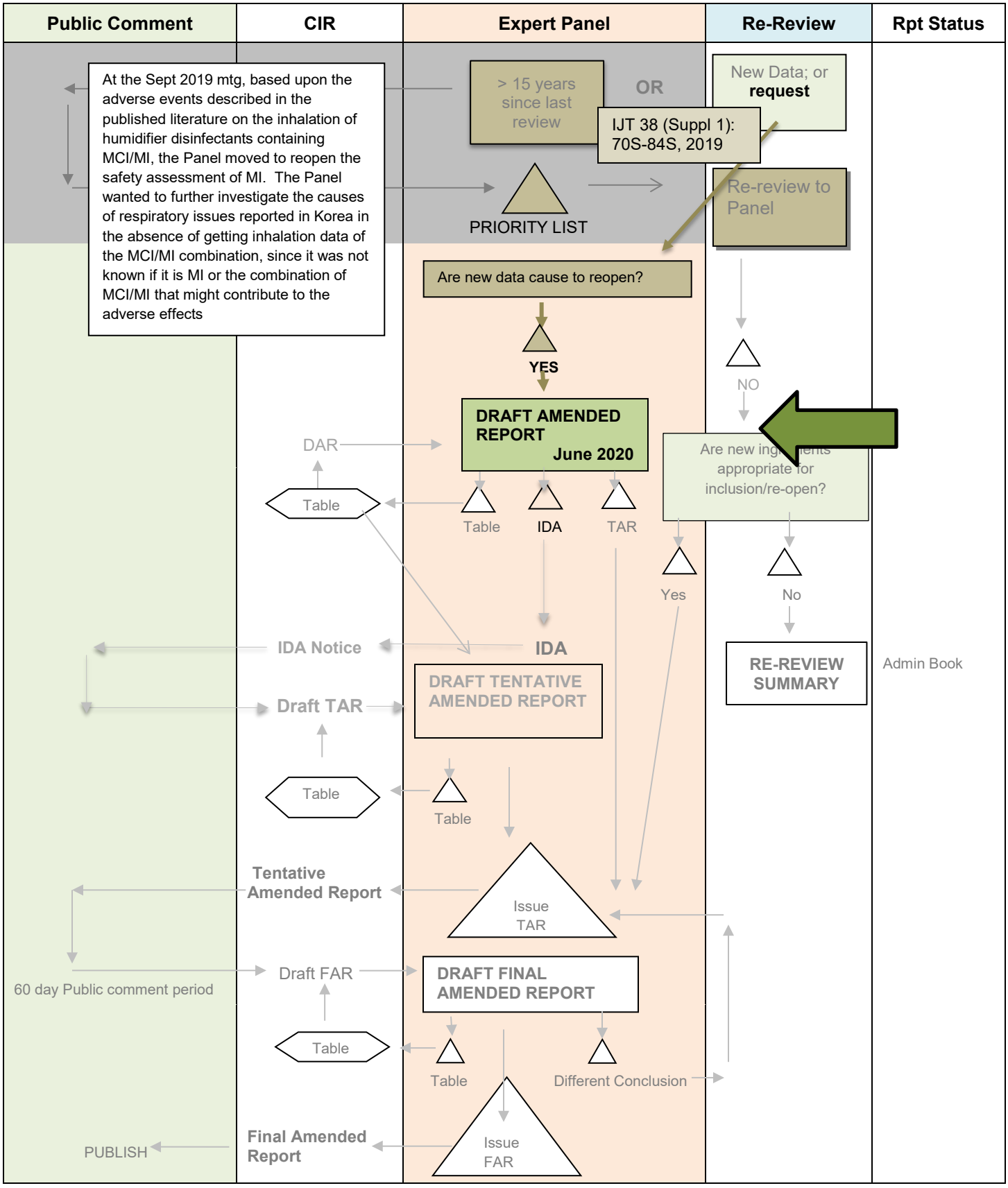
Minutes from the Panel discussions from 2008, 2013-2014, and 2019 (where MI was re-opened) are identified as *MI062020min* in the pdf document. Other supporting documents for this report package include a flow chart (*MI062020flow*), report history (*MI062020hist*), the two previous safety assessments (*MI062020_2010origrep* and *MI062020_2019amendrep*), a search strategy (*MI062020strat*), and a data profile (*MI062020prof*).

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Amended Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an Insufficient Data Announcement (IDA).

# RE-REVIEW FLOW CHART

**INGREDIENT/FAMILY** Methylisothiazolinone

**MEETING** June 2020




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

# Methylisothiazolinone History

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

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

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

**March 2014** – The Expert Panel tabled their discussion on MI to allow a QRA to be performed using corrected $EC_3$ values from a local lymph node assay that the Panel had previously considered when this ingredient was reviewed in 2008. A QRA is being prepared by Cosmetics Europe and is anticipated to be available mid-May. If it is not available by this time, the Panel strongly recommended that a QRA be performed in the United States in a timely manner to ensure that the Panel can evaluate this ingredient at the June 2014meeting.

**June 2014** – The Expert 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 reviewed the results of QRAs performed by Cosmetics Europe and the CIR Science and Support Committee using $EC_3$ values (the effective concentrations of the test substance required to produce a three-fold increase in the stimulation index, compared to vehicle-treated controls) from local lymph node assays (LLNAs), which were corrected in the literature since the Panel previously considered this ingredient in 2008, 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 substantially lower concentrations.

**September 2014** – The Expert Panel issued a final amended safety assessment on MI 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 quantitative risk assessment (QRA)."

**2019** – The amended safety assessment was published in the International Journal of Toxicology.

**September 2019** - Based upon the adverse events described in the published literature on the inhalation of humidifier disinfectants containing MCI/MI, the Panel moved to reopen

the safety assessment of MI. The Panel wanted to further investigate the causes of respiratory issues reported in Korea.

| Methylisothiazolinone Data Profile – June 2020 – Christina Burnett | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Toxico-kinetics | | Acute Tox | | | Repeated Dose Tox | | | DART | | Genotox | | Carci | | Dermal Irritation | | | Dermal Sensitization | | | | Ocular Irritation | | | Clinical Studies | |
| | Reported Use | log P/log $K_{ow}$ | Method of Mfg | Constituents/ Impurities | Dermal Penetration | ADME | Dermal | Oral | Inhalation | Dermal | Oral | Inhalation | Dermal | Oral | In Vitro | In Vivo | Dermal | Oral | In Vitro | Animal | Human | In Vitro | Animal | Human | Phototoxicity | In Vitro | Animal | Human | Retrospective/ Multicenter | Case Reports |
| **2010 Report Data** | X | X | X | X | X | X | X | X | X | | X | | | X | X | X | | X w/MCI | X | X | X | | X | X | X | X | X | | | X |
| **2019 Amended Report** | X | | | | | | | | | | | | | | | | | | | | | | X | X | | | | | X | X |
| **2020 Current Review** | X | | | | | | X | X | X | | X | | | X | X | X | | | | X | | | X | X | | | | X | X | X |

"X" indicates that data were available in a category for the ingredient

**Methylisothiazolinone – Christina Burnett**

| Ingredient | CAS # | PubMed | FDA | EU | ECHA | SIDS | ECETOC | HPVIS | NICNAS | NTIS | NTP | WHO | FAO | NIOSH | Web |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methylisothiazolinone | 2682-20-4 | results below | Approved for indirect food products in combo w/MCI | Annex V | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |

**Search Strategy/PubMed – Search Performed in December 2019-January 2020**

Methylisothiazolinone NOT Methylchloroisothiazolinone – 111 hits

With "Inhalation" – 0 hits

27 references ordered or downloaded.

## LINKS

**Search Engines**

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)
- Scifinder (https://scifinder.cas.org/scifinder)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

**Pertinent Websites**

- wINCI - http://webdictionary.personalcarecouncil.org

- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- EAFUS: http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- OTC ingredient list: https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/

- HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
- NTP (National Toxicology Program ) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/

- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/

- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/

- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information – not as a scientific source, purely for informational reasons

## APRIL 2008 PANEL MEETING – REVIEW OF DRAFT REPORT

### Belsito's Team Meeting – April 14, 2008

Dr. Belsito reminded the team that Methylisothiazolinone is a new stand-alone report. Dr. Belsito commented that he appreciated Rohm & Haas' comments. He noted that one issue for discussion was how to abbreviate Methylisothiazolinone in the report, if at all.

Ms. Burnett noted that MI/MCI is the common abbreviation for the United States and MIT/CMIT is common in Europe. Most publications use MI/MCI.

Rohm & Haas told the team that Europe's adoption of the abbreviations "MIT" and "CMIT" are a move toward world-wide standardization.

Dr. Klaassen felt that the new abbreviations should be used in the report. The name of the ingredients is too long to use constantly throughout the report.

Dr. Snyder recommended adding a paragraph in the Introduction explaining the change from using "MI" to "MIT".

Drs. Snyder and Belsito noted that the description of the concentrations used in the studies in the report were often confusing. The active ingredient was 100 ppm, not 9.5% - 9.9%.

George Hazelton from Rohm & Haas noted that animal studies throughout the report did not show neurotoxic effects. He asked that a prepared statement be included in the report stating this.

Dr. Belsito asked that the background information on MI/MCI be removed from the report.

Dr. Belsito asked if the team wanted to declare Methylisothiazolinone "safe as used " or "safe up to 100 ppm. The representatives from Rohm & Haas asked that the limit of 100 ppm be used in the conclusion for the sake of clarity. Dr. Andersen responded that either way works, but the Consumer Federation of America may want the concentration stated in the conclusion.

The team agreed that Methylisothiazolinone is safe in cosmetic products at concentrations up to 100 ppm. The report goes forward as a Tentative Safety Assessment.

### Marks' Team Meeting – April 14, 2008

Dr. Marks recused himself from discussion of Methylisothiazolinone due to his ties with Rohm & Haas.

Dr. Bergfeld took over as team leader for this ingredient discussion. She introduced the ingredient by going over the history of Methylisothiazolinone.

Dr. Bergfeld noted that Rohm & Haas has requested that the Panel consider the abbreviations "MIT" and "CMIT" instead of "MI" and "MCI" for Methylisothiazolinone and Methylchloroisothiazolinone, respectively.

Dr. Shank felt that the abbreviations for the ingredients should stay as-is in the report.

Ms. Burnett noted that MI/MCI is the common abbreviation for the United States and MIT/CMIT is common in Europe. Most publications use MI/MCI.

The team agreed that the ingredient should be described by its INCI name and not be abbreviated in the report.

Dr. Bergfeld asked that the background information on the mixture be used for Methylisothiazolinone's discussion.

Sensitization and neurotoxicity should be incorporated in the discussion.

The representatives from Rohm & Haas asked that the comments they had on the neurotoxicity section be incorporated into the report. They had drafted a statement noting that other animal toxicity studies in the report did not find evidence of neurotoxicity.

Dr. Shank asked that the discussion section include a paragraph on cross-contamination sensitization from Methylchloroisothiazolinone. Dr. Bergfeld added that the discussion should have language on the threshold dose response.

Dr. Shank noted that the descriptions on the concentration of the substance in several of the studies were rather confusing since a concentration range of the test product and the active ingredient concentration after dilution were both included. The representatives from Rohm & Haas agreed and asked that the report only discuss the substance's concentration as active ingredient (100 ppm in most cases).

Dr. Bergfeld noted that predictive and provocative studies were commingled in several areas of the report, specifically in the neurotoxicity study. She asked Ms. Burnett to group the studies together to avoid confusion.

Ms. Burnett asked the representatives from Rohm & Haas to clarify what the Mintel database is.

Rohm & Haas replied that it is an industry database. The use data was supplied to CIR since the FDA use database commingled Methylisothiazolinone and Methylchloroisothiazolinone data.

Dr. Bronaugh from the FDA said that FDA has hopefully resolved the commingling issue.

The team agreed that Methylisothiazolinone is safe in the present practices of use and concentration as described in this safety assessment. The report goes forward as a Tentative Safety Assessment.

**Full Team Meeting – April 15, 2008**

A Scientific Literature Review on this ingredient was announced on December 13, 2007.

Dr. Belsito stated that this ingredient is being reviewed for the first time by the Expert Panel and thanked industry for providing data. He also noted that, after reviewing all of the data on this ingredient, his Team concluded that Methylisothiazolinone is safe for use up to a concentration of 100 ppm in both leave-on and rinse-off cosmetic products.

Dr. Marks said that it should be noted that he is recusing himself from deliberations on the safety of this ingredient and voting, considering that he has been a consultant to Rohm and Haas and that Rohm and Haas supports a meeting in Hershey, Pennsylvania that he directs.

After considering the Belsito Team's 100 ppm concentration limit, Dr. Shank wanted to know if the conclusion should only state that Methylisothiazolinone is safe under the current practices of use.

Dr. Belsito said that his Team was comfortable with Dr. Shank's proposed conclusion, but noted that industry prefers that the conclusion contain a concentration limit and may wish to comment.

Dr. Snyder said that the standardized nomenclature for Methylisothiazolinone should be adopted throughout the safety assessment.

Dr. Belsito agreed and noted that MIT should be used as the abbreviation for Methylisothiazolinone.

Dr. Shank said that his Team agreed that Methylisothiazolinone should not be abbreviated in the report text, considering that is identified as MIT in Europe and as MI in the United States.

Dr. Klaassen said that Methylisothiazolinone has a rather long name and that it should be abbreviated, provided that the MIT abbreviation is clearly defined at the beginning of the report.

Dr. Shank recalled that in the published CIR Final Report on Methylisothiazolinone/Methylchloroisothiazolinone, MI is the abbreviation for Methylisothiazolinone.

Dr. Klaassen said that the United States is switching to the new abbreviation (MIT).

Dr. Bergfeld said if the MIT abbreviation is going to be used throughout the safety assessment, it should be redefined in the report discussion.

Dr. Belsito said that, at various locations in the safety assessment, Methylisothiazolinone (commercially available as 9.5 to 9.9% solution) was diluted to a concentration of 100 ppm and studied. However, he noted that the reference implies that it was tested at concentrations of 9.5 to 9.9%, and the test concentration needs to be changed to 100 ppm.

Dr. Belsito also said that a blend of Methylisothiazolinone with Methylchloroisothiazolinone is commercially available, and that this could be a source of confusion. With this in mind, he noted that the background information on Methylisothiazolinone/Methylchloroisothiazolinone in the report text should be deleted from the safety assessment on Methylisothiazolinone because this information is not necessary for reviewing the safety of this ingredient.

Dr. Shank said that his Team agrees.

Dr. Andersen wanted to know whether it is acceptable to include historical information relating to Methylisothiazolinone/Methylchloroisothiazolinone in the report Introduction.

Dr. Belsito said that the inclusion of such a statement in the Introduction is acceptable, but that all of the data summaries relating to test results for Methylisothiazolinone/Methylchloroisothiazolinone should be deleted from the report text.

Dr. Bergfeld said that issues relating to the skin sensitivity threshold, cross-reactivity, (i.e., cross-reactivity of MIT with Methylchloroisothiazolinone), and neurotoxicity should be addressed in the report discussion. She noted that the skin sensitivity threshold has already been addressed with the proposed 100 ppm concentration limit. Dr. Bergfeld said that reorganization of the human data under predictive and provocative testing subheadings was requested.

Dr. Bergfeld also noted that references to total % and the active ingredient % are made in the report text, which is rather confusing, and that only the active ingredient % tested should be stated.

The Expert Panel voted unanimously in favor of issuing a Tentative Final Report with a conclusion stating that Methylisothiazolinone is considered safe for use in cosmetics at concentrations up to 100 ppm.

Dr. Bergfeld noted that all discussant points elaborated on at today's meeting will be incorporated.

## SEPTEMBER 2008 PANEL MEETING – REVIEW OF DRAFT FINAL REPORT

### Belsito's Team Meeting – September 22, 2008

- requested the inhalation boiler plate be added
- some wording changes were requested on pages 57 and 57
- Final Report

### Marks' Team Meeting – September 22, 2008

- Dr. Marks recused himself from the discussion of this ingredient)
- the Team would like (0.01%) to be added to the Conclusion following the term 100 ppm in order to define that number clearly
- there were some editorial changes noted

**Full Team Meeting – September 23, 2008**

Dr. Marks stated that he had recused himself from the Panel's discussions on Methylisothiazolinone, due to a conflict of interest.

Dr. Belsito stated that, at the April 14-15, 2008 Panel meeting, the Expert Panel issued a Tentative Report with a conclusion stating that Methylisothiazolinone is considered safe for use in cosmetics at concentrations up to 100 ppm. He noted that new data were not received during the comment period and, therefore, that a Final Report with this conclusion should be issued.

Dr. Shank requested that the ppm unit in the concentration limit be changed to %. Thus, 100 pm should be expressed as 0.01%. He noted that ingredient use concentrations in the safety assessment are expressed as %, not ppm.

Referring to the report discussion, he stated that the following sentence in the last paragraph is not correct: Individuals sensitized with CMIT, however, do not cross-react with MIT. He said that this statement is incorrect because these individuals do cross-react, but with very high concentrations of MIT. Thus, the statement should be revised to read as follows: However, most individuals sensitized with CMIT do not cross-react with MIT. A minor revision of the last sentence in the discussion was also made.

Dr. Bergfeld asked for any comments on the neurotoxicity statement in the second paragraph of the discussion, and the Panel agreed that the statement does not need to be revised.

The Panel voted unanimously in favor of issuing a Final Report with a conclusion stating that Methylisothiazolinone is safe for use in cosmetic formulations at concentrations up to 100 ppm (0.01%).

**MARCH 2013 PANEL MEETING – ADMINISTRATIVE REVIEW TO REOPEN**

**Belsito's Team Meeting – March 18, 2013**

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

DR. LIEBLER: I think so.

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

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

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

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

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

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

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

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

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

DR. BRESLAWEC: We would support reopening this.

COURT REPORTER: Speak up, please.

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

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

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

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

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

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

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

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

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

DR. BELSITO: All right.

DR. LIEBLER: So it make sense to reopen.

DR. KLAASSEN: Sure.

DR. BELSITO: Okay.

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

DR. BELSITO: With methyliso?

DR. ANDERSEN: Yeah.

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

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

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

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

DR. BELSITO: Right.

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

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

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

DR. LIEBLER: Can I ask one question?

DR. BELSITO: Sure.

DR. LIEBLER: Have we surveyed for new use?

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

DR. LIEBLER: Yeah. So we have that?

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

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

DR. BELSITO: With methylisos?

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

**Marks' Team Minutes – March 18, 2013**

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

DR. SHANK: I object to that acronym.

DR. MARKS: Yes.

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

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

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

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

DR. SLAGA: I agree with that.

DR. BERGFIELD: I do, too.

DR. HILL: Yes.

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

(Laughter)

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

DR. ANSELL: Yeah, we agree.

DR. SHANK: Next?

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

**Full Team Meeting – March 19, 2013**

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

DR. ANDERSEN: Well, I can

DR. BERGFELD: Introduce it anyway.

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

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

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

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

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

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

SPEAKER: Thank you.

SPEAKER: MI.

DR. SHANK: We had a lengthy discussion about this. It was nice to have Don reflect some of his personal experiences and things and some other issues that were brought up were there appears to be disagreement among dermatologists actually what concentration you test and he expressed that some results may be negative because some people are only testing at 200 parts per million, some are testing at 2,000 parts per million. He himself is testing at 1,000 parts per million and they get a positive result relatively quickly, within 48 hours.

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

DR. BERGFELD: Is there a second?

SPEAKER: Second.

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

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

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

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

DR. HILL: Who me?

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

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

SPEAKER: We'll need a vote.

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

(Hands raised.)

## MARCH 2014 PANEL MEETING –REVIEW OF DRAFT AMENDED REPORT

### Belsito's Team Minutes – March 17, 2014

DR. BELSITO: So methylisothiazolinone, Christina. So everyone got my Wave 3 stuff that we had the wrong EC3 value? First let me start by saying that as opposed to our more emotional colleagues in Europe, we cannot go about banning this in leave-ons because we would essentially ban MCI/MI because MI is a component. Number two, I am privy to the QRA that was run by Cosmetics Europe, but I cannot share any of the information, the specific information, with you. They promise to get us their actual values within the month, they're hoping. But they're not ready to release them to us even though they presented them to the European Society of Contact Dermatitis, and I didn't really understand why they would do that and not present it to us. But be that as it may, that's where they're at.

I can tell you that the QRA will tell you that every leave-on use below where industry feels this could be used as an appropriate biocide, which is 75 ppm -- so 75 ppm is where MI alone would be an active biocide. Below that it starts to become less than functional. So I'm not going to tell you what the EC3 for leave-ons is other than it is below that 75 ppm. So when we look at the QRA for MI, we will essentially end up banning it from every leave-on product. But it should be sufficient to allow us for it to continue to be used in most wash-off products. That's all I can tell you. I can't tell you the levels.

So my recommendation would be that we table it to get the QRAs from Cosmetic Europe, or alternatively, we have the new EC3 value that theirs was based off. That's the David Roberts commentary. We can do our own QRA. So instead of just saying "tabling it" and letting the Europeans do our work, simply say insufficient for a QRA on the new EC3 values as set in the paper that I sent you, the editorial, the correction that David Roberts did.

So I would say that since I'm a little bit annoyed that Cosmetics Europe can share their data with an organization of dermatologists in Europe and not share it with an organization in the United States that looks to set safety standards, I'm not convinced that they're necessarily going to give us their data in a month. So my recommendation would be that we go insufficient to calculate our own QRA based off of the new EC3 values.

DR. SNYDER: Tabled to calculate.

DR. BELSITO: Tabled to calculate or insufficient? I don't care, however the protocol is, but I don't -- I like these people, but again I was just very annoyed that they've already presented the data to the European Society of Contact Dermatitis Committee that is looking at MI and wants to ban MI without even realizing that if they ban MI, they ban MCI/MI. I mean they don't really necessarily think out the consequences in Europe of everything they're doing. So I don't think we want to ban MI from leave-ons. We want to set a leave-on limit for MI, which we can do from the QRA. It's going to be a limit, which is going to be useless to industry for use as a biocide alone in a cosmetic product. That's what I've been told. I don't know, but I've been told by the industry people that they need 75 ppm of MI alone to preserve a product.

DR. LIEBLER: So if we're going to need one QRA or another, what's the most expeditious course for us?

DR. BELSITO: Is to calculate our own because I'm not willing to trust Cosmetics Europe to release it.

DR. LIEBLER: So not to table it.

DR. BELSITO: No, to go insufficient for CIR to do their own QRA based upon the article that I provided from Dave Roberts that sets the new EC3. It's a simple calculation.

DR. LIEBLER: I'm fine with that.

DR. BELSITO: So that's all I have to say. Comments? Yes?

DR. WATT: Since I'm comfortable that I can take information back to Cosmetics Europe and say that this information does need to be supported within one month, could you leave it open? That if that is not forthcoming, that your own QRA would be put in place? But I think it would be helpful to have one single document that would support --

DR. BELSITO: Well, I mean I agree.

DR. BRESLAWEC: We certainly from PCPC agree, and we have requested that information as Ian knows from Cosmetics Europe. Cosmetics Europe has its own justification for not providing that either to CIR or to the American Dermatologists who are scheduled to meet and discuss this next week. And so I appreciate that, but I also recognize that CIR needs to move ahead and if that information is not forthcoming, I would recommend that CIR do something.

DR. BELSITO: It's a quick calculation. I mean we don't meet again until June. It's March 16. So if we don't have the information from Cosmetics Europe by April 16, by the day after U.S. taxes are due, then I would say that CIR needs to go ahead and do their own calculation. I mean it's just very annoying. If you're not going to share your data that's one thing, but if you're going to share your data to an organization of dermatologists in Europe -- and I know why they did it because they were afraid they were going to push to ban the whole thing and it's dermatologists that are sitting there who are going to make the recommendations. So they wanted those dermatologists to see the data. I'm sure that's the reason. But if you're going to share it with them, then why don't you share it with your U.S. equivalent, which is us. That's just upsetting to me.

DR. WATT: I can understand that.

DR. BELSITO: And so I'm not convinced that they will necessarily share. What Nicki told me was if it's ready for publication -- we will share it with you as soon as it's ready for publication, and I believe that will be within the month. Well, I believe it will be within a month and it will be -- I don't know.

DR. WATT: Yeah, there's a meeting at Cosmetics today. It's already happened.

DR. BELSITO: Yes, Petra's presenting the information that she derived.

DR. WATT: And to discuss an action plan. And my feedback from this discussion will be that Europe needs to do that.

DR. BELSITO: Well, let's see. Then maybe we'll have some information today.

DR. BRESLAWEC: We all strive for alignment, but at some point --

DR. WATT: You need to move ahead. I fully support that.

DR. LIEBLER: Maybe if somebody other than Don asks.

DR. BELSITO: Yeah, maybe I'm just not a good asker. No, Halyna has asked.

DR. LIEBLER: Well, then that's the perfect control.

DR. BELSITO: So we're going to go insufficient for the QRA, and we're going to allow Cosmetics Europe about a month to share their data with us. And if by mid to end of April we don't have that data, then we're asking CIR, PCPC, someone to go ahead and do the QRA for us based upon the EC3 levels that were set by or reevaluated and corrected by Dave Roberts.

DR. SNYDER: And so we also wanted them to confirm the 0.6 percent contamination issue because if that can be clarified, make sure that that's --

DR. EISENMANN: That's a different report.

DR. SNYDER: Oh, was it? Sorry, never mind.

DR. BRESLAWEC: I have a procedural question for the Panel. I take it you would like to be able to make a decision or a recommendation on this by June?

DR. BELSITO: Yes.

DR. BRESLAWEC: Then the question is procedurally, does CIR have adequate time to move ahead with this?

MS. BURNETT: Well, if it's tabled then it doesn't really -- if it's insufficient, it's not really going out for comment either. So the chronology is --

DR. BRESLAWEC: If you table it, then you can deal with it in June. If you do insufficient data, you can't deal with it in June.

MS. BURNETT: Yeah, we could.

DR. BELSITO: Comment periods are now 60 days, no?

MS. BURNETT: Right, but I don't know if insufficient -- I mean we don't have to post anything other than the notice for insufficiencies. So I think either way it would come back in June.

DR. BELSITO: Good. Well, I mean Lillian will be there tomorrow and if we go insufficient and she thinks tabling is more -- I don't care which way we go, but basically we just need the QRA because the most important thing is that we not ban MI in all product categories because then we're essentially banning MCI/MI.

MS. BURNETT: I think that will be okay. Either way it comes back as a direct --

DR. BJERKE: I'd just like to make one comment. When you get the C E QRA, let's make sure we customize it for the VCRP data that we have on the table that you're looking at right now so it meets both European and North American needs. So we may need a little bit of time to do that.

DR. BELSITO: To tweak it, yeah.

MS. BURNETT: And you did get -- I don't remember which Wave, but we had the email from FDA that they didn't give us the break-apart, but they gave us the overall number of total uses and it's not -- for MI low. It's not 3,000. It's 800.

DR. BELSITO: Yeah, but 800 very important uses, including not only baby wipes, but the other thing that you're going to see that we're seeing is Neutrogena put it in their ultra-sheer sunscreen. And we're seeing a ton of reactions from Neutrogena Ultra Sheer.

MS. BURNETT: And hopefully we're going to talk back and forth with PCPC people and get the actual breakouts for next time.

DR. BELSITO: So we're going insufficient, but if Lillian says table is a better way, we're comfortable, but basically for the QRA based upon the corrected EC3 value.

**Marks' Team Minutes – March 17, 2014**

DR. MARKS: So, tomorrow I'll move the final report be issued on the alkyl betaines, with a conclusion that these are safe when formulated to be non-irritating. Okay. The last ingredient I have is methylisothiazolinone. Is that correct? That's the last one?

As in the past, I will recuse myself from this since, historically, I actually came to the CIR and was one of the investigators for MCI, methylchloroisothiazolinone and methylisothiazolinone for Roman-Hawes when they existed, and they're now part of Dow, and also Rohm and Hass supported multiple meetings in Hershey that I ran. So, I think it's appropriate that I continue to recuse myself from the discussion of this. Ron Shank, I think, is going to take over facilitating this ingredient again as he's shaking his head. So, I will not make any comments. I may or may not stick around for the discussion. We'll see.

DR. BERGFELD: Hey, hey. We stood around for you.

(Laughter)

DR. SHANK: (inaudible) Please stick around. This is an ingredient we've met before. It is one that the European Union says not to use on leave-ons because of sensitization, but we have two studies, one by Lundov et al, on the ingredient with 11 patients, so they're already allergic to MI, and they found that 15 parts per million of methylisothiazolinone did not sensitize but 49 did. So, that might be used as a cut-off. Then, in Wave 2, we had a table that showed the chloro isomer and MI, so MCI and MI, that the use concentrations did not exceed 15 parts per million. Then, in Wave 3, we had a (inaudible) local lymph node assay that concluded this is a strong sensitizer. So, does anybody on our team have a comment?

DR. SLAGA: How about setting it at 15 parts per million? I think that's where I would go, because we have data to support it. I have one question that bothers me. The use concentration, according to our tables, varies a millionfold. So, if MI is effective as a preservative at 10 to the -8 percent, why do people use it a million times higher? That doesn't seem to make any sense to me. Can anybody explain why we need a millionfold range for a preservative?

MR. WATT: My name is Ian Watt. I represent the Dow Chemical Company. The data that you show, I think, demonstrates that when companies are requested to provide information on use levels, one thing that they're providing is CMIMI, the mixture that Dr. Marks talked out. When you include that in the responses, then you'll find that the MI component, which is one quarter of that, then CMIMI is allowed up to a maximum of 15 ppm. Therefore, you will find that the MI that is reported is at very low concentrations. MI alone is allowed up to 100 ppm and so that's why you see this very large range, because people are reporting the MI from CMIMI and MI alone.

DR. SHANK: But, still, that's not a millionfold difference. If you say MI is a quarter, 25 percent of the mixture, how can you go to either the mixture of MI at 10 to the -8 percent and then somebody else needs 10 to the -2 percent. I don't understand why such a range is used.

MR. WATT: I would certainly agree with that comment that -- in fact at those low levels MI will not be effective. It's probably there, I believe, as an artifact of being used in raw materials, because it is also used to preserve raw

materials that are supplied to cosmetic formulators, and, so, it's likely that the reporting is actually a calculation based on raw material usage.

DR. SHANK: Thank you. I think that clears that up.

MR. ANSELL: Well, we have a number of -- well, clearly this has been a topic of some great interest to the industry, and we've had a number of companies undertake their QRA methodology, the quantitative risk assessment, and pretty much conclude that, based on all of the HRIPT data available and other LLNA data, we think for rinse-off products 100 ppm would be sustainable. The safe level for rinse-off is -- or the NESIL and no effective sensitization threshold for leave-on is quite a bit lower, but we would argue at least for rinse-off products that 100 ppm, which is the number which is floating around in Europe, would be sustainable.

DR. SHANK: Dr. Hill? Dr. Slaga? Any comments?

DR. SLAGA: We could make that further. The rinse-off could be 100 ppm, because that is correct (inaudible). The other would be 15 parts per million, the leave-on.

DR. SHANK: We have data, HRIPT data, on MI, normal people, 100 ppm was not sensitizing, so hard data to support that. That was our conclusion in 2008 also. And, the new data are on patients, so this is called provocative test. These people are already allergic to MI, and 15 ppm was okay. So, would we say 100 ppm for rinse-offs and 15 for leave-on?

MR. ANSELL: Yes. (Laughter) We spent a lot of time with the rinse-off number, and I think we're pretty confident in the 100 ppm. I think the leave-on becomes more complicated and comes up at -- borders on ineffective concentrations, but I think that the real focus has been on the rinse-off. Do we have a sense of what we would recommend from the leave-on? I mean, 15 seems to be the number that was discussed last week as well.

MR. WATT: I would certainly support your comment that 15 ppm in either leave-on or in rinse-off applications will not be effective. So --

DR. SHANK: Will not be effective.

MR. WATT: Will not be effective as a preservative.

DR. SHANK: Oh, as a preservative.

MR. WATT: Yeah.

MR. ANSELL: But, at least in terms of the panel, I don't know that that's really within your purview. I think if you establish a safe level, it would be up to industry to formulate within those constraints or alternatively develop additional data. I think the 100 ppm rinse-off was really something we were much more confident in from a data standpoint. And, I know that Dr. Belsito had some discussions last week, and perhaps we'll have some more insight in terms of what would be an appropriate leave-on number tomorrow.

DR. HILL: Well, 15 ppm, even though it alone wouldn't be effective, it would still allow for 15 ppm in conjunction with the combination, for example, with the methochloro, so that's one thing, and the second thing is it would allow it to be in there. If the MI was used as a preservative in a raw ingredient where it was diluted down, it wouldn't be effective as a preservative. I don't know if this would ever occur, but you mentioned. So, I don't know if that helps at all.

DR. BERGFELD: Is it not true that most of the preservatives in cosmetic products are mixtures?

MR. WATT: Companies tend to formulate, and we're a raw material supplier of preservatives, so, but, my understanding is that formulators will tend to coformulate preservatives. That is true. Again, whether MI would be effective enough, given that MI is a broad spectrum preservative, so it has efficacy against a broad range of microorganisms, and it is effective at low concentrations, but, having said that, 15 ppm is still probably inefficient from a formulation point of view, even with other copreservatives, because copreservatives tend to have their own spectra of performance, and MI tends to be a broad spectrum performance, and, therefore, it kind of underlays the preservation, and the other preservatives that it's used in combination with affect the rest of the spectrum.

DR. BERGFELD: What is the lowest concentration that is effective?

MR. WATT: Most of the usage today of MI alone is towards the upper end of the approved use level of 100 ppm.

DR. HILL: Just a random sampling of the bottles in my shower and my wife's shower and bathroom is most often combined with methylchloro, like the isothiazolinone. So, those activities should, I presume, be at least additive, because I'm assuming the spectrum of that chlorinated compound should be pretty similar to MI itself, maybe even broader.

MR. WATT: It's important to recognize that CMIMI is a preservative in its own right. In fact, most of the efficacy does come from the CMI. As we've already heard, CMIMI, the maximum use concentration for that in rinse-off is 15 ppm. Virtually all of the efficacy does come at those use levels. I mention that it's a 3:1 ratio product. Pretty much all of the efficacy comes from the CMI in that product. The reason that you see CMI and MI on the same label, it's because it's a product which is CMIMI. That's actually a manufactured product. It's not combined as CMI produced and then MI produced. It's actually --

DR. HILL: Partially chlorinated when it's produced is what you're saying.

MR. WATT: Yeah. CMIMI is the product that's produced and placed on the market at 15 ppm.

DR. HILL: I've suspected that for a long time. I just didn't know it for sure until now.

DR. SHANK: Okay. What do we want to do? Who presents this? Dr. Belsito? Tomorrow?

DR. BELSITO: Yeah.

DR. SHANK: So, we respond or do I have to present it?

DR. BERGFELD: He presents.

DR. SHANK: He presents it. Okay. So, it seems for rinse-off 100 ppm. We all agree? Is that right?

SPEAKER: Mm-hmm.

SPEAKER: Yes.

DR. SHANK: So, the only question is what about leave-ons. What concentration?

MR. ANSELL: Whether you want to make a statement as it relates to leave-on concentrations.

DR. BERGFELD: Are you referring to non- sensitizing?

MR. ANSELL: I'll be interested in the discussion tomorrow. The concern was that the mixture has a long history of use, going back, I guess, to the '80s, and it's really been quite a new phenomena associated with the use of MI by

itself in leave-on products. So, that's really the focus. I'll huddle with some folks, who I thought were going to be here now, about whether there's a recommended threshold for the leave-on, but the recommendation is that it be --

DR. SHANK: The 15 ppm.

MR. ANSELL: Right.

DR. SHANK: Okay. Because, the Europeans apparently say no MI in leave-ons. Is that correct?

MR. ANSELL: That's correct.

DR. SHANK: Okay. All right. So, we'll respond to the Belsito team tomorrow.

MR. ANSELL: At this point, it's not really -- I mean, it's perhaps pedantic, but it's a recommendation of one European expert group. It's not really a European level yet.

DR. SHANK: Oh. Okay. Thank you. Okay?

DR. BERGFELD: Onward till tomorrow.

**Full Team Meeting – March 18, 2019**

DR. BERGFELD: Okay. Moving on to the reports, advancing to the next level, Dr. Belsito on the MI.

DR. BELSITO: Okay. Methylisothiazolinone. This has been a major issue and a major concern that, unfortunately, we got wrong. But we and Europe and the world got wrong in terms of the leave-on capacity. Part of that I think was due to the fact that the EC3, there was a miscalculation in the value. David Roberts, in late 2013, published in Contact Dermatitis the corrected EC3. Cosmetics Europe has run the QRA on that. Unfortunately, to date, they have not been willing to share it with CIR. They did share it with me but I cannot comment specifically on it other than to tell you that when you run it, you will find that the leave-on -- what is the acceptable leave-on use will not be an acceptable bias side. There is an acceptable leave-on use, so we should not rush like some Europeans are to ban MI because MI is part of MCIMI, which would effectively ban that preservative.

So I think we should table this. I was told yesterday that we should have the Cosmetics Europe QRA in our hands in about a month. It was actually presented by Petra -- I'm just blanking on her last name -- yesterday in Europe. Hopefully, as soon as the paper is available we will get it. However, we do have the EC3 that was published. We can calculate our own QRA, and I would recommend we table it. If we've heard nothing from Cosmetics Europe by the middle to end of April that PCPC or CIR calculate our own. I think that we will find it safe for use in rinse-offs. We will find a safe level in leave- ons, but it will be a level that industry cannot use as a preservative. But it won't end up banning MCIMI. So table it.

DR. BERGFELD: Is there a second to table?

DR. SHANK: Yes, second.

DR. BERGFELD: Second. There's no discussion on the table and we have to vote it.

All those in favor of tabling under the circumstances given?

Unanimous.

(Motion passed)

**JUNE 2014 PANEL MEETING – REVIEW OF DRAFT TENTATIVE AMENDED REPORT**

**Belsito's Team Meeting – June 9, 2014**

DR. BELSITO: …So now we move on to the real bone of contention, and that's methylisothiazolinone.

DR. BERGFELD: I thought we had solved that bone.

DR. BELSITO: Well, we have and we have not, because unfortunately, I have not had time to calculate out what the acceptable level of use in a leave-on is, and no one -- none of the data we had has given that to us because we cannot say that – methylisothiazolinone is safe in rinse-offs, which I think we can say up to 100 parts per million – but what we cannot do is say that it is not safe in leave-ons, because then we effectively ban MCI/MI and get rid of a whole other group of preservatives, and pretty soon we're not going to have any preservatives. Because even those that are safe, like parabens and formaldehyde releasers, companies are bowing to NGOs and saying they're taking them out of their products. So, you know, what I really would like to do is table this and have the Scientific Committee or someone in PCPC do a QRA for methylisothiazolinone, put into a diaper cream or an underarm deodorant, the one that has the highest SAF value, and show us at what level it could be used in a product that would be -- would allow -- would have to have the lowest concentration not to induce sensitization. So then we could say that methylisothiazolinone is safe as used up to 100 parts per million in rinse-off products and safe when used at a level of this and below in leave-on products. And from what I heard and saw from the Cosmetics Europe data, that level in leave-ons is not going to be sufficient to allow it to be used as a preservative alone, but it's not going to restrict its use in the combination MCI/MI product.

DR. EISENMANN: But the problem is the use amount in products varies so, you know, like in the RIFM they come up with various levels, so I wonder if you need to -- we've never done this before, set a limit. I mean, you know what the NESIL is and you know the highest SAF is 300, so --

DR. BELSITO: Right.

DR. EISENMANN: So it would be 300 times below whatever the NESIL is. If I remember right, it's 15. Is that correct for this one?

DR. BELSITO: Fifteen micrograms per centimeter squared. Yes.

DR. EISENMANN: So it would be 300 lower. So I wonder if you --

DR. BELSITO: But then you need product application, so you need product type and the amount that's applied and the frequency of application, so you need all that.

DR. EISENMANN: Well, in other words, your limit would be in terms of micrograms per centimeter squared rather than a concentration in a product, 300 fold below 15 micrograms per centimeter squared.

DR. BELSITO: We could do it that way, I suppose. But I think we need to be very careful, you know, what we do, and, you know, in Europe there are a certain group of dermatologists who feel that to protect consumers they not only need to protect against induction, but once induction occurs, they need to protect everyone who is sensitized, and that is so extreme, and you see that coming out in part of the SCCS opinion that there is no safe level of use in a leave-on product is essentially what they say. And that's perhaps true for people who are already sensitized, but that's why you have labeling. And I just, you know, I'm very concerned as I'm looking at, you know, the absence of being able to preserve cosmetic products, resulting in infections and, you know, I mean, there was an epidemic -- or not an epidemic. There were several women who were blinded by a mascara in the 1970s that was contaminated with pseudomonas. And so if we want cosmetic products, we need preservatives. And we're slowly getting rid of, you know, methyldibromo glutaronitrile is gone now, essentially. You know, and we need to be very careful to preserve MCI/MI, I think, because MI is, you know, except in wash-offs, essentially gone from leave-ons, but we

need to specify what a safe level is for use of MI in a leave-on, even though it means that it can't be used as an individual preservative in a leave-on.

DR. EISENMANN: I think we'd be able to do that. He probably can do it today because Don has been doing the calculations for us.

DR. BELSITO: Okay. You know, I would just like to come up and say, you know, in a leave-on we feel that this would be a safe limit to, you know, we could even say in the discussion, you know, that it may not -- it could be an issue for individuals who are already sensitized, but it would be a safe limit to prevent induction of new sensitization, and that's why we have labeling. So if you're allergic to something, you can read a label and avoid it.

DR. SNYDER: So the last sentence under the QRA is actually not correct. Right? I mean, that has to come out?

DR. BELSITO: I'm not even on that report yet. Let me save the last one. So what page are you on, Paul?

DR. SNYDER: Well, I have the word document. I'm on -- it's on PDF 19, Don, first paragraph. It's the last sentence, the QRA predicted that consumer exposures to 100 parts per million in skin leave-on products and cosmetic wet wipes could induce skin sensitization. Well, that doesn't --

DR. LIEBLER: What about 50? What about --

DR. BELSITO: Well, yeah. I mean, that's --

DR. LIEBLER: That's a non-statement.

DR. BELSITO: Right. That's the point. I mean, but, you know, and I do know, I mean, I saw the Cosmetics Europe data. You know, they have gone through all their classifications and they actually do have, you know, QRA-based limits for leave-ons as well. But the issue is, at least from what Nicky Gilmour, who is with Unilever told me is that those levels would be below what would be adequate as a preservative in a leave-on. So it makes it essentially useless.

DR. SNYDER: Okay.

DR. BELSITO: But I think we need to know what those levels are because, you know, if we simply say it's not safe in a leave-on, then we've effectively banned MCI/MI as well. And I just don't want to do that. And, you know, so I would like to see those calculations so we can say, you know, even under the, you know, worst-case scenario of using this in a leave-on product, at this level it would not induce sensitization based upon the QRA. And so we find that it's safe in leave-ons up to whatever level, and then if industry can use that, fine. And if they can't use it, fine. But it will be at a level where MCI/MI is used at 15 parts per million in rinse-offs and 7.5 parts per million in leave-ons it'll be safe.

DR. LIEBLER: So if we assume that we would have a number by the time we discuss this in full panel tomorrow, could we have a tentative conclusion then?

DR. BELSITO: Safe up to 100 parts per million in rinse-offs and safe up to whatever in leave-ons.

DR. SNYDER: I mean, as it currently stands we're insufficient for leave-ons and we're safe at 100 parts per million for rinse-offs.

DR. BERGFELD: You have some data from the European group at 15 parts per million that's safe in leave-ons, I thought. Did we not, Carol?

DR. SNYDER: No, that was their limit for rinse-offs, 15 parts per million.

DR. BERGFELD: I thought it was 100.

DR. SNYDER: They said there is no safe level for leave-on, and they said it was safe up to 15 parts per million for rinse-offs. That's EU's. So we were going to go higher on the rinse-offs at 100.

DR. BERGFELD: Well, here it says --

DR. BELSITO: Well, and then the question becomes -- go ahead, Wilma. I'm sorry.

DR. BERGFELD: Well, it says under QRA in the last sentence, "The QRA predicted that consumer exposure --"

DR. BELSITO: What page?

DR. BERGFELD: What page? It's just about phototox. Let me see what page is it? Difficulty. I don't know what page it is.

DR. LIEBLER: It's probably that same PDF 19.

DR. BERGFELD: Yeah. Exposure to 100 parts per million MI in skin leave-on products and cosmetic wipes could induce skin sanitization, while exposure to the same concentration in rinse-off products, in rinse-off products and hair care leave-on products, would not induce skin sensitization. So the 100 parts per million has been sort of clarified for rinse-offs. Now, there's somewhere else where there's 15.

DR. SNYDER: It's under the cosmetic use section, way at the beginning, the next (inaudible) paragraph talks about the EU's.

DR. BELSITO: What page?

DR. SNYDER: I have a Word document, so it's --

DR. BELSITO: Cosmetic use?

DR. SNYDER: Yeah.

DR. LIEBLER: PDF page 19, where they say the 15 parts per million for rinse-offs, but no safe limit for leave-ons for induction or elicitation in leave-ons.

DR. BELSITO: Yeah. So what you have to understand is that the SCCS is an advisory board, and it is composed of individuals who look and make recommendations. So this has not yet been approved as part of an EU directive. It's a recommendation that goes to DG SANCO [*Directorate-General for Health and Consumers*] and then DG SANCO put -- makes their decision and then puts it in front of the EU Parliament to pass as a directive. So what I can tell you is that this recommendation that is coming from the SCCS is being based upon trying to protect consumers who are already sensitized. It's not about preventing new sensitization. So that's all of that data where they went through and they got, what, 12 patients who were hypersensitive to MI and they did dilutional ROATs on them and they found that, you know, they could get responses down to one part per million or whatever. It's not based upon QRA for induction of sensitization. It's based upon elicitation. So I -- that's, and I pointed that out to other of my European colleagues, because I'm involved in a QRA workshop that RIFM is sponsoring in Brussels. That is a very dangerous approach because if they do that, they're going to be banning MCI/MI. And they don't necessarily think about the consequences of their actions as we've seen from the fact that they outlawed animal testing in cosmetics when they didn't have good alternatives.

So I don't think -- we can certainly put that in, you know, that statement, because it has been made. It has been, you know, drafted and sent to DG SANCO, but it's not an EU directive at this point. And it's being very carefully looked

at by the people at DG SANCO because they realize what is going to happen here.

So I would like to take the other precedence and say, yeah, we probably can't protect people who are already sensitized to MI, you know, but they can read a label. But, you know, MCI/MI is an important preservative. MI can be safely used in rinse-off products. The question becomes do we also want to say in certain hair products, because they're leave-on and the QRA would suggest that, at least in some of those product categories, or do we really want to punt it and say that MI may be safe in leave-on products based upon an AEL [*acceptable exposure level*] over CEL [*consumer exposure level*] value of greater than one, which is what is used in the QRA to say that it won't induce sensitization.

However, that begs the question as to whether the QRA has been validated, and the people on the SCCS will tell you that it's not been validated, and we're relying on an invalidated methodology to set limits. So, I would be comfortable just saying 100 parts per million is safe in rinse-offs because those AEL/CEL values are all extremely high. And then coming up with the highest value that could safely be used in any leave-on product and say anything, you know, at that level or below would be safe in a leave-on.

DR. EISENMANN: Don did some calculations, 4 ppm and 13 ppm in a baby wipe is what he got.

DR. BERGFELD: Okay. So 4 ppm would be the critical thing if you put in a lipstick, and that's not going to restrict it from MCI/MI because in a leave-on, MCI/MI is 7.5 parts per million divided by 4 is like 2 parts per million. So if we say MI is safe in any application, you know, it can be used at various levels, but the highest level that it could be used in a lipstick, which would be the most restrictive product, would be 4 parts per million. And so we find it would be safe as used in a cosmetic product. Certainly safe as used in a cosmetic product up to 4 parts per million or something like that in the discussion, leave-on.

DR. EISENMANN: But it would be nice also in the discussion to recognize --

DR. BELSITO: Show the different categories could have higher levels.

DR. EISENMANN: Right.

DR. BELSITO: Yes. You know, I just point that out, you know, but, you know, I think that somehow we need to convey the fact that we don't need to go back and look at MCI/MI and that, you know, MCI/MI in leave-ons, the amount of MI in that is, you know, going to be 7.5 divided by 4 and that that's a safe level in a leave-on. And here's the reason. Because in the MCI/MI report we didn't, you know, we just said, you know, safe as used, you know, up to 15 parts per million in rinse-off. We didn't go into product categories, which means that, you know, lipsticks are a product category where it could be used. So that's why I wanted to go to the lowest number that would be safe in any product category, or the highest number that would be safe in any product category, and that's 4 parts per million in lipstick. We can point out in the discussion that you could use it in a body lotion at a higher level or whatever, but to err on the side of being conservative, we're going to say 4 parts per million, and then we don't have to touch MCI/MI. We don't need to go back to that report.

DR. BJERKE: Let me double-check to make sure I've got all the products.

DR. BELSITO: Okay. I mean, we can have this discussion tomorrow but, I mean, that's where I'm most concerned about is that we don't need to go back and look at MCI/MI and we don't effectively ban it by saying MI can't be used in a leave-on, because it is used in MCI/MI in leave-ons.

DR. BERGFELD: Can you make a table on the different product lines and the maximum concentration it could be used?

DR. BJERKE: The QRA that we provided has that table in there, and what I looked at is the AEL/CEL ratio. And

if you take that lowest ratio, you can make it -- you can determine the level of MI that's appropriate to get that to one. What I want to do is make sure that -- and so I've got all the product categories covered for the current uses as reported in the VCRP data. I just want to make sure, I go back and make sure all the FDA VCRP categories are in there.

DR. BELSITO: I mean, I think that would be great so we have a ppm level where the AEL/CEL would get to one for that product category, and then we could actually include that as part of the report, and then in the discussion say, you know, that MI can safely be used in leave-ons from a product range of a low of 4 parts per million in lipstick to a high of whatever in a whatever. And that, you know, in the conclusion, you know, say something to the effect that, you know, MI is safe in rinse-offs up to 100 parts per million in leave-ons. It depends upon product category, but overall, the panel, you know, felt that certainly it was safe at levels up to 4 parts per million.

But I would like us to try and be sort of quick with posting this, so at least the European authorities can somehow see what the Americans are doing because I'm just afraid that there are a few individuals in Europe who are trying to be very persuasive in getting this banned, and they're just not seeing that bans MCI/MI or maybe they want to ban that too. I don't have a clue. Anything else? I've done all the talking. Okay.

DR. KLAASEN: Well, I guess according to the theory of the SCCS we would have to ban peanuts from the world.

DR. BELSITO: Well, yeah.

DR. BERGFELD: They have a lot in it.

DR. LIEBLER: That's half the M&Ms in the world, by the way.

DR. BELSITO: Okay, I'm still trying to figure out how to navigate through PDFs here. Is there anything else?

SPEAKER: Do you want to tackle --

DR. BELSITO: Just to clarify, it says on PDF page 18, MI was named "Society Contact Allergan of the Year by 2013 by the American Contact Dermatitis Society." It was named Allergen of the Year for 2013 by the American Contact Dermatitis Society, and those are the reasons, just to clarify that it's just called Allergan of the Year.

Again, I think, in the discussion if we get around to setting a safe level in leave-ons, we need to discuss the ROAT in 7 patients. This is on page 18 -- that that ROAT was obviously done on only 11 patients, 7 of whom reacted to very low levels, but again we're setting levels that would prevent induction, and we're not looking at individuals already sensitized to or are who able to avoid it by reading labels, so that we don't look like we're just ignoring those individuals. And is there anything else? And just throughout its -- just Contact Allergen of the Year. That was the only other comments that I had. And what are we moving onto now?

**Marks' Team Minutes – June 9, 2014**

DR. MARKS: … I have to recuse myself. I've done this consistently, so I will again with, methylisothiazolinone. So, Ron Shank and Tom Slaga are going to have an arm wrestling match and whoever wins gets the (inaudible).

SPEAKER: I don't know (inaudible)

DR. MARKS: Ron Shank is going to facilitate.

DR. SHANK: I thought the -- well, do I need to review the history like you do? We've had this before us, before, and the compound -- the issue, the toxicological issue has been sensitization, but I think the report is in good shape,

and that we can go to a conclusion that MI is safe to a 100 parts per million in rinse-offs, and 15 parts per million in leave-on. Now I think my understanding is the 15 percent -- the 15 ppm may not be effective as a preservative, but that's not our concern.

DR. SLAGA: It's (inaudible) that 100 is --

DR. SHANK: It's (inaudible) that 100 --

DR. SLAGA: Yeah.

DR. SHANK: -- but apparently not at 15.

DR. SLAGA: Below -- below 100 it is --

DR. SHANK: So those were the only comments I have.

DR. SLAGA: Mm-hmm. And that's basically what we agreed to, and we -- this group agreed to once --

DR. SHANK: Yeah. This is a final report, is it not?

MS. BURNETT: No.

DR. SHANK: No?

MS. BURNETT: This would be (inaudible), no this.

DR. SHANK: Tent.?

MS. BURNETT: Yes.

DR. SHANK: Oh, yeah.

MS. BURNETT: If they'll expand on the discussion that would be great.

DR. SHANK: Oh, okay.

MS. BURNETT: Because it has a -- it has the -- you know, we amended this -- this is the amended report, so I have the original discussion but this is going to -- what's going on today and tomorrow will supersede that discussion.

DR. SHANK: I understand -- oh, I see. Yes.

MS. BURNETT: Sorry, it's a bit hard --

DR. SHANK: No. That's all right. I see in your -- in your letter to us, Christina --

MS. BURNETT: Mm-hmm.

DR. SHANK: -- presented basically what we wanted in our discussion.

MS. BURNETT: The QRA discussion?

DR. SHANK: Mm-hmm. Let me find it. I think the discussion can just say that this has been reviewed before.

MS. BURNETT: Mm-hmm.

DR. SHANK: The Panel requested a QRA, it was prepared by Cosmetics Europe.

SPEAKER: Cosmetics Europe -- huh?

DR. SHANK: Isn't that what it's called? Anyway, we've got one.

MS. BURNETT: Mm-hmm.

DR. SHANK: And based on the data available we determine that for rinse-offs it's 100 part per million, and for leave-ons 15 parts million, would be safe as used.

MS. BURNETT: Mm-hmm.

DR. LORETZ: With the other product category, what's a concern is -- it's a leave-on but the leave-on hair products were some of those under the QRA, were cleared at a much higher level, and there's still interest in using those.

DR. SHANK: Okay. I'll have to find that.

DR. LORETZ: And that's why, because to make sure, was separating out skin leave-on products from hair leave-on products. So the Panel specifically pulls out wipes?

MS. GILL: You know, it's not intuitive, so if you are wiping something on, it seems like it's not staying but it is, you know, for some of the sensitization I've seen in (inaudible).

DR. SHANK: Mm-hmm.

MS. GILL: When you wipe it, it's not staying on.

DR. LORETZ: No. It is staying on, but when you're wiping it, you know, the consumer doesn't necessarily think of it that way, they are thinking it's evaporating for us.

MS. GILL: I see.

DR. LORETZ: It's not necessarily --

DR. SHANK: Right.

DR. LORETZ: -- conclusive.

DR. SHANK: -- pardon me.

MS. GILL: If the wipe-ons or if it's a leave-on.

DR. LORETZ: It's a wipe-on.

DR. HILL: We actually have a statement in here on page 15, and my comment was, I wasn't quite sure I understood the sentence, but -- so I was asking for clarification I think, pertaining to wet wipes, it says, it's founded in leave-on cosmetic products, including wet wipes, no safe concentration has been adequately demonstrated for induction or elicitation of contact allergy.

DR. LORETZ: That's the SCC's opinion?

DR. HILL: Yeah, but -- so what does that mean? What are they trying to say -- that no levels have been established as safe at this point for leave-on? That's the way I read it.

MS. BURNETT: Or believe --

DR. SHANK: Well from test data we have weight of evidence. No expected sensitization induction level of 15 micrograms per square centimeter. I guess that was translated to 15 parts per million in a formulation.

DR. HILL: And they say that's okay for rinse-off?

DR. SHANK: No, for leave-on.

DR. HILL: I thought the European Group said no safe concentration has been demonstrated for leave-on, of any kind, including wet wipes.

DR. SLAGA: So they said not to use it in leave-on.

DR. HILL: That's what they are saying?

DR. LORETZ: Mm-hmm.

DR. HILL: Which, when you consider that, there is no chronic dermal or inhalation tox to fit.

DR. SHANK: So what do you want to do? We asked for the QRA, we got -- pardon me -- we got one, and there seems to be no problem with the 100 part per million in rinse-offs. And if you say it's unsafe for leave-ons what data are you going to use?

DR. HILL: Well, there is no data, so that means it's insufficient for leave-on use.

DR. SHANK: Even with the QRA, that was based on sensitization studies, repeated insult patch and lymph node.

DR. HILL: Okay. So where are we setting it then?

DR. SHANK: So rather than, say, 15 -- for leave-on, rather than saying 15 parts per million, say 15 micrograms, huh. We've never done that before. And exposure, that would produce no more than 15 micrograms per square centimeter.

DR. HILL: Okay. I don't see why we -- why that would be unreasonable.

DR. SHANK: Tom, do you?

DR. SLAGA: No. No. I agree.

DR. LORETZ: And does that -- just to go back to the hair leave-on, like the hair conditioner under the QRA is fine at 100 ppm, is that something that can mean --

DR. SHANK: Isn't that -- isn't that rinse-off?

DR. LORETZ: No. The leave-on conditioner, hair conditioner.

DR. SHANK: It is the leave-on?

DR. LORETZ: Right. There are a few leave-on hair products, only in hair products, not skin products that are okay at 100 by the QRA, and for which there is, interest in, continuing use of that concentration.

DR. HILL: And I think that's how this long trail -- rabbit trail got started actually.

DR. LORETZ: Mm-hmm.

SPEAKER: Hmm?

DR. HILL: This leave-on, I always think, if the people I see who were -- you see a lot of them where I live, so they are working in a very hot environment in food service, usually, and then they have the hairnet on, and they've got these products on, so then they are sweating, and it's not just on their hair, it's undoubtedly reaching their scalp, and it's sweaty, hot scalp, which probably enhances penetration, so.

But you think you'd be seeing a lot of clinical incidents if that was what they -- I don't know; Down South, if they are or they aren't. I have no idea.

DR. SHANK: Then we could say that MI is safe at 100 parts per million in rinse-offs and leave-on hair products, and 15 micrograms per square meter for leave-on skin products.

DR. LORETZ: Or, although I caution that the hair -- like some of the permanent hair dyes, so that's a leave-on hair product, and now it's not okay by the QRA. The hair leave-ons are the most complicated of the categories, because there some that fall okay, and some don't. Whereas those -- skin products are simpler.

DR. HILL: Would it be possible to run a calculation, we had a certain ppm level, which is, of course, significant for preservative function, assuming that's what they are using for? Run a calculation, this is the amount of product we'd expect to put on maximum exposure, there's so many square centimeters, the scalp, some of you know that number off the top of your head, I don't know it. And then see where we are at in terms of that microgram number; microgram per square centimeter number?

DR. LORETZ: That's what the QRA is trying to calculate (inaudible).

DR. HILL: Yeah. Okay.

DR. LORETZ: It's the sensitization, so that's based on what --

DR. HILL: Okay. And so it came up with mixed results.

DR. LORETZ: Yeah. Just, again, for the hair leave-on.

DR. HILL: Okay.

MS. BURNETT: So the hair -- according to what we sent out, Wave 2, it's like hair sprays, where we are flagged.

MS. GILL: Semi permanent hair dye?

MS. BURNETT: Right.

DR. LORETZ: It's not okay, right. With the hair conditioner leave-on hairstyling products, those are the ones that came out okay.

DR. HILL: You could think they would have a big (inaudible). It doesn't make sense, unless the MI is being doubled in the dying process, and you really don't have as much MI as what's applied.

MS. GILL: But the dying process is the one that isn't safe?

DR. HILL: I know -- okay, right, so --

MS. GILL: It's in a sense just the (inaudible).

DR. HILL: It's the other way around, so I don't get it.

MS. BURNETT: I think the gentleman from SSC Committee was going to run some more numbers?

DR. BOYER: Yes.

MS. BURNETT: That he'll have available tomorrow?

DR. BOYER: Yeah. And he's actually going to attempt to calculate a worst-case concentration for leave-on products. Based on the worst of all those categories, and we'll go with that as the basis.

DR. SHANK: So you'll go with that tomorrow?

DR. BOYER: Yeah.

MS. BURNETT: Yes.

DR. SHANK: Good. So, I think right now we can see for rinse-offs, 100 ppm, safe. And we'll wait to see what he has to say about leave-ons. Because for leave-ons the only thing I could go to would be the quantitative risk assessment that we got.

DR. SLAGA: And that's 15, right?

DR. SHANK: That's 15 micrograms per square centimeter, which is usually not for (inaudible), these set levels. Anymore discussion on that one?

SPEAKER: Mm-hmm.

DR. SLAGA: No.

DR. SHANK: Okay.

MS. BURNETT: I'll fish out something more for you guys tomorrow then, after the presentation from the numbers so I can beef it up a little bit.

DR. SHANK: Okay. All right, Dr. Belsito starts off on this right?

DR. MARKS: Correct.

DR. SHANK: Okay.

DR. SLAGA: So we are listening to what he says?

**Full Team Meeting – June 10, 2014**

DR. BELSITO: ...I am not longer thrilled with HRIPT data because MI came out of that data, looking pretty clean at 100 parts per million and has proved not to be and had the QRA been used, we would have realized immediately that 100 part per million on almost every leave-on product would cause sensitization…

… I mean, everything virtually, can be safely used, if you use a low enough concentration. So that's why I think that you need to look at what the NESIL is, what kind of product it's going to be used in, what is the amount of that product that is typically applied, where it's applied to, what are the number of times a day the product is applied to. These are all things that the QRA looks at, that a single HRIPT on the back of a patient, you know as well as I that

the genital area is much more absorptive, the underarm area is occluded and absorptive, so HRIPT on the back may not predict what happens. I mean the issue with MI; the baby wipes -- that passed an HRIPT. But at 100 parts per million in baby wipes, at whatever concentration that's being used, it's creating huge problems, so I don't think that, I mean the HRIPT is nice but it, I think that if the QRA predicts that a lower level should be used, then that perhaps should take precedence because the QRA looks at more factors than just nine applications over three weeks and a challenge a week later on the back. It looks at how much product is applied, where it is applied, safety factors, is it shaved skin, unshaved skin.

…Various companies, including Unilever and Procter and Gamble have done the same thing for MI and predicted exactly what we are seeing for MI. So certainly compared to the HRIPT, based upon those two preservative systems, one of which has already been totally banned in Europe, methyldibromo glutaronitrile, and one that is certainly going to be significantly restricted, if not potentially banned in Europe, mainly Methylisothiazolinone, the QRA, even version 1, that doesn't look at cumulative exposure, worked much better than HRIPT…

DR. BERGFIELD: … MI. Dr. Belsito.

DR. BELSITO: Okay, well.

DR. BERGFIELD: After all that discussion on QRA, I thought we'd done it. Okay. Sorry. Thank you.

DR. BELSITO: So MI is clearly causing a problem in the marketplace, and when you do QRA on MI, what you find is that it can be safely used in rinse-off products up to 100 parts per million, and in perhaps a few selected categories of hair leave on products, but in all of the other leave-on product categories, it will potentially cause issues with sensitization. However with this particular preservative, we need to be very careful as to what we do, because MI is also a component of Methylchloroisothiazolinone and Methylisothiazolinone, so we need to be cognizant of the fact that we could impact that preservative as well, by our decision on Methylisothiazolinone. I've been told that the highest level at which it could be used in any leave-on product, and that's a lipstick category, is four parts per million, which would preserve its use in Methylchloroisothiazolinone/Methylisothiazolinone, because we allow that at 7.5 parts per million in leave-ons, and Methylisothiazolinone is just one fourth of that mixture, so certainly below four parts per million, so I think that our team felt that we would go with, safe as used up to 100 parts per million in rinse-off products, safe for use in all leave-on cosmetics at four parts per million, and potentially at levels higher than that, based upon QRA, but at this point, we have not yet been able to see the QRA data for all of the various leave on products and get a sense as to what that range might be. So the question in my mind becomes, how quickly do we want to act and make a statement, because I am quite concerned from my dealings with my people in Europe that they may do something unreasonable like ban MI in leave-on products, which would effectively ban MCI/MI, so we'd certainly like a statement to come out from this panel that MI can be safely used at some concentration in leave-ons that would preserve its use in MCI/MI. So safe up to 100 parts per million in rinse-offs and I would say at this point, safe in all leave-on product categories at four parts per million, which will preserve the MCI/MI use but essentially we'll take MI out of, as a sole preservative system, out of leave-ons.

DR. BERGFIELD: Jim, comments?

DR. MARKS: Yes, I have to recuse myself so --

DR. BERGFIELD: Okay.

DR. MARKS: Dr. Shank will represent our team.

DR. BERGFIELD: Uh-huh.

DR. SHANK: Oh, we had the same struggle with the leave-on. rinse-off at 100 ppm and that's fine. And we were

unable to come to a very clear statement as a safe level in leave-on. Can you repeat for me the basis of the four parts per million? Is that a test data, or based on use?

DR. BELSITO: It's a QRA data, looking at what is the concentration, what is lowest concentration that could be safely used across, or the highest concentration rather, that could be safely used across all product categories. And we were told yesterday by individuals who have looked at the QRA and, that, in a lipstick at four parts per million MI, so that's the most sensitive category with lipstick, that at four parts per million, the AEL over CEL would be greater than one. So that would be a safe leave-on unit, leave-on concentration. Now in other product categories, you could probably go higher than four parts per million. I think I mentioned at the March meeting that I had the opportunity to briefly glance at data, therefore all the leave-on products, and I was told by the individual that was showing me that data, that at the concentrations that could be used in leave-ons, MI would not be an effective preservative system. I don't know. I mean, I'm not a preservative expert. I'm just trying to look and be very proactive that the U.S. comes out and says that MI can be safely used in a leave-on, that would allow MCI/MI to continue to be used, that we don't need to reopen that document, and so I thought four parts per million was a good start. If industry wants to come back to us and give us their full QRA data for all the different leave-on products, perhaps we could say a range of four parts per million to 55, I think was the highest I saw, parts per million, based upon QRA in leave-on products. We could make that conclusion much more specific, but I would like to go out with a conclusion, a final conclusion, that we could always amend. Just so it's out there. Because this is going to be looked at very quickly, and Cosmetics Europe has already presented to DG SANCO. There are going to be recommendations made in Europe and so I would like at least the authorities there to see what we're doing here.

DR. SHANK: Okay, well the QRA value for leave-on was not 15 micrograms per square meter.

DR. BELSITO: 15 micrograms per centimeter squared. Correct.

DR. SHANK: Yeah. And that converts to 4 ppm.

DR. BELSITO: Depends again with, the 15 micrograms per centimeter squared is the NESIL. Then the NESIL is subjected to various safety assessment factors. Immediately it's reduced by 10 for inter-individual variability. Then there are factors of 1, 3 or 10, for how much is applied, where it's applied, so lip is mucous membrane, it's more absorptive so it's a factor of 10, so you could have anywhere from a safety assessment factor of one to a thousand, depending upon the product type, and that's why lipstick ends up at a four parts per million, because it's mucous membrane. It's absorbed at the amount that you put on. It's not a thin layer that you rub in completely so it disappears. There's obviously product left on the lip.

DR. SHANK: Okay, good. I think that needs to be made very clear in the discussion, if the 4 ppm is based on a lipstick formulation.

DR. BELSITO: Right.

DR. SHANK: Okay.

DR. BELSITO: Based on a lipstick formulation, and is the highest level that could be used across all product categories without inducing sensitization.

DR. SLAGA: Would that have to be in a discussion?

DR. BELSITO: You know, it would --

DR. SLAGA: With lipstick, I mean. It's very specific.

DR. BELSITO: No, I think it could be put in the discussion and then I'm told that the information that we were

provided yesterday needs to be vetted by Carol, help me out.

DR. EISENMANN: The CIR SSC will discuss it in July and bring me more details.

DR. BELSITO: Okay. So it needs to be vetted, and then we perhaps will, at the September meeting, when we see this, okay, we'll have a range that says that it can be safely used in leave-ons from four to whatever, based upon QRA or other data, you know, how we use Cocamidopropyl Betaine. Assuming CIR's calculations are the same as Cosmetics Europe; my understanding will be that the range we will give them will not be an acceptable range for MI to act as a preservative alone in a leave-on. So we will effectively have banned the use of MI in a leave-on, without touching MCI/MI.

DR. BERGFIELD: Will you also state that in your discussion, that it could be used as a mixture?

DR. BELSITO: I don't think we need to confuse MCI with MI.

DR. BERGFIELD: Okay.

DR. BELSITO: I think that it will be quite clear, that it will be quite clear, that if we come out with four parts per million as the highest at this point that we think could be safely used, that we're not touching MCI/MI at all. I mean, that's my concern. I don't want to reopen that report.

DR. BERGFIELD: Okay. Linda, do you have anything to say, or Carol, in addition? A comment on this?

DR. LORETZ: Just that the chance to come back in September is great, and to vet it, so that's good.

DR. BELSITO: Right.

DR. BERGFIELD: Thank you.

DR. BELSITO: And if we become less restrictive, it still can go out as a final, correct? Which we will obviously, since we restricted it at the maximum at this point, the less restrictive we can be done with it, at least now it will go out for public comment, people will get to see how our group has handled this.

DR. BERGFIELD: Ron, do you want to comment again?

DR. SHANK: As an alternative, could one say the ingredient at 100 parts per million is safe in rinse off and for leave-ons when formulated to be non-sensitizing?

DR. SLAGA: Then you wouldn't have all the different uses, lipstick, et cetera, to deal with.

DR. BERGFIELD: It would be in keeping what you've done in the rest, the earlier conclusions today.

DR. BELSITO: And so the Cocamidopropyl Betaine was as based upon the QRA or other methods, or which may be based on the QRA? I'm okay with that, I mean that's essentially what we're saying.

DR. BERGFIELD: So we have a motion, we have no second. We have some alterations or amendments to the motion. Can we restate the motion?

DR. BELSITO: So MI is safe as used up to 100 parts per million in rinse-offs and may be safely used in leave-ons when formulated to be non-sensitizing --

DR. GILL: Which may be based on the QRA?

DR. BELSITO: Which may be based on the QRA.

DR. SHANK: Second.

DR. BERGFIELD: Second. Any further discussion? Dan? Paul? Curt? Lillian, do you have a question?

DR. GILL: Will Linda, will the CIR SSC continue to look at this and provide information?

DR. LORETZ: Yes, definitely.

DR. BERGFIELD: Paul?

DR. SNYDER: I think as we go through this new process of the different language for the conclusion about non-sensitizing, we have to be, I think we want to still be very detailed in the discussion section about what the constituents as far as contaminants and sensitizers that we're talking about, and still provide examples on how to use the QRA to get the safe levels and things like that. I think we still need to do that, even though it may not be transferred directly to the conclusion. I just don't like, I don't want it ended up that we just go with this catch all of non-sensitizing and not provide any information about why we're concerned about sensitization in general.

DR. BELSITO: I would hope that the detailed table that gets vetted through the CIR process gets published with the report, showing product categories and the AEL/CEL ratios for those categories and then showing where you've gone below an AEL/CEL of one, what you would have to reduce the concentration to, to get it. So the current table was nice, but I would like a last column that would say, AEL over CEL greater than or equal to one, and put the concentrations there.

DR. SNYDER: In addition to that, it seems like it's almost going to be a new data set that we're going to almost require, in addition to where we used to require repeat patch testing up to concentrations of use. Well we still like to see that support, but also the generation of this ratio to show other data important for our consideration.

DR. BERGFIELD: Dan, did you have a comment? I saw you commiserating there. Ron?

DR. HILL: I think especially if perhaps smaller companies, new outfits and so forth, if people need to be, that are actually making cosmetic products need to be educated, that the conclusion doesn't stand alone without the discussion and I know most people know that, but I think that needs to be -- it's like the home use of nail gels, but in this case, companies, that they need to be very clear that discussion is part of it. Without that, the conclusion has limited meaning -- not limited meaning, but it's not enough.

DR. BERGFIELD: Thank you. Ron Shank? Tom? Everyone all right? Any other discussion? Seeing none, call the question. All those in favor? Please indicate by raising your hand. Thank you. So Lillian, tell me exactly what will happen here with this report?

DR. GILL: Sounds like this report can now go, tentative amended report, with the conclusions stated in the motion.

DR. BERGFIELD: Okay.

DR. SHANK: And a new --

DR. GILL: And a new discussion that captures the use of the QRA.

DR. BERGFIELD: I personally would like to have Don record it, how that QRA is actually conformed, and the formulation. That would be very helpful to keep reflecting on, how they put that together, if that could be done. We could have a resource for that.

DR. BELSITO: Right, yeah. The paper has been published in 2008, so there will be a new publication looking at QRA 2.0.

DR. BERGFIELD: Mm-hmm.

DR. BELSITO: QRA 2.0 is based upon aggregate exposure.

DR. BERGFIELD: Mm-hmm.

DR. BELSITO: That's being put together by a company in Ireland called Creme, and they're doing aggregate exposure for the U.S. and for Europe separately, so the fragrance industry will be using the QRA 2 with the aggregate exposure in looking at Europe and the U.S., and have been setting limits based upon what would be the lowest that would protect both populations. since things are used somewhat differently in the two areas. They will not have aggregate exposure for Asia, South America at this point. But yeah, I think it would be very helpful to have Ann Marie or one of the individuals very involved with the QRA come and re-address the panel, because I do think that it certainly is proving to be a more valuable tool for predicting sensitization than what we've had previously.

DR. BERGFIELD: Thank you. That's what it sounds like. Curt?

DR. KLAASEN: How about sending out the 2008 article to the members, maybe along with the next mailing?

## SEPTEMBER 2014 PANEL MEETING – REVIEW OF DRAFT FINAL AMENDED REPORT

### Belsito's Team Meeting – September 8, 2014

DR. BELSITO: Methylisothiazolinone. So that's Monice. Is she --

DR. BOYER: Actually I worked on that one.

DR. BELSITO: Pardon?

DR. BOYER: I worked on the latest version.

DR. BELSITO: You worked on the latest version, okay, Ivan. So at the June panel meeting we said that it was safe for use in rinse-off cosmetics at concentrations of up to 100 parts of a million. It's safe in leave-on products when formulated to be non-sensitizing which may be determined based upon a quantitative risk assessment.

I think that Ivan and whoever else was responsible is fairly, accurately told us what cosmetics Europe is going to do. I honestly, from my standpoint, think we're doing the more responsible thing of saying that it's okay in rinse-offs. That even those rinse-offs will be issues for people who are sensitized. No doubt about it but it's labeled. I think the universe of cosmetic preservatives is shrinking and we need to be very careful and, you know, not to bend things that can be safely used.

And I feel -- and we got it wrong. We sensitized people. We can't do anything but it's labeled and 100 parts per million will prevent, I think, new sensitization and leave-on products. And I think you have to use the QRA for, I mean, for wash-off products and you have to use the QRA for leave-on products. And I think what you'll find is for many leave-ons that it's not going to be a reasonable preservative. For some it will be when combined with the phenoxyethanol and for some it will be okay.

But the companies have to decide. I don't want to -- Europe is going to effectively ban it by limiting it to 15 parts per million in wash-offs and that's a mistake that's going to affect us but I don't we should reduplicate it. So I'm very comfortable with this conclusion.

DR. SNYDER: The discussion was pretty good. I'd like, Don, to get to the next to last paragraph of the discussion. It begins with in addition. Do you have any comments there?

DR. BELSITO: Let me get to it.

DR. SNYDER: Okay. Not only that, I have to get through all of them. Just trying to get through all the discussion part, okay. I'm here. So that paragraph there, it wasn't really clear to me what we're trying to say there.

DR. BELSITO: In addition, where are you?

DR. SNYDER: The next to last paragraph there it says in addition, it is important to note that the limits for MI recommended by the panel as based on anticipated non-inducing exposures.

DR. BELSITO: Yes, so that's what we're trying to say. Europe has basically taken the approach not only for preservatives but a number of other ingredients that they went to protect the consuming public that has already been sensitized from elicitation. And what we know is that it takes a higher concentration to sensitize and in many cases, a significantly lower concentration and that's very variable.

And the data suggests that the higher the concentration that you were sensitized with, the lower the concentration that will cause elicitation.

DR. SNYDER: Okay.

DR. BELSITO: So for example, with hexavalent and chromium there are -- you can see studies where there's one person who was sensitized that can be elicited by one to two parts per million of hexavalent chromium. So what Europe is trying to do is they have introduced this concept of minimal elicitation threshold 10 and they want to protect 90 percent of the people who are already sensitized by coming in with a level where only 10 percent of the sensitized population will react.

What we're saying is that we're not -- it's not that we're not concerned about people who are sensitized. People who are sensitized can read a label, see it has methylisothiazolinone and avoid it. So the limits we've set are not going to protect sensitized people. The limits we set are hopefully going to prevent new sensitization and allow this to be used on the 90 percent of the population that either would never become sensitized because they don't have the genetic predisposition or have not yet been sensitized and they may be able to use this product.

So it's a very important paragraph.

DR. SNYDER: Okay, but that doesn't make --

DR. ANSELL: It's a different risk management approach.

DR. BELSITO: Right.

DR. SNYDER: Yeah, but my question is what's the difference between inducing and elicitation?

DR. BELSITO: The difference is in the level of concentration that's needed to induce versus elicit.

DR. SNYDER: But in the context of this isn't non-inducing mean non-sensitizing exposure?

DR. BELSITO: Yeah, you could change it to non-sensitizing exposure.

DR. SNYDER: Yeah, because I think, because to me the induction is the same as the elicitation, it can be.

DR. BELSITO: No. Induction is causing the allergy. Elicitation is bringing it out.

DR. SNYDER: Okay, so I think --

DR. BELSITO: Non-inducing is a proper term but if it's clearer --

DR. SNYDER: Should we parenthetically say sensitization levels or something? Because I don't understand the non-inducing exposures --

DR. BELSITO: Are based on anticipated exposures that will not induce sensitization.

DR. SNYDER: Okay.

DR. BELSITO: To MI will that make it clearer to you?

DR. SNYDER: Yes, yes.

DR. BELSITO: Okay, so that would be just a minor editorial change. I mean it says the same but if it wasn't clear to you it should be clear to everyone. So exposures that would not induce sensitization to --

DR. SNYDER: And then the second half of that is there's a double negative. Not on concentrations that will preclude, so I don't like the not and preclude elicitation. So what is that actually saying.

DR. BELSITO: It's saying that we know that the levels we're allowing could cause people who are sensitized to have reactions.

DR. ANSELL: Who are allergic.

DR. SNYDER: Right. So I think we need a middle because I don't like that not preclude and elicitation. I think it's confusing there, too.

DR. BELSITO: Yeah, but you need the not there if you're going to keep it in this same sentence otherwise you need to make a different sentence. Something to the effect that the panel acknowledges that the concentrations that --

DR. SNYDER: In cosmetics.

DR. BELSITO: In cosmetic that the panel recognizes that the levels that they're allowing in cosmetics could result in elicitation reactions in individuals already sensitized. I mean, that's what we're saying.

DR. SNYDER: Right. I'd just like it to be a little more clear because it --

DR. BELSITO: I mean, I think it's clear here but if it's not --

DR. ANSELL: Yeah, it's very precise of an issue that although it may be terms of art that are confusing. But it's a very precise statement as it is.

DR. SNYDER: And then the panel recommends that individuals sensitized to MI, you know, I think we need to put something not just recommends that sensitized individual. It doesn't make sense. Sensitized to what?

DR. BELSITO: Well, we're talking about MI but it would be more specific in that individuals sensitized to MI read product labels and avoid products that contain MI. I think one of the, you know, I mean, the -- if there were a huge universe of preservatives that could be used, broad spectrum preservatives, I might be a little bit more rigid with this in allowing it to go out as it is.

But the problem is is that there are preservative systems there that we know are safe like parabens that are getting pulled off because marketers are making decisions that they don't want to deal with the non-issue of endocrine disruption. J& J has come out and said they're going to remove formaldehyde and formaldehyde releasers from their products.

We're going to be left with no broad spectrum effective preservatives. In the 1970s there were women blinded by pseudomonas in mascaras. I mean, this is a real issue. And most contamination of cosmetic products comes from use by consumers. I mean, it doesn't come out of the factory contaminated. It's contaminated, you know, so I -- we need to keep the preservatives we have. We just need to keep them safe. And to be able to use MI at a safe level, you are going to cause elicitation reactions in people who have been sensitized. That's, you know, that's going to happen.

So but I think we need to allow this to go forward, you know, because that you need to assume some, I think, the benefit of keeping this on the market far outweighs the risk. People can read labels. It's there.

DR. BOYER: So for clarity, what if we change that not on concentrations that would include elicitation to simply not on concentrations that will elicit reactions in previously.

DR. BELSITO: Yeah, I mean, well however you want to, yeah.

DR. SNYDER: I go back to the beginning. As I said, I think the limits that we put are not based on anticipated. They're based on calculated. We did a QRA, right?

DR. BELSITO: Right.

DR. SNYDER: It's a QRA calculation. So we should still specify that it's --

DR. BOYER: Predicted.

DR. SNYDER: Yeah.

DR. BOYER: Predicted.

DR. SNYDER: Yeah.

DR. KLAASSEN: Okay, I need to talk about this a little bit. So Ivan sent this method of quantitative risk assessment which sounds very sophisticated and if you read the methodology all the way through it sounds very scientific until the last step. And then, this magic comes in. And what is that magic that comes in at the end? Divide by 100.

DR. BELSITO: Well it's based upon, I mean, toxicological principles and it's not always divided --

DR. KLAASSEN: That's not a toxicological principle of dividing what happens in laboratory animals by 100 and that's what going to happen in man. So if we did that, we, you know, what the safety -- that's often called a safety factor. You know what the safety factor is for acetaminophen and aspirin? Three. We have no aspirin or acetaminophen or 99 percent of the drugs.

DR. BELSITO: So in other words, this is ultraconservative?

DR. KLAASSEN: Yes.

DR. BELSITO: Well, but that's the point. It's made to be ultraconservative to --

DR. KLAASSEN: No, we don't want it ultraconservative. We want to have the response that we want by using our brain and not putting a hundredfold safety factors. I mean, we've done this in other parts of toxicology and the problems it's raised for our discipline is enormous.

DR. BELSITO: Well, but the conversion from animal data to human data is not 100, Curt. It's 10.

DR. KLAASSEN: Oh, yeah, 10 times 10 is 100.

DR. BELSITO: No, but there are a number of other factors. What --

DR. KLAASSEN: But you don't --

DR. BELSITO: -- the principle --

DR. KLAASSEN: -- do it with aspirin or acetaminophen.

DR. BELSITO: I understand that, Curt. But the principle that really defines the QRA and why we got it wrong when we simply look at human repeated cell patch testing that is done on the back, our safety assessment factors that look at frequency and site of use. So where we run into problems with MI are especially in baby wipes. So here you're getting 100 parts per million being wiped onto mucus membranes --

DR. ANSELL: Over large area.

DR. BELSITO: -- four times a day in occluded space and sometimes in damaged space. Where you, you know, other areas where we ran into problems, eyes, very thin- skinned, semi-occluded because they blink up. So what QRA really does, and actually there's going to be a QRA version two, the exact format is going to be is yet to be defined. But it's going to look at combinations of products.

So for instance, a woman who might shave her underarm with a shaving cream that contains chemical X and then put a deodorant on the chemical X. So there's a group in Ireland called KREM that's look at consumer practices and habits that will add another factor to these. So yeah, they're meant to be very conservative. But quite clearly, using the old method we got MI wrong. We got it terribly, terribly wrong. Was it our fault? No, I mean it was 2005. We didn't have a QRA and we looked at even repeat and cell patch testing and it flew by at 100 parts per million.

DR. KLAASSEN: I have no problem of taking the animal data --

DR. BELSITO: Well, this is --

DR. KLAASSEN: -- with all of these caveats of surface area what have you.

DR. BELSITO: Right.

DR. KLAASSEN: And the thinness of the skin and put that in the formula which they have done but then they divided by 100. It wouldn't make it so ultraconservative that we aren't going to be able to use anything and this is a precedent.

DR. ANSELL: I think QRA 2 goes down to a factor of 10 because they're improving the precision of some of the (inaudible) assumptions. But, you know, your point is well taken. At some point there's just a conservative factor that's added in.

DR. BOYER: Yeah, and actually this particular approach and we're just -- we just presented this document as an example, is based on that copy 2008 paper, and that's the RIFRAM approach that it seems to be fairly widely accepted at least in that particular world. I guess it's still undergoing --

DR. BELSITO: By the fragrance industry.

DR. BOYER: Right.

DR. BELSITO: Widely accepted by the fragrance industry. We'll see how widely it's accepted -- and widely accepted by more reasonable people and cosmetics Europe and DG Sanco but not necessarily widely accepted by the SEC advisory committee.

DR. SNYDER: So I think going back to this further, I think we need to say that the limits recommended by this report by the panel --

DR. BELSITO: We say that such as QRA. I mean, you can use QRA. You can use some other safety assessment. I don't -- I think we're very careful not to endorse QRA but to simply say formulated to be non-sensitizing. You can use QRA. You can use other methods.

DR. BOYER: And we also stipulate that if you do use QRA you need to be transparent about your assumptions and your safety factors and so on. So if you can show that your formulation is not going to be a problem, using these extremely conservative safety factors then and you're home free. Maybe the next formulator is going to have to think a little harder about what might be better, more refined as to what's -- what would be better to use as a safety assessment, safety assessment factors.

DR. SNYDER: You know, because the limits really are based upon the calculation and in addition the safety factor. So it's not -- it really isn't on --

DR. BELSITO: Right. First of all, there is the no expected sensitization level, okay? Usually in the fragrance industry now not necessarily here. So they take it from an LLNA and then they round it down. So if it's 655 micrograms per centimeter squared, they'll say 650 micrograms per centimeter squared. And then, they'll do a confirmatory HRIPT of 100 people just to confirm that the animal data, that you're not dealing with some oddball chemical that was fine in animals and comes out not good in humans.

So that's -- the NESIL is usually confirmed. If there's good human data that already exists then they use that. Human data always precludes animal data but then the next set of animal data that they want, at least if it exists now because if it's only a cosmetic product, you can't do any animal testing is going to be the LLNA. So that's where the NESIL comes from.

And then, the safety assessment factors are, is it used on damaged skin? You know, it is used on eyelids. It is used on perianal skin. It is used as a whatever. So those are the factors. The matrix, is it used with a propylene glycol ester that's going to increase penetration that we're going to need to be concerned about in a clone.

Is it, I mean, so what are the other things that are going to go into a product that would be with this fragrance that might change absorption or whatever, percutaneous penetration. So what are all those safety assessment factors go into.

DR. KLAASSEN: And those are okay but then when they get that, everything they could think of, then they divide that by --

DR. BELSITO: No, they don't.

DR. KLAASSEN: Yes, they do.

DR. BELSITO: No, they don't.

DR. KLAASSEN: Yes, they do.

DR. BELSITO: They don't, Curt. I've sat on the --

DR. KLAASSEN: Well, that's not what that paper said.

DR. BELSITO: I'm on the QRA panel. Once you add in the safety assessment factors, the safety assessment factors can range up to 100 when all multiplied out. But they don't add another hundred. They don't go beyond that. They are ultraconservative.

DR. ANSELL: Each of the elements has an uncertainty factor.

DR. BELSITO: Of 1 to 10.

DR. ANSELL: Or 3.

DR. BELSITO: 1, 3 or 10 and when multiplied out can become 100. But 100 is, you know, for underarm deodorants, I think eyes, you know, intimate hygiene products. That's -- there's not another hundred added to that.

MR. BJERKE: I think that it's actually 300 is the max.

DR. BELSITO: 300 is the max, yeah. But that may change now that we're looking at cumulative exposure. That max may get lowered down because of an additional factor of looking at multiple products at the same site. So QRA 2 may reduce to 100. It's yet to be determined.

MR. BJERKE: So for the QRA each individual product type, the SAFs are considered independently so it's inter-individual variability which is 1 to 10 which is -- I think Curt is locked into 10 there.

DR. BELSITO: Right.

DR. KLAASSEN: Then you got the species.

DR. BELSITO: No, no.

MR. BJERKE: No, we don't do that.

DR. KLAASSEN: You don't do that? Okay.

DR. BELSITO: No, inter-individual variability includes species. That's 10. Site.

MR. BJERKE: Site of contact, you know, mucosal membranes stuff like that and then matrix and that's based on the difference in the matrix between the data that you have, whether some LLNA or an HRIPT versus what your product formula is.

DR. BELSITO: And that's 1, 3 or 10 and then, nothing else is -- there's no other factor once you've looked at those safety assessment factors.

MR. BJERKE: That's correct. That's correct.

DR. KLAASSEN: So they probably can go up to 300?

DR. BELSITO: Yes.

MR. BJERKE: Yes.

DR. ANSELL: Well, 3 is a value in making a recommendation to the community as to this product.

DR. BELSITO: But you know, the interesting thing, Curt, is that there have been MI is a good example. Had we used QRA we would have obviated a lot of the problems we saw and within the fragrance industry cinnamic aldehyde, ic-eugenol, hydroxycitronellal which we ran into problems with, had we used QRA we probably would have solved those problems.

So yes, is it ultraconservative but in going back and looking at chemical or cosmetic ingredients that have been problematic and created issues with sensitization, again, that's all we're talking about, QRA would have obviated a lot of those problems had it been used. So is it perfect? Probably not. Does it need to be improved? Probably yes. But it's probably the best thing we have right now.

DR. KLAASSEN: Well, one thing better is just have zero. I mean, that's kind of what it's boiling down to. If you decrease things enough you won't have a problem with anything.

DR. BELSITO: I understand.

DR. KLAASSEN: And one of these days, we're going to have something that we want and need and we have a precedent of using something that is too darn conservative. Then what do we do?

DR. LIEBLER: So here's how I've been looking at this and Curt's comment raises a point that I think is a little different but I think we maybe consider it. So I don't have a problem with the phrase, which may be determined based on a QRA. I don't think the issue is whether or not to use a QRA. Is that -- I don't want to put words in your mouth, Curt, but as I see it that's not the issue.

The issue may be how do you do the QRA because you could be, in principle, you could be overly conservative or not conservative enough. But by endorsing the idea of applying a QRA, which is indeed an evolving approach, that provides some kind of yardstick and basis for taking something where the difference between what is the effective concentration of this preservative and the risk of inducing adverse reactions is a potentially fairly narrow window. And you're going to navigate that based on some quantitative calculation with the best, most justifiable assumptions. So that's the part I'm okay with and that's essentially what our conclusion says as written. The thing I think that potentially is problematic is if we use whatever the QRA is it's as of the time this report is written to come up with maximum recommended amounts in individual product types, then I think we potentially cross the line into what Curt's worried. And I actually share that concern a little bit and because then I think we can end up boxing ourselves in to particular numbers.

DR. BELSITO: So you're concerned about the table where we put safe numbers?

DR. LIEBLER: Yeah.

DR. BELSITO: Where the AL and CEL becomes one?

DR. LIEBLER: Right.

DR. BELSITO: So eliminate that part of the table. I don't have a problem with that.

DR. LIEBLER: Because I see that as potentially the precedent that turns around and bites us in the butt so to speak.

DR. BELSITO: Yeah, I mean, if you want to -- so what you're saying particularly since we said such as QRA --

DR. LIEBLER: Yeah.

DR. BELSITO: -- get rid of the portion of the table where they then go out and calculate what would be the acceptable concentration on a lipstick to produce an AEI, CEL of one?

DR. LIEBLER: Right.

DR. BELSITO: Just simply point out where currently at 100 parts per million the AEL/CELs are. Point out, okay, boom, boom, boom, boom and this is exactly where we've had consumer problems. You know, particularly in the baby wipes where you're getting down to AEL/CELs of, you know, .5 and things like that.

DR. LIEBLER: And I'm okay with pointing that out, taking that calculation that far. It's that last step where we have a prescriptive kind of number. I think that maybe is a step too far for us.

DR. BELSITO: So Ivan, get rid of that portion of the table where we redo the calculations to say what would be safe based upon a QRA?

DR. BOYER: Uh-huh.

DR. BELSITO: Just simply show that based upon the QRA, there are these, you know, the rinse-offs are fine. The leave-ons, a number of leave-ons seem to be problematic and you could even make a comment, for example, baby wipes which has been the hugest issue, baby wipes have created significant -- a significant number of case reports of

induction due to exposure to baby wipes has been reported as noted in the enclosed table. The AEL to CEL for baby wipes is boom.

DR. SNYDER: So a couple of things. So in the paragraph preceding that in addition paragraph we say that the MI concentration never exceed 100 parts per million in any hair product, leave-on product or rinse-off product. But we just said that we were going to go safe in rinse-off as long as labeled as desensitizer?

DR. BELSITO: No. Safe -- we had already limited it to 100 parts per million. That was the level that we had.

DR. SNYDER: Right.

DR. BELSITO: So and it's use in rinse-offs is up to 100 parts per million. So we're going safe as used and rinse-offs. Okay.

DR. SNYDER: Labeled as a sensitizer.

DR. BELSITO: No.

DR. SNYDER: No? I thought that's what you said before.

DR. BELSITO: We're not labelling anything as a sensitizer. We're simply saying that people --

DR. ANSELL: No, no, it's (inaudible).

DR. BELSITO: -- we're saying that the levels that we're going to allow in both rinse-offs and leave-ons are going to cause problems in people who are already sensitized. And those people will have to learn to read methylisothiazolinone on their cosmetic products to avoid it.

So we are, as opposed to cosmetics Europe and it remains to be seen what DG Sanco will do but I think they probably will follow those recommendations. We are not saying that you have to limit to prevent elicitation. In Europe they are trying to set limits to prevent people who are sensitized from having problems with products that are marketed that contain MI.

DR. SNYDER: Yeah, I understand all that.

DR. BELSITO: In the process of that, they are reducing MI to 15 parts per million in rinse-offs and banning it in leave-ons which is going to be funny because it's going to be MCI/MI is going to be in leave-ons so I don't quite know how they're going to do that. I've already had discussions with Ian White that it makes no intellectual sense to me to do it but he feels that it's a different product.

Be that as it may, what's essentially going to happen is that MI will be banned in Europe because it's not going to work at 15 parts per million as a preservative even when combined with phenoxyethanol. At least, that's what I'm told. And in the United States, hopefully, I mean, this will come out. I don't think -- it's been recommended but nothing has been passed in Europe yet. Is that correct? The EU has not set a defined regulation yet? Does anyone know? My assumption is they haven't gotten it.

DR. ANSELL: The SCCS is an opinion.

DR. BELSITO: The SCCS opinion has gone in but the Commission has not ruled on that opinion right? DG Sanco has not weighed in.

MS. KOTKOSKIE: Not yet. From my understanding.

DR. ANSELL: Yeah, they have not issued the adaption to technical.

DR. BELSITO: So what I'm hoping is that we can get a document out there that they may want to look at before they go ahead and just rubber stamp the opinion of the SECS. Anyway.

DR. SNYDER: So again, the language in that paragraph that originally -- so the non-inducing exposure, is that not -- is that really the consumer exposure level?

DR. BELSITO: No, the consumer exposure that we're setting presumably will not cause any new induction of allergy to MI.

DR. SNYDER: So then in exposures we should specify that it's related to the consumer exposure level as used to calculate the QRA above rather than just say non- inducing exposure because I don't know what that means. Are based upon anticipated non-inducing exposures.

DR. BELSITO: Right.

MR. BJERKE: That's the acceptable exposure level.

DR. BELSITO: Right.

MR. BJERKE: So it's the NESIL divided by the sensitization accept for the factors --

DR. SNYDER: So we need to clarify what that exposure level is because we have three different exposure levels in a previous thing so I was --

DR. BELSITO: No, it says --

DR. BOYER: It's predicted by the QRA example.

MR. BJERKE: Because the SAFs are going to vary depending on the product type.

DR. ANSELL: So that's all baked into the QRA.

DR. BELSITO: Right.

DR. ANSELL: Already.

DR. BELSITO: So what we're saying is that across the universe of wash-off, rinse-off products, 100 parts per million is safe for individuals who are not sensitized.

DR. SNYDER: Cumulative? Is that cumulative then? So if you use three products that have MI?

DR. BELSITO: Yes. Well, cumulative exposure has not been added in to QRA yet. That's QRA version two.

DR. LIEBLER: Going all botanical on us.

DR. SNYDER: Well, I mean, this is really important, I think because we are going out a limb. We have to get it right.

DR. BELSITO: Your chances -- yeah, the -- thinking about it, I mean, if you look at where the reports have been, there really have been virtually no reports on wash-off products causing issues. The reports have been paint, cosmetic products that are leave-ons, baby wipes. My experience, my big products are baby wipes and a company that decided to put it in their sunscreens.

So where I see it all the time are baby wipes and sunscreens. I see it in wash-off products but only once they've been sensitized to another type of product. So cumulative exposure is not in QRA 1 and it will probably be in QRA version two but that meeting is not until 2015. So I don't know what will happen.

But I think, you know, we're okay right now. We need to monitor it but so what we're very clearly saying is we're not protecting the sensitized individual. And I think that's okay because it's labeled because I think we need these preservatives --

DR. ANSELL: We're taking a different approach.

DR. BELSITO: Correct.

DR. ANSELL: A different approach to protection of sensitized individuals. The risk management that we've done in the US is a different approach, the risk management undertaken but we don't believe we're providing a lesser degree of safety. We're doing it through labeling and through prevention of induction. The Europeans have decided to take a different approach.

DR. BELSITO: You know, and it's not a logical approach, Paul, because every preservative creates sensitization in some people. And parabens are actually the safest and they're the ones the marketers are going after. But preservatives by their very nature tend to be small, electrophilic reactive products. That's how they preserve. And allergens, by their very nature, are small, electrophilic products.

So if you wanted a group of chemicals that would cause the greatest amount of allergy, you've got them in preservatives. And you're going to have to accept some allergy or get rid of preservatives and accept infection.

DR. ANSELL: And we do like the idea of dropping the table which gives calculated concentrations.

DR. BELSITO: Yeah, I think we should do that because we seem to be endorsing levels and I don't think we should be doing that. So that's a very good point, Dan.

DR. ANSELL: And that's carried through to the discussion so that it would need to be modified as well.

DR. BOYER: So would you want to remove the example that's presented in the discussion as well? The lipstick calculation.

DR. SNYDER: So I think maybe if we did eliminate it, then we just have a greater expanded discussion about the risk management aspect that we recognize.

DR. BELSITO: You know, if you were going to do an example, I would do an example with baby wipes. Simply because that's the product that has really gotten everyone up in arms. Oh my God, all these babies that have been sensitized, you know, so just take a good example. I mean, do it for baby wipes and say quite clearly we got it wrong and so all the case reports on baby wipes.

DR. LIEBLER: Instead of, I'm okay with that of having an example but I would recommend that you don't go the very last step of saying here's a number that shouldn't exceed in baby wipes.

DR. BELSITO: Right, I wouldn't say that.

DR. LIEBLER: Just simply say the EAL of --

DR. BELSITO: I would say, you know, as based on the QRA quite clearly should not exceed this but we don't know if that's the real level either. This is just using a QRA.

DR. LIEBLER: Or a level used in baby wipes.

DR. BELSITO: Right, we know what the level used in --

DR. LIEBLER: And the AEL versus whatever it is and that that's problematic and that says all we need to say there. We don't need to go the last step and say here's your number limit.

DR. BELSITO: Do the level. Baby wipes we're up to 90 parts per million most baby wipes 90 to 100. They are on the high end.

DR. ANSELL: We don't typically, though, you know, we point to based on lack of sensitization and lack of irritation. We really go through and give a protocol for how to determine sensitization or irritation and I wonder whether this has gone a little too far.

DR. LIEBLER: You mean the QRA?

DR. ANSELL: Well, in any of the methods where we put in a qualification. We don't tell them using OECD 403 or go through the calculations on how to calculate the LLNA. You know, I don't object to it I'm just curious.

DR. LIEBLER: Well, the only protocol we're showing here is this discussion section on using the QRA approach calculating the maximum acceptable which I think needs to be deleted.

DR. ANSELL: Okay.

DR. LIEBLER: Right? That's the only protocol we're showing.

DR. BELSITO: Right, and the original discussion obviously needs to be deleted.

DR. LIEBLER: Yeah.

DR. BELSITO: Fine. I'm fine with getting rid of the in the example the discussion. I certainly agree that we should get rid of the calculations we did to make AEL/CEL one and just -- but do show there that at 100 parts per million that the rinse-offs all seem to clear QRA. We're not endorsing that. It's one way of looking at risk of induction. But, you know, therefore, in as safe as used and rinse-offs and safe when formulated to be non-sensitizing using methods such as the QRA and not endorsing the QRA just saying such as.

DR. LIEBLER: So the discussion doesn't need to have an example calculation but it can refer to the baby wipes as shown in table two where the AEL/CEL is particularly low, you know, had a particularly low value of.13 which the panel recognizes consistent with the high incidents of reports of problem. And that way, you don't peg a number and say this is your limit for baby wipes.

DR. BELSITO: Right.

DR. LIEBLER: Because we don't need to do that. And then, as QRA evolves, we don't have to suddenly reopen to change the report because our numbers are out-of-date by two years.

DR. BELSITO: Right. Any other comments?

DR. SNYDER: So are we going to expand that little paragraph about the limits recommended by the panel?

DR. BELSITO: No, we're going to shorten it. We're going to get rid of the calculations.

DR. SNYDER: So what are the limits based on?

DR. BELSITO: Well, we -- so in going through this discussion it says --

DR. SNYDER: (Inaudible) a little bit.

DR. BELSITO: Okay, the risk of inducing sensitization depends upon the dose of MI, one type can differ from the other. So that paragraph stays in. The paragraph, I'm on 29, using the QRA approach exposure assumptions, that paragraph goes away, correct?

DR. LEIBLER: Right, and all the calculations below.

DR. BELSITO: And all the calculations below it go away. So all of those calculations go away and then moving on to page 30 of the pdf, it's important to note that appropriate exposure to substances can vary depending on factors, blah, blah, blah. That would stay. Right? And then, the next paragraph as indicated would go out.

DR. SNYDER: Strike that paragraph with the panel determined? Take out the however, start that next paragraph? Because somewhere we have to --

DR. BELSITO: Right, yeah, the panel determined that maximum MI concentration should never exceed 100 parts per million in any hair product, leave-on product or rinse- off product.

DR. SNYDER: Based on?

DR. BELSITO: Based on sensitization.

DR. SNYDER: Data?

DR. BELSITO: Induction of sensitization. And then, continuing that paragraph, it is important to note that the limits for MI recommended by the panel are based on anticipated non-inducing exposures to MI and not on concentrations that would preclude elicitation reactions. Or rinse-off products based on the induction --

DR. SNYDER: Based on the induction of sensitization data.

DR. BELSITO: Based upon the induction of --

DR. SNYDER: Sensitization data or --

DR. BELSITO: -- of sensitization not data.

DR. SNYDER: Yeah.

DR. BELSITO: Okay, and then you don't need to get do import -- it is in addition rather, you just get rid of that. Continue the same paragraph, it is important to note that the limits for MI recommended by the panel are based on anticipated non-inducing exposures and not on concentrations that will preclude elicitation of reactions of previously sensitized individuals.

DR. SNYDER: And then, I think we need to take out read product labels. The panel recommends that sensitized individuals avoid products that contain MI.

DR. BELSITO: Right, I mean, by reading labels if you want to let people know how they can avoid it.

DR. ANSELL: There are other ways.

DR. BELSITO: Simply not using cosmetic products at all.

DR. ANSELL: That's one way.

DR. LIEBLER: Recommends that individuals avoid products that contain MI. Good. That's fine.

DR. SNYDER: I think that's better.

DR. KLAASSEN: The other one, nobody reads it.

DR. LIEBLER: Yeah, that's great.

DR. BELSITO: Okay.

DR. LIEBLER: All right.

DR. BELSITO: We don't recommend. We -- recommend is like I recommend you fly Delta not United. I mean, do we want a stronger word than recommend?

DR. SNYDER: No, we don't want to say that. Well, yeah.

DR. BELSITO: The panel noted that --

DR. LIEBLER: Sensitized individuals should avoid products that contain MI.

DR. BELSITO: Yeah.

DR. SNYDER: Yeah, there you go. Even shorter than it, good.

DR. LIEBLER: You got that Ivan?

DR. BOYER: Yeah.

DR. LIEBLER: Okay, good.

DR. BOYER: I got it all.

DR. BELSITO: Okay, anything else?

DR. LIEBLER: Let's get out of here.

DR. BELSITO: So --

DR. SNYDER: I think we have the conclusion right then?

DR. BELSITO: I hope so.

DR. KLAASSEN: It hasn't changed.

DR. BELSITO: CIR expert panel safe for use in rinse-off products in concentrations up to 100 parts. Safe in leave-on cosmetics when they are formulated to be non-sensitizing which may be determined based on --

DR. SNYDER: No, but that's different than what we said up here in this previous part. We said it's never exceed 100 parts per million in any hair product, leave-on product or rinse-off product.

DR. BELSITO: Yeah, that's what we said, the concentrations up to 100 parts per million.

DR. SNYDER: Did we say on rinse-off products?

DR. BELSITO: Yeah.

DR. SNYDER: Safe for use in rinse-off. The other one we're saying all products.

DR. BOYER: That was a suggested change, addition to the conclusion. Basically, one would be -- when Don did the QRA and to estimate concentrations for particular product types that would not likely be inducing, he found that for some product types, the concentrations were actually higher than one PPM -- than 100 PPM.

DR. BELSITO: For rinse-offs.

DR. BOYER: Right and so, the concern, I think, was that if -- you can't -- they didn't want us to leave into the impression that --

DR. BELSITO: They could go higher than that --

DR. BOYER: -- you could go above 100 PPM based on QRA.

DR. BELSITO: Right.

DR. SNYDER: So is that congruent with what we have up there with the new paragraph we just did. The panel determined that the maximum MI concentration would never 100 parts per million in any hair product, leave-on product or rinse-off product based on induction sensitization?

DR. BELSITO: Yeah, but it -- I see what you're saying, Paul, but if you do the QRA you're not going to get a level of even close to 100 parts per million for any leave-on product. So in essence, by saying such as or determined based on QRA, you have taken leave-ons to below 100 parts per million.

So what we wanted to do, as Ivan just said is, that if you do QRA, there are some products you could use at 12,700 parts per million would seem to be safe. We don't want that --

DR. SNYDER: Restriction.

DR. BELSITO: We want a restriction at 100 parts for all rinse-offs and then leave-ons do a risk assessment where you'll find that you can't get to 100 parts per million for most of them. For a few hair products I think you can, right?

MR. BJERKE: Yeah.

DR. LIEBLER: So conclusion stays as written?

DR. BELSITO: Yeah, unless you want to be really, you know, and say for use in cos -- that the MI is safe for use in cosmetic products at --

DR. LIEBLER: Concentrations of 200 PPM. I don't see any reason to change this. I think we're getting into this kind of negative discussionary spiral that's turning into deep bullshit so.

DR. BELSITO: Right.

DR. LIEBLER: Pardon my French.

DR. BELSITO: Yeah.

DR. ANSELL: I like the first paragraph. I like the way you wrote it the first time. I mean, we don't go through and explain a lot of the terms of art that appear throughout our reports. And if you don't know if there's confusion as to the difference between induction and sensitization and elicitation, look it up.

DR. BELSITO: Okay. I'm fine with the conclusion. Paul, do you want to --

DR. SNYDER: I just was asking about whether it was consistent with what we wrote above.

**Marks' Team Meeting – September 8, 2014**

DR. MARKS: Let's start our team meeting, and the first ingredient is methylisothiazolinone, and as previously I have recused myself, since I had Rohm and Haas, which was bought out by Dow sponsor, the meeting is held in Hershey, so I feel like I could have a conflict of interest. So, Ron Shank, if you would lead our team's discussion --

DR. SHANK: Okay.

DR. MARKS: -- with MI.

(Discussion off the record)

DR. SHANK: This MI is report is now a final report, and we were asked to review the abstract discussion and conclusion. And I thought the report was in very good shape and had a few editorial comments, but that's all. I think the abstract -- the discussion and the conclusion are fine. How about the rest of you guys?

(No response heard)

DR. SHANK: Anybody have a problem with any part of this report?

SPEAKER: Not here.

DR. SHANK: No? Dr. Hill?

SPEAKER: Not here.

DR. HILL: No. I flagged something in the discussion that now I don't see why I flagged it. I think I had a momentary brain glitch. So, no. And then, I had a couple of editorial things, too.

DR. SHANK: Okay. Then, I guess we're ready to discuss it with the other team tomorrow. But it looks like this report is ready to -- I called it a final report. It's a final amended report. I guess that's it? Dr. Marks?

**Full Team Meeting – September 9, 2014**

DR. BERGFELD: Then moving on to MI, Dr. Belsito.

DR. BELSITO: Yeah, at the June 2014 meeting we concluded that MI's safe for use in rinse off cosmetic products with concentrations up to 100 parts per million and safe in leave on products when they are formulated to be non-sensitizing, which may be determined based on a QRA and relooking at this document, we felt we could go ahead and make that a final.

DR. BERGFELD: Is there a second?

SPEAKER: Second.

DR. BERGFELD: Thank you. Any further discussion before I call for the vote?

DR. BELSITO: Yeah, just, in the sensitization, on page 26 of the pdf, this was very poorly written and I rewrote it because it, really the author seemed to be confused on SIs and EC3s and really what they just needed to report were the EC3s and so there's some substantial rewriting in that, and in the discussion, but otherwise, just some typos on that.

DR. BERGFELD: Anything else? All right, I'll call the question. All those in favor of this conclusion, please raise your hands, thank you, unanimous.

DR. MARKS: No, I recused (sic) myself because of conflict of interest so it isn't unanimous.

DR. BERGFELD: Thank you, I thought your hand went up, sorry. All right, so we have one recused or sustained, abstaining (sic).

**SEPTEMBER 2019 PANEL MEETING – REVIEW OF MCI/MI LEADING TO REOPEN MI**

**Full Team Meeting – September 10, 2019**

DR. SHANK: This methylchloroisothiazolinone and methylisothiazolinone were first reviewed in 1992, by the panel. And then there was another review this year in April and June. In June the panel asked for information on inhalation toxicity and that data have not been provided.

I would suggest we have the conclusion that the mixture of MCI/MI 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; this is the original conclusion. But there is insufficient data to support the safety of the mixture of MCI/MI for use in cosmetic products that may be inhaled.

DR. BERGFELD**:** Is there a second to that, or a comment?

DR. BELSITO: Well, actually, if you look at the QRA there are some rinse-off products that would go above QRA limits if allow at 15 parts per million, in leave-ons as well.
So, we had said formulated to be non-sensitizing using QRA or other methodologies. But in no case should levels exceed 7.5 parts per million in leave-on, including baby wipes, or 15 parts per million in rinse-off. And as you said, insufficient for products with potential for inhalation.

DR. BERGFELD**:** So you've expanded the conclusion; that is what's going to be in the conclusion, all that?

DR. BELSITO: Yes, that a QRA be run, but in no instances should they exceed the prior limitations that were set. Because according to the QRA, in some categories, you could exceed 15 parts per million. But we felt that it should not.

DR. BERGFELD**:** Is there a second to this motion?

DR. SNYDER: Seconded.

DR. BERGFELD: Any further comments regarding this motion? Seeing none -- Ron you have a comment?

DR. SHANK: No. That's okay. It's more convoluted, but it's okay.

DR. BERGFELD: I'm going to call for the question.

DR. BELSITO: So, we're voting on my motion?

DR. BERGFELD: Yes. It's been seconded; Dr. Marks is recluse from this due to conflict of interest. I'll call for the vote, all those in favor? Unanimous and Dr. Marks is recused. All right.

DR. BELSITO: Comment?

DR. BERGFELD: Comment, please.

DR. BELSITO: Based upon the information we have on the inhalation of the MCI/MI, was there a need to reopen the MI report in the absence of getting inhalation data for this combination; since we don't know which if not both ingredients might contribute to the respiratory issues that were reported in Korea?

DR. BERGFELD: Any comments from the other panel members? And you desire that just to figure out --

DR. BELSITO: I think we have to. I mean, you know, we don't have any inhalation information. We don't know whether it's the methylchloroisothiazolinone or the methylisothiazolinone that's causing these issues. We really don't have any data and we need to look at that again and look at inhalation uses; and restrict those if we don't get any additional inhalation data or further details on these Korean issues.

DR. BERGFELD: So you're actually proposing a motion to reopen the individual ingredient MI?

DR. BELSITO: MI.

DR. BERGFELD: Oh, just MI?

DR. BELSITO: Yeah, we've already taken care of MCI/MI. And there's no use of MCI alone.

DR. BERGFELD: Okay. Do we need to have a motion and vote on that, or could we just make a recommendation?

DR. HELDRETH: No. If the panel request that we take another look at it we can certainly do that.

DR. BERGFELD: Okay, it's done. We're going to reopen MI. All right?

DR. HELDRETH: I would ask, is that something you would like to see once we've concluded the MCI/MI report? Or do you want us to get to work on that right away and not wait for us to get a final report on the mixture?

DR. BELSITO: I mean, I think that industry has gotten a heads up from this. I don't think putting the MI report, since we just reviewed it, would take a lot of effort. It basically would pretty much be the same report. There's really no new data on it other than, you know, continuing reports of contact sensitization that we already dealt with, other than for this information about inhalation.

DR. HELDRETH: As soon as feasible?

DR. BELSITO: Yeah.

DR. BERGFELD: But you're proposing the end result might be a restriction on inhalation for MI?

DR. BELSITO: Yes, unless we get the data we asked for this time and didn't get it.

DR. BERGFELD: Jay?

DR. ANSELL: So, we would support that and leave it up to the staff to determine procedurally how to put it into the hopper for review to align the MI and the MCI/MI conclusions.

DR. HELDRETH: Procedurally, a final report can be reopened for cause at any time; it certainly seems like a cause.

DR. BERGFELD: We have voted on this and this was an extra comment in addition for a review of MI. So then, any other comments before we move on to Wheat? None, Dr. Belsito, you're up for Wheat.

# Amended Safety Assessment of Methylisothiazolinone as Used in Cosmetics

| | |
|---|---|
| Status: | Draft Amended Report for Panel Review |
| Release Date: | May 15, 2020 |
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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer, CIR.


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## INTRODUCTION

In 2019, the Expert Panel for Cosmetic Ingredient Safety (Panel) published an amended safety assessment of the preservative Methylisothiazolinone with the conclusion that "Methylisothiazolinone 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)."[1] This conclusion superseded the findings of the Panel's earlier safety assessment that was published in 2010.[2] At the September 2019 Panel meeting, during the re-evaluation of the mixture methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), the Panel reopened the amended safety assessment of Methylisothiazolinone to gather and evaluate additional data, with particular regard to inhalation toxicity.

In 2019, the Panel issued an amended safety assessment of the mixture MCI/MI (supplied as a ratio of 3:1), with the conclusion that the mixture "is safe in cosmetics when formulated to be non-sensitizing, based on the results of a QRA or similar methodology; however, at no point should concentrations exceed 7.5 ppm in leave-on products or 15 ppm in rinse-off products."[3]

Data from the original Methylisothiazolinone safety assessment that was published in 2010 and the amended safety assessment that was published in 2019 are summarized in *italics* in each appropriate section of this report.[1,2]

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the data included in this safety assessment was obtained from the European Chemicals Agency (ECHA).[4] These data summaries are available on the ECHA website, and when deemed appropriate, information from the summaries has been included in this report.

## CHEMISTRY

### Definition and Structure

Methylisothiazolinone (CAS No. 2682-20-4) is the heterocyclic organic compound that conforms to the structure depicted in Figure 1.[5]

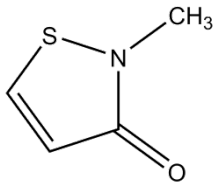


**Figure 1**. Methylisothiazolinone

### Physical and Chemical Properties

*Methylisothiazolinone has a molecular weight of 115.2 Da and a density of 1.02 g/ml at 25° C.[2] The ultraviolet/visible spectrum for a tradename Methylisothiazolinone product had peak wavelengths at 274 nm for a neutral solution, 266 nm for an acidic solution, and 274 nm for a basic solution. Additional properties are described in the original safety assessment.*

### Method of Manufacturing

*Methylisothiazolinone is produced by the controlled chlorination of dimethyldithiodipropionamid in solvent.[2] Methylisothiazolinone is then neutralized and extracted into water followed by a solvent strip.*

### Composition and Impurities

*The composition of technical grade Methylisothiazolinone was 96.8% Methylisothiazolinone, 0.1% 5-chloro-2-methyl-4-isthiazoline-3-one, 0.1% 4,5-dichloro-2-methyl-4-isothiazolinone-3-one, 0.2% N, N'-dimethyl-3,3'-dithiodipropionamide, 0.5% N,N'-dimethyl-3,3'-trithiodipropionamide, 0.1% N-methyl-3-chloropropionamide, 0.3% ammonium chloride, 0.2% water, 0.1% ethyl acetate, 0.1% acetic acid, and 1.5% unknown compounds.[2] Impurities of a tradename Methylisothiazolinone product (9.5% active ingredient) included 79-103 ppm N,N'-dimethyl-3,3'-trithiodipropionamide, 44-79 ppm 5-chloro-2-methyl-4-isothiazolin-3-one, and 490 ppm N, N'-dimethyl-3,3'-dithiodipropionamide.*

## USE

### Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2019 VCRP survey data, Methylisothiazolinone (when not used with MCI) is used in a total of 915 formulations; the majority of the uses are in bath soaps and detergents (Table 1).[6] These uses have increased since the last review where 745 uses were reported; the majority of the uses reported then were in non-coloring hair conditioners and shampoos.[1] In the amended safety assessment published in 2019, the maximum concentration of use range was reported to be 3.5 x $10^{-8}$% 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). A survey of the present concentration of use range is currently being conducted by the Council.

Methylisothiazolinone may be used in products that can come into contact with the eyes or mucous membranes; for example, it is reported to be used in eye lotions, mascaras, and in bath soaps and detergents.[6] Additionally, Methylisothiazolinone is used in cosmetic sprays and could possibly be inhaled; for example, it is reported to be used in hair sprays and 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.[7,8] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[9,10]

Under regulations governing the use of cosmetic ingredients in the European Union, Methylisothiazolinone is listed under Annex V, the list of preservatives allowed in cosmetic products, with the restriction that it may only be used in rinse-of products at up to 0.0015% (15 ppm). [11] The most recent opinion on Methylisothiazolinone by the European Union's Scientific Committee on Consumer Safety (SCCS) 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.[12] In rinse-off cosmetic products, the SCCS has concluded that concentrations up to 0.0015% (15 ppm) Methylisothiazolinone 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 Methylisothiazolinone should not be added to cosmetic products that already contains MCI/MI.

### Non-Cosmetic

*The uses of Methylisothiazolinone in paints and other non-cosmetic products were described in the original safety assessment.*[1,2]

## TOXICOKINETIC STUDIES

### Absorption, Distribution, Metabolism, and Elimination (ADME)

*The percutaneous absorption of [$^{14}$C]-Methylisothiazolinone (99.88% radiochemical purity) was determined using rat skin mounted on diffusion cells.*[2] *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 Methylisothiazolinone (96.90% radiochemical purity) in human epidermis was 29.8, 38.0, and 54.7% for 52.2, 104.3, and 313 μg Methylisothiazolinone/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 Methylisothiazolinone/ml was 29.5%, 8.98%, and 19.6%, respectively. The rates for absorption of Methylisothiazolinone 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 Methylisothiazolinone (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 Methylisothiazolinone (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 Methylisothiazolinone (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 Methylisothiazolinone was recovered and the main metabolites were NMMA and 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propionamide.*

## TOXICOLOGICAL STUDIES

### Acute Toxicity

*Methylisothiazolinone at 97.5% was slightly toxic in rats in an acute dermal toxicity study.[2] 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% Methylisothiazolinone was corrosive to rat skin, but no deaths occurred during the study. The $LD_{50}$ was greater than 484.5 mg/kg body weight.*

*In acute oral toxicity studies, Methylisothiazolinone was slightly toxic in rats in concentrations ranging from 9.69% to 99.7%.[2] At 9.69%, the $LD_{50}$s for male and female rats were 274.6 and 105.7 mg/kg body weight, respectively. Rats that died during these studies had reddened intestines and/or stomach mucosa, clear or red/yellow fluid in the intestines and/or stomach; blackened intestines and distended stomachs. Studies in rats on body lotion, shampoo, and sunscreen formulations containing 100 ppm Methylisothiazolinone 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% Methylisothiazolinone. The $LD_{50}$ was 167 mg/kg body weight. An acute oral toxicity study of the metabolite NMMA in rats found the substance slightly toxic. The calculated oral $LD_{50}$s for NMMA in males and females were 3550 and 4100 mg/kg body weight, respectively.*

*Acute inhalation toxicity studies in rats found that 53.52% and 97.8% Methylisothiazolinone were slightly toxic after 4 h exposures.[2] The $LC_{50}$s were 0.35 and 0.11 mg/L, respectively. Rats that died during these studies had reddened lungs and distended gastrointestinal tracts. Mice exposed to 10 minutes of atomized 98.6% Methylisothiazolinone had up to 47% decrease in respiratory rates that equated to moderate responses for sensory irritation.[2]*

Acute toxicity studies are summarized in Table 2. In a dermal study in rats, the $LD_{50}$ for 49.0% Methylisothiazolinone was greater than 2000 mg/kg bw.[4] In oral studies, the $LD_{50}$ for a 1% solution of Methylisothiazolinone in rats was 148.0 mg/kg, while the $LD_{50}$ for a 50% solution of Methylisothiazolinone in rats was 232 - 249 mg/kg in males and 120 mg/kg in females. The $LC_{50}$ of aerosolized 49.8% Methylisothiazolinone in rats was 0.422 mg/L in males and 0.354 mg/L in females.

### Short-Term Toxicity Studies

#### Oral

In a 28-day oral toxicity study performed in accordance with Organization for Economic Co-operation and Development (OECD) test guideline (TG) 407, groups of 5 male and 5 female Wistar rats received 0, 10.03, 28.59, or 71.21 mg/kg bw Methylisothiazolinone in water daily via gavage.[4] The study included high-dose and control recovery groups that were observed for an additional 14 days following completion of the dosing period. Terminal studies included measuring organ weight and relative organ weight, and performing gross pathological and histopathological assessments. The number of mortalities were not reported. In males, the absolute and relative weights of the prostate in the low and high dose group, and the heart in the mid dose group were significantly reduced when compared to the control group. However, no lesions were found in the prostate. Absolute weight of the testes and epididymides was significantly less ($p < 0.05$) in the high dose recovery group when compared to the control recovery group; however, the relative weight of these organs was comparable to the control recovery group. Relative weight of the liver in the mid and high dose groups was significantly increased as compared to the control group; however, there was no significant variation in the high dose recovery group and no treatment-related lesions were observed in the liver. In females, the absolute weights of the organs in the treated animals were comparable to the controls, but there were statistically significant increases in relative weight of the kidneys in the low and mid dose groups. These observations were considered incidental as thee high dose group and high dose recovery group were comparable to the control groups. While pathological and histopathological changes were observed, the study summary did not detail the differences between the control and dose groups. The no-observed-adverse-effect level (NOAEL) was 28.6 mg/kg bw/day in males and females based on the combined assessment of clinical signs, mortalities, and pathological and histopathological findings; the lowest-observed-adverse-effect level (LOAEL) was 71.2 mg/kg bw/day in males and females was based on lethargy and mortality. No further details were provided.

### Subchronic Toxicity Studies

#### Oral

*No toxic effects were observed when 97.5% Methylisothiazolinone was administered to rats in drinking water for 13 weeks at concentrations of 0, 75, 250, or 1000 ppm.[2] Dogs that were fed diets prepared with 51.4% Methylisothiazolinone 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 up to 500 ppm NMMA and 100 ppm MA in their diets for 3 months had no systemic toxicity.*

In a 90-day oral toxicity study performed in accordance with OECD TG 408, groups of 10 male and 10 female Wistar rats received 0, 7.52, 15.05, or 30.09 mg/kg bw Methylisothiazolinone in water daily via gavage.[4] The study included high-dose and control recovery groups that were observed for an additional 28 days following completion of the dosing period. The animals were observed for mortalities, clinical signs of toxicity, ophthalmological changes, and feed consumption. Hematology values and clinical chemistry measurements were taken. Sperm were analyzed for motility, number, and morphology (results reported in the section below). Terminal studies included measuring organ weight and relative organ weight, and performing gross pathological and histopathological assessments. No treatment-related mortalities, clinical signs of toxicity, ophthalmological changes, or changes in feed consumption were observed. There were no significant treatment-related changes in hematological values or clinical chemistry. No significant adverse effects were reported in terminal studies. The NOAEL was 30.09 mg/kg bw/day in males and females based on no treatment-related mortality or clinical signs of toxicity.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

*In a teratogenicity study, Methylisothiazolinone 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 NOAELs were 20 mg/kg/day and 40 mg/kg/day, respectively. In a teratogenicity study of Methylisothiazolinone in rabbits, pregnant females received daily single oral doses of 3, 10, or 30 mg/kg/day Methylisothiazolinone 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 Methylisothiazolinone in drinking water at concentrations up to 1000 ppm was not a reproductive toxicant.[2]*

In the 90-day oral toxicity study described above, no adverse effects were observed on the male rat reproductive system after Wistar rats received up to 30.09 mg/kg bw Methylisothiazolinone in water.[4]

The teratogenic potential of 49.8% Methylisothiazolinone was studied in Wistar rats in accordance with OECD TG 414.[4] Groups of 25 pregnant rats received 33.4, 49.8, or 74.7 mg/kg of the test material in water via gavage once daily on days 6 through 15 of gestation. Slight maternal toxic effects, including depressed body weight gains and feed consumption, were observed at 49.8 mg/kg and 74.7 mg/kg. A significant increase in the number of visceral anomalies were observed at 74.7 mg/kg, which were likely due to maternal toxicity. No teratogenic effects on fetuses attributed to the test material could be verified. The NOAEL and LOAEL for maternal toxicity were 33.4 mg/kg bw/d and 49.8 mg/kg bw/d, respectively; the NOAEL and LOAEL for embryotoxicity were 49.8 mg/kg bw/d and 74.7 mg/kg bw/d, respectively.

## GENOTOXICITY

*Methylisothiazolinone (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 (CHO) cell assay, 97.5% pure Methylisothiazolinone was non-mutagenic when tested with and without metabolic activation (0.5 - 40.0 µg/ml). However, another CHO assay that studied Methylisothiazolinone at 97.5% active ingredient (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. Methylisothiazolinone was non-mutagenic in an unscheduled DNA synthesis assay and in a micronucleus test.[2]*

Genotoxicity studies are summarized in Table 3. Methylisothiazolinone (49.0% - 49.8%) was not mutagenic in an Ames study, chromosome aberration study, or in a mammalian cell gene mutation assay, nor was it mutagenic in an in vivo micronucleus assay in mice.[4]

## CARCINOGENICITY

*Studies of the carcinogenicity of the sole ingredient Methylisothiazolinone 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.[2]*

## OTHER RELEVANT STUDIES

### Neurotoxicity

*An acute in vitro neurotoxicity study of Methylisothiazolinone (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 Methylisothiazolinone (up to 3.0 µM) from the same laboratory concluded that prolonged exposure to Methylisothiazolinone and related isothiazolinones may damage developing nervous systems. However, no evidence of neurotoxicity has been observed in vivo.[2]*

## DERMAL IRRITATION AND SENSITIZATION

*In EpiDerm™ skin constructs, 1.7% Methylisothiazolinone applied for 3 or 60 minutes was non-corrosive.[2] In the same study, 51.5% Methylisothiazolinone was non-corrosive in the 3-minute exposure but corrosive at the 60-minute exposure. Undiluted 97.8% Methylisothiazolinone was corrosive to intact rabbit skin after an exposure period of 1 h. Rabbit dermal irritation studies of Methylisothiazolinone at 9.69% and 10% concluded the chemical was non-irritating. A single 24-hour application of 100 ppm Methylisothiazolinone in 40 volunteer subjects did not produce skin irritation. Respective skin irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm Methylisothiazolinone also found Methylisothiazolinone to be nonirritating.*

*In a guinea pig maximization test, 0.076% w/v Methylisothiazolinone was a weak sensitizer and a follow-up study found that 0.015% Methylisothiazolinone produced no sensitization.[2] An investigation using the Buehler method found that 99.8% Methylisothiazolinone was a sensitizer at concentrations ≥ 1000 ppm. Another maximization test that evaluated the sensitization potential of 99.7% Methylisothiazolinone concluded that the chemical was not a sensitizer at concentrations up to 800 ppm. Methylisothiazolinone was a sensitizer at concentrations ≥ 1.5% in an open epicutaneous test. Results from a local lymph node assay (LLNA) indicated that 99.8% Methylisothiazolinone produced sensitization at >10,000 ppm. In one LLNA, the effective concentration inducing a stimulation index (SI) of 3 ($EC_3$) for Methylisothiazolinone 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 Methylisothiazolinone, the $EC_3$ for Methylisothiazolinone diluted in acetone/olive oil was 0.4% (4000 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% Methylisothiazolinone in acetone/olive oil was not typical for respiratory allergens, and the authors concluded that Methylisothiazolinone was not likely to cause sensitization of the respiratory tract. The metabolite NMMA did not induce hypersensitivity in a LLNA up to and including 30% concentration.*

*A re-evaluation of the LLNA results reported in the published literature in an editorial article indicates that Methylisothiazolinone should be categorized as a strong sensitizer, and not a moderate sensitizer as previously reported.*[1]

*In a cumulative irritation/ sensitization study of Methylisothiazolinone in 80 subjects, the sensitization threshold was determined to be at or around 1000 ppm.[2] A human repeated insult patch test (HRIPT) in 98 subjects tested with 100 ppm Methylisothiazolinone concluded that Methylisothiazolinone did not induce skin sensitization in humans. A series of HRIPTs evaluating the sensitization of 50% Methylisothiazolinone at concentrations of 200, 300, 400, 500, or 600 ppm concluded that Methylisothiazolinone up to 600 ppm was not a dermal sensitizer.*

*In sensitization studies conducted in 11 Methylisothiazolinone-allergic patients, the lowest eliciting dose in a patch test was 1.47 µg Methylisothiazolinone/$cm^2$ (49 ppm). No reactions were observed at 0.441 µg Methylisothiazolinone/$cm^2$ (15 ppm) or lower, nor were there any reactions in the controls. In a HRIPT of 100 ppm Methylisothiazolinone, with or without various glycols, no evidence of induced allergic contact dermatitis was observed in any of the subjects.*[1]

Dermal irritation and sensitization studies are summarized in Table 4. In a rabbit irritation study, 49.0% Methylisothiazolinone in water was corrosive.[4] Methylisothiazolinone was sensitizing in a guinea pig maximization test and in a local lymph node assay (LLNA) when tested at up to 10.0%; however, it was not a sensitizer in another LLNA at up to 4.5%. In human sensitization studies, dose-dependent sensitization was observed to Methylisothiazolinone at up to 2500 ppm in a cumulative irritation study and human repeated insult patch tests (HRIPTs).

### Phototoxicity

*Methylisothiazolinone at 100 ppm was not phototoxic or photosensitizing in guinea pig studies. No phototoxic effects were observed in a study of 200 ppm Methylisothiazolinone in 12 female subjects.[2] A photosensitization study of 200 ppm Methylisothiazolinone in 32 subjects did not produce photoallergic reactions.*

## OCULAR IRRITATION STUDIES

*A bovine cornea study classified Methylisothiazolinone [neat] as mildly irritating. Ocular irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm Methylisothiazolinone found the formulations non-irritating in rabbit eyes.*[2]

### Human

In an ocular irritation study, 12 human subjects received 100 ppm Methylisothiazolinone in buffered physiological saline received a single 10 µl drop in the eye on 5 consecutive days.[4] An ophthalmologist performed eye examinations and the subjects subjectively rated the irritation. Mild pink in the bulbar and palperbral conjunctiva and slight lacrimation were noted 30-60 seconds after instillation of the test material, but not after 60 min and the results were comparable to the control subjects. No more than slight/mild stinging/burning/pain were reported for both the test material and the control. Three adverse events were reported by 2 subjects: one subject reported mild bilateral ocular discharge and stinging, which were possibly related to the test material, and the other subject reported mild bilateral ocular discharge which was unlikely related to the test material. The test material was considered safe and well tolerated in this study.

## CLINICAL STUDIES

### Retrospective and Multicenter Studies

*In a clinical study of 22 patients tested with fractions isolated from a tradename mixture of MCI/MI, only 2 patients had positive reactions to Methylisothiazolinone.[2] Sensitization may have been due to cross-reactions to MCI. Methylisothiazolinone was determined to be a weak sensitizer in a study of 12 patients. Eighty-five patients with pre-determined sensitization to MCI/MI were tested epicutaneously to 500 or 1000 ppm Methylisothiazolinone. The results show that at high concentrations of Methylisothiazolinone (500 to 1000 ppm), 32% of the subjects with known sensitivity to MCI/MI reacted to Methylisothiazolinone. In a repeat open application test (ROAT), 7 patients (64%) reacted to 0.105 and 0.21 µg Methylisothiazolinone/cm$^2$ and 2 patients (18%) reacted to 0.0105 µg Methylisothiazolinone/cm$^2$.*

*Incidences of contact allergy to Methylisothiazolinone, tested separately from MCI/MI, appear to be increasing in Europe since the start of the use of Methylisothiazolinone as a stand-alone ingredient.*[1]

*Methylisothiazolinone 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.*[1] *A standard series of patch testing includes the mixture MCI/MI, which may miss 40% of contact allergy to Methylisothiazolinone alone due to the relatively low concentration of Methylisothiazolinone in the mixture. Recommendations have been made to test for Methylisothiazolinone contact allergy separate from the MCI/MI, although there currently is no consensus of about the concentration of Methylisothiazolinone that should be tested.*

A selection of the numerous baseline and retrospective studies on Methylisothiazolinone that have become available in the published since 2014 are summarized in Table 5. These studies show that sensitization to Methylisothiazolinone is still found world-wide.[13-22]

### Case Studies

*Three cases of allergic contact dermatitis were reported in patients that had come into contact with coolant solutions containing biocides.[2] Patch testing in 2 of the patients revealed ++ and +++ reactions to Methylisothiazolinone, respectively. An investigator in this study developed eczematous dermatitis while isolating coolant components and had a ++ reaction to Methylisothiazolinone 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 tradename mixtures of MCI/MI biocides. Positive reactions to Methylisothiazolinone were observed with patch testing. Two cases of occupational contact allergy and dermatitis were reported in patients exposed to compounds containing the biocide Methylisothiazolinone. Patch testing revealed +++ reactions to Methylisothiazolinone. Four out of 14 workers at a Danish paint factory were observed with contact dermatitis after exposure to paint additives containing 7-10% Methylisothiazolinone. Positive reactions were observed in all 4 patients during patch testing. Numerous other reports of contact allergy, particularly to toilet wipes and water-based wall paint containing Methylisothiazolinone, have been reported.*

A sampling of case studies that report adverse effects to Methylisothiazolinone from various exposures is summarized in Table 6. Cases include reports of Methylisothiazolinone sensitization from a wide range of materials, including personal care products, paints, photographic processing agents, glues, eye glass frames, and cleaners.[23-32]

## QUANTITATIVE RISK ASSESSMENT

*Cosmetics Europe and the CIR Science and Support Committee (SCC) conducted QRAs of Methylisothiazolinone in response to the increased incidences of contact sensitization to Methylisothiazolinone in Europe.*[1] *The QRA, which used a conservative no expected sensitization induction level (NESIL) of 15 µg/cm$^2$/day that was derived based on a weight of evidence (WoE) evaluation of data from 5 HRIPTs and 4 LLNAs, predicted that consumer exposures to 100 ppm Methylisothiazolinone 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.*

## SUMMARY

In 2019, the Panel published an amended safety assessment of the preservative Methylisothiazolinone with the conclusion that this ingredient "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." This conclusion superseded the findings of the Panel's earlier safety assessment that was published in 2010. At the September 2019 Panel meeting during the re-evaluation of the mixture MCI/MI, the Panel reopened the amended safety assessment of Methylisothiazolinone to gather and evaluate additional data, with particular regard to inhalation toxicity.

According to 2019 VCRP survey data, Methylisothiazolinone (when not used with MCI) is used in a total of 915 formulations; the majority of the uses are in bath soaps and detergents. These uses have increased since the last review where 745 uses were reported; the majority of the uses reported then were in non-coloring hair conditioners and shampoos. In the amended safety assessment published in 2019, the maximum concentration of use range was reported to be 3.5 x 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). A survey of the present concertation of use range is currently being conducted by the Council.

In a dermal study in rats, the $LD_{50}$ for 49.0% Methylisothiazolinone was greater than 2000 mg/kg bw. In oral studies, the $LD_{50}$ for a 1% solution of Methylisothiazolinone in rats was 148.0 mg/kg, while the $LD_{50}$ for a 50% solution of Methylisothiazolinone in rats was 232 - 249 mg/kg in males and 120 mg/kg in females. The $LC_{50}$ of aerosolized 49.8% Methylisothiazolinone in rats was 0.422 mg/L in males and 0.354 mg/L in females.

In a 28-day oral toxicity study in rats tested with 0, 10.0, 28.6, or 71.2 mg/kg bw Methylisothiazolinone, the NOAEL was 28.6 mg/kg bw/day and the LOAEL was 71.2 mg/kg bw/day based on lethargy and mortality. When Methylisothiazolinone was tested at up to 30.09 mg/kg bw in a 90-day oral toxicity study in rats, the NOAEL was 30.09 mg/kg/day based on no treatment-related mortality or clinical signs of toxicity.

In the 90-day oral toxicity study, no adverse effects were observed on the male rat reproductive system after rats received up to 30.09 mg/kg bw Methylisothiazolinone in water. In a study that investigated the teratogenic potential of 49.8% Methylisothiazolinone in rats, no teratogenic effects on fetuses attributed to the test material could be verified. The NOAEL and LOAEL for maternal toxicity were 33.4 mg/kg bw/d and 49.8 mg/kg bw/d, respectively; the NOAEL and LOAEL for embryotoxicity were 49.8 mg/kg bw/d and 74.7 mg/kg bw/d, respectively.

Methylisothiazolinone (49.0% - 49.8%) was not mutagenic in an Ames study, chromosome aberration study, or in a mammalian cell gene mutation assay. Additionally, it was not mutagenic in an in vivo micronucleus assay in mice.

In a rabbit irritation study, 49.0% Methylisothiazolinone in water was corrosive. Methylisothiazolinone was sensitizing in a guinea pig maximization test and in an LLNA when tested at up to 10.0%; however, it was not a sensitizer in another LLNA at up to 4.5%. In human sensitization studies, dose-dependent sensitization was observed to Methylisothiazolinone at up to 2500 ppm in a cumulative irritation study and HRIPTs. Methylisothiazolinone (100 ppm in saline) was considered safe and well tolerated in an ocular irritation study of human subjects.

A sampling of the numerous baseline and retrospective studies on Methylisothiazolinone that have become available in the published literature since 2014 indicate that sensitization to Methylisothiazolinone is still found world-wide. A selection of case studies that report adverse effects to Methylisothiazolinone from various exposures included reports of Methylisothiazolinone sensitization from a wide range of materials, including personal care products, paints, photographic processing agents, glues, eye glass frames, and cleaners.[23-32]

## PREVIOUS (2019) DISCUSSION[1]

*The Panel noted the numerous reports of contact allergy to Methylisothiazolinone in Europe and the increased incidences of contact allergy to Methylisothiazolinone observed in their own clinical experience. The Panel also noted that Methylisothiazolinone 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 Methylisothiazolinone in rinse-off product categories at concentrations up to 100 ppm. However, the QRA indicated that Methylisothiazolinone use in many leave-on product categories would be safe only at concentrations lower than 100 ppm. For example, the AEL/CEL calculated for 100 ppm (0.01%) Methylisothiazolinone 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 Methylisothiazolinone in wet wipes.*

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

*The risk of inducing sensitization depends on the dose of Methylisothiazolinone per unit area of the skin exposed (e.g., expressed in units of µg/cm$^2$/day). One type of cosmetic product will differ from another in the potential to cause sensitization at a given Methylisothiazolinone 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 Methylisothiazolinone in rinse-off products are less than those associated with leave-on products and, for instance, why the risks associated with exposures to Methylisothiazolinone in leave-on hair conditioners would likely be substantially lower than those associated with Methylisothiazolinone 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 Methylisothiazolinone unlikely to induce sensitization from the use by consumers of a specific cosmetic product or product category.*

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

*The Panel discussed the issue of incidental inhalation exposure to Methylisothiazolinone in non-coloring hair sprays and hair tonics or dressings. There were no chronic inhalation toxicity data identified or provided. Methylisothiazolinone 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.*

## DISCUSSION

To be determined…

## PREVIOUS (2019) CONCLUSION[1]

*The Panel concluded that Methylisothiazolinone 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.*

## CONCLUSION

To be determined…

# TABLES

**Table 1. Frequency and concentration of use according to duration and type of exposure for methylisothiazolinone.**

| | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* |
|---|---|---|---|---|
| | **2019[6]** | | **2014[1]** | |
| **Totals†** | **915** | **NS** | **745** | **0.000000035-0.01** |
| ***Duration of Use*** | | | | |
| Leave-On | 559 | NS | 478 | 0.000000035-0.01 |
| Rinse Off | 345 | NS | 260 | 0.00000025-0.01 |
| Diluted for (Bath) Use | 11 | NS | 7 | 0.0002-0.01 |
| ***Exposure Type*** | | | | |
| Eye Area | 28 | NS | 22 | 0.00019-0.01 |
| Incidental Ingestion | 1 | NS | 1 | 0.0048 |
| Incidental Inhalation-Spray | 3; 278[a]; 168[b] | NS | 3; 268[a]; 114[b] | 0.00018-0.01; 0.0002-0.01[a] |
| Incidental Inhalation-Powder | 168[b] | NS | 114[b] | NR |
| Dermal Contact | 679 | NS | 544 | 0.000000035-0.01 |
| Deodorant (underarm) | NR | NS | NR | 0.0095 |
| Hair - Non-Coloring | 224 | NS | 190 | 0.000004-0.01 |
| Hair-Coloring | NR | NS | NR | 0.000056-0.0095 |
| Nail | 3 | NS | 5 | 0.0002-0.006 |
| Mucous Membrane | 124 | NS | 103 | 0.0000009-0.01 |
| Baby Products | 5 | NS | 6 | 0.0002-0.0075 |

NR = Not reported; NS = Survey not completed

† 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 may be sprays, but it is not specified 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.

**Table 2. Acute toxicity**

| Concentration | Dose | Species/Strain | Method | Results | Reference |
|---|---|---|---|---|---|
| | | ***Dermal*** | | | |
| 49.0%; no vehicle used | 2000 mg/kg bw; no control dose | 5 male and 5 female Wistar rats | Acute dermal toxicity study in accordance with OECD TG 402; 24-h patch was occluded | $LD_{50}$ > 2000 mg/kg bw; strong irritation of the treated skin was observed | 4 |
| | | ***Oral*** | | | |
| 50% solution of active ingredient in distilled water | 150, 180, 225, or 300 mg active ingredient/kg | CD(BR) rats; 6 males each in 180, 225, and 300 mg/kg dose groups and 6 females each in 150, 180, and 225 mg/kg dose groups (36 rats total) | Animals received test material in a single 10 ml/kg dose via gavage | $LD_{50}$ was 232-249 mg/kg in males and 120 mg/kg in females | 4 |
| 49.0% in water | 110.3, 165.6, 247.9, 371.9, or 558.1 mg active ingredient/kg bw | 6 male and 6 female Wistar rats per dose group | Acute oral toxicity study in accordance with OECD TG 401 via gavage | $LD_{50}$ was 285.5 mg/kg bw for both sexes | 4 |
| 1% w/v solution in water | 100, 126, 160, 200, or 251 mg/kg | 3 male and 2 female Sherman-Wistar rats per dose group | Animals received a single dose via gavage | $LD_{50}$ was 148.0 mg/kg for both sexes | 4 |
| | | ***Inhalation*** | | | |
| 49.8%; vehicle not reported | Calculated atmospheric doses were 0, 0.127, 0.252, or 0.504 mg active ingredient/L | 5 male and 5 female Wistar rats | Acute inhalation toxicity study in accordance with OECD TG 403; animals were exposed nose-only to aerosol for 4 h | $LC_{50}$ was 0.422 mg/L in males, 0.354 mg/L in females | 4 |

**Table 3. Genotoxicity studies**

| Concentration | Dose | Species/Strain/Cell | Method | Results | Reference |
|---|---|---|---|---|---|
| | | ***In Vitro*** | | | |
| 49.0% in DMSO | 3.9, 11.8, 35.3, 105.8, or 317.5 µg/plate, with and without metabolic activation | *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 | Ames study in accordance with OECD TG 471 | Not mutagenic | [4] |
| 49.8%; vehicle not reported | 0.0013, 0.0025, or 0.005 mg/ml, with and without metabolic activation | Human lymphocytes | Chromosome aberration study in accordance with OECD TG 473 | Not mutagenic | [4] |
| 49.8%; vehicle not reported | 0.125-2.490 mg/ml; with and without metabolic activation | Chinese hamster ovary cells | Mammalian cell gene mutation assay in accordance with OECD TG 476 | Not mutagenic | [4] |
| | | ***In Vivo*** | | | |
| 49.8% in 0.9% NaCl | 0, 49.8, 74.4, 99.6 mg/kg bw | 5 male and 5 female NMRI mice per dose group | Micronucleus assay in accordance with OECD TG 474; single oral gavage treatment | Not genotoxic | [4] |

**Table 4. Irritation and sensitization studies of Methylisothiazolinone**

| Concentration/Dose/Vehicle | Test System | Method | Results | Reference |
|---|---|---|---|---|
| | | ***Irritation – Animal*** | | |
| 49.0% in water | 3 New Zealand White rabbits; sex not reported | Dermal irritation study in accordance with OECD TG 404; patches were semi-occluded and were of 4 h duration; test material was not diluted | Corrosive; moderate dermal irritation and eschar formation was observed; primary dermal irritation index was 2.9, erythema score was 2, edema score was 1; erythema and edema were not fully reversible within 14 days | [4] |
| | | ***Sensitization – Animal*** | | |
| 49.0% in water; 1st induction was 0.1%, 2nd induction was 10%, challenge was 1% | Female Dunkin-Hartley guinea pigs; 10 test and 5 control animals | Guinea pig maximization test in accordance with OECD TG 406; challenge patch was occluded | Sensitizing; erythema observed in all treated animals at up to 72 h post-challenge patch, no reactions in control group | [4] |
| 0.75%-4.5% in water | Groups of 5 female CBA/J mice | LLNA in accordance with OECD TG 429; positive control group received 25% α-hexylcinnamalde-hyde in DMSO; negative control was tissue culture water | Not sensitizing; the SI values were less than 3 at all concentrations; controls yielded expected results | [4] |
| 50.5% in ethanol/water (1:1, v/v) tested at 2.5%, 5%, and 10% (w/v) | Groups of 4 female CBA mice | LLNA in accordance with OECD TG 429 | Sensitizing; SI values were 1.9, 6.5 and 16.0 at 2.5%, 5.0%, and 10.0%, respectively | [4] |
| | | ***Sensitization- Human*** | | |
| 51.4% active ingredient tested at 1000, 1500, 2000, or 2500 ppm in water | Groups of 12 male and female subjects; total completed through challenge was 43 | Cumulative irritation study for 21 consecutive days except Sundays, total of 18 patches; challenge patches were performed 2 weeks after the final irritation patch; 0.2 ml of test material was applied on the back of each subject with occlusive 2 $cm^2$ patches; SLS was the positive control and distilled water was the negative control | Sensitizing with number of sensitizing reactions increasing with increasing concentration of active ingredient; irritation scores of the test material were below that of the SLS control | [4] |
| 500, 750, 1000, 1500, or 2000 ppm in aqueous solution | 115 male and female subjects divided into 5 groups | HRIPT; induction phase consisted of daily patches for 14 days followed by a challenge phase conducted after a 2-week rest period; 0.15 ml of test material was applied on the back of each subject with occlusive patches; SLS was the positive control, negative control was physiological saline | Minimal sensitization was observed in the 500 ppm dose group, but a clear dose-response relationship was not observed; irritation responses were observed in a dose-dependent manner | [4] |
| 300 ppm active ingredient with 300 ppm propylene glycol in water | 98 subjects completed study | HRIPT; 0.2 ml test material was applied on the back of each subject with 2 $cm^2$ occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 weeks followed by a challenge phase conducted after a 2-week rest period | Not sensitizing | [4] |
| 400 ppm active ingredient with 400 ppm propylene glycol in water | 13 subjects completed study | HRIPT; 0.2ml test material was applied on the back of each subject with 2 $cm^2$ occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 weeks followed by a challenge phase conducted after a 2-week rest period | Not sensitizing | [4] |
| 600 ppm active ingredient with 600 ppm propylene glycol in water | 108 subjects completed study | HRIPT; 0.2ml test material was applied on the back of each subject with 2 $cm^2$ occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 weeks followed by a challenge phase conducted after a 2-week rest period | Not sensitizing | [4] |

**Table 4. Irritation and sensitization studies of Methylisothiazolinone**

| Concentration/Dose/Vehicle | Test System | Method | Results | Reference |
|---|---|---|---|---|
| 500.1 ppm active ingredient in water | 109 subjects completed study | HRIPT; 0.2ml test material was applied on the back of each subject with 2 $cm^2$ occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 weeks followed by a challenge phase conducted after a 2-week rest period | Not sensitizing | [4] |
| 300 ppm active ingredient with 300 ppm propylene glycol in water | 98 subjects completed study | HRIPT; 0.2ml test material was applied on the back of each subject with 2 $cm^2$ occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 weeks followed by a challenge phase conducted after a 2-week rest period | Not sensitizing | [4] |
| 500 ppm active ingredient with 500 ppm propylene glycol in water | 101 subjects completed study | HRIPT; 0.2ml test material was applied on the back of each subject with 2 $cm^2$ occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 weeks followed by a challenge phase conducted after a 2-week rest period | Sensitizing | [4] |

OECD TG - Organization for Economic Co-operation and Development test guideline
LLNA – local lymph node assay
SI – stimulation index
HRIPT – human repeated insult patch test

**Table 5. Baseline and retrospective studies**

| Number of Patients | Clinical Testing Type | Location and Time Span | Results | Reference |
|---|---|---|---|---|
| 4857 patients | Patch tested with screening series of 70 allergens, including 0.2% Methylisothiazolinone aq.; patches were Finn chambers | 13 centers in North America; January 1,2013 to December 31,2014 | 10.9% (527) patients had positive reaction to Methylisothiazolinone | [13] |
| 79 out of 9037 patients which had allergic reactions to allergens identified with wet wipes | Retrospective review of patients tested with the North American Contact Dermatitis Group coded with wet wipes as source of allergen; 0.2% Methylisothiazolinone aq. | North America; January 1, 2011 to December 31, 2014 | Out of the reactions associated with wet wipes, 59% had positive reactions to Methylisothiazolinone | [14] |
| 324 patients | Retrospective study of patients tested with European baseline series, including 0.2% Methylisothiazolinone aq.; patches were IQ Ultra changers | Turkey; January 2016 to June 2018 | 8.02% (26) patients had positive reaction to Methylisothiazolinone | [15] |
| 264 patients with suspected eyelid allergic contact dermatitis | Prospective study of patients tested with an eyelid series, the European baseline series, the French additional series, and personal products; additional testing with additional series and repeated open application tests were performed if necessary; concentration of Methylisothiazolinone tested not reported | France; September 2014 to August 2016 | 10.2% (27) patients had positive reactions to Methylisothiazolinone; these results may include reactions to MCI/MI | [16] |
| 798 patients | Testing in consecutive dermatitis patients with diagnosed Methylisothiazolinone contact allergy; Croatian baseline series that included 0.2% Methylisothiazolinone aq. and 0.01% MCI/MI aq.; patches were 8 mm Finn chambers | Croatia; November 2, 2015 to November 3, 2016 | 13.2% (105) patients had positive reactions to Methylisothiazolinone | [17] |
| 2787 patients | Retrospective study of patients tested with allergen including 0.2% Methylisothiazolinone aq.; taches were Finn chambers or Allergeaze test chambers | Australia; January 1, 2011 to December 31, 2017 | 14.5% (404) patients had positive reactions to Methylisothiazolinone | [18] |
| 1142 patients | Retrospective study of patch test cases of children with known atopic dermatitis | United States; January 1, 2015 to December 31, 2015 | 3.2% (14/429) patients had positive reactions to Methylisothiazolinone | [19] |
| 139 patients | Retrospective study of patients with Methylisothiazolinone-induced allergic contact dermatitis; European baseline series, targeted complementary series, and personal products used; 200, 500, or 2000 ppm Methylisothiazolinone ; patches were IQ chambers | France; January 2010 to December 2015 | Relapses observed in 64% of patients and were severe in 18%; rinse-off cosmetics were responsible for 27% of the relapses | [20] |
| 2028 patients | Testing in consecutive dermatitis patients; Methylisothiazolinone tested at 0.2% aq. | Italy; January 2012 to December 2014 | 5.2% (106) patients had positive reactions to Methylisothiazolinone overall; prevalence of Methylisothiazolinone sensitization increases from 2.3% in 2012 to 6.9% in 2014 | [21] |
| 99 patients | Retrospective study of patients that underwent cutaneous allergy testing for perianal and/or genital symptoms; patch testing with British Society for Cutaneous Allergy standard series with additional series in some patients; patches were IQ Ultra chambers or Finn chambers; 0.2% Methylisothiazolinone | Ireland; January 2013 to December 2015 | 5% (5) patients had positive reactions to Methylisothiazolinone | [22] |

**Table 6. Case reports**

| Suspected Sensitizing Material | Patient(s) | Presentation | Patch Test Results | Reference |
|---|---|---|---|---|
| Multiple personal care products, and wall paint containing Methylisothiazolinone | 51-year-old atopic woman | Pruritic eczema dermatosis of the face, ears, cheeks, neck, forearms, elbow folds, and back that evolved over a time span of 6 years | ++ reaction to Methylisothiazolinone and + reaction to 2-n-octyl-4-isothazolin-3-one in the European contact allergen series | [23] |
| Hair care products (gel and conditioner) containing Methylisothiazolinone | 60-year-old man | Allergic contact dermatitis presenting over 3 years, and involving dorsal hands, forearms, torso, and face | +++ reaction to Methylisothiazolinone and a ++ reaction to MCI/MI in the North American Contract Dermatitis Group standard series and preservatives series | [24] |
| Photograph developing stabilizing agents containing isothaizolinones | 61-year-old man | Itchy erythematous and vesicular lesions presenting for 1 year on the dorsa of the hands, progressively extending to the neck, neckline and face | + and ++ reactions to MCI/MI (200 ppm), + and ++ reactions to Methylisothiazolinone (2000 ppm), and + and ++ reactions to octylisothiazolinone (1000 ppm) on Day 2 and Day 3, respectively, when tested with the European baseline, additive series, photographic chemical series, dyes, and personal photographic developing chemicals; patches were IQ Ultra chambers that were occluded for 2 days | [25] |
| Wall paint | 66-year-old man | Pruritic, erythematous and edematous lesions on the face following sleeping in a freshly painted house; prior to the allergic contact dermatitis, patient was under treatment for plaque psoriasis | Positive reactions were observed on Day 2 and Day 4 to Methylisothiazolinone, MCI/MI in the TRUE Test series and to 2 of the 4 paints that were used in the house, which contained MCI/MI | [26] |
| Wall paint and façade renders | 26-year-old man | Persistent dry cough and rhinitis, followed a few days later by eczematous eruptions on face, eyelids, chest, nape of neck, and elbow folds | When tested with European baseline series, preservatives series, and occupational products, ++ and +++ reactions were observed on Day 2 and Day 4 to Methylisothiazolinone (2000 ppm aq.), MCI/MI (200 ppm aq.) and indoor façade render ("as-is"); + and ++ reactions were observed to water-based paint ("as-is") | [27] |
| Eye lash extensions | 34-year-old atopic woman | Immediate pain when product was directly applied; within 12 h, pruritic, edematous, and eczematous rash developed around eyes; after 4 months, patient still had periorbital eczema | +++ reactions on Day 3 to Methylisothiazolinone at 62, 250, and 2000 ppm as well as to the eyelash products; ++ reaction to Methylisothiazolinone at 125 ppm, and + reaction to Methylisothiazolinone at 31 ppm | [28] |
| Adhesive labels | 28-year-old woman with history of atopic dermatitis | Hand eczema of 1-year duration | + reaction to Methylisothiazolinone (0.2% aq.) on Day 2 and Day 3 when tested with the German baseline series | [29] |
| Eyeglass frames | 48-year-old man | Eczema on ulnar aspects of both hands on the right lower leg; one month later, severe facial dermatitis | +++ and ++ reactions to Methylisothiazolinone and ++ and ++ reactions to eyeglass frame scrapings on Day 3 and Day 7 following testing with TRUE Test, additional allergen series, and personal products; further testing showed the glass frames contained 11.8 µg/g Methylisothiazolinone | [30] |
| Facial sponges | 38-year-old woman | Vesicular pulpitis on fingers of both hands; facial dermatitis | ++ and + reactions to Methylisothiazolinone (0.02% aq.) in the European baseline series supplemented with baseline allergens; further testing showed the facial sponge contained 387 ppm Methylisothiazolinone | [31] |
| Household detergent | 60-year old non-atopic woman and a 36-year-old atopic woman | Eyelid and facial dermatitis | Both patients had had positive reactions to 0.2% Methylisothiazolinone aq. (+ and ++, respectively) and 0.002% MCI/MI aq. (++ each) following patch tests with the Belgian baseline and additional series; patients had used a household detergent containing 200 ppm Methylisothiazolinone to clean eyeglasses | [32] |

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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]

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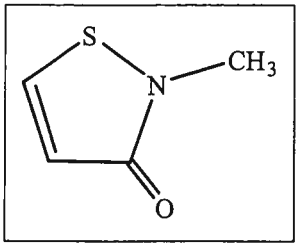


**Figure 1.** Methylisothiazolinone.

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

| | |
|---|---|
| Synonyms | 3(2H)-Isothiazolone, 2-methyl-<br>2-Methyl-3(2H)-isothiazolone<br>2-Methyl-4-isothiazolin-3-one |
| Trade names | Microcare MT<br>Neolone 950 preservative<br>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<br>Mostly soluble in acetonitrile, methanol, hexane<br>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 ($6.7 \times 10^{-3}$ mole $\times$ $1^{-1}$; CMIT 0.100% wt/vol and MIT

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

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

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

0.076% wt/vol). Twenty-four hours prior to topical sensitization, animals were treated with sodium lauryl sulfate (SLS) solution (200 μL). For the topical sensitization, 200 μL of the suspected sensitizing test chemical in 99.5% ethanol (0.050% wt/vol for CMIT and 0.038% wt/vol for MIT) was placed on a 2 × 4-cm patch at equimolar concentrations ($3.3 \times 10^{-3}$ 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 ($1.3 \times 10^{-3}$ 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 $5.21 \times 10^4$ cells per square centimeter and treated with 0.1, 0.3, 1.0, and 3.0 μM MIT for 14 hours in serum-containing media. Cell viability was determined after the incubation with MIT using an LDH-based in vitro toxicity assay. The cells were analyzed for morphological changes, and immunoprecipitation, electrophoresis, and immunoblotting were performed. A cell-free tyrosine kinase assay was also performed.

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

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

### *In Vivo*

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

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

## Clinical Assessment of Safety

### Dermal Irritation

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

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

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

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

### Dermal Sensitization

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## Phototoxicity

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

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

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

## Case Reports

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

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

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

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

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

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

## Margin of Safety

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

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

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

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

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

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

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

## Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## Discussion

In 1992, the CIR Expert Panel concluded that the mixture MIT/CMIT (23.3% MIT and 76.7% CMIT) may be safely used in rinse-off products at a concentration not to exceed 15 ppm and in leave-on cosmetic products at a concentration not to exceed 7.5 ppm. Currently, MIT is used as a standalone biocide. Accordingly, it was considered necessary to evaluate the safety of MIT alone.

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

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

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

## Conclusion

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

### Authors' Note

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

### Declaration of Conflicting Interests

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*Article*

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# Amended Safety Assessment of Methylisothiazolinone as Used in Cosmetics

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**Abstract**

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

**Keywords**

methylisothiazolinone, cosmetics, safety

## 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 Cosmetic Ingredient Review (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 (MCI)/MI. The Panel reopened this safety assessment to gather and evaluate additional data.

In June 2014, the Panel reviewed the results of quantitative risk assessments (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 previously reviewed the safety of the mixture MCI/MI (sold at a ratio of 3:1) 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]

Extensive data from the original MI safety assessment report, which was finalized in 2008 and published in 2010, were considered by the Panel during the review of this amended safe assessment. Because those data are included in the report th was published in 2010[1] (and can be found on the CIR websi [https://www.cir-safety.org/ingredients]), only new informatic will be included in this report. However, notes have been add in text, referring to the original safety assessment, to identify tl types of data that were available in that original report.

## Chemistry

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

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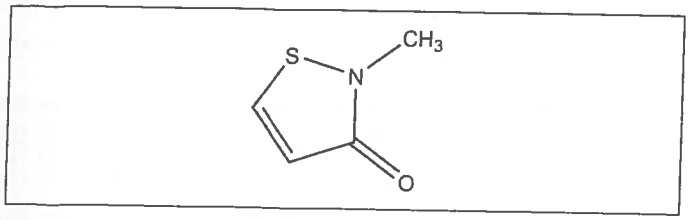


**Figure 1.** Methylisothiazolinone.

## Use

### Cosmetic

Table 1 presents the historical and current product formulation data for MI. Methylisothiazolinone 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 1,125 reported uses, with the majority of the uses reported in noncoloring 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 $4 \times 10^{-6}$% to 0.01%, with 0.01% reported in both leave-on and rinse-off baby, noncoloring 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 $3.5 \times 10^{-8}$% 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]

Methylisothiazolinone was reported to be used in noncoloring 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 acrodynamic 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 (ie, they could 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. For 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]

### Noncosmetic

The noncosmetic uses of MI include use in water-based paints, which has been noted in a number of case studies of sensitization reactions (eg, see Table 2). The uses of MI in paints and other noncosmetic products were described in the original safety assessment of MI that was published in 2010.[1]

## Toxicokinetics

### Absorption, Distribution, Metabolism, and Excretion

Absorption, distribution, metabolism, and excretion studies are summarized in the original safety assessment of MI that was published in 2010.[1]

## Toxicological Studies

### Acute Toxicity

Acute oral and dermal toxicity studies are summarized in the original safety assessment of MI that was published in 2010.[1]

### Repeated Dose Toxicity

Oral repeated dose toxicity studies are summarized in the original safety assessment of MI that was published in 2010.[1]

## Reproductive and Developmental Toxicity

Reproductive and developmental toxicity studies are summarized in the original safety assessment of MI that was published in 2010.[1]

## Carcinogenicity

Carcinogenicity studies of the sole ingredient MI were not discovered in the published literature, and unpublished data were not submitted to CIR.[1]

## Genotoxicity

In vitro genotoxicity studies are summarized in the original safety assessment of MI that was published in 2010.[1]

## Neurotoxicity

Neurotoxicity studies are summarized in the original safety assessment of MI that was published in 2010.[1]

**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[a] | 2014[b] | 2007 | 2014 |
| Totals[c] | 1125 | 745 | $4 \times 10^{-6}$-0.01 | $3.5 \times 10^{-8}$-0.01 |
| Duration of use | | | | |
| Leave-on | 236 | 478 | 0.002-0.01 | $3.5 \times 10^{-8}$-0.01 |
| Rinse-off | 807 | 260 | $4.0 \times 10^{-6}$-0.01 | $2.5 \times 10^{-7}$-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[d]; 54[e] | 3; 268[d]; 114[e] | 0.005; 0.008-0.009[d] | 0.0002-0.01[d]; 0.0002-0.0 |
| Incidental inhalation—powder | 1; 2[g] | 114[e] | NR | NR |
| Dermal contact | 469 | 544 | 0.0008-0.01 | $3.5 \times 10^{-8}$-0.01[h,i] |
| Deodorant (underarm) | 2[d] | NR | NR | 0.0095[j] |
| Hair—noncoloring | 579 | 190 | $4.0 \times 10^{-6}$-0.01 | $4.0 \times 10^{-6}$-0.01 |
| Hair—coloring | 76 | NR | NR | $5.6 \times 10^{-5}$-0.0095 |
| Nail | 1 | 5 | NR | 0.0002-0.006 |
| Mucous membrane | 241 | 103 | 0.0015-0.01 | $9.0 \times 10^{-7}$-0.01 |
| Baby products | 14 | 6 | 0.002-0.01[k] | 0.0002-0.0075 |

Abbreviations: MCI, methylchloroisothiazolinone; MI, methylisothiazolinone; NR, not reported.
[a] Data provided are not clear as to whether uses are MI alone or include uses of MI/MCI.
[b] Data provided are for uses of MI alone.
[c] 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.
[d] Includes products that can be sprays, but it is not known whether the reported uses are sprays.
[e] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
[f] 0.01% in an aerosol hair spray; 0.0002% to 0.01% in a pump hair spray; 0.006% to 0.0095% in a pump hair tonic or dressing.
[g] Includes products that can be powders, but it is not known whether the reported uses are powders.
[h] 0.00023% to 0.01% in a hand soap; 0.01% in a foot scrub.
[i] 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 concentrati of 0.005%.
[j] Not a spray deodorant.
[k] 0.01% in baby wipes.

## Irritation and Sensitization

### *Irritation*

Nonhuman and human dermal irritation studies, and nonhuman ocular irritation studies, are summarized in the original safety assessment of MI that was published in 2010.[1]

### *Sensitization*

*Nonhuman.* A letter to the editor reporting the reevaluation of published local lymph node assay (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.* Methylisothiazolinone 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 conta allergy to MI, alone, because of the relatively low MI conce tration in the MCI/MI mixture tested (approximately 25 pp MI in a 100 ppm MCI/MI test solution).[17,18] Recommendatio have been made to test for contact allergy to MI alone, althou there currently is no consensus about the concentration of M that should be used in such testing.[13,19-24]

The dose–response relationship of contact allergy to MI w investigated in 11 MI-allergic patients.[25] The patients we patch tested with 2 dilution series of 12 doses of MI (Neolo 950 9.7% active ingredient) in 10% ethanol and 90% aqua a 12 doses of MI with 9.26 µg phenoxyethanol/cm$^2$ in 10% eth nol and 90% aqua. (Phenoxyethanol may increase antimicr bial efficacy of MI and was tested to determine whether influenced reactivity to MI.) The MI doses with and witho phenoxyethanol were 0.0105, 0.105, 0.147, 0.21, 0.441, 1.4 2.94, 4.41, 8.82, 15, 30, and 60 µg MI/cm$^2$. Controls (n = 1 who were not MI-allergic patients were patch tested with 60 MI/cm$^2$ and 9.26 µg phenoxyethanol/cm$^2$. Each test s received 15 µL of each dilution applied by filter disc in a Fi Chamber and were occluded for 2 days. Readings were p formed on days 2, 3 or 4, and 7. The subjects also underwen repeated open application test (ROAT) with a cream that co tained 0, 0.0105, 0.105, or 0.21 µg MI/cm$^2$ (0, 5, 50,

**Table 2.** Quantitative Risk Assessment of Methylisothiazolinone (MI) at Highest Maximum Use Concentration (100 ppm) in Cosmetic Products.[a,28]

| Product category[b] | Product amount applied/day (μg/cm$^2$) | Consumer exposure level (CEL; μg/cm$^2$/d) | Sensitization assessment factor (SAF) | Acceptable exposure level (AEL; μg/cm$^2$/d)[c] | AEL/CEL |
|---|---|---|---|---|---|
| Baby shampoo | 200 | 0.02 | 100 | 0.15 | 7.50 |
| Baby lotions, oils, powders, creams | 2,200 | 0.22 | 300 | 0.05 | 0.23 |
| Baby wipes | 4,000 | 0.40 | 300 | 0.05 | 0.13 |
| Other baby products (powders and talc's) | 4,200 | 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 | 2,200 | 0.22 | 300 | 0.05 | 0.23 |
| Eyeliners | 2,170 | 0.22 | 300 | 0.05 | 0.23 |
| Eye shadow | 2,170 | 0.22 | 300 | 0.05 | 0.23 |
| Eye lotion | 2,170 | 0.22 | 300 | 0.05 | 0.23 |
| Eye makeup remover | 900 | 0.09 | 100 | 0.15 | 1.67 |
| Mascara | 2,170 | 0.22 | 300 | 0.05 | 0.23 |
| Other eye makeup | 2,170 | 0.22 | 300 | 0.05 | 0.23 |
| Cologne and toilet waters | 1,7700 | 1.77 | 100 | 0.15 | 0.08 |
| Blushers | 1,000 | 0.10 | 100 | 0.15 | 1.50 |
| Other fragrance products | 2,200 | 0.22 | 100 | 0.15 | 0.68 |
| Hair conditioners | 200 | 0.02 | 100 | 0.15 | 7.50 |
| Hair sprays (aerosol fixatives) | 1,390 | 0.14 | 100 | 0.15 | 1.08 |
| Hair sprays (pump) | 2,200 | 0.22 | 100 | 0.15 | 0.68 |
| Hair straighteners | 4,200 | 0.42 | 100 | 0.15 | 0.36 |
| Permanent waves | 4,200 | 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 |
| Wave sets | 4,200 | 0.42 | 100 | 0.15 | 0.36 |
| Other noncoloring hair products | 1,000 | 0.10 | 100 | 0.15 | 1.50 |
| [d]Hair dyes and colors | 1,000 | 0.10 | 100 | 0.15 | 1.50 |
| [d]Hair tints | 990 | 0.10 | 100 | 0.15 | 1.52 |
| Hair rinses (coloring) | 200 | 0.02 | 100 | 0.15 | 7.50 |
| [d]Hair bleaches | 1,000 | 0.10 | 100 | 0.15 | 1.50 |
| Other hair coloring preparations | 1,000 | 0.10 | 100 | 0.15 | 1.50 |
| Face powders | 1,000 | 0.10 | 100 | 0.15 | 1.50 |
| Foundations | 3,170 | 0.32 | 100 | 0.15 | 0.47 |
| Lipsticks | 11,460 | 1.15 | 300 | 0.05 | 0.04 |
| Other makeup preparations | 4,200 | 0.42 | 100 | 0.15 | 0.36 |
| Other manicuring preparations | 1,000 | 0.10 | 100 | 0.15 | 1.50 |
| Other personal cleanliness products | 4,400 | 0.44 | 300 | 0.05 | 0.11 |
| Aftershave lotions | 2,210 | 0.22 | 100 | 0.15 | 0.68 |
| Preshave lotions (all types) | 2,200 | 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 | 2,200 | 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 | 2,700 | 0.27 | 100 | 0.15 | 0.56 |
|  | 1,120 | 0.11 | 300 | 0.05 | 0.45 |

*(continued)*

**Table 2.** (continued)

| Product category[b] | Product amount applied/day (μg/cm$^2$) | Consumer exposure level (CEL; μg/cm$^2$/d) | Sensitization assessment factor (SAF) | Acceptable exposure level (AEL; μg/cm$^2$/d)[c] | AEL/CE |
|---|---|---|---|---|---|
| Body and hand creams, lotions, and powders | | | | | |
| Moisturizers | 2,700 | 0.27 | 100 | 0.15 | 0.56 |
| Nail care creams and lotions | 970 | 0.10 | 100 | 0.15 | 1.55 |
| Deodorants (underarm) | 8,500 | 0.85 | 300 | 0.05 | 0.06 |
| Night creams, lotions, powders, and sprays | 3,170 | 0.32 | 100 | 0.15 | 0.47 |
| Paste masks (mud packs) | 4,200 | 0.42 | 100 | 0.15 | 0.36 |
| Skin fresheners | 150 | 0.02 | 100 | 0.15 | 10 |
| Other skin care products | 2,200 | 0.22 | 100 | 0.15 | 0.68 |
| Suntan gels, creams, liquids, and sprays | 2,200 | 0.22 | 100 | 0.15 | 0.68 |
| Indoor tanning preparations | 2,200 | 0.22 | 100 | 0.15 | 0.68 |
| Other tanning preparations | 2,200 | 0.22 | 100 | 0.15 | 0.68 |
| Foot powders and sprays | 2,200 | 0.22 | 100 | 0.15 | 0.68 |

Abbreviations: AEL, acceptable exposure levels; CEL, consumer exposure level; NESIL, no expected sensitization induction level; QRA, quantitative ris assessment.

[a] Shaded rows indicate the ratio of AEL × CEL$^{-1}$ is less than 1.

[b] Exposure values assumed for each product category were from the IFRA RIFM QRA Information Booklet (2011)[50] and Api et al. (2008).[51]

[c] Based on NESIL of 15 μg/cm$^2$/d.

[d] Note that this product category may be diluted prior to application.

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 human repeated insult patch test (HRIPT) of 226 subjects performed in accordance with the International Contact Dermatitis Research Group 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. Additional nonhuman and human sensitization studies are summarized in the original safety assessment of MI that was published in 2010.[1]

## 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 contac sensitization to MI in Europe.[27,28] Both of these QRAs wer conducted using the same no expected sensitization inductio level (NESIL = 15 μg/cm$^2$/d) and sensitization assessmer factors (SAFs).

Table 2 summarizes the QRA conducted by the CIR SSC. A conservative NESIL of 15 μg/cm$^2$/d was derived for MI base on a weight-of-evidence (WoE) evaluation of data from HRIPTs and 4 LLNAs. The NESIL was then used to calculat acceptable exposure levels (AELs) for the potential for th induction of sensitization from dermal exposure to MI in cos metic products, assuming the maximal use concentration o 100 ppm MI and product category–specific SAFs. The rati of the AEL and the consumer exposure level (CEL) was the calculated for each of many cosmetic product categories, rang ing from hair conditioners (CEL = 0.02 μg/cm$^2$/d) to lipstick (CEL = 1.15 μg/cm$^2$/d). The concentration of an ingredient i considered to be acceptable in a product when AEL/CEL ≥ (ie, AEL ≥ CEL).

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

## Phototoxicity

Nonhuman and human phototoxicity and photosensitizatio studies are summarized in the original safety assessment o MI that was published in 2010.[1]

**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 nonatopic 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<br>– 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<br>– Previous patch tests with a baseline and cosmetic series were negative<br>– 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<br>– 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<br>– 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 | 29 |
| Toilet wipes that contain 90 ppm MI and water-based paint that contained 0.01% MI and 0.01% MCI/MI | 62-year-old nonatopic 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<br>– Symptoms occurred 2 months following the initial use of a toilet wipe<br>– 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)<br>– 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<br>– Patch testing with paint produced a ++ reaction | 29 |
| Toilet wipes that contain 90 ppm MI | 50-year-old nonatopic female | – Patient presented with a 1-year history of perianal dermatitis following the use of moist toilet paper to control anal pruritus<br>– 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 | 29 |
| Toilet wipes that contain 90 ppm MI | 43-year-old nonatopic female | – Patient presented with a 3-month history of eczematous lesions on the genital and perianal area<br>– Patch testing with European baseline, 1000 ppm MI, and toilet wipe yielded a + (day 2) and ++ (day 4) reaction to 1000 ppm MI | 29 |
| Toilet wipes that contain 90 ppm MI | 20-year-old nonatopic 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<br>– 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 | 29 |

*(continued)*

**Table 3.** (continued)

| Mode of contact | Patient(s) | Indication | Referenc |
|---|---|---|---|
| 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<br>– Patch testing with European baseline, cosmetic series, and 1000 ppm MI yielded + reactions (days 2 and 4) to 1000 ppm MI | 29 |
| 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<br>– Patient also had reactions previously to perfumed bath salts and has experienced severe scalp itch<br>– 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 | 29 |
| 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<br>– 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;<br>– Repeated open allocation test on the volar forearm with the deodorant was strongly positive on day 2<br>– Patch testing with 200 ppm MI yielded a ++ reaction on day 2 | 31 |
| 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<br>– Rash "waxed and waned" for about 4 weeks with corticosteroid treatment while patient continued to sleep in painted bedroom and then started to clear<br>– Patch testing with adapted European baseline series for children had a + reaction on D4 for MCI/MI at 0.01% or 100 ppm<br>– Child had a history of extensive dermatitis following use of a moist toilet paper that contained MI but not MCI | 30 |
| 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<br>– Patch testing revealed + reaction to MCI/MI and ++ reaction to MI<br>– During treatment, patient also developed a 5-cm eczematous reaction on left inner thigh extending to the buttock<br>– Patient had a new toilet cleaner in home toilet that contained both MCI and MI at 11 ppm and 10 ppm, respectively<br>– Eczema improved after removal of toilet cleaner from home | 32 |
| Wall paint containing MI | 23-year-old nonatopic 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<br>– Patient also experienced burning sensation of the cheeks, malaise, and dizziness that worsened the more | 33 |

(continued

**Table 3.** (continued)

| Mode of contact | Patient(s) | Indication | Reference |
|---|---|---|---|
| | | consecutive days she worked and improved during days off<br>– 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<br>– After initial airborne exposure, patch testing and onset of dermatitis, patient was reexposed to MI in a cleansing product to which she had never been exposed and immediately experience marked aggravation of facial dermatitis | |
| Wall paint containing MI | 36-year-old nonatopic 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<br>– Initial patch testing showed ++ reaction to 2% formaldehyde and +? reactions to fragrance and 0.2% MI<br>– Symptoms disappeared after 2.5 months of sick leave, but reappeared after patient moved to a newly refurbished apartment<br>– Both the apartment and casino (workplace) had been painted with a paint that contained MI | 34 |
| Wall paints containing 1.2-187 ppm MI, 0.3-10 ppm MCI/MI, and 8.5 -187 ppm benzisothiazolinone (BIT) | 57-year-old nonatopic 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<br>– During the same time period, the patient was participating in a clinical investigation of the dose–response relationship of MI in MI-allergic patients<br>– Patient previously had positive patch tests to formaldehyde, quaternium-15, DMDM hydantoin, *p*-phenylenediamine, melamine formaldehyde, urea formaldehyde, MCI/MI, and MI<br>– 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 | 34 |
| Wall paint containing MI | 53-year-old nonatopic 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<br>– Patch testing with the European baseline series, an extended standard, and a paint series yielded + reactions to 2000 ppm MI and 5% farnesol<br>– Symptoms resolved after the patient moved out of her apartment | 35 |
| "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<br>– Patient used the device 3×/day for 10 minutes each for a few days before developing progressive skin changes<br>– 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 | 36 |

*(continued)*

**Table 3.** (continued)

| Mode of contact | Patient(s) | Indication | Refere |
|---|---|---|---|
| Household wipes and skin cleansing products containing MI | 39-year-old nonatopic female employed as a neonate nurse | – Labeling of the contact gel used by patient indicated the presence of both MCI and MI<br>– Patient presented with eczematous skin lesions on the arms, neck, and trunk of 7 months duration<br>– Patient also developed palmar hand dermatitis 2 months later, after receiving treatment for the initial symptoms<br>– Patient had previously developed a severe eczematous reaction on the hands to water-soluble paint and eyelid dermatitis while her house was being painted<br>– Patient had daily contact to nitrile gloves, hospital soap, skin cleansing products, baby wipes, household wipes, and rubber<br>– 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"<br>– 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. | 37 |

Abbreviations: BIT, benzisothiazolinone; DMDM, 1,3-Dimethylol-5,5-dimethyl; MCI, methylchloroisothiazolinone; MI, methylisothiazolinone.

## Clinical Use

### *Case Reports*

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.[29-37] Incidences of contact allergy to MI, tested separately from MCI/MI, appear to be increasing in Europe in recent years.[38-49] Additional case reports are summarized in the original safety assessment of MI that was published in 2010.[1]

## 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 1,125 reported uses, with the majority of the uses reported in noncoloring hair conditioners and shampoos. Industry reported the maximum use concentration range to be $4 \times 10^{-6}$% to 0.01%, with 0.01% reported in leave-on and rinse-off baby, noncoloring 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 wi out MCI. This safety assessment addresses the use of MI cosmetic products that do not also contain MCI. In 2014, VCRP database indicated that MI was used as an ingredient 745 cosmetic products that do not also contain MCI, with majority of the uses reported in leave-on products such as sl moisturizers. A survey of use concentrations conducted by Council in 2014 reported a maximum concentration of range of $3.5 \times 10^{-8}$% to 0.01%, with 0.01% reported in m tiple product categories including eye makeup remover, h shampoos and conditioners, and skin care products (both lea on and rinse-off).

The European Union's SCCS has a recently updated opini on the use of MI and has found that in leave-on cosme products (including "wet wipes") no safe concentration l been adequately demonstrated for induction or elicitation contact allergy. In rinse-off cosmetic products, the SCCS concluded that concentrations up to 0.0015% (15 ppm) MI safe, in terms of induction of contact allergy, but recogniz that there is no information available to evaluate the potent for this ingredient to elicit contact allergy. Furthermore, SCCS states that MI should not be added to cosmetic produ that contain MCI/MI. A reevaluation of the LLNA resu reported in the published literature in an editorial article in cates that MI should be categorized as a strong sensitizer, a not a moderate sensitizer as previously reported.

Methylisothiazolinone was named Allergen of the Year 2013 by the American Contact Dermatitis Society due to rise of use of the preservative and the increased incidences contact allergy being reported, especially in the Europe Union. A standard series of patch testing includes the mixt MCI/MI, which may miss 40% of contact allergy to MI alc

**Table 4.** Retrospective and Multicenter Studies.

| Number of dermatitis patients tested; location | Concentration of MI tested | Years analyzed | Results | Reference |
|---|---|---|---|---|
| 2,536; Gentofte, Denmark | 2000 ppm in supplemented European baseline series | May 2006 to February 2010 | – 1.5% (37/2536) of the patients patch tested with MI had contact allergy<br>– MI contact allergy more often associated with occupational exposure, hand eczema, and age above 40 years<br>– 12/37 cases (32%) were cosmetics exposure and 11/37 cases (30%) were occupational exposure, with half of these occurring in painters | 38 |
| 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<br>– 66% of those who were MI-positive were also positive to 100 ppm MCI/MI | 39 |
| 653; Australia | 200 ppm in the Australian baseline series; testing with 100 and 200 ppm MCI/MI also performed | January 1, 2011 to June 30, 2012 | – Of 33 patients who submitted to a use test, 10 had positive results<br>– 43 (7%) reactions were observed, 23 (4%) of which were deemed relevant<br>– 7 of the patients were parents of young children with hand dermatitis caused by allergic contact dermatitis to MI in baby wipes<br>– Remaining patients reacted to MI in shampoos, conditioners, deodorants, moisturizers, a skin cleanser, and a facial wipe<br>– 3 patients had occupational exposure to hand cleansers<br>– 34/43 patients (79%) had concomitant reactions with MCI/MI | 40 |
| 2,766 to MI, 2,802 to MCI/MI, and 2,413 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<br>– Contact allergy to MCI/MI increased from 1.0% in 2010% to 2.4% in 2012<br>– MI-allergic patients tended to have occupational exposure, hand and face dermatitis, and were > 40-years-old<br>– Cosmetic products were the most common substances causing relevant exposure in both MCI/MI- and MI-allergic patients | 41 |
| 1,289; 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<br>– In 2011, 18/521 patients (3.5%) had a positive reaction to MI<br>– In 2012, 33/584 patients (5.7% had a positive reaction to MI)<br>– Reactions appeared to be more prevalent in patients ≥40 years old | 42 |

(continued)

**Table 4.** (continued)

| Number of dermatitis patients tested; location | Concentration of MI tested | Years analyzed | Results | Referer |
|---|---|---|---|---|
| 219 painters and 1,095 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 ($P < 0.0001$)<br>– 11/41 (27%) of painters had positive reactions to MI<br>– 5/21 (25%) of painters had positive reactions to octylisothiazolinone<br>– 7/37 (19%) of painters had positive reactions to benzisothiazolinone (BIT) | 43 |
| ~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<br>– 1.54% of patients had positive reactions to MI in preservative series<br>– 67% (134/199) of MI-positive patients also reacted to MCI/MI<br>– MI sensitization observed more often with occupational dermatitis | 44 |
| 563 and 2,056 for 2 different concentrations of MI, 2,489 for MCI/MI; Leeds, United Kingdom | 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<br>– Percentage of patients positive to 0.02% MI increased from 0.6% in 2009 to 2.5% in 2012<br>– percentage of patients positive to 0.02% MCI/MI increased from 0.9% in 2008 to 4.9% in 2012 | 45 |
| 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<br>– 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<br>– For MCI/MI, an average of 2.5% of the patients across 11 countries had positive reactions | 46 |
| 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<br>– Prevalence of MI sensitization reported to have increased from 1.94% in 2009 to 6.02% in 2012<br>– Increases observed in female patients ≥40 years old, patients with face dermatitis, and use of cosmetics | 47 |
| 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 2 concentrations of MI, 10 patients had relevant reactions<br>– All 10 patients reacted to 0.05% MI, while only 5 reacted to 0.02% MI | 48 |

(continu

**Table 4.** (continued)

| Number of dermatitis patients tested; location | Concentration of MI tested | Years analyzed | Results | Reference |
|---|---|---|---|---|
| 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 | – Only 1 patient of the 10 reacted to 100 ppm MCI/MI<br>– All 5 patients who had been tested with personal care products containing MI reacted<br>– 1.9%, 3.4%, and 4.4% positive reactions in 2009, 2010, and 2011, respectively<br>– Proportion of MI-positive patients in those reacting to MCI/MI increased from 43% to 59% between 2009 and 2011 | 49 |

Abbreviations: MCI, methylchloroisothiazolinone; MI, methylisothiazolinone.

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 (64%) patients reacted to 0.105 and 0.21 μg MI/cm$^2$ and 2 (18%) patients 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$^2$/d 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 SSC using an appropriate NESIL (ie, 15 μg/cm$^2$/d) 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 3, 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 nonsensitizing, as demonstrated, for example, by QRA estimates of safe exposures (typically expressed in μg/cm$^2$/d) for the relevant cosmetic product category.

The risk of inducing sensitization depends on the dose of MI per unit area of the skin exposed (eg, expressed in units of μg/cm$^2$/d). 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 noncoloring hair sprays and hair tonics or dressings. There were no chronic inhalation toxicity data identified or provided. Methylisothiazolinone reportedly is used at concentrations up to 0.01% in cosmetic products that may be aerosolized. The Panel noted that 95% to 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 https://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 nonsensitizing, which may be determined based on a QRA.

### Authors' Note

Unpublished sources cited in this report are available from the Executive Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

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C. L. Burnett contributed to conception, design, acquisition, analysis, and interpretation, drafted the manuscript, and critically revised the manuscript. I. Boyer contributed to analysis and interpretation. W. F. Bergfeld, D. V. Belsito, R. A. Hill, C. D. Klaassen, D. C. Liebler, J.G. Marks, R. C. Shank, T. J. Slaga, P. W. Snyder, and L. J. Gill contributed to conception, design, analysis, and interpretation, critically revised the manuscript, and gave final approval. B. Heldreth contributed to analysis and interpretation, critically revised the man script, and gave final approval. All authors agree to be accountable f all aspects of work ensuring integrity and accuracy.

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| CAS # | Ingredient | Count |
|---|---|---|
| 2682-20-4 | METHYLISOTHIAZOLINONE | 6403 |
| 2682-20-4, but not 26172-55-4 | METHYLISOTHIAZOLINONE, but not METHYLCHLOROISOTHIAZOLINONE | 915 |

**VCRP Frequency of use of products that contain METHYLISOTHIAZOLINONE, but not METHYLCHLOROISOTHIAZOLINONE**

| VCRP Product Category Description | Product Counts |
|---|---|
| Aftershave Lotion Count | 2 |
| Baby Shampoos Count | 2 |
| Bath Soaps and Detergents Count | 45 |
| Beard Softeners Count | 1 |
| Body and Hand (exc shave) Count | 39 |
| Bubble Baths Count | 8 |
| Cleansing Count | 71 |
| Douches Count | 1 |
| Eye Lotion Count | 12 |
| Eye Makeup Remover Count | 2 |
| Eye Shadow Count | 1 |
| Eyeliner Count | 2 |
| Face and Neck (exc shave) Count | 128 |
| Foot Powders and Sprays Count | 1 |
| Foundations Count | 1 |
| Hair Conditioner Count | 46 |
| Hair Spray (aerosol fixatives) Count | 1 |
| Hair Straighteners Count | 1 |
| Indoor Tanning Preparations Count | 20 |
| Leg and Body Paints Count | 3 |
| Lipstick Count | 1 |
| Makeup Bases Count | 1 |
| Mascara Count | 7 |
| Moisturizing Count | 144 |
| Nail Creams and Lotions Count | 1 |
| Night Count | 13 |
| Other Baby Products Count | 3 |
| Other Bath Preparations Count | 3 |
| Other Eye Makeup Preparations Count | 4 |
| Other Fragrance Preparation Count | 2 |
| Other Hair Preparations Count | 42 |
| Other Makeup Preparations Count | 8 |
| Other Manicuring Preparations Count | 2 |
| Other Personal Cleanliness Products Count | 66 |
| Other Shaving Preparation Products Count | 9 |
| Other Skin Care Preps Count | 19 |
| Other Suntan Preparations Count | 1 |
| Paste Masks (mud packs) Count | 40 |
| Preshave Lotions (all types) Count | 1 |
| Rinses (non-coloring) Count | 2 |
| Shampoos (non-coloring) Count | 45 |
| Shaving Cream Count | 11 |
| Skin Fresheners Count | 13 |
| Suntan Gels, Creams, and Liquids Count | 5 |
| Tonics, Dressings, and Other Hair Grooming Aids Count | 82 |
| Wave Sets Count | 3 |