Amended Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	May 10, 2019
Panel Meeting Date:	June 6-7, 2019

The 2019 Cosmetic Ingredient Review Expert Panel 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.; Ronald A. Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett, Senior Scientific Writer/Analyst
Date: May 10, 2019
Subject: Draft Amended Report on the Safety Assessment on Methylisothiazolinone and Methylchloroisothiazolinone

Enclosed is the draft amended report of the safety assessment of Methylisothiazolinone and Methylchloroisothiazolinone (MCI/MI) as used in cosmetics. (It is identified as *mcimi062019rep* in the pdf document.) This ingredient combination functions as a preservative in cosmetics. In 1992, the final report on MCI/MI was published with the conclusion that this mixture 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. (Minutes from the Panel discussions from 1987 to 1990 are identified as *mcimi062019min_orig* in the pdf document, while minutes from the more recent Panel discussions are identified as *mcimi062019min*.)

At the April 2019 meeting, the Panel voted to re-open this safety assessment to reassess the conclusion based on the numerous sensitization studies and reports that have been published since 1992. The relevant data from these studies have been included in this draft report, along with summary information from the original report (indicated by *italics*).

According to 2019 VCRP data, MCI and MI are reported separately and not as a mixture. The total number of uses reported for MCI are 5137; 480 of these are in leave-on products. MI has 6037 reported uses; 1042 of these are in leave-on products. The uses have increased significantly since the original report on MCI/MI was published; the 1986 total number of uses for the ingredient mixture was 381. Currently, the Council has reported the results of their survey that indicate MCI/MI (3:1) is used at up to 7.5 ppm in leave-on products and at up to 15 ppm in rinse-off products. In the original report, the ingredient combination was reported to be used at up to 1% in both leave-on and rinse-off products.

Comments from the Council on the re-review document have been addressed and are included in this report package (*mcimi062019pcpc*). Data received since the April meeting include an open human repeated insult patch test of 12 ppm MCI/MI in a hand wash, updated concentration of use data, and the QRA2 risk assessment of MCI/MI performed by the CIR Science and Support Committee (*mcimi062019data1* through *mcimi062019data4*). These data have been incorporated into the report and highlighted in yellow.

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).

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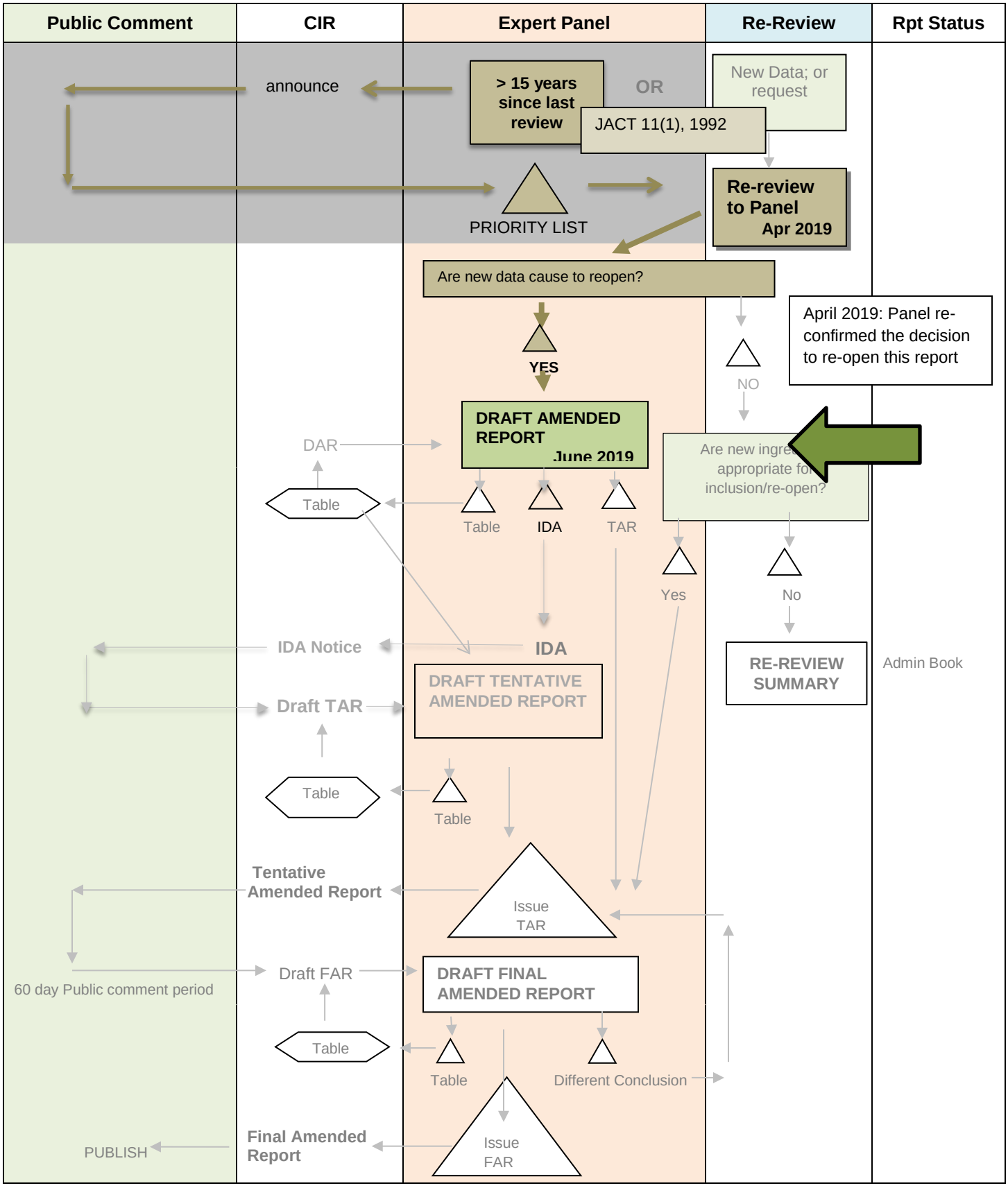
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Methylisothiazolinone and Methylchlorothiazolinone

MEETING June 2019



MCI/MI History

1992 – Safety Assessment of Methylisothiazolinone/Methylchloroisothiazolinone is published in the Journal of American College of Toxicology.

April 2019 – Based on the multiple reported incidences of sensitization reported globally since the original report was published and the large number of uses being reported to the VCRP database, the Panel re-opened the safety assessment on MCI/MI to amend the current conclusion. Prior to determining the new conclusion, however, the Panel is awaiting the results of a second-generation quantitative risk assessment (QRA 2.0) calculation to be performed by industry stakeholders.

MCI/MI Data Profile* – June 2019 – Christina Burnett																													
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Constituents/ Impurities	log P/log K_{ow}	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	Retrospective/ Multicenter	Case Reports
1992 Report Data	X	X	X			X	X	X	X	X	X	X		X	X	X	X			X	X			X	X	X	X	X	
2019 Re-review	X					X					X				X									X	X			X	X

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

MCI/MI – Christina Burnett

Ingredient	CAS #	SciFin	PubMed	FDA	EU	ECHA	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	Web
Methylchloroisothiazolinone/ Methylisothiazolinone	26172-55-4 and 2682-20-4	similar to PubMed results	results below	Approved for indirect food products	Annex V	Dossier only for MI										

MCI/MI – published in 1992 with the conclusion that this mixture 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.

Search Strategy/PubMed – Search Performed in February 2019

Methylchloroisothiazolinoe OR Kathon – 541 hits

90 references ordered or downloaded.

Search updated - April 24, 2019

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

Methychlorloisothiazolinone/Methylisothiazolinone (MCI/MI)
April 8-9, 2019

Belsito's Team Minutes

DR. BELSITO: No brainer. It has to be opened.

MS. BURNETT: We were just bringing this for you to look at, what we have so far and to get an update from counsel on where we are with the calculations.

DR. BELSITO: Yeah. So, I mean, as some of you may know, some of you may not, Europe has also banned this in leave-ons. And they've actually added further restrictions -- no, they've kept the same on rinse-offs, right? It's 15 parts per million. They haven't changed the rinse-off concentration, right? They didn't reduce it, or did they?

DR. BJERKE: You're right.

DR. BELSITO: They kept it, in 15 parts rinse-offs, but completely banned in leave-ons. So I mean, it's going to be the same thing. I mean, Europeans have moved to the idea that their public can't read a label. And so they're looking at not sensitization levels, reduction of sensitization, they're looking at elicitation. And that's why they've essentially banned MI in everything, and MCI/MI in leave-ons.

The problem, I guess now, is that it sounds like -- well, we're getting reports of MCI and MI, but we're not getting actually the MCI/MI which is used. So when we're getting them, we're getting a mixture of everything. Is that correct? Did I interpret that correctly?

MS. BURNETT: For the use or --

DR. BELSITO: For VCRP data.

MS. BURNETT: So VCRP data not report it as a mixture. It reports it as --

DR. BELSITO: The number with MCI.

MS. BURNETT: And then MI. So we cannot for certain state that --

DR. BELSITO: You could almost though.

MS. BURNETT: -- MCI is the mixture, because that doesn't seem to be a standalone ingredient.

DR. BELSITO: MCI is not a standalone ingredient. So you could take the level of MCI, you could take the number with MCI and you could subtract out the MI, because everything with MCI will have MI.

MS. BURNETT: Okay.

DR. BELSITO: And then that will give you the individual ingredients out there still with MI.

DR. LORETZ: And then we still have the council survey, which will --

DR. BELSITO: Right. And then that will give you the range of concentrations of MCI/MI.

MS. BURNETT: So, based on that -- the Wave 2 memo had the updated use table.

DR. BELSITO: Right.

MS. BURNETT: So the 5,000 uses for MCI/MI. And then we have --

DR. BELSITO: And MI has probably gone way down.

MS. BURNETT: We're still about 1,000 over --

DR. SNYDER: The majority of those are leave-ons. 1,042 leave-ons for MI and 480 for MCI. Is that right? That's right, isn't it?

MS. BURNETT: I did want to note that when we received the council survey on the concentration of use, there are a few product categories that we did not receive concentration for that there's recorded uses for, like moisturizers, obvious leave-on uses.

DR. BELSITO: Well, the crazy one was mucous membrane at 15 parts per million. So it's rinse-off, okay.

MS. BURNETT: So the 15 was in hair conditioners, shampoos, soaps and skin cleansing.

DR. BELSITO: And just as a comment -- I thought I printed it out. Just another criticism that we got as a group. I'm not seeing it here. What page in Wave 2 is -- maybe it's -- it's here. I'm sorry. It wasn't in Wave 2. I put the comment here, I think. I didn't put who it was quoted for.

But anyway, we were quoted in a European paper that says, "By July 16, 2015, cosmetic manufacturers we will be prohibited from marketing leave-on products in Europe that contain a mixture of methylchloro and methylisothiazolinone under a ban. The Cosmetic Ingredient Review Expert Panel anticipated with concern, in reading recent meetings, these restrictions."

Which the implication is we thought they weren't necessary, we were poo-pooing. So a little bit more bad press, not in New York Times, but in the European literature, about our function as a panel.

DR. KLAASEN: That statement came from what?

DR. BELSITO: I'd have to look it up again, Curt. I think it came out of a newspaper report in the UK.

DR. KLAASEN: Okay.

DR. BELSITO: So that's MI. So the question becomes, Christina, how you're going to deal with all of this new data. And the other issue is that we have seen if you start looking at the North American group data, we've seen a significant uptick in MCI/MI.

Now there's a two-tailed issue to that. The level of MI is such in MCI/MI that you get patients who are positive to MI but negative to the combination. But you also get people who were strongly sensitized to MI that are reacting to the concentration that we test at, which is 100 parts per million, leave-on, which is significantly higher than you're getting in a cosmetic product.

So when you start looking at it, you're going to see a significant uptick in the amount of reactions to the MCI/MI mixture. Unfortunately, there is no way to separate out what percentage of that, even in like the North American Contact Dermatitis Group data, because we don't say, you know, these MCI/MIs were questionable reactions, but they were two plus to MI, so we called them positive, right?

So you are going to see an uptick. And that will have to be discussed. And we'll need to really closely look at QRA levels for those.

I would just really briefly summarize the reports. And you know, I think in our discussion, depending upon what we conclude, that we point some of the uptick in MCI/MI is likely due to sensitization to MI and not to MCI/MI as used in cosmetics.

MS. BURNETT: Okay. I was putting it in a table.

DR. BELSITO: I would just say that, you know, since 2000, was it 2009, the first reports of MI sensitization started coming out of Europe. There's been increasing numbers of reports of sensitization particularly due to MI, and here's a brief summary.

In fact, I would not do case reports. I would you know, say that there are an extraordinarily large number of case reports. You can get data. You can get the North American Contact Dermatitis Group data for, I think, two cycles, which will give you like 10,000 Americans. You can get the IVDK data, I think GIRDAC (phonetic), which is the French group has data, the Spanish group. Just put the large bodies of data. If you start doing every case report, we're going to have a 500-page document.

MS. BURNETT: So delete that table I already started?

DR. BELSITO: I would just say that there been a large number of case reports and that this has been an issue and so we've elected to concentrate on, you know, population-based surveys as done by the North American group, the IVDK, which is the German speaking nations, GIRDAC, which is the French, GODAC (phonetic), which is the Spanish, I think the Italians have published something, and just look at that and look at the numbers.

And when you're doing it, do it for both MCI/MI and MI, because I think in the discussion we have to point out that the uptick that we're seeing in MCI/MI is at least partially due to the fact that we all got MI wrong, unfortunately, you know, due to a typo in the EC3 value from the LLNAs, and the fact that we had one nice HIRPT that came to clean it 100 parts per million. And Jim will end up recusing himself from this because he was a consultant to the manufacturer.

Marks' Team Minutes

DR. MARKS: Okay. Any other comments? Christina, Monice, Ron, Tom? So second an IDA, insufficient data announcement, tomorrow, and we'll see how things go.

MI/MCI, and I will recuse myself from this discussion as I consistently have done. If you look on page 54, you'll see the reason, since I was a consultant at Roman Hall (phonetic), which were the original manufacturers of MCI/MI. So Tom, you graciously consented to lead. Usually, Ron Shank is, but he got off the hook.

DR. MARKS: Christina, you might want to start since, in your memo, the last arguments that's on the table.

MS. BURNETT: Right. Well, we've already started discussion on this at the September 2018 meeting. A strategy memo went out, and I believe the panel at that time agreed that a rereview would be sought and that it would likely be reopened to amend the conclusion. In the meantime, we are waiting on a NESIL to be provided so that a QRA2 can be performed on this.

So what we're asking the panel to do right now is just review what we have in the document so far. If you like how we're presenting the data -- because I can tell you that most of the data is either going to be some sort of retrospective study or case report of either a cosmetic use or a non-cosmetic use that caused sensitization.

There really isn't anything other. I think I might have found a genotoxicity study, but I don't think it's anything different than what was already out there. The next time the panel sees this rereview package -- the summary -- we will move on with including the summarized sections of the original report, and it will look -- we'll prepare it as if it's going to go ahead and be amended.

DR. EISENMANN: I'll just add the status of the QRA. It's not going to be -- it's going to include the safety factors that are going to be used in the new approach to QRA, but it's not going to be an aggregate exposure. We don't have enough information to do an aggregate exposure.

So it's going to include the new safety factors, but it's not going to be an aggregate exposure. So it's not really necessarily correct to call it a QRA2. It's underway, but it still needs to be reviewed with in industry before we provide it to you. We needed to complete the concentration of use survey before we could complete the QRA.

DR. SLAGA: But we do need that second-generation quantitative risk assessment, right?

DR. EISENMANN: You're going to have the safety factors -- the newer safety factors, but we're not going to be able to do the clause on aggregate exposure because we don't have enough information on other things outside of cosmetics to complete that.

DR. SLAGA: So you recommend we don't table it, or we do?

DR. EISENMANN: Yes. Table it because it's still underway. We're working on it. It's been drafted and we're going to be reviewing it with the committee. And then we'll give it to you.

MS. BURNETT: Do you believe this will be available before the June meeting? No?

DR. EISENMANN: No, we would hope to have it available for the June meeting.

MS. BURNETT: For May?

DR. EISENMANN: Well, that's a question of how soon before the June meeting. We meet at the end of this month, so it's going to be close, if you want it three weeks before the meeting.

MS. BURNETT: Okay. So heads up, it will be a Wave 2 submission for MI/MCI.

DR. SLAGA: So any other comments? Do we all agree that the MCI/MI should be tabled until the additional information?

DR. HILL: Yes.

DR. SLAGA: It's gone.

MS. BURNETT: I have a further question. Did you say that there's going to be another concentration of use survey update, as there was -- the VCRP database, there's reported uses for leave-on products that we don't have corresponding concentrations of use?

DR. EISENMANN: That's all I've received. Because of the changes in regulation in Europe, I think a lot of companies are currently changing. So the VCRP may not have caught up with what -- in response to concentration of use survey I sent out, I got a fair number of companies saying they're not using this mixture any more. And I also got suppliers that said they're not using it to preserve botanicals or whatever types of materials that they need to preserve anymore. So people are moving out of this mixture.

DR. HILL: So in table one in this document, on the maximum concentration of use, it just has a bunch of S's.

MS. BURNETT: A Wave 2 supplement has an updated table.

DR. HILL: Okay. Maybe I missed that.

DR. SLAGA: So we can go on then, if we're going to table this.

DR. MARKS: Sure. I'm going to move on to -- Thank you. We'll move on to -- I'll try this the third time. That's the charm. We'll move on to benzyl salicylate, unless Ron Hill, you had one more comment about MI/MCI, mehtylisothiazolinone/methylchloroisothiazolinone.

DR. HILL: I did not. I was now looking at the -- so we have the updated table in Wave 2 for concentration.

Full Panel Minutes

DR. MARKS: So as I've done in the past, I'll be consistent in recusing myself. The reason is that I'd previously consulted with Rohm & Haas. And if you look at page 54, you'll actually see me in the minutes of that meeting in the CIR. So, Tom's going to take over for me.

DR. SLAGA: So this is a re-review of the safety assessment of both the methyl and the methylchloroisothiazolinone. And in 1992, there was a final report on this mixture where it was safe if the rinse-off did not exceed 15 parts per million and leave-ons didn't exceed 7.5. Since then, we have reviewed MI, the methyl, several times, and there have been a number of case reports on the sensitization aspects of this.

And then in September 2018, we reviewed a strategy memo and we came up with that we thought that some information and what's called a second-generation risk assessment, be done. And so far, we haven't received the information or data or that quantitative risk assessment, the second generation one. And so we suggest that it should be tabled.

DR. BELSITO: I don't think it's a question of tabling or not. The question is do we reopen it, or do we stand by our prior conclusion? And we think, given the issues that have gone on in Europe, the fact that MI/MCI has been banned in leave-on products in Europe, and allowed only in rinse-off, really, we would be remiss if we did not reopen and look at this as a new safety assessment.

DR. BERGFELD: So you're requesting to reopen?

DR. BELSITO: Yes, reopen it.

DR. BERGFELD: Tom?

DR. SLAGA: That's fine.

DR. BERGFELD: Okay, so this will be reopened. Paul, do you have something to say?

DR. SNYDER: No. I was saying second; it needed to be seconded.

DR. BERGFELD: Oh, you're seconding it. Okay, thank you.

DR. SNYDER: Sorry.

DR. BERGFELD: All those in favor of reopening please indicate by raising your hand. Thank you. So we're going to reopen this.

All right, moving on to the next report, Dr. Belsito, the Alkyl Amide MIPA.

MINUTES OF THE THIRTY-SECOND MEETING OF THE EXPERT PANEL

November 16-17, 1987
Key Bridge Marriott Hotel
Rosslyn, Virginia

Expert Panel Members

William O. Berndt, Ph.D., Chairman
Wilma F. Bergfeld, M.D.
Roswell K. Boutwell, Ph.D.
William W. Carlton, D.V.M., Ph.D.
Dietrich K. Hoffmann, Ph.D.
Arnold L. Schroeter, M.D.
Ronald C. Shank, Ph.D.

Liaison Representatives

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Ms. Mary Ellen Fise, Esq.

Industry
Gerald N. McEwen, Jr., Ph.D.

FDA Contact Person
John Wenninger

CIR Staff

Robert L. Elder, Sc.D., Director/
Scientific Coordinator

Elizabeth M. Santos,
Senior Scientific Analyst

Adopted _____
(Date)

_____
William O. Berndt, Ph.D.
Chairman

Methylisothiazolinone and Methylchloroisothiazolinone

Dr. Bergfeld reported that her team was recommending an insufficient data conclusion for Methylisothiazolinone and Methylchloroisothiazolinone, with a request for a chronic skin painting study in mice. She noted that the skin painting study included in the report was conducted with an inappropriate vehicle. Her team also recommended a concentration limit of 0.001% active ingredient due to the sensitizing potential of these compounds.

Dr. Hoffmann commented that positive mutagenic results were obtained in the Ames test with strain TA100 without S-9 activation, an indication of a direct alkylating agent. Addition of S-9 resulted in detoxification. He noted that globin adducts were formed upon metabolic activation in the liver, thus supporting the positive result obtained with TA100. He also noted that the carcinogenicity study was conducted with MI/MCI-CG applied in water, which is considered an inappropriate vehicle, as water runs off the skin of mice. He noted that this was probably an unintentional error as MI/MCI-CG (the commercial biocide product) is used in water.

Dr. Schroeter indicated that his team concurred with the insufficient data conclusion. He was still concerned that some sensitization would occur even at 0.001%. He noted that these ingredients were used more extensively in Europe than the U.S. and much more sensitization was seen there. Referring to the carcinogenicity study, the fact that necrosis was seen on the mouse skin told him that an adequate amount of MI/MCI-CG was reaching the skin, although there were other defects in the study.

Dr. McEwen expressed concern about the principle being expounded; that is, even though a preservative is used in aqueous solution, it should not be tested as such.

Dr. Boutwell stated that MI/MCI-CG decomposes in water by hydrolysis; however, it does not decompose in nonaqueous solution.

Dr. McEwen emphasized that these compounds are supplied in aqueous solution and obviously contacted the skin enough to cause necrosis (in the carcinogenicity study). He said that the Panel could request full details of the study from Rohm and Haas.

A motion was made to approve an insufficient data conclusion.

Discussion again ensued on the limit of 0.001%. Dr. Schroeter noted that this may limit the usefulness of these compounds as antimicrobials in that this concentration was encroaching on the MICs (minimum inhibitory

concentrations). However, 0.001% as an induction concentration does produce some sensitization. He indicated that on dermatitic skin with a reduced barrier function, some sensitization will occur; he questioned if this level of sensitization was acceptable. He was concerned about extensive sensitization in the future because MI/MCI-CG is an excellent preservative.

Dr. McEwen noted that almost all preservatives are sensitizers.

Dr. Schroeter stated that you have to look at the incidence of sensitization versus use levels. The NACDG shows a 0.5% incidence; therefore, MI/MCI-CG is a much safer preservative than formaldehyde although it has to be used at a lower concentration. He indicated that there was no problem limiting the concentration, as they had with formaldehyde. He also noted that some patients patched with MI/MCI-CG reacted at first exposure. This would indicate that MI/MCI-CG is either a very provocative ingredient or that very few people have not already been exposed to it. Dr. Bergfeld noted that a third choice is that it is an irritant. Dr. Schroeter commented that even with a biopsy, it is hard to tell if a reaction is due to irritation or sensitization.

Dr. McEwen commented that a conclusion with a concentration limit would help Rohm and Haas in their efforts to promote the proper use of this biocide.

Dr. Hoffmann inquired if the results of the skin painting study were negative, would the Panel then approve a safe conclusion. If not, then it would not be fair to ask for a long-term study. Dr. Schroeter responded affirmatively, although he still expressed reservations about the sensitization potential of these compounds. He indicated that the data were not available as yet to support his suspicions; however, he feels that it is just a matter of time.

Dr. McEwen asked why the Panel had selected the limit of 0.001% rather than 0.0015% active ingredient. He indicated that the data support a limit of 15 to 20 ppm.

At this point, Jack N. Moss and John C. Harrington of the Rohm and Haas Co. arrived and were introduced to the Panel. Dr. Hoffmann gave a brief synopsis of the Panel's problems with MI/MCI-CG and specifically the carcinogenicity study. They offered to send the complete report and pathology to the Panel. They stated that all of the treated animals that had died prior to termination did not have tumors different than the controls. Tumors in the major organs had been looked at as well. They explained that water had been used as the vehicle because it is the vehicle of use and MI/MCI-CG is water

soluble. Erema and erythema were observed, indicating irritation. No evidence of preneoplastic activity was found. Dr. Hoffmann noted that standard procedures call for acetone as the solvent. It was asked why carcinogenicity would be expected in one solvent and not the other. It was noted that the solvent increases penetration. Then the Panel asked how the MI/MCI-CG had been applied. Rohm and Haas responded that 25 µl of MI/MCI-CG had been brushed on with either a cotton swab or a small brush (like a nail polish brush). The skin of the mice had been shaved prior to application. Dr. McEwen emphasized that the key was that there had been an effect, and it was an effect (necrosis) not caused by water.

Rohm and Haas noted that they have some in vivo absorption data. MI/MCI-CG is absorbed, but is transformed in the skin.

Dr. Boutwell expressed concern about the solvent despite the effects observed and also that two tumors were observed at the treatment site but were not considered treatment-related as they had also been observed in the controls; however, those observed in the controls were not at the treatment site. He noted that these tumors were not commonly seen in the skin.

Dr. Carlton stated that these tumors were seen in the skin.

Dr. Boutwell repeated that he has seen thousands of animals and hemangiosarcomas are not usually observed.

Rohm and Haas then addressed the genotoxicity tests. These were mostly negative and it is their position that the likelihood of carcinogenicity is remote.

Dr. Shank commented that MI/MCI-CG had been orally administered and the testicular DNA examined; however, MI/MCI-CG (as an entity) woould not survive long enough to reach the testicles. He indicated that MI/MCI-CG should be applied topically and the skin DNA examined.

Dr. Schroeter asked Rohm and Haas to comment on the effectiveness of the Kathon biocide at concentrations of less than 15 ppm. They responded that most cosmetics could be adequately preserved with 3 to 15 ppm MI/MCI-CG. Hair care/shampoo products could be preserved with 10 ppm as the matrix was not complicated; however, many other products would need a higher concentration. If 15 ppm was used as the standard, 95% of all products would be adequately preserved. They have advised manufacturers that if they have to go above 15 ppm for effective preservation, MI/MCI-CG is probably the wrong preservative for their system. Rohm and Haas had, in fact, sent direct notification to all customers (both U.S. and European) in February or March of

1987 setting 15 ppm as the maximum limit.

Dr. Schroeter inquired if some companies are using more than 15 ppm. Rohm and Haas responded affirmatively; however, this has occurred primarily in Europe because the EEC had approved a level of 30 ppm active ingredient. They noted that in the U.S. they sell directly to the formulator and so have fairly good control over where MI/MCI-CG goes; however, in Europe they have to sell to distributors. Also, there are no labelling requirements in Europe and so that makes it difficult to ascertain which products actually contain MI/MCI-CG. They indicated that two products had been identified as having a concentration of MI/MCI-CG higher than 15 ppm and that Rohm and Haas had taken what action they could. The companies had apparently used a higher concentration because they could not get preservation at the lower concentrations; Rohm and Haas informed them that they should use a different preservative system.

Rohm and Haas agreed to forward to the Panel the full report of the 30-month skin-painting study, a copy of the letter to customers recommending a 15 ppm maximum usage, and any additional biochemical data available.

This document and the new data will be reviewed by both teams in January and will return to Panel in April. No vote was taken at this time.

Adjournment

The Expert Panel meeting adjourned at approximately 12:00 p.m., November 17, 1987. The next meeting of the Expert Panel is scheduled for April 14-15, 1988.

Respectfully submitted,

Elizabeth M. Santos

Elizabeth M. Santos
Senior Scientific Analyst

Attachments: Meeting agenda (Nov. 16-17, 1987)
Chevron's Comments on Captan

C I R

MINUTES OF THE THIRTY-THIRD MEETING OF THE COSMETIC INGREDIENT REVIEW EXPERT PANEL

April 14-15, 1988
Key Bridge Marriott Hotel
Rosslyn, Virginia

Expert Panel Members

William O. Berndt, Ph.D., Chairman
Wilma F. Bergfeld, M.D.
Roswell K. Boutwell, Ph.D.
William W. Carlton, D.V.M., Ph.D.
Dietrich K. Hoffmann, Ph.D.
Arnold L. Schroeter, M.D.
Ronald C. Shank, Ph.D.

Liaison Representatives

Consumer
Ms. Mary Ellen Fise, Esq.

Industry
Gerald N. McEwen, Jr., Ph.D.

FDA Contact Person
Heinz Eiermann

CIR Staff
Robert L. Elder, Sc.D., Director/
Scientific Coordinator

Elizabeth M. Santos,
Senior Scientific Analyst

Adopted_____
(Date)

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William O. Berndt, Ph.D.
Chairman

the discussion of the report and was the basis for a recommendation of a concentration limit of 0.1% for use in cosmetics. A UV spectrum also had been requested and received; Benzalkonium Chloride had a peak absorption maximum of 262 nm and did not absorb UV light at wavelengths of 300 nm and above.

Dr. Schroeter noted his team's concern with the fact that, in solution, Benzalkonium Chloride bears a net charge and therefore might be bound by proteins or other agents, possibly leaving no free Benzalkonium Chloride to act as a preservative. His team concurred with the 0.1% concentration limit but wanted the limit to apply to the free active ingredient.

After noting that the discussion of the report should reflect the Panel's concerns as to Benzalkonium Chloride's irritation and sensitization potential and its behavior in solution, the Panel unanimously approved Benzalkonium Chloride as safe as a cosmetic ingredient at concentrations up to 0.1% of the free, active ingredient. The revised and corrected report will be mailed to the Panel for a two-week review, after which the tentative final report will be announced for a 90-day comment period.

Methylisothiazolinone and Methylchloroisothiazolinone

Dr. Bergfeld opened the discussion by noting that this was the second time the Panel had reviewed this report and read the proposed conclusion for Methylisothiazolinone and Methylchloroisothiazolinone of safe as cosmetic ingredients at concentrations not exceeding 15 ppm of the active ingredients. She noted that the 15 ppm limit was due to irritation and sensitization concerns.

Dr. Schroeter noted that the biocide mixture of these two ingredients is a preservative which primarily has been used in rinse-off products; however, it is now being used in many more leave-on products. He stated that the data in the document could be interpreted differently. Low concentrations of the biocide had caused sensitization in specialized cases. He noted that a test concentration of 100 ppm may also cause sensitization. He noted that De Groot

had found 4% sensitivity in their clinics probably because of previous use of this biocide at high concentrations in Europe. Dr. Schroeter expressed concern that many leave-on products, primarily moisturizers, are applied to dry skin, which, having an altered barrier function, may absorb more of the product than normal skin. In this case, 15 ppm may be in excess in leave-on products. He recommended that the Panel analyze the data to see if they justified limiting the concentration to 15 ppm and for use in rinse-off products only.

The Panel then discussed at length the clinical data in the report. It was concluded that, in the professional judgement of the Panel, these data indicate that Methylisothiazolinone and Methylchloroisothiazolinone may be emerging contact sensitizers of significant potential.

The Panel was also concerned with the genotoxic potential of this biocide. It was noted that positive results have been obtained under certain test conditions; however, flaws in both a DNA binding study (DNA binding measured at a distal site using a relatively insensitive method) and a carcinogenicity bioassay (inappropriate solvent; too few animals) that were conducted did not allow for appropriate scientific interpretation. Dr. McEwen requested the minutes reflect that the carcinogenicity bioassay should have been terminated at 18 months. By continuing the study to 30 months, the animal population was ageing and tumors were seen that would not have developed had the study been terminated earlier. There was some disagreement as to the significance of the hemangiosarcomas seen in one mouse, but the concensus was that these probably were not significant. It was concluded that the genotoxicity data were insufficient to approve these ingredients as safe for leave-on products.

Jack Moss and John Harrington of the Rohm and Haas Co. then addressed the Panel regarding its concerns with these ingredients. They noted that, in the opinion of Rohm and Haas as well as other outside genotoxicity experts, the data do not indicate that Methylisothiazolinone and Methylchloroisothiazolinone possess any genotoxic potential. They explained that water had been the solvent of choice for the carcinogenicity bioassay because the

biocide mixture is very soluble in water and is supplied in aqueous solution. The study had been carried to 30 months to detect any possible latent effects.

The Panel noted that the solvent of choice for carcinogenicity bioassays is acetone because its boiling point is just above skin temperature and thus evaporates quickly, leaving the test compound deposited on the skin. Water does not evaporate so quickly and can be licked off by the animal. They also noted that, as for the mutagenicity data, no single mutagenicity test is sufficient. A battery of tests must be used and if any have positive results, then further testing should be conducted.

Dr. Bergfeld suggested that since the Panel was going to request more genotoxicity data, perhaps clinical data should also be requested to address the sensitization problems.

The representatives of Rohm and Haas stated that they were planning to reassess the diagnostic patch test and conduct a new multi-clinic prevalence study hopefully to be started before the end of 1988. The Panel requested that results of this study be forwarded to them.

Therefore, in light of the Panel's concern with the sensitization potential of this biocide and the inadequacy of the genotoxic data, it was concluded that:

a) limiting the concentration of the active ingredients to a maximum of 0.0015% (15 ppm) as well as limiting its cosmetic use to rinse-off products only would effectively reduce the genotoxic and sensitization risk associated with these ingredients.

b) for cosmetic uses other than rinse-off products, additional testing is required. The types of data required to establish safety are:

1) Repeated insult patch tests using 250 individuals with normal skin.

2) An *in vivo* DNA binding study conducted in mice with a topical application of the biocide in an appropriate solvent to the skin and the DNA binding measured at the site of application. If results of this study are negative, no further testing would be required. If results of this study are positive, an 18-month carcinogenicity bioassay in mice would be required using a non-water solvent and an appropriate number of animals.

3) The CIR Expert Panel has been advised that a multi-clinic study to assess the sensitization rate of these two ingredients in dermatological patients is also being planned. The results of this study should be submitted to the Panel.

The Panel then unanimously approved a conclusion for Methylisothiazolinone and Methylchloroisothiazolinone 1) as safe for use as cosmetic ingredients in rinse-off products only at concentrations not exceeding 15 ppm of the active ingredients and 2) data are insufficient to judge the safety of these ingredients at any concentration in leave-on cosmetic products.

The revised and corrected report will be sent to the Panel for a two-week mail review and will shortly thereafter be announced for a 90-day comment period as both a tentative final report and an insufficient data announcement.

1-Naphthol

Dr. Schroeter gave a brief synopsis of the data on 1-Naphthol and noted that this ingredient is used as a coupler in hair dyes. He noted that the EEC had set a limit of 0.5% for cosmetic use and that the Panel had requested, received, and reviewed the data they had used to establish the limit. He noted that a UV spectrum for 1-Naphthol had been received and the data would be incorporated into the report.

The Panel then unanimously approved 1-Naphthol as safe as a cosmetic ingredient in the present practices of use and concentration. The tentative final report will shortly be announced for a 90-day comment period.

General Comments

Dr. Bergfeld expressed her concern that the work load was excessive for this meeting.

MINUTES OF THE THIRTY-FOURTH MEETING OF THE EXPERT PANEL

August 29-30, 1988

Key Bridge Marriott Hotel

Rosslyn, Virginia

Expert Panel Members

William O. Berndt, Ph.D., Chairman
Wilma F. Bergfeld, M.D
Roswell K. Boutwell, Ph.D.
William W. Carlton, D.V.M., Ph.D.
Dietrich K. Hoffmann, Ph.D.
Arnold L. Schroeter, M.D.
Ronald C. Shank, Ph.D.

CIR Staff

Robert L. Elder, Sc.D., Director/
Scientific Coordinator

Liaison Representatives

Consumer

Ms. Mary Ellen Fise, Esq.

Industry

Gerald N. McEwen, Jr., Ph.D.

FDA Contact Person

Heinz J. Eiermann

Adopted _____
(Date)

William O. Berndt, Ph.D.
Chairman

Priority List will also be discussed at the January Panel Meeting. Dr. Elder reported that Dr. McEwen had sent CIR an updated risk assessment on Methylene Chloride. This report will also be discussed at the January meeting. Currently there are eight delayed reports which Dr. Elder hopes will be available for review at the January meeting. He also mentioned that there would be a Congressional Hearing on September 15 on cosmetic safety. He and Dr. McEwen would both be involved with these hearings. Dr. Hoffman requested to see the Diethanolamine report. Dr. Berndt requested a copy of the Final Report on Methylene Chloride be sent to each Panel member along with any new data.

Methylisothiazolinone and Methylchloroisothiazolinone

The public comments received from Rohm and Haas Co. and Dr. Storrs of the North American Contact Dermatitis Group were discussed. The safe conclusion for the rinse-off products versus the insufficient data conclusion for the leave-on products were re-evaluated. Dr. Carlton referred the Panel to the sentence in the first paragraph of the Discussion "Recent provocative clinical test data have indicated that MI/MCI-CG may cause sensitization in a large proportion of the exposed population." Dr. Schroeter proposed to change the word "large" to "a significant portion" in the previous sentence. Dr. Bergfeld agreed with Dr. Schroeter. The Panel was in agreement.

Dr. Berndt referred to the comments received from the Rohm and Haas Company on the use of a water vehicle in the requested DNA Binding study. Dr. Moss who was in attendance from Rohm and Haas questioned the decision made by the Expert Panel to require that a non-water solvent be used; noting that the ingredients were not soluble in acetone. Dr. Boutwell did not concur with the adequacy of the dermal test conducted after the rabbit skin was moistened with water. He suggested that Rohm and Haas re-evaluate the preparation of the test substance for application in a more appropriate solvent. He thought an acetone and/or alcohol in water mixture might be more effective. Dr. Boutwell noted that the study was valid, up to a point, but he was concerned that the actual concentration was something less than 400 ppm. He noted that one could not neglect the fact that the sebaceous glands repel water. Dr. McEwen suggested the test was a valid chronic study since concentration was high enough to cause dermal irritation. Dr. Hoffman discussed the problems involved in making a water/acetone mixture, and stressed that skin irritation was not a good indication that the ingredient was tested at a concentration high enough to determine whether an ingredient was, or was not, a potential carcinogen. Dr. Berndt suggested the Panel refer to the Haas' in vivo study in which ^{14}C Kathon-CG in water and ^{14}C Kathon-CG in acetone and water data could be available in 6-8 weeks, and that a bioassay would take much longer.

Dr. Shank resummarized why his request that the DNA binding study be performed first. A bioassay would be necessary only if the results of the skin binding assay were positive. He noted that DNA adducts test results were generally not accepted as an indicator of chemical carcinogenesis, but

when positive data were obtained it indicated that DNA damage had occurred. Dr. Shank also noted that HPLC would have been a more acceptable method to detect the adducts. Dr. Boutwell agreed that the formation of a DNA adduct alone was not sufficient for the determination that an ingredient was or was not a carcinogen, but it was helpful in interpreting the data.

Dr. Hoffman suggested that a long-term carcinogenesis test may be needed even if the DNA binding study was negative. After a general discussion, the Expert Panel decided against requiring a carcinogenesis bioassay independent of the binding study; and that Rohm and Haas should proceed with the dermal bioassay (Therefore the original Panel request in the Insufficient Data Announcement was not changed).

Dr. Berndt then asked for comments on the skin sensitization issue. Dr. Schroeter stated that he was concerned that the concentration of Kathon (15 ppm) used in the challenge dose was the same as that used in the induction phase of the study. He noted that usually a higher concentration, perhaps 100 ppm, should be used for the challenge dose. Dr. Bergfeld requested two specific tests, one for sensitization and one for irritation. She noted the difficulty in determining a threshhold level for sensitization concentration level if one could not separate irritancy from sensitization. Dr. Moss (Rohm and Haas) agreed to incorporate a challenge concentration of 100 ppm, or at least, a rechallenge at this concentration. He also noted that the individuals chosen to be used in this testing program would be screened toeliminate any with clinical problems. He was concerned that he might obtain a false positive response at 150 ppm. Both Dr. Bergfeld and Dr. Schroeter noted that these ingredients were of late, frequently reported as allergenic and that the protocol should be adequate to determine a sensitization threshold value. Dr. Moss then offered to incorporate two challenge concentrations into the protocol. Dr. Berndt reminded the Panel that it is not responsible for designing test protocols, and that Rohm and Haas would have to take the Panel's concern for establishing the sensitization threshhold concentration when an ingredient was also a possible sensitizer.

Dr. Bergfeld referred the Panel to attachment II, section E2, noting that phototoxicity was not addressed. She felt that phototoxicity data were necessary for leave-on products. Dr. Berndt also referenced page 71, the Draize sensitization test, in which the results of the test were not detailed enough.

Dr. McEwen noted that if carcinogenicity testing in animals was required by the panel it would be 2-3 years before the data from a 15-month bioassay would be available. However, the skin binding study could be available by mid-1989. Dr. Schroeter then made a motion that the Panel delay any further review until the July 1989 Panel Meeting. Dr. Bergfeld seconded the motion. The Panel members then agreed to delay its review of the full report until the July 1989 meeting. The Panel noted that the repeat insult patch test would be tested at 150 ppm and an in vivo binding study would be completed and available for review at that time. It was again suggested that Rohm and Haas re-evaluate the solvent to be used in the DNA binding study. A planned multiclinical human test study would also be made available to the Expert Panel when it is completed.

MINUTES OF THE THIRTY-SIXTH MEETING OF THE EXPERT PANEL

July 24-25, 1989
Key Bridge Marriott Hotel
Rosslyn, Virginia

Expert Panel Members

Ronald C. Shank, Ph.D., Acting Chairman

Wilma F. Bergfeld, M.D.

Roswell K. Boutwell, Ph.D.

William W. Carlton, D.V.M., Ph.D.

Dietrich K. Hoffmann, Ph.D.

Arnold L. Schroeter, M.D.

Liaison Representatives

Consumer

Ms. Mary Ellen Fise, Esq.

Industry

Stephen Gettings, Ph.D.

FDA Contact Person

Heinz J. Eiermann

CIR Staff

Robert L. Elder, Sc.D., Director/
Scientific Coordinator

Adopted _____
(Date)

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William O. Berndt, Ph.D.
Chairman

study in mice at 3 dose levels. A tumor initiation/promotion study on mouse skin where glutural is tested as both an initiator and a promoter would suffice instead of the 18-month mouse carcinogenic study. If a commitment to test is made during the 90-day comment period, then the report will be tabled until such test data are available.

A discussion was introduced by Dr. Boutwell on the problem of antimicrobials and other preservatives in cosmetics. He noted that antimicrobials by their nature are reactive compounds; they have to react with DNA and proteins to be toxic to microbiological organisms, and as such, are also reactive with human protein and DNA. He requested that in the future the Panel should discuss a way of dealing with antimicrobials in cosmetics, with hopes of resolving the problem of the effectiveness of an antimicrobial being related to its potential deleterious effects on humans.

Dr. Hoffmann suggested that testing was the means of determining the safest of the antimicrobials, and that those antimicrobials which had toxic effects in mammals should not be used.

Methylisothiazolinone and Methylchloroisothiazolinone

Dr. Bergfeld opened the discussion by summarizing the Panel's concern about the potential for these ingredients to act as sensitizers. She noted that the Panel had previously agreed that these ingredients were safe in rinse-off products at concentrations not exceeding 15 ppm of the active ingredient. She stated that the Panel had been asked to rereview the material in consideration of the availability of new data, namely an in vitro DNA binding study and a repeat insult patch test in 250 subjects with normal skin. She thought that the Panel had several choices in dealing with these ingredients:

keep the conclusion that is already in the report (that the ingredients are safe in rinse-off products only), reevaluate for both rinse-off products as well as "stay-on" products, or deny the request for additional hearings and go ahead with the final report. She stated that she was quite uneasy with the results of the patch tests in which a fairly large number of individuals were sensitized.

Dr. Schroeter remarked that European products have had excessive amounts of preservatives used in leave-on products. This has not been the experience in the United States; it is possible that researchers in the U.S. have learned from this, and certain clinical investigators are now hesitant to use these leave-on products. He reiterated that the data from Shelanski's study appeared to indicate a sensitization of normal skin at 15 ppm.

Dr. Bergfeld noted that the incidence of sensitization increased with increasing concentrations of the test product.

Dr. Schroeter then mentioned that Shelanski questioned the relevance of the evidence of sensitization in subjects with normal skin. He noted that there is no evidence indicating that the subjects who were sensitized in Dr. Shelanski's study would have allergic reactions to products containing methylisothiazolinone or methylchloroisothiazolinone. He remarked that the potential for sensitization by these ingredients may all be a matter of degree depending on how much of the ingredients are applied, how long they remain in contact with the skin, and the condition of the skin to which they are applied. He stated that he has reservations about the use of these ingredients in leave-on products, and that he believes that there will be continued incidences of sensitization, albeit at a low level, in our population.

Dr. Bergfeld asked Dr. Schroeter what he thought about the options that she had mentioned previously. She questioned whether the rinse-offs should be separated from the leave-ons and whether one study with a population of 250 subjects was enough to say that the ingredients were potent sensitizers posing a great risk to the general population.

Dr. Schroeter replied that he thought there was a risk to the general population, but that he did not know how great was the risk. He thought that the use of these ingredients in leave-ons should be discouraged, and thus that the two uses should be separated. He then noted that there was an additional area of concern with the use of these ingredients and that concern was carcinogenic potential. He stated that if there were little chance of carcinogenic potential for rinse-off products, then he would agree with separating the two uses, but that if the risk were great for both rinse-offs and leave-ons, then there would be no reason to separate them.

Dr. Elder reminded the Panel that they had requested the DNA binding study because of concern over the leave-on products rather than over the rinse-off products.

Dr. Boutwell wondered why the ingredients were tested in the presence of high concentrations of magnesium chloride and magnesium nitrate; he asked the representatives of Rohm and Haas if it were not possible to dissolve the ingredient in acetone or to stabilize the ingredient in some other way.

Dr. Hoffmann noted that the group advising Rohm and Haas suggested an acetone/water mixture which would still contain some of the stabilizer. He stated that he thought that perhaps if a straight acetone solution were used, then a new solution would have to be made for each time the ingredient was tested.

Dr. Boutwell noted that in the data request for glutural, the Panel specifically requested that the 18-month carcinogenicity study be performed with fresh solutions of glutural made daily.

Dr. Hoffmann stated that possibly the only way to determine the stability of an acetone solution would be to perform an NMR analysis over time and track the half-life of the ingredient in the acetone solution. He commented that Rohm and Haas' first recommendation was for an a initiation/promotion study and that he was inclined to accept that.

Dr. Boutwell added that the Panel's first recommendation was for a very short term DNA binding study and that he always accepted short term tests before going on to the long term studies. He noted that the short term study was on the effects of the ingredients on skin morphology, dark cells and the potential to cause inflammation and hyperplasia. He stated that dark cells were basal cells which are rich in ribosomes and which tend to be present whenever skin cells are stimulated to divide. There are a certain number of these cells present all of the time, but in the presence of a tumor promoter, their numbers tend to increase. He remarked that though he thought short term studies and initiation/promotion studies were good, he and and his colleagues discovered 30 years ago that when dermal studies were performed with mice that were able to groom themselves, skin absorption of the test materials did not occur, rather the material was ingested. Thus the dermal carcinogenicity tests came out negative, not necessarily because the material was nontoxic, but rather because the test procedure was poor. This is especially a problem when the solvent is water because water tends to remain on top of the skin with the compound and the magnesium salts dissolved in it long enough for the mouse to lick it off. He stated that he felt that the existing skin carcinogenicity study had been performed poorly.

A representative from Rohm and Haas reported his company had determined over the years that the magnesium salts were the most suitable stabilizers. He mentioned that the ingredient was quite soluble in water, while when the sole solvent was acetone some precipitation of both the magnesium salts and the parent compound occurred. Under those circumstances, Rohm and Haas did look at combinations of water and acetone that would retain the good properties of the individual solutions. A 75:25 percent acetone:water solution seemed to be suitable. He noted that the important factor was that the acetone/water solution had the same absorption rate in multiple dose testing as did the water solution.

Dr. Boutwell commented that he did not notice the same trend in the new report from Rohm and Haas. He noted that variation among individual mice was great and for this reason he could not be sure of the validity of the statement that the absorption rates were essentially the same for the two solutions. He reiterated his belief that the reason for the variations were the result of the mice having cleaned the test material from their skin.

Dr. Schroeter asked whether the carcinogenicity data was necessary to make a decision on rinse-off products.

Dr. Boutwell replied that, in general, if something were proven to be carcinogenic it did not matter whether the products containing it were leave-ons or rinse-offs, the ingredient would be considered unsafe.

Dr. Schroeter then remarked that the report need not be divided if the carcinogenicity issue also pertained to the rinse-off products.

Dr. Bergfeld noted that the report was currently tabled and could remain tabled. She mentioned that the other possibilities were to rule the ingredients unsafe or insufficient.

Dr. Elder commented that the ingredients could be ruled unsafe because of the sensitization data and or insufficient with respect to the carcinogenicity data. He reminded the Panel that the ingredients had already been considered safe for rinse-offs and that the Panel had been asked to delay the report until data for the leave-on products were available.

Dr. Bergfeld commented that there seemed to be enough concern among the Panel members over the carcinogenic potential of these ingredients that perhaps they were unwilling to consider rinse-off products safe without those data.

Dr. Carlton remarked that the carcinogenicity study would not be of use in the evaluation of the leave-on products if the dermatologists on the Panel still considered the ingredients to be sensitizers, and if this were the case then the carcinogenicity study was really for the safety evaluation of the rinse-off products.

Dr. Schroeter stated that the concern over sensitization would probably prevent the approval of these ingredients for leave-on products, but that data on the relevancy of the positive results in the Shelanski study has yet to be released. These results could have a bearing on the decision on the safety of the leave-on products.

Dr. Bergfeld noted that the sensitization capability of the ingredients was fairly significant in the Shelanski study and that the results of Shelanski's relevancy study may not be significant. She questioned whether there was any background information to support Dr. Shelanski's relevancy study (a base on which he can establish his data).

Dr. Elder asked the Rohm and Haas representative why seven people had been disqualified from the study.

A representative from Rohm and Haas replied that those individuals appeared to have been presensitized to the ingredient being tested.

Dr. Bergfeld then asked how often did sensitization during premarket testing occur.

The Rohm and Haas representative replied that they had not seen similar results before.

Dr. Schroeter noted that the Panel was dealing with a prejudiced population. This ingredient is commonly used in rinse-off and leave-on products. In Europe, the ingredient is well documented to be used in 20 percent of the products.

Dr. Carlton asked whether much of the product remained behind after being rinsed off.

Dr. Bergfeld replied that some of the compound is incorporated into the stratum corneum, and that which is not bound would be rinsed off. There was general consensus among the Panel that the ingredients would bind to the stratum corneum, especially since the ingredients are, as preservatives, biologically active.

Dr. Schroeter noted that alot of the so-called rinse-off products could technically be considered leave-ons since the cold cream cleansing preparations are generally only wiped off, leaving a residue behind; the face, body, and hand cream preparations are all leave-on products.

Dr. Bergfeld motioned that the Panel keep the document tabled until the results of the DNA in vitro studies are available for review. Dr. Schroeter seconded the motion.

Dr. Boutwell called for more discussion. He stated that the reason the DNA study was requested was because it is as a predictor of whether the

compound is apt to be carcinogenic whereas the Panel has recommended that the DNA study be bypassed in favor of a carcinogenicity study.

Dr. Hoffmann remarked that the DNA study was fine as a predictor of dermal carcinogenicity. He also noted that mutagenicity studies indicated that these are reactive compounds and as such, the rinse-off products ought to also be reevaluated with regard to carcinogenic potential.

Dr. Schroeter commented that the separation of the ingredients by use as either rinse-offs or leave-ons is unnecessary.

Dr. Elder asked whether the restriction of the ingredients in rinse-offs to 15 ppm would minimize the risks of both sensitization and carcinogenicity.

Dr. Bergfeld commented that she believed the risk of sensitization would be reduced in rinse-off products containing less than 15 ppm of the active product.

Dr. Boutwell noted that since these ingredients are used in wetting agents such as shampoos, which strip the skin of oil while the process of shampooing works the ingredients into the epidermis, there is a greater possibility of reactivity of the ingredients in the skin. In contrast, he noted, the vehicle used in the mouse study did not strip the skin of oil and did not bring the ingredient into intimate contact with the skin (in addition to the probability that the mice removed at least some of the test material through grooming) and thus the reactivity seen in the study results was probably lower than what actually occurs in normal rinse-off use situations.

Dr. Bergfeld stated that the lower the concentration of the ingredient in product the lower the chance of sensitization occuring.

Dr. Boutwell agreed that this was probably also true of carcinogenic potential.

Dr. Elder commented that this meant the ingredients could be used in rinse-off products.

Dr. Schroeter noted that the motion was to table the document rather than change it.

Dr. Bergfeld stated that the Panel ought to go ahead and say that the ingredient is a sensitizer in leave-on products, but that dilution to less than 15 ppm effectively reduces the risk of sensitization. The question is with the carcinogenic potential; until the carcinogenicity data is available we must table the document as the data may have a bearing on the safety of rinse-off products.

Mr. Eiermann commented that it would be helpful to industry if the Panel would make a declaration regarding the leave-on products, since industry would then be aware that the ingredients should not be used in such a manner.

Dr. Bergfeld then withdrew her motion to table the document.

Dr. Hoffmann agreed with Mr. Eiermann's comments, noting that the manufacturer had supplied data that the Panel requested and thus if a decision can be reached for at least one of the uses, that decision should be made public at this meeting.

Dr. Bergfeld suggested that the Panel divide its vote, voting on the leave-on products and deciding what course to take for the rinse-off products.

Dr. Schroeter noted that he thought the discussion of the report should contain a statement about the relevancy of the sensitization data even though the Panel agrees that the ingredients are sensitizers.

Dr. Carlton commented that it wasn't the positive result of the sensitization test that was being questioned, but rather the relevancy of the positive results.

Mr. Eiermann noted that while most sensitization studies are performed with exaggerated concentrations of the test material, the Shelanski study was performed using concentrations close to those actually used in product formulations.

Dr. Schroeter remarked that the relevancy question had to do with the fact that normal patients were sensitized to the ingredients, but that there were no data on whether these patients would have sensitization reactions while using products containing these ingredients. He noted that these patients had undergone induction and challenge, and had positive reactions upon challenge, but that there was still no evidence that these subjects would go on to develop contact dermatitis under in-use conditions.

Dr. Bergfeld remarked that the question of relevancy was a new area and a statement regarding it could be included into the discussion of the report, but that regardless of the relevancy of the positive test data, she considered the ingredients sensitizers in leave-on products, and as such, they should not be used in leave-on products, and that for rinse-off products, the only question was that of carcinogenicity.

Dr. Schroeter stated that if Dr. Bergfeld would move that statement, he would second it.

Dr. Moss from Rohm and Haas remarked that in a North American Contact Dermatitis Group study in patients with allergic contact dermatitis, the incidence of sensitization for these ingredients was actually quite low. He suggested that though the Shelanski study was important, the Panel should not overlook the North American Contact Dermatitis Group study using clinical patients.

Dr. Schroeter replied that it's possible that because of a low degree of sensitization in clinical populations leads to a peak of use of the

ingredients resulting in a rise in incidences of sensitization, leading to a prejudiced population, and subsequently, decreased use of these ingredients. He stated that he would not like to see an increase in sensitization to these ingredients and a corresponding prejudice in the population because the compound is an excellent preservative.

Dr. Bergfeld then clarified the new motion: that these ingredients in rinse-off products at concentrations of less than 15 ppm reduced the risk of both sensitization and carcinogenic potential, and that for leave-on products, the ingredients are sensitizers, but that a final conclusion on the safety of these ingredients rested on the results of the carcinogenicity studies.

Dr. Elder asked if the Panel was issuing an insufficient data report, and if so, was it insufficient for both uses or just for use in rinse-off products.

Dr. Boutwell noted that the insufficiency was basically for rinse-offs, though in actuality if the carcinogenicity test results were positive, then the ingredients would be considered unsafe for any use.

Dr. Bergfeld then agreed that these ingredients would be considered unsafe for use in leave-on products due to the potential for sensitization.

A representative from Rohm and Haas then noted that a battery of mutagenicity studies have been published; results in the Salmonella study were positive with a steep dose-response curve, with mutagenicity occurring at near toxic levels. He noted that the ingredients were negative for mutagenicity in Drosophila and in cytogenetic studies, and in hepatocytes. Peer reviewers do not believe that these ingredients will be positive in carcinogenicity studies. He also mentioned the current carcinogenicity study being reviewed by the Panel, which had also been peer reviewed. He said that he felt that

for rinse-off products, the data indicated that there was not a potential for carcinogenicity.

Dr. Bergfeld asked the representative if he was saying that for use in rinse-off products the available data, including data on sensitization, was sufficient to document the safety of the ingredients. She also asked if he agreed with the Panel that the ingredients were unsafe for leave-on products.

The representative stated that he personally thought that they were safe for both rinse-offs and leave-ons. He went on to say that under use conditions of less than 15 ppm, he did not believe that the human detoxification system would be overwhelmed by the amount of ingredient coming into contact with the body.

Dr. Boutwell commented that industry did not have the data to support the idea that the body's detoxification system would not be overwhelmed.

The representative replied that there is a large amount of metabolism data and mutagenicity data that would indicate that the ingredients would not have an adverse effect under in-use conditions.

Dr. Boutwell noted that metabolism studies were not a good basis for prediction because the radioactivity recovered from various routes of elimination does not account for the small fraction of one percent which is not recovered and which is all that is necessary to do genetic damage.

Dr. Bergfeld then noted that the Panel seemed to have come to the opinion that for rinse-off products containing the ingredients at less than 15 ppm, are safe, and that in leave-on products the ingredients are unsafe at any concentration due to sensitization potential and carcinogenic potential.

Mary Ellen Fise asked for a clarification as to whether the motion stated that rinse-off products were safe or whether there was insufficient data to make a decision on these products.

Dr. Schroeter commented that the data were insufficient for both rinse-offs and leave-ons.

Dr. Bergfeld reminded the Panel that their discussion had indicated that the dilution of the ingredients in the rinse-off products also decreased the carcinogenic potential.

Dr. Elder remarked that his understanding of the motion was that these ingredients when limited to a concentration of less than 15 ppm in rinse-off products, effectively removing the risk of sensitization or carcinogenic effects, were safe and that for leave-on products, the ingredients were significant sensitizers, and therefore unsafe for that use.

After some discussion, the Panel removed the word "effectively" from the initial statement.

Mary Ellen Fise then asked what would happen to the existing draft conclusion on p. 84 of the draft report. Ms. Fise suggested that the phrase "other than rinse-off" be changed to "leave-on"; this was generally agreed to.

Dr. Bergfeld remarked that the Panel was making two conclusions: that the rinse-offs were safe and that leave-ons were unsafe.

Dr. Boutwell stated that if something were found to be carcinogenic, then it could not be considered safe for any use, and that it is unknown whether these ingredients are carcinogenic, and thus there must also be concern for the rinse-off products with respect to this matter. He noted that unless there was a good carcinogenicity study available, the Panel could not say that these ingredients are safe even for rinse-off products.

Dr. Bergfeld remarked that Dr. Boutwell had agreed that the risk of carcinogenic effects was reduced by dilution.

Dr. Boutwell stated that he did agree with this, and that dilution would also help to reduce the risk of overwhelming the body's detoxification system, but that his concern was that data on carcinogenicity was just not avaialble.

Dr. Bergfeld then asked if Dr. Boutwell was suggesting that the data were insufficient for both uses, and he replied that since the Panel was already saying that the ingredients were unsafe for leave-ons due to sensitization potential, then the carcinogenicity data were only necessary to support the safety of rinse-off products.

Dr. Hoffmann remarked that the Panel should just say unsafe for leave-ons rather than consider it insufficient for that use.

Mr. Eiermann noted that if the carcinogenicity study was positive, then a risk assessment could be performed.

Dr. Shank called for a final motion.

Dr. Schroeter suggested separating the question into two parts, first for the rinse-offs then for the leave-ons.

Dr. Bergfeld stated that the motion for leave-ons was that it was unsafe due to sensitization potential.

Dr. Shank called for a vote on the ingredient in leave-on products.

Dr. Elder noted that this report would go final as unsafe for leave-on products.

Dr. Shank called for a vote for those in favor of Methylisothiazolinone and Methychloroisothiazolinone being unsafe for use in leave-on products.

Dr. Elder read the statement as it would appear in the report: "The CIR Expert Panel concludes that Methylisothiazolinone and Methylchloroisothiazolinone are significant sensitizers and are unsafe for use in cosmetic

products designed to remain in contact with the skin for prolonged periods (leave-on products)."

After some discussion, the motion was carried by a unanimous vote.

Dr. Shank asked for a restatement of the second motion to be voted upon.

Dr. Bergfeld responded by reading the amended motion: "there are insufficient data to determine the safety of use of Methylisothiazolinone and Methylchloroisothiazolinone in rinse-off products due to the lack of data on carcinogenic potential".

Dr. Boutwell seconded this motion.

Dr. Shank called for any discussion before taking the vote.

Dr. Elder noted that since this is an insufficient data report with respect to carcinogenic potential; a statement could be made that a second report will be issued when the carcinogenicity data are made available and have been evaluated by the Expert Panel. Dr. Elder commented that the Panel did not really need to request data if it was satisfied with the study the industry had indicated that it would undertake. The Panel agreed with this statement.

The representative from Rohm and Haas stated that his company would carry through with both the initiation/promotion study and with the Shelanski study to determine relevance.

Dr. Elder requested a statement from the Panel on what the Panel was expecting with respect to the carcinogenicity study.

Dr. Shank, Dr. Boutwell and Dr. Hoffmann stated that the Panel wanted the results of a mouse skin initiation/promotion study in which the ingredients are tested as both tumor initiators and tumor promoters.

Dr. Shank called for those in favor of the motion. The motion was carried with a unanimous vote.

Dr. Hoffmann moved that future Expert Panel meetings be nonsmoking. The motion was seconded and unanimously carried.

The Panel also agreed to a mail review of the Methylisothiazolinone report before announcing it as a Final Report.

Ammonium Thioglycolate, Thioglycolic Acid, Glyceryl Thioglycolate,

Dr. Schroeter stated that the Thioglycolates report was a Tentative Final Report and that a final decision on the report had been delayed in response to an industry request to be allowed extra time to test at higher concentrations. He noted that the data were supposed to be available for this meeting, but were not.

Dr. Bergfeld asked whether the Panel was to continue to delay the decision while awaiting the additional test data.

Dr. Schroeter mentioned an address by Marvin Rappaport, M.D., and said that there is some validity to his observations, but that they were speculative at best. Dr. Schroeter noted that these are very irritating compounds. He noted that he had had clinical experience with these compounds. They were so irritating to the skin that it was difficult to determine if these ingredients were also sensitizers. He remarked that he felt that the Panel would not be able to differentiate between severe irritant reactions caused by the ammonium thioglycolates and possible sensitizing reactions. He commented that he thought the Panel would have to take the available data at face value. He asked the Panel whether it would prefer to continue to delay the report or to vote on it as a Tentative Final Report.

MINUTES OF THE THIRTY-SEVENTH MEETING OF THE EXPERT PANEL

NOVEMBER 13, 1989
Key Bridge Marriott Hotel
Rosslyn, Virginia

Expert Panel Members

William O. Berndt, Ph.D., Chairman
Wilma F. Bergfeld, M.D.
Roswell K. Boutwell, Ph.D.
William W. Carlton, D.V.M., Ph.D.
Dietrich K. Hoffmann, Ph.D.
Arnold L. Schroeter, M.D.
Ronald C. Shank, Ph.D.

Liaison Representatives

Consumer

Ms. Mary Ellen Fise, Esq.

Industry

Gerald N. McEwen, Jr., Ph.D.

FDA Contact Person

John Bailey

CIR Staff

Robert L. Elder, Sc.D., Director/
Scientific Coordinator

Adopted _____
(Date)

_____
William O. Berndt, Ph.D.
Chairman

Referring to one of the "Wyden" ingredients, Dioctyl Phthalate, Dr. Hoffmann mentioned that there is no cosmetic product that does not contain traces of this ingredient, and questioned why it had been deleted from the review process.

Dr. Elder stated that in order for an ingredient to be reviewed, it must be specifically added to a cosmetic formulation for specific cosmetic purposes. CIR has been advised that there are no direct reported uses of Dioctyl Phthalate; therefore, the ingredient was deleted from the review process.

Dr. Elder agreed to send copies of his introductory report to each Panel member, as requested by Dr. Bergfeld.

Methylisothiazolinone/Methylchloroisothiazolinone

Dr. Boutwell moved that the Panel reconsider its vote and reopen the case on Kathon relative to rinse-off products. The motion was seconded and unanimously approved.

Dr. Berndt stated that the motion was to reconsider the previous Panel action on rinse-off products. He reminded the Panel that during earlier discussions, it was indicated that there were not sufficient data for determining whether Kathon is safe for use in rinse-off products.

Dr. Boutwell noted that skin absorption studies, using radioactive Kathon, had been done by Rohm and Haas. The studies were done in a way that was analogous to their carcinogenicity studies. Namely, small amounts (25 µl) of the unlabeled product, in water, were applied six times, and, ^{14}C-Kathon, during the final application. There was much evidence that the chemical had been absorbed: skin-bound material that couldn't be rinsed off, and the presence of radioactivity in the excreta and in the carcass. With this in mind, Dr. Boutwell concluded that the negative carcinogenicity skin painting study was adequate.

Dr. Bergfeld asked Dr. Boutwell if he considered Kathon to be a non-carcinogen.

According to Dr. Boutwell, one cannot say that Kathon is a non-carcinogen. However, one can say that it did not produce tumors. Dr. Boutwell reiterated that the skin painting study was adequate, and that his concern that water was an improper solvent was a false concern. He acknowledged that the radioactivity data indicated that the material, applied in water, did get into the system of the animal, even though he had previously emphasized that water is a very poor solvent for skin carcinogenicity studies. He also cited previous discussions of sensitization reactions as evidence that Kathon had gotten into the cells.

Dr. Shank agreed that water usually is an ineffective solvent for use in skin carcinogenicity studies. However, he concluded that water was not an ineffective solvent in the skin painting study because necrosis and hyperplasia were observed.

Dr. Schroeter asked how one could be sure that all of the Kathon found in the carcass was not due to ingestion.

In response, Dr. Boutwell noted that after the skin was washed and swabbed, radioactivity remained. So, this observation, along with the histological data, indicated that the material had reacted with the skin. He also noted that the quantity that remained in contact with the skin in some of the studies was as high as 34.0%, only 29.0% was excreted, and 10.0% was found in the carcass. Although the skin comprises a relatively large proportion of the body mass, the percentage that remained in contact with the skin was way out of proportion. So, the radioactivity could not have come from the gut and then become distributed throughout the body.

Dr. Hoffmann asked Dr. Boutwell if he would expect tumors to develop in areas of the skin where major damage had occurred.

Dr. Boutwell stated that it is not likely that one would observe carcinogenesis without also observing a range of responses, such as: epidermal necrosis, eschar, hyperplasia, and hyperkeratosis.

Dr. Hoffmann stated that there are many skin carcinogens, such as nitroso-diethanolamine, that do not cause hyperplasia.

Dr. Boutwell emphasized that without increased cell division there can be no tumors.

Dr. Schroeter stated that Dr. Boutwell's comments concerning cell division and the development of tumors may be true in animals. However, in humans, hyperplasia does not necessarily have to occur. Only in repeated scar formation or burn formation are hyperplasia, and then squamous cell carcinoma, observed.

Dr. McEwen stated that the 30-month skin painting study is a competent study indicating that Kathon is not a carcinogen.

Dr. Schroeter expressed his acceptance of the skin painting study, as interpreted by Dr. Boutwell. However, he also admitted that he still had lingering doubt about Kathon.

Dr. Shank said that several of the Panel members had expressed doubt about Kathon, but emphasized that the discussion was concerning Kathon in rinse-off products.

Dr. Carlton asked the dermatologists whether they were concerned about potential sensitization reactions to rinse-off products containing Kathon.

Dr. Schroeter indicated his acceptance of the fact that sensitization can occur, but that the incidence of sensitization is very low. He said that, based on the data, sensitization probably is not a relevant factor for consideration.

Dr. Elder verified that the discussion was about reconsidering the need for an insufficient data report relative to rinse-off products. In other words, the Panel had accepted the minutes but wanted to reconsider whether there was a need for further studies relative to rinse-off products.

Dr. Berndt stated that the Panel was reconsidering the issue of whether or not additional data are needed or whether there are insufficient data.

Dr. Carlton moved that Kathon is safe for use in rinse-off products. The motion was seconded by Dr. Boutwell.

Dr. Berndt reiterated that a motion had been made to say that Kathon is safe in rinse-off products at the use limitation concentration, and that the motion had been seconded.

Dr. Elder emphasized that the Panel reconsidered minutes from the last Panel meeting in which it had decided on an insufficient data report relative to carcinogenesis. He added that the vote, first of all, should concern whether or not the Panel needs a carcinogenicity study. This would establish whether or not the skin painting study is adequate relative to rinse-off products.

Dr. Berndt stated that the first motion was to reconsider the issue of carcinogenicity, or the adequacy of the carcinogenicity study for rinse-off products. He noted that the motion was carried, and that the Panel was in the process of reconsidering that issue. Out of that discussion came a motion that Kathon is safe in rinse-off products under whatever limitation on the concentrations of use that had been stipulated. This motion was seconded.

Relative to sensitization, Dr. Shank noted that it seemed that two kinds of populations, European and American, were dealt with. Furthermore, in the United States, these studies don't make a very compelling argument that Kathon is not a sensitizer. Dr. Shank also wanted to know how he should handle what is being observed in Europe. He mentioned that if Kathon-induced sensitization is a problem that will continue to increase in the United States, then the United States will have the same problem that exists in Europe.

Dr. Schroeter responded to Dr. Shank's concern that the cumulative data from Europe indicate a higher sensitization rate for Kathon than that found in the United States. He noted that there were preservative concentrations used in Europe that were above

what had been recommended by Rohm and Haas, and that these concentrations were used for a substantially longer period of time than they would have liked. Furthermore, in Europe, more leave-on products are used. Also, patch testing in Europe is done at higher concentrations, above 200 ppm. Concerning the United States, Dr. Schroeter is under the impression that Rohm and Haas has tried to standardize testing by providing the North American Contact Dermatitis Group with a patch test concentration of 100 ppm. He emphasized that the higher sensitization rate in Europe may be explained on the basis of one or all of the preceding reasons mentioned.

Dr. McEwen emphasized that if one looks at the data, it is not all of Europe that has a high sensitization rate. Observations are very refined in terms of particular countries with a high sensitization rate, and there has been no adequate explanation as to why this is observed. It may be due to the type of testing; however, this has not been shown. He noted that Germany, Sweden, Denmark, and, possibly, Italy and France have high sensitization rates.

Ms. Fise expressed concern about whether there are any differences in the uses of rinse-off products containing Kathon in America, compared to Europe. She also wanted to know if there was reason for concern that certain rinse-off products, like cold creams, are left on the skin for longer periods of time relative to other rinse-off products.

Dr. Bergfeld stated that the frequency of use of rinse-off products containing Kathon may vary culturally from one country to another, and that if a product is used more frequently, there would be residue remaining. She emphasized that directions are often overlooked and that one would anticipate that certain rinse-off products would be left on the skin. Particularly, body lotions for emollient use or lubrication are frequently used as astringents.

Dr. Schroeter asked whether CIR has definitions of rinse-off and leave-on products.

Dr. McEwen stated that there are, more or less, general definitions. A rinse-off product is one that is customarily applied and then rinsed off. A leave-on product is one that

is customarily applied and not rinsed off.

On the subject of genotoxicity, Dr. Hoffmann noted that there are a number of genotoxicity tests that are positive, and that, in the past, these results were not disregarded.

Dr. Berndt stated that positive results from genotoxicity tests had not been disregarded and that, after all, a long-term test for carcinogenicity is available.

Dr. Slaga, with the University of Texas, gave a presentation on genotoxicity tests involving Kathon that had been completed. He noted, as had been pointed out by Dr. Hoffmann, that the Salmonella mutagenicity test and the mouse lymphoma assay yielded positive results. In both tests, positive results were reported under the conditions of extreme toxicity. He noted that in the Salmonella assay, the only positive result was with strain TA100, in the absence of metabolic activation. He emphasized that strain TA100 has been constructed to be extremely sensitive, and, according to Dr. Bruce Ames, high toxicity is almost always accompanied by DNA damage when this strain is tested. For this reason, EPA has recommended that the Salmonella test not be used as a mutational endpoint when testing biocides. Concerning the mouse lymphoma assay, he said that there are a number of ways in which one can evoke false positives with mouse lymphoma cells. Anything that affects pH, osmolality, etc. will cause an adverse response in this assay. For example, sucrose and common table salt yield positive responses. This assay system has been, more or less, discredited for its indicative capabilities. Data from the NTP data base indicate that 80.0% of the carcinogens are positive and 60.0% of the noncarcinogens are positive in the mouse lymphoma assay. Furthermore, in October of 1989, EPA's scientific advisory panel recommended that the mouse lymphoma assay no longer be used in a battery of tests. Dr. Slaga also mentioned that results were negative in the Drosophila test, DNA repair assay, chromosomal aberrations assay, and ß cell transformations assay. He concluded that Rohm and Haas has a valid group of genotoxicity assays that indicate that Kathon is not a

mutagenic hazard, and a valid skin painting study indicating that Kathon is not a carcinogen.

Dr. Hoffmann added that there are chemicals that are known to be tumorigenic in experimental animals that do not yield positive results in genotoxicity assays, and that Dr. Slaga had failed to mention this.

Dr. Slaga acknowledged that each of the genotoxicity tests mentioned had the potential for false positives.

Dr. Hoffmann mentioned that in the Salmonella assay, there was more activity without the S9 fraction; this indicated that Kathon is a direct alkylating agent that can be deactivated by metabolism. He also said that when one observes activity with S9 that is not observed in its absence, detoxification has taken place.

Dr. Slaga agreed that there are many detoxifying systems. So, if a compound like Kathon is administered, it is going to be detoxified, and this will either lead to a less mutagenic state or the compound will not be mutagenic.

Dr. Berndt restated the motion to approve Kathon as safe in rinse-off products at the use limitation concentration. The Panel voted five to one in favor of the motion. Dr. Hoffmann opposed the motion.

Drs. Bergfeld and Boutwell confirmed that the use concentration limit was 15 ppm.

After the vote, Dr. Elder asked the Panel if their decision was based solely on the material in the report, or if additional information should be included. In other words, he wanted to verify that the report that the Panel had voted on is the one that will be announced to the public.

Dr. Berndt said that the Panel's decision regarding the use of Kathon in rinse-off products was based solely on the data in the report.

Dr. McEwen stated that during the public comment period for Kathon in leave-on products, studies that support the safety of Kathon in leave-on products were received.

Dr. Elder emphasized that any additional data received will have to be analyzed, formatted, and submitted to the Panel.

Dr. Bergfeld noted from an earlier discussion that a review of the mouse lymphoma assay and the Ames test had been done by a credible group, and requested that this information be included in the report.

Dr. Hoffmann thought that the comments made by EPA concerning the two genotoxicity assays should also be included in the report.

Dr. Elder indicated that he was willing to include any additional information in the report. He emphasized that until April, 1990, the action of the Panel is that Kathon is unsafe for use in leave-on products and safe for use in rinse-off products. A Tentative Final Report on Kathon bearing this conclusion will be announced. Data received from the cosmetics industry will be incorporated, and the document will be reviewed at the April, 1990 Expert Panel Meeting.

Dr. Schroeter complemented Rohm and Haas for their cooperation.

Dr. Shank noted that one can find much information in the scientific literature indicating that each of the genotoxicity assays performed by Rohm and Haas has limitations, and did not understand why the Ames Test and the mouse lymphoma assay were singled out for false positives.

Dr. Bergfeld noted that EPA's comments on the Ames Test and the mouse lymphoma assay are included in the Federal Register and reiterated that this information should be included in the report.

Dr. Berndt emphasized that EPA's position concerning the two assays does not mean that these tests will not be used in non-EPA laboratories. He agreed with Dr. Shank relative to the fact that each of the genotoxicity tests discussed earlier has limitations.

Dr. Elder mentioned that because additional data from the cosmetics industry are going to be included, the document will also have to be updated with respect to any current

published data that are available. He emphasized that the Panel will be reviewing an updated document at the April, 1990 Panel meeting, and that all new data and textual decriptions will be underlined in text.

Dr. McEwen stated that the additional data received from the cosmetics industry to date were submitted in response to the Tentative Final Report stating that Methylisothiazolinone and Methylchloroisothiazolinone are unsafe for use in leave-on products.

Dr. Berndt asked what was going to be done concerning the decision on rinse-off products.

Dr. Elder indicated that the Tentative Final Report will be announced with an additional conclusion concerning use in rinse-off products. He stressed that the document will not be reviewed by the Expert Panel until the April, 1990 Panel meeting.

Dr. Berndt stated that the document reviewed at this meeting will be announced as a Tentative Final Report. The forthcoming data on Kathon, whether concerning leave-on or rinse-off products, should be received before the deadline. He noted that there are different deadlines for submissions concerning the use of Kathon in rinse-off and leave-on products, respectively. An updated document containing all of the additional data incorporated, published and unpublished, will be be reviewed at the April, 1990 Expert Panel meeting. The new data will be underlined in text.

Dr. Schroeter asked if the document had been released to the public for comments. He also wanted to know whether, with the exception of Rohm and Haas, other comments had been included in the document.

Dr. McEwen indicated that data and a comment had been received. The comment was that the Panel should not take any final action on leave-on products until they have had a chance to thoroughly review the additional data that have been submitted. The new data largely have not been from Rohm and Haas.

One of the representatives from Rohm and Haas stated that there should be some type of definition, quantitative definition, for leave-on and rinse-off products.

Dr. Elder stated that according to the procedures, it is the responsibility of the cosmetics industry to supply the Panel with this type of information.

Dr. Schroeter suggested that definitions of rinse-off and leave-on products should be available for the April, 1990 Panel meeting.

Dr. McEwen agreed to provide definitions of rinse-off and leave-on products for the April, 1990 Expert Panel meeting.

Pyrogallol

Dr. Berndt opened the discussion on Pyrogallol by referring to the Panel's action at the 24-25 July Panel Meeting, subsequent to which CIR determined that cosmetic grade Pyrogallol contained a minimum of 99.0% Pyrogallol. He suggested that the Panel rethink its request for additional data on Pyrogallol, taking into consideration the purity of the cosmetic grade product.

Dr. Hoffmann noted that in the synthesis of Pyrogallol, as in the synthesis of certain other organic compounds, the reaction may result in the formation of dibenzylpuranes and dibenzyldioxanes, which are extremely toxic. He suggested that the second sentence of the impurities section of the report should be changed to state: "data on possible organic impurities in cosmetic Pyrogallol, especially on chlorinated hydrocarbons, are not available."

Dr. McEwen suggested that wording other than especially be used, noting that though these possible impurities are of concern, they might not be the most important items of concern.

The Panel agreed and decided to reword the statement, replacing the word "especially" with "such as."

Dr. Boutwell commented that in the section on cocarcinogenicity, the VanDuren and Goldsmith study, which shows positive cocarcinogenicity when Pyrogallol together

C I R

MINUTES OF THE THIRTY-EIGHTH MEETING OF THE EXPERT PANEL

APRIL 16, 1990

Key Bridge Marriott Hotel
Rosslyn, Virginia

Expert Panel Members

William O. Berndt, Ph.D., Chairman

Wilma F. Bergfeld, M.D.

Roswell K. Boutwell, Ph.D.

William W. Carlton, D.V.M., Ph.D.

Dietrich K. Hoffmann, Ph.D.

Arnold L. Schroeter, M.D.

Ronald C. Shank, Ph.D.

Liaison Representatives

Consumer

Ms. Mary Ellen Fise, Esq.

Industry

Gerald N. McEwen, Jr., Ph.D.

FDA Contact Person

Heinz Eiermann

CIR Staff

Robert L. Elder, Sc.D., Director/
Scientific Coordinator

Adopted _____
(Date)

_____
William O. Berndt, Ph.D.
Chairman

available to the Panel industry's descriptions of "rinse-off" and "leave-on" products, and acknowledged that these descriptions have been received. A "rinse-off" product is one designed to be applied to the hair or body in diluted or undiluted form for a short period of time (less than 1 hour) followed by thorough rinsing. Operational examples include shampoos, cleansers, hair conditioners, and depilatories. A "leave-on" product is a product intended to be applied to the skin and left in place for a long enough period to achieve the desired benefit. Dr. Berndt stated that, based on the Panel's discussion during the executive session, it appears that the policies followed by the Panel in the past are consistent with these definitions.

Dr. Elder recalled that there was also a discussion concerning data submittals.

Dr. Berndt confirmed that the Panel had discussed data submittals: "Again, consistent with what the Panel has done in the past, we reaffirm that we will continue to examine data on a case by case basis if the data are submitted late."

Dr. Elder stated that there would be no mailing of raw data to the Panel after mail date. However, copies of information submitted to CIR after mail date, which is about three weeks in front of the meeting, will be delivered to the Expert Panel meeting room.

Methylisothiazolinone/Methylchloroisothiazolinone

Dr. Berndt recognized Dr. John Harrington, with Rohm and Haas. Dr. Harrington introduced the representatives from Rohm and Haas who were to make presentations to the CIR Expert Panel: Dr. John Wilkinson, Chairman of the Department of Dermatology at Wickham General Hospital, United Kingdom (to address questions concerning the European perspective and give comments on his personal perspective); Dr Jim Marks, with the Division of Dermatology, Penn State College of Medicine (to give the North American perspective and his own observations on Kathon CG use in North America); Dr. Thomas Slaga, University of Texas Department of Carcinogenicity; Philip Lewis, M.D., Dermatologist and Rohm and Haas Corporate Medical Director and Director of Safety, Health, and Environmental Affairs (to give components of new data presentation);

Dr. Harvey Scribner, Department Manager for the Department of Toxicology, Rohm and Haas; Dr. Mike Jayjock, with Project Integrity Department of Rohm and Haas; and Mr. Morse, Section Manager, Department of Toxicology, Rohm and Haas.

Dr. Wilkinson addressed the concern about sporadic reports of high levels of sensitivity to Isothiazolinone in some European countries: There is not and there never has been a problem with Isothiazolinones in the United Kingdom, Ireland, Belgium, Denmark, France, or in Spain. Even in countries where there was a problem, such as in Finland, levels of sensitivity returned to normal once the problem product was identified and the concentration levels of Isothiazolinone reduced to below 15 ppm. High levels of sensitivity do, of course, still persist in some countries. Where there is a relative shortage of good biocides, if a biocide such as Isothiazolinone is removed from the market, we will, I guess, see rising levels of sensitivity to other biocides. There will also be less of a choice for those who are sensitized, particularly those who are sensitive to formaldehyde. At this moment in time in my contact dermatitis clinic, the leading biocide sensitivity that I see is formaldehyde, with an incidence of 3.8% (males and females combined). The second highest level of prevalence is to Quaternium-15, a formaldehyde releaser, with a prevalence figure of 2.8%. Parabens is third, with a level of 2.1%. Isothiazolinone (Germall 115) is fourth, with a prevalence figure of 1.3%. I think that our figures are probably very similar to those seen in the United States and in the other European countries that I mentioned. Personally, I would still like to see Isothiazolinones available for preservation in cosmetics.

Dr. Carlton wanted to know if the problem of sensitization in European countries is a matter of misuse in terms of the concentration of Kathon or of a particular type of product.

Dr. Wilkinson stated that in Finland, the problem was traced back to one product that contained 19 ppm Isothiazolinone. This was a product that was in widespread usage, and contained Isothiazolinone above the level that was recommended. When that was rectified, the incidence of sensitization decreased to approximately 1.0%. The EEC had set an initial level of 13 ppm for Isothiazolinone. History has shown that that was not wise.

Mr. Eiermann wanted to know the type of product that had been used in Finland, whether or not it was a cream, lotion, or shampoo.

Dr. Wilkinson stated that Finland and Sweden had very similar problems. In each country, it was a hand cream. Both creams had similar concentrations of Isothiazolinone, concentrations above 15 ppm.

Dr. Berndt wanted to know the level that Kathon was reduced to when the problem was corrected.

Dr. Wilkinson stated that he was not involved with that decision. He indicated that the level that caused the problem was 19 ppm for one product and about 15 ppm for the other product. He added that the contact sensitivity figures in those countries have returned to acceptable levels since the level of Kathon was reduced.

Dr. Berndt wanted to know the incidence of sensitization in Finland before the correction was made.

One of the representatives from Rohm and Haas stated that the incidence was 5.0% or 7.0%.

Dr. Hoffmann recalled that at the last meeting, the Panel had been informed that Germany, Sweden, Denmark, and, possibly, Italy and France have high sensitization rates.

Dr. Wilkinson said that the sensitization rate in Germany is still high, above 5.0%. The rate was high in Sweden, but has decreased. The sensitization rate in Denmark has never been high, and is high in Italy, but not in France.

A representative from Rohm and Haas stated that Italy is one of the countries where the highest concentrations of Isothiazolinone in leave-on products are found, some as high as 17 ppm.

Dr. McEwen wanted to know whether the EEC or enough countries would pay attention to any concentration limit on Kathon that would be set by the CIR Expert Panel.

Dr. Wilkinson indicated that the EEC is becoming increasingly more uniform. When there is a problem, it may be possible to determine the levels that companies are using and reduce them.

Dr. Bergfeld noted that the Panel had received several communications concerning Kathon and wanted to know if Dr. DeGroot had changed his position within the last year.

Dr. Wilkinson said that he thought that Dr. DeGroot would say that Isothiazolinone is

a very active chemical, as are many of the biocides, and that it has shown a significant capacity to sensitize, and, because of that, it should not be used. Dr. Wilkinson also stated that, in terms of contact dermatitis, he and probably the majority of dermatologists would not take such a harsh stand. He said that when Parabens was first introduced, it was used at a concentration of 5.0% in an anti-athlete's foot cream and caused an absolute epidemic of contact dermatitis. Declaring Parabens an unsafe chemical would have been a mistake, for it is known that it has been a remarkably safe preservative when used at a concentration of 0.15%. Recommending the proper concentration for the proper usage is important.

Dr. Schroeter said that Dr. Wilkinson had inferred that use of Kathon at a concentration of 19 ppm was the reason why certain countries had a high incidence of contact dermatitis. He wanted to know if the problem had been solved by decreasing the concentration of the biocide to 15 ppm.

For the one product that contained 19 ppm, Dr. Wilkinson recalled that the problem no longer existed after the product had been changed. He did not know how the product had been changed.

Dr. Schroeter stated that physicians, as well as the public, would not recommend a product that causes contact dermatitis. They will cease to use a product or industry will change the biocide, and, therefore, the incidence of that particular contact allergy will dissipate in the community. He asked if use of the biocide was discontinued or if there was some other reason why the incidence of contact dermatitis had decreased. He also said that it is inconceivable that decreasing the concentration of Kathon to 15 ppm would have a significant impact. Referring to Cransway's data from the Mayo Clinic, he said that Kathon was initially tested at a high concentration, and it was brought to the attention of the staff, which he at that time was part of, that Kathon was indeed an active preservative that would cause contact dermatitis. Dr. Schroeter recalled his observation of a flux away from the use of products that contained Kathon, and that the level of contact sensitization receded slowly.

A representative from Rohm and Haas stated that the level that Dr. Wilkinson was referring to, 19 ppm, was discovered in a product, at which time the manufacturer was advised that the concentration be dropped to 7 ppm. He then noted that the prevalence rate

in Finland has decreased significantly, even though the number of products coming into Finland is quite the same as it was in the past. The only exception is that in one product, the level of Kathon was reduced to 7 ppm. He also said that he did not know the concentration of Kathon in all of the other products, but, for most of them, the concentration is below 15 ppm.

Dr. Wilkinson stated that there are two key issues that explain why the problem began, one of which is the road product issue. In many of the countries, for example, Sweden, Finland, and Italy, there are a handful of road products in which the concentration of Kathon is beyond what is recommended. The problem was solved by the removal of one product. A similar problem occurred with another biocide in the United kingdom, Quaternium-15, that began to get too much of the market share on leave-on products. It was even used in Johnson and Johnson baby creams; in fact, the concentration exceeded 4.0%. The manufacturers became aware that the levels were becoming too high, and some of the large manufacturers began using other biocides. As a result, the level has slimmed down a bit. Quaternium-15 is still used widely in the United Kingdom. So, apparently, there are no problems when this biocide is used within good guidelines. If use of a particular biocide predominates, like in the leave-on cosmetics market, then, perhaps the allergenic weight on a population goes beyond a particular level. Thus, Dr. Wilkinson is in favor of the availability of as many biocides as possible.

Dr. Jim Marks said that no one in the North American Contact Dermatitis Group is clamoring to ban Kathon CG. His opinion is that Kathon CG is safe for use in leave-on products. This position is based on his review of the literature, personal experience in his patch testing clinic, and the collective experience of the North American Contact Dermatitis Group between 1988 and 1989, and prior to this period.

Dr. Bergfeld wanted to know at what concentrations Dr. Marks considered Kathon to be safe.

Dr. Marks stated that based on his communications with the cosmetics industry, both verbal and written, some level below 15 ppm is safe for use in leave-on products. Concentrations below 15 ppm either did not cause increased adverse reactions per unit

dispensed over the last few years or did not cause positive reactions when patients who were patch-test sensitive to 100 ppm Kathon were rechallenged. In addition to his own experience with patients, he mentioned that the number of positive reactions to Kathon CG was not great in the North American Contact Dermatitis Group study. Specifically, 1.9% of the 949 subjects tested with 100 ppm Kathon CG had positive reactions. This was not a significant increase compared to previous data from the North American Contact Dermatitis Group. In looking at North American Contact Dermatitis Group data from 1985 to 1989, one does not see either a significant rise in prevalence or an epidemic in North America. If one compares the sensitization rate for Kathon CG to that of other biocides that are commonly used in cosmetics, it is either slightly greater, in terms of parabens, or less, meaning from one-half to one-third the rate seen for some other popular cosmetic biocides. Dr. Marks reiterated that his personal approach would be not to ban Kathon in leave-on products. He said that he would leave the decision of whether or not to use Kathon in cosmetic products to the individual cosmetic companies that are formulating. However, his personal opinion is that of approving Kathon for use at a low level. He emphasized that in the future, Kathon, as well as other contact allergens, will be continually monitored by the North American Contact Dermatitis Group and by the cosmetics industry.

Dr. Bergfeld noted that in one publication, North American patch test study, 100 ppm Kathon was used to elicit the reaction. She wanted to know if Dr. Marks could identify the product and concentration of Kathon in that product in those individuals who were patch test positive to Kathon.

Dr. Marks said that Dr. Bergfeld's concern is about what is commonly known as relevance, specifically, the relevance that the positive patch test has to both the presenting dermatitis and the products that person is using at that time to lend significance to that positive reaction. About half of the time, maybe 44.0% of the time, a product could be identified which seemed relevant to that positive patch test reaction. The percentage was also similar, close to 50.0%, in the open repeat test. So, it seems that about half of the positive patch tests to 100 ppm Kathon were relevant.

Dr. Bergfeld wanted to know the concentration of Kathon that was used in those products.

Dr. Marks said that testing was a multicenter operation, and that he did not look specifically at the composition and ingredient concentrations of the products tested.

A representative from Rohm and Haas stated that some of the products identified may have contained between 7.5 and 15 ppm Kathon. One of the products that was used in a provocative test was a leave-on product.

Dr. Bergfeld wanted to know if she was correct in saying that American products contain no more than 15 ppm Kathon.

The same Rohm and Haas representative said that American products contain less than 15 ppm Kathon. He emphasized that the major leave-on products that are in use in the United States contain less than 15 ppm.

Dr. Bergfeld recalled that Dr. Wilkinson had stated earlier that in Finland and, perhaps, in Sweden, a certain product had been identified as the culprit. She wanted to know if that had been done in the United States.

Dr. Marks stated that there was nothing in the publication, in the discussion of the publication, indicating that one product stood out as being the bad actor and all of the reactions were to that one product.

Dr. Berndt wanted to know if Dr. Marks would, in his clinic, tell sensitive individuals to stay away from Kathon completely or to find a lower concentration.

Dr. Marks said that in the actual clinical practice, how to tell a patient to find a lower concentration would be difficult. In contrast to Europe, where there is no labelling, the main problem in the United States concerning cosmetics is fragrances, because, of course, of the huge number of fragrances. He said that he actually allows patients to try fragrances, but by means of an open test conducted in a very controlled manner.

Dr. Carlton wanted to know the percentage of Kathon-sensitive patients who are also positive to formaldehyde-containing biocides.

Dr. Marks said that close to 60.0%, maybe 58.0%, had other positive reactions. So, it is not uncommon to have individuals who will have multiple positive reactions.

Dr. Carlton wanted to know if, in interpreting the data, Dr. Marks thinks that there is a population of individuals that is making up the positive population for essentially all of the biocides.

Dr. Marks said it may be that a sensitive population is being dealt with, and there may be a genetic basis for this sensitivity. For example, he noted that half of the individuals in the meeting room are sensitive to poison ivy or poison oak and half are not; this could be due either to exposure or genetic makeup.

Dr. Carlton wanted to know the effective concentration of Kathon as a biocide. Kathon is believed to be very effective at 15 ppm. However, there seems to be an undercurrent that Kathon is very effective at a concentration of 7.5 ppm.

A representative from Rohm and Haas said that of the many products that are preserved with the compound, skin care products are probably more difficult to preserve than the other products. A concentration of 7.5 ppm will preserve the majority of them. There are many products in which Kathon may not be used at 7.5 ppm, but, for the majority of the products, the major skin care products, 7.5 ppm is an adequate concentration. These same products can be preserved at even lower concentrations.

Mr. Eiermann wanted to know the context in which the term adequate was being used.

The representative from Rohm and Haas explained that adequate meant adequate in terms of antimicrobial activity. This is based upon the information that they receive from the formulator in terms of the shelf life. For some formulators, the shelf life is one year. Other formulators may have different criteria.

Dr. McEwen mentioned that CTFA had received data on a number of major products; the names of the products were kept confidential at the request of the manufacturer. The products were preserved at 7.5 ppm, 7.0 ppm, and two products were preserved at 8.0 ppm. These were major market products that had been on the market for some time. Based on this information, one may assume that the products are adequately preserved.

Mr. Eiermann said that anyone who knows product formulation and is involved in microbiological testing knows that in most cases, a system, rather than one preservative, is used. More than likely, a preservative system would consist of parabens, Kathon, and,

maybe, a formaldehyde donor; it depends. The preservatives needed must be determined on a product by product basis. Each product behaves differently. The issue is, in the case of leave-on products, contamination of the product with the fingers. Not only is it an issue of product integrity, which means that the preservative remains active, as formulated, but also preservation of the product during use. Another issue is use around the eye area if the pH is considered to be safe. For a leave-on product, one would want to make a special exception with regard to the eye area.

The assessment of the recent data generated by Rohm and Haas was presented by Dr. Lewis, and is as follows: Generally, of course, microbiocides have been of special interest to me. The present data that I am going to present to you are a couple of repeat insult patch test studies that were done at a couple of labs, one in New York and one in San Francisco. The idea here was to have a double blind controlled study of about 200 patients in each test group and each control group, looking at Kathon at concentrations of 7.5 ppm and 15.0 ppm. Then, we had specifically requested to put in the 100 ppm patch test diagnostically after the routine RIPT to get a handle on any reactions that might occur during induction or challenge phases. The data, particularly for the 7.5 ppm, the San Francisco study, is still pending. There is still a bit of that to come in yet, as is clear from the controls, and what I have here is the listing of the number of subjects, the reactions during induction, and reactions during positive challenge. As one can see, there is a significant difference, based on chi square, between the reactions at 7.5 and 15 ppm during the induction phase. One of the things that is a little surprising in this study, of course, is the number of reactions in the water control in the 15 ppm study. We are looking back at that now to try and determine what might have been the cause there. Certainly, one of the things is that the distribution of reactions, that is, +1, +2, +4, is reasonably similar. So, it may either be a problem in terms of difference in reading, which we have certainly seen in doing previous prevalence studies, meaning that there is a difference between the two clinics in terms of degree of reading, or there may have been some other factor; some people have suggested bacterial growth in the water control, but we will be looking at that. Again, in the San Francisco study, things have been clean all the way through, and, as I said, there is clearly a significant difference between these two labs.

Dr. Berndt said that if he had controls in any of his experiments like those indicated by Dr. Lewis, the study would have to be discontinued and then repeated. He wanted to know if the data were going to be accepted, assuming that there is a difference between water in New York and water in San Francisco.

Dr. Lewis said that his company is in the process of determining which factors are most important in determining the difference between the controls in San Francisco and the controls in New York. He also said that it had been noticed during previous prevalence studies that there is a significant difference in the way reading is done. There is greater sensitivity in some clinics compared to others. Until the results from some of the microbiological assays are received, his suspicion is that, more often than not, the explanation is going to be in the difference in the degree of reading.

Dr. Hoffmann questioned whether, despite the high background, the difference between experimentals and controls is statistically significant.

Dr. Lewis said that this was not the case and offered an explanation. The difference between the control in New York and the exposed group in New York is not statistically different. The difference is between the lab in San Francisco, both either looking at both the controls and the exposed compared to both the control and the exposed in New York.

Dr. Hoffmann said that he had done a student's t-test and found the difference between 45 and 55 to be statistically significant
(P = .05). He was speaking of the difference between experimentals and controls.

Dr. Lewis said that the chi square done did not suggest that there is a statistically significant difference between the two.

Dr. Carlton said that in any case, there were no sensitization reactions.

Dr. Lewis confirmed that there were no sensitization reactions during the challenge phase.

Dr. Schroeter wanted to know what had been used for the test of positive challenge.

Dr. Lewis said that in New York, it was the 15 ppm material and, in San Francisco, the 7.5 ppm material. Subjects were challenged with the material used during induction.

Dr. Lewis said that he had been particularly interested in looking at results for the 100 ppm challenge dose so that one has an idea of what Rohm and Haas has observed in a number of studies, which has been a difference in reading, and what would eventually occur in people who seem to have some sensitivity in a 100 ppm patch test, and, later on, sensitivity to products used. Referring to the data, he explained that there are a couple of differences in terms of the number of people who react, but the chi square value is based upon the whole group of positive reactions that were observed at 100 ppm. This suggests that at greater than 40.0% of the time, one would expect to see a difference in distribution of that size based on chance alone. So, there are not significant differences between the numbers. The question about the positive result in the water control in New York again may be a question of reading. The 100 ppm patch test is at the highest non-irritating dose. In some situations, it is right at the border; so, sometimes one may probably get some mild irritation that is misread. There have been suggestions, particularly from some of the studies in Germany, that the patch test really needs to be lowered to 50 ppm so that one gets better specificity of the patch test. All in all, the findings suggest that there isn't a significant difference and that while both of the concentrations seem to reasonable, one might get some increased safety by moving to a level of 7.5 ppm.

Dr. Schroeter said that Dr. Lewis had suggested that if a patch test of 50 ppm had been used, there would have been more specificity. He emphasized that Rohm and Haas has that data as well as data at lower concentrations, and sensitivity is lost. If the level of contact patch test is raised to 150 ppm, greater sensitivity can be attained. Some specificity may be lost, but on the other hand, a significant amount of positivity may result. Dr. Schroeter did not advise Dr. Lewis to test at 150 ppm, because, at that concentration, there is a greater chance of induction. He said that one should not go to the other extreme and infer that the positive patch tests should be wiped out and that they are not as specific.

Dr. Lewis agreed with Dr. Schroeter. He said that the argument would eventually be at what point the test will be used and what the test is being used to determine. At a point in which a diagnostic look at something is being done, one would rather have a highly sensitive test and then move to a more specific test later on. He also said that the idea of moving to

titrations of lower levels is a way of getting the specificity to weed out the question of false positives that might occur when using a highly sensitive test. The suggestion of moving to a lower concentration in the patch test course has been resisted because of the same reason mentioned by Dr. Schroeter. Rohm and Haas has suggested, rather, the idea of moving to titrations to try and increase the specificity.

Dr. Bergfeld said that Dr. Lewis had presented data suggesting that there is no greater risk with the concentration of 15 ppm than with 7.5 ppm. However, emotionally the Panel is being guided to choose 7.5 ppm as a safer concentration.

Dr. Lewis said that the data suggest that there is a reasonable degree of safety at concentrations of 15 ppm and below. Furthermore, the data suggest that as concentrations closer to 7.5 ppm and below are approached, questions about the possibility of a safety question become fewer and fewer. Though anything below 15 ppm is a reasonable dose, a reasonable concentration to think of, one may indeed have a margin of safety by going to 7.5 ppm. In doing risk assessments, modeling plays a major role. The dose response curve in the modeling for Kathon at the levels indicated is very steep, and one gets a significant margin of safety by going to 7.5 ppm.

Dr. Berndt wanted to know why the studies were done at two different locations. He also wanted to know why the readings for both laboratories were not done by the same individuals.

Dr. Lewis said that the fact that more than one laboratory was used was related to how many patients a given laboratory could handle, and did not address the issue of different locations, New York and San Francisco. He said that, from the standpoint of clinical design, one would rather have a situation where there is one group of readers that has a handle on the variability of its reading routinely doing the same reading. This would certainly reduce the variability.

Dr. Schroeter said Dr. Lewis indicated in his interpretation of the data that the Panel should be ruling in favor of a 7.5 ppm concentration limit for the biocide on the basis of induction data, whereas, all previous decisions by the Expert Panel have always been based on patch test data. If one looks at the data, it is hard to determine that there is a difference

in the positive patch test data, notwithstanding the exception that Dr. Lewis said that if a more specific test at 50 ppm were used, maybe there would have been less than that at 7.5 ppm. But, in reality, this is not known. Dr. Schroeter wanted to know the reasoning as to why the Panel was being asked to make a decision based on induction data rather than patch test data.

Dr. Lewis said that there is a link between the kind of data that can be generated on induction, a kind of situation that would be reasonably equivalent to the incidence data that could be generated. This has a logical plus, in that it introduces a degree of risk directly. He prefers prevalence data because it allows one not only to look at the inherent difference in toxicity of the materials, but also allows one to look at such things as, for example, exact use in the environment.

Dr. Schroeter said that use in the environment is not of concern. He noted that the data, as reported by Dr. Lewis, indicated that at 15 ppm there were positive tests, in the 100 ppm patch test there were three, and at 7.5 ppm, there were three. There is no difference between the 15 ppm and 7.5 ppm groups, yet, Dr. Lewis pointed to the induction and said that because there is a significant difference between the induction data at 15 ppm and 7.5 ppm, it may be concluded that it is safer to use the 7.5 ppm concentration.

Based on Dr. Schroeter's interpretation of what had been said, Dr. Lewis said that he had perhaps miscommunicated what the data suggest. The data suggest that there is a fairly good degree of safety once the concentration is below 15 ppm. However, one could and could not look at the data per se and say that there really is much difference between 7.5 and 15 ppm. From the standpoint of modeling of the elicitation data from the prevalence studies, this suggests that at 7.5 ppm, there is an additional margin of safety. Dr. Lewis said that based on his discussions with Bjorkner, Krager, or other people, the arguments begin once concentrations are in the 15 ppm to 19 ppm range. At lower concentrations, which is true for most products, there have been significantly fewer questions or problems. So, based on the prevalence data per se, one would suggest, in conjunction with the modeling, that there is an additional margin of safety when the concentration is decreased to 7.5 ppm. From a purely scientific view, the dose should be lowered to below 15 ppm.

Mr. Eiermann said that from a purely scientific view, one would justify the opposite. In 45 people, water controls caused positive reactions. Since there is no significant difference between 45 and 55 during induction at the 15 ppm level, one could argue that there was greater sensitivity for positive reactions. Therefore, if one looks at 7.5 ppm, the three positives showed up in the 100 ppm test because, perhaps, that investigator was less sensitive with respect to getting a positive reaction. This is perhaps more meaningful than the two or one at the 15 ppm level.

Dr. Lewis said that what Mr. Eiermann said is a possibility.

Dr. Carlton said that if Mr. Eiermann is correct, the degree of sensitivity is terribly complicating and of no benefit. One would not want anyone to be sensitive to the extent that water would elicit a reaction.

Dr. Berndt wanted to know if there were enough patients on the west coast to bring the total to relatively 200.

Dr. Lewis said that he thought so. He agreed that there is a reasonable amount of variability in the way reading is done. Significant attempts, particularly in the prevalence studies, have been made to standardize the way that dosing, using patches, is done, the systems that are being used, and the way that reading is done.

Dr. Carlton said that the individual who was in charge of the study must have made some observation as to why so many subjects had positive reactions to water.

A representative from Rohm and Haas said that the water used in New York and the water used in San Francisco were the same. Though neither investigator knew the nature of the material being tested, both knew that they were testing water and a concentration of Kathon. Each test was done using two separate panels; one group was tested with water and the other group was tested with Kathon. When the investigator first noticed reactions to both materials, he immediately asked if both contained Kathon. Analyses of both materials indicated that one of the samples was water and the other was Kathon. The reactions observed were classified as edge erythema or edge edema, which, for some investigators, are not uncommon clinical findings. The reasons for reactions to water may have been bacterial contamination of the water or the extraction of some material from the actual patch material

itself. The fact that in a blind study, reactions were observed in both groups suggests that the reactions were nonspecific, that is, induction was not attributed to anything other than water. The issue of aquagenic urticaria was brought up, and maybe this is an explanation for the reactions observed. He emphasized that in using the RIPT and the criteria for a positive or negative response, none of the subjects had positive responses when challenged with either water or Kathon. The responses observed during induction were not fully understood.

Dr. Carlton wanted to know if the investigator was able to differentiate in terms of the reaction to water versus Kathon.

Dr. Lewis said that the distribution, in terms of the character of the reactions, was reportedly the same.

Dr. Carlton asked if one would expect the reactions to look different.

Dr. Lewis said that one would expect the reactions to look different, but no differences were reported.

Dr. Bergfeld wanted to know the grade of the reaction.

Dr. Lewis said that most of the reactions generally were +1 reactions, on a +3 system, to begin with.

Dr. Bergfeld mentioned that Dr. Lewis had used what was referred to as modeling data and asked for an explanation of this method.

Dr. Lewis said that modeling suggests that one can get some view as to the degree of reaction that will be observed at fairly low doses. The modeling data suggest that as concentrations decrease to below 15 ppm, there is a significant decrease in the degree of reaction; at 7.5 ppm, there is even more of a decrease. In fact, while there is is a two-fold difference between 7.5 and 15 ppm, there is a ten-fold decrease in terms of the degree of risk of reaction, because the curve is fairly steep in that area.

Dr. Bergfeld wanted to know if modeling is an accepted practice, and if there are many statisticians and epidemiologists who would use this method.

Dr. Lewis said that modeling is commonly used, more particularly in terms of dealing with risk for carcinogenicity in terms of environmental antigens. It is something that one more generally does in terms of looking at risk for materials in the environment. On the

subject of monitoring the prevalence data, he said that he has worked with several dermatologic groups to insure that Rohm and Haas will continue to communicate with clinicians and will have knowledge of any developments in the future. Particularly, the prevalence data have been very helpful in the identification of areas where problems have existed concerning the location of materials or products in which a material has been used inappropriately, and getting those products reduced to a level that has minimized their risk in the environment.

Dr. Bergfeld wanted to know if any of the systems mentioned can be used to evaluate the environmental load if an intermediate concentration of Kathon is used in a majority of cosmetic products in the United States.

Dr. Lewis said that a model for such an evaluation could be developed. He also said that Rohm and Haas has been interested in looking at the question of antigen load. He then referred to a slide relating to the amount of antigen, based on the amount of material that is being sold into the North American market, and the amount of antigen that a population is exposed to compared to the prevalence rate. The amount of antigen exposure would be one of the factors that would influence prevalence rates, and clinic variability would be the other thing. The slide suggests that while there has been a several-fold increase in the amount of antigen in the environment, there has been little difference, from a statistical standpoint, between the points through 1985 and 1989. Also, at present use levels, there has been little change in terms of prevalence of sensitization seen in clinics, even though there has been a significant amount of antigen increase. This may be seen more easily with regard to other prevalence rates, particularly, with regard to consumer products, which would basically include the leave-on materials. While there is a slight, if any, statistically significant increase in terms of prevalence rate, there has at least been a leveling in terms of the amount of material used specifically.

Dr. Bergfeld wanted to know if the information presented suggests that there is a population that is at risk, a total body that is countable. She said that if contact allergy or irritancy is represented by a straight line, then there is a growing amount of antigen present. That would suggest that there may be only one population at risk or a limited population at

risk.

Dr. Lewis said that from the standpoint of genetics, the data suggest that there seems to be a population that is more likely to have difficulty with groups of antigens. That population will be the population that one sees routinely. Once that population has been identified, unless there is either a significant change from the way that the material is used in the environment, that is, a significant increase in concentration or some other factor, there is likely not to be a change in the prevalence rate over a significant period of time.

Dr. Bergfeld said that Dr. Lewis' other slide demonstrates that there is increased industrial exposure, and wanted to know if this is true in the United States.

Dr. Lewis indicated that the slide refers to the United States.

Dr. Bergfeld knew that increased industrial exposure was occurring in Europe, and noted that this was the first time that she had seen this data for the United States. So, environmental load in the United States is growing even greater than the consumer cosmetic load.

Dr. Lewis said that for industrial uses, environmental load is growing significantly. Many of those uses are in air handling and in many systems where the concentrations are certainly less than in some consumer products. So, yes, there is a good deal of increase there. For one of the industrial uses, in metal-working fluids, there has been no problem in terms of the degree of prevalence of sensitization in the industrial environment.

Dr. Carlton asked what level of Kathon should be recommended that could be considered safe.

Dr. Marks said that his recommendation for cosmetic use of Kathon would be less than 15 ppm. This is based on challenges involving individuals who are sensitive and, also, the preservation level in cosmetics and reactions per unit dose. Below 15 ppm and at 7.5 ppm seem to be safe levels. Based on interviews with the patients he has seen, he has concluded that there is no epidemic, because most of the patients are using Kathon in that range.

Dr. Carlton said that in terms of the study done in Denmark, he is concerned about changes in the ratio of chlorinated to non-chlorinated compounds and reactions to one or both compounds.

A representative from Rohm and Haas said that Dr. Carlton was referring to the study by Dr. Estoge. Dr. Estoge raised the question some time ago concerning what happens to Kathon simply because the ratio of the chlorinated compound to the non-chlorinated compound changes in some preparations. He referred the Panel to a copy of the letter explaining some of the problems associated with stability, particularly of the chlorinated analog, that is, the lesser stable of the two isothiazolinones. In some systems, one finds degradation of the chlorinated analog. Also, in some of these systems, the by-products have been isolated. These are primarily small organic acids that have been tested in terms of their sensitization potential. It may be that there is a nucleophile that will attack the S-N bond, open it up very quickly, and, since it is the least stable of the two species, one will find a decrease in the chlorinated component in some preparations. Certain amines will attack the S-N bond.

Dr. Hoffmann said that the chlorinated compound is the most active.

The representative from Rohm and Haas then said that in some preparations, one finds that the chlorinated analog has actually disappeared. When that happens, many of the products are no longer protected; they don't have the preservation activity.

Dr. Schroeter said that he had expected Dr. Lewis to address the Shelanski study. He was referring to the Shelanski human repeated insult patch test done by Rohm and Haas that had been found to be flawed.

A representative from Rohm and Haas said that one of the major problems was the use of non-occlusive patches. Most of the subjects were patched with other material, and he did not know the number of subjects involved or the nature of the material that the patch was made of. One of the major criticisms concerning the protocol was the scoring system. Shelanski's 4+ reaction was what the ICDRG would have classified as 1+ erythema. Shelanski identified that reaction as a 4+ reaction because his scoring system goes up to maybe a score of 7+.

Dr. Lewis said that Shelanski has a seven level grading system. The first four are essentially subdivisions of a +1 kind of reaction, which made looking at the results more complicated.

The representative from Rohm and Haas continued by saying that that was one of the reasons why the company subsequently asked the subjects to participate in the equivalent of a provocative test involving Kathon. Rohm and Haas was not satisfied that the scoring system that Shelanski was using was comparable to the classical HRIPT scoring systems that are standard for the industry.

Dr. Lewis said that the comparison included in the data submitted indicates that the ICDRG score of +1 is reasonably equivalent to Shelanski's score of +4.

Dr. Schroeter said that based on the comparison, 1+ does not equal 4+, and expressed concern about the ICDRG scoring system: ? to 1+. The ? refers to a doubtful reaction, a faint, macular erythema, and 1+ refers to erythema + infiltration, induration, and possible papules. The 1+ reaction is extremely different from the ? reaction. He said that there is much confusion associated with comparing the two scoring systems, but emphasized that the system of scoring is not the only issue. This issue should not be used as a criterion for throwing out the Shelanski study.

Dr. Berndt agreed that use of a different rating scale doesn't flaw a study.

Dr. McEwen said that as the Shelanski methodology versus what is normally done is discussed, another difference is the contact time. There is difficulty in having anything to compare the Shelanski methodology to simply because it is different. Then again, one is dealing with subjective measurements.

Dr. Berndt suggested that the Panel consider the conclusions in the Kathon document.

Dr. Hoffmann said that throughout the document, Kathon CG and Kathon 886 are mentioned and wanted to know the difference between the two.

A representative from Rohm and Haas said that both names refer to the same active ingredient. Kathon 886 refers to the industrial grade for industrial applications. It is available in liquid concentrations, and, compared to Kathon CG, it has essentially the same active ingredients in the same ratio.

Dr. Hoffmann wanted to know if he had understood correctly what had been said, specifically, that the tentative indication that the aging of chlorinated MCI in the cosmetic boiler, which limits its shelf life, is that there is some indication of reactive S-H groups.

The representative from Rohm and Haas said that that may be one of the reactants.

Dr. Hoffmann asked if the S-H groups could react in the same way that S-H groups in proteins on the skin react.

The representative from Rohm and Haas could not answer that question. He could only say that there are a number of ingredients in various cosmetic and toiletry preparations that will react with the chlorinated analogs, and had no knowledge of what the actual reaction products are. However, some of the degradation products of the material have been examined in a variety of matrices and have been found to be essentially the same, regardless of the matrix. The most significant end product is N-methyl melanomic acid. The process involves ring opening and loss of the salt and chlorine.

Dr. Berndt said that the Panel should think about conclusions.

Dr. Bergfeld said that the conclusion that Kathon should be limited to a concentration of 15 ppm in rinse-off products is reasonable and should stand. She recalled that the Panel had already stated that Kathon is unsafe for use in leave-on products, and that the Panel could review that decision. Based on the information submitted to the Panel and the presentations made, leave-on products would be safe at the upper limit concentration of 7.5 ppm Kathon. This decision is based on two issues, and not specifically on the patch test data supplied by Rohm and Haas. She noted that these data are incomplete and that further studies should be done that may be considered the larger pool of patients defining the 45 person group in the water patch test system. First of all, the decision is based on recommendations concerning the prevalence of contact dermatitis to Kathon, at 15 ppm, in this country and in Europe, specifically, that the relative risk of contact allergy and irritation is intermediate as compared to other biocides. The other important issue is environmental load to the consumer, coming from cosmetic products as well as industry. Dr. Bergfeld feels somewhat comfortable with the observation that for the consumer, risks are not growing and are somewhat at steady state, and that some type of risk group exists. These issues contributed to her suggestion that 7.5 ppm be the upper limit concentration for Kathon in leave-on products. She noted that even if the problem of contact dermatitis has been solved, the problem surrounding the activity level, meaning genotoxicity and carcinogenicity, is yet to

be resolved.

Dr. Hoffmann said that his point of view that Kathon CG, especially the chlorinated Kathon product, is highly reactive is supported by a very recent study that has been discussed by Dr. Estoge, who has products on the market in Denmark. According to this study, 1990 publication in *Contact Dermatitis,* even in products, the chlorinated isothiazolinones react with other ingredients that are present. Dr. Hoffmann noted that the major cosmetic ingredients are inert, but is still concerned about how easily Kathon reacts with proteins and with the skin. He added that the slide presented by Dr. Lewis indicates that sensitivity increases with industrial exposure. The manner in which the population that is susceptible to Kathon will grow is not known.

Dr. Bergfeld asked for Dr. Hoffmann's opinion concerning the potential genotoxicity and carcinogenicity of Kathon, in light of its chemical and biological activity.

Dr. Hoffmann said that the carcinogenicity test supplied by Rohm and Haas does not meet the standards of the National Toxicology Program. Whatever the finding, NTP will not accept this study because of the limited number of mice involved and because of the solvent used. Their methodology is based on what the federal government has decided to do with respect to carcinogenicity testing. The carcinogenicity test supplied by Rohm and Haas was negative, however, it was not done as it would be done by NTP today.

Dr. Bergfeld asked Dr. Hoffmann if he would suggest that because Kathon is highly reactive, the carcinogenicity test should be repeated using the guidelines established by NTP.

Dr. Hoffmann said that if Kathon were found to be non-carcinogenic, his conscience would be clear.

Dr. Bergfeld wanted to confirm that the other statement made by Dr. Hoffmann was that Kathon is so active that he is not worried about genotoxicity, but is worried about contact dermatitis.

Dr. Hoffmann agreed that Dr. Bergfeld had quoted him accurately.

Dr. Bergfeld also said that Dr. Hoffmann had suggested from the graph that the population that is sensitive to Kathon is growing exponentially.

Dr. Hoffmann agreed.

Dr. Bergfeld said that she did not interpret the graph that way and asked Dr. Lewis to discuss the graph again.

Dr. Lewis said that the population certainly was growing several fold relative to the prevalence rate.

Dr. Hoffmann said that the population that was sensitive had doubled between the years 1985 to 1989.

Dr. Lewis agreed with Dr. Hoffmann.

Dr. Berndt asked for a copy of the graph for the record.

Dr. Tom Slaga, with University of Texas M.D. Anderson Cancer Center, made statements on carcinogenicity: Although NTP does have regulations for dietary relationships of different chemicals to see if they cause cancer, in terms of dermal relationships, they are only setting up guidelines now. Most of the studies that are done with dermal applications of chemicals are done using only one sex because there are really no data to support that we have to use two sexes, in that there is really no sex difference of any magnitude by any chemical on the skin. So, in this particular study, long-term use, 40 males are tested with a very high level of Kathon, 400 ppm. I think that it is true, speaking in terms of needing at least a high dose level, that there were not multiple doses, but if they were given another dose, it probably would have been a lower dose. The high dose was totally negative. Concerning the review committee that I would have gone to to review this data, Dr. Henry Peto of McArdle Laboratories and Dr. Steve Hecht of the American Health Foundation, we all agreed that they did use enough animals to determine if it was carcinogenic, because they did use a dose-finding range, coming up with this particular high dose. So, I think the data leading up to it was very strong in terms of picking an adequate dose to perceive as carcinogenic. We did have some concerns in terms of the solvent, and we asked Rohm and Haas if they had additional data on absorption, which they did supply. That particular committee was actually very impressed with the absorption studies that they did comparing water, water and acetone, and acetone as solvent combinations. Water was an adequate solvent to be used in this particular case. It did penetrate the skin, and penetrated basically as well as the acetone and water. It did, in the long-term studies, lead to some inflammation

and hyperplasia, suggesting that it did have some effect on the skin. This was a concern of one of the Panel members last time, Dr. Boutwell, but it is my understanding that he was convinced with the additional data that I presented. I had done some studies with water soluble compounds and found that they do penetrate the skin. Water is an adequate solvent. Although Dr. Hoffmann may think that this does not meet the NTP standards, there are really no actual standards for dermal application of compounds. They are coming up with that.

Dr. Hoffmann questioned the sizes of the animal groups.

Dr. Slaga said that if one uses one particular sex, 40 or 50 animals are usually sufficient in any study.

Dr. Hoffmann said that males are not used in skin carcinogenicity studies because there is a high tendency for them to bite and scratch their skin.

Dr. Slaga stated that the animals were individualized, one per cage.

Dr. Hoffmann asked Dr. Slaga if he would have designed the carcinogenicity study differently.

Dr. Slaga said that Kathon is reactive. However, considering that it was administered to the skin three times per week for 30 months, if the reactivity were doing something to the skin that could lead to a cancer or toxicity, these effects would have been seen. If Kathon had caused cancer, the next step would have been to determine if it reacted with genetic material or proteins. A binding study would then have been set up to see if there was any covalent binding and if it were more of a genotoxin, tumor initiator, or tumor promoter. Since the data indicate that it does not cause cancer of the skin, binding does not seem to be relevant to carcinogenicity.

Dr. Hoffmann quoted from the minutes a statement that was made by Dr. Boutwell: "One cannot say that Kathon is not carcinogenic. However, one can say that it did not produce tumors."

Dr. Slaga agreed with Dr. Boutwell's quote. He also said that one could say that about any particular chemical. One has to come up with the best possible test relationship and dose range for the compound, as NTP does in determining its two doses for feeding studies.

Even after those studies have been done, one cannot say that if the results are negative, Kathon cannot cause cancer under some other kinds of conditions.

Dr. Bergfeld asked Dr. Slaga if the dose-finding and absorption studies had been submitted to the Panel for inclusion in the report.

Dr. Slaga said that that information had been given to the Panel, and Dr. Berndt concurred.

Dr. Schroeter wanted to know if Dr. Hoffmann's opinion had been changed regarding the necessity of more carcinogenicity data.

Dr. Hoffmann said that if a proper carcinogenicity test were designed and done by an outside group, NTP, or an advisory group to NTP, and the test turned out to be negative, his conscience would be clear. He emphasized that Kathon is highly reactive and that without the proper bioassay, he could not conclude that it is a non-genotoxic agent.

Dr. Shank said that he had reviewed the raw data from the carcinogenicity study. His first concern was the low animal numbers and that the time limit was long. A time period of 2 to 2.5 years is a very long study for a mouse. However, he concluded that the study is a valid bioassay for skin carcinogenicity. He does not have any concern over it being genotoxic, and does not think that another carcinogenicity study is needed.

Concerning Dr. Elder's draft of the analysis of comments, Dr. Schroeter noted that several studies are mentioned. Specifically, he had reviewed summaries for two of the studies, but was unable to locate the raw data. He asked if anyone in the audience could elaborate on those particular studies.

Lincoln Crockmall, board certified dermatologist and Vice President of Dermatology Research and Development for Bristol Meyers - Squibb and for the Westwood Dermatological Division, responded to Dr. Schroeter's question: These studies that summarize a total experience of 2,335 patients or volunteers were performed under the auspices of Westwood Pharmaceuticals for the purpose of building a data base such that the true incidence and the true concern for Kathon CG is understood. These studies, in total, represent a number of independent RIPT studies that were performed by a number of investigators across the country at different sites and at different times during the year. If

one looks at the totality, there were approximately 25 different products, final formulations, that were tested on volunteers. Concerning the 25 formulations, when one looks at the number of volunteers who had initial reactions during induction stages and were then challenged with the final formulations, at the end of the study, these final formulations contained 7.5 ppm. The total number of volunteers that reacted was 31, which gave an incidence of 1.3% with final formulations containing no more than 7.5 ppm Kathon. So, the two studies that Dr. Schroeter was referring to were included as part of the independent RIPT's. I think that the crucial point to focus on here is the summation, rather than any particular study, because one could have selected the study involving 216 people, where there are absolutely no reactions. On the other hand, one could have selected the study where there were, at worst case scenario, I think, 13 or 14 reactions. But, if one looks at the totality of it, the different times of the year, the different investigators, and the different environments in which the studies were performed, then I think that one is able to conclude that based on the 1.3% incidence in the United States with products that are formulated for this population, Kathon is considered to be a safe preservative. That gives us the ability to choose various preservatives, whether in combination or singly, that will not only give micropreservative purity to the final product, but there will also be safe and routine usage in the general population.

Dr. Gibson, Director for Clinical Research at Westwood Department of Bristol Meyers - Squibb, the department that oversaw the independent RIPT studies, made comments: I think it would also be fair to specifically address the question as given. Very specifically, in response to Dr. Schroeter's question, I can speak to one of these studies, which was, I think, the group of 212 patients where there were 14 positives. I did review this and, essentially, when one looks closer one finds, first of all, that there was no proof that Kathon was implicated in all of these anyway. Secondly, of the 11 people who were rechallenged, only two actually gave an indication that it may be relevant. If one focuses on the data, one finds that in the end, there was only one in which it was probably relevant to Kathon. In other words, out of the group of 212 with 14 positives, there is essentially one, or, at most, two who are probably positive on rechallenge with Kathon. Although I did not have a chance to

review the other study in as much detail, one will find a similar situation. First of all, in this circumstance, one has got to screen out all of those reactions that may not be due to Kathon, and then when one physically looks at the numbers who respond when rechallenged, they are actually even smaller than the 1.3%. As for what Lincoln Crockmall was talking about, 1.3%, one is really looking at all those who reacted at all, not absolutely probably all of those who reacted to Kathon.

Dr. Crockmall said that the 1.3% represents the total number of responders who reacted, on rechallenge, to the final formulation. Those patients were not then taken to the next step of having every single ingredient in the formulation tested. So, the worst scenario is 1.3%. He believes that if one had gone to the individual ingredients, at the levels in which they are found in the final product, one would have indeed found the incidence to have been even less.

Dr. Elder said that in the report, it was concluded that Kathon could neither be implicated nor ruled out. He commented that what he referred to as "clustering" was observed in the new studies, in the work done in France, and in Dr. DeGroot's data. This observation is, in fact, another area that cannot be explained. For the record, he explained that when the CIR staff does an analysis, that document does not exist until it is edited by the Panel. The document being reviewed is only a draft and does not exist until the Panel accepts it.

Dr. Carlton wanted to know if material is available concerning what was presented by Drs. Gibson and Crockmall, specifically, the analysis.

Dr. Gibson said that the analysis was not included in the materials that he had submitted, but that it had been included as a summary.

Dr. Elder quoted from the summary as follows: "Of the 31 subjects experiencing sensitization type reactions, one was rechallenged with Kathon CG at 7.5 ppm in water with no evidence of allergic reaction; none of the others was rechallenged with Kathon CG. Therefore, Kathon CG can be neither implicated nor ruled out as a cause of the allergic reactions."

Dr. Crockmall said that the subjects were not patch tested specifically with a single ingredient. They were patch tested with the final formulation containing 7.5 ppm Kathon CG. So, they were exposed in the patch test to Kathon, but it was in the final formulation.

Dr. Schroeter said that the issue was that a challenge patch test at even 50 ppm or 100 ppm had not been done.

Dr. Gibson said that he had gotten up to specifically answer Dr. Schroeter's question about the facial moisturizer study involving 212 subjects.

Dr. Schroeter said that there were two studies.

Dr. Berndt said that there were two panels that contained 70.0% of the reactors out of the 2,000 plus subjects.

Dr. Schroeter said that one panel contained 223 subjects and the other panel contained 212 subjects. He also said that Dr. Gibson had focused on the study involving the 212 subjects.

Dr. Crockmall said that of the 223 subjects, there was a total of another ten subjects. These ten subjects were once again patch tested with the final formulation that contained 7.5 ppm. They were not patch tested specifically with the single ingredients. Assuming that all of the ten were reactive to Kathon is the worst scenario, one has to look at the entirety, because there are other panels with absolutely no reactors. If one recalls the comparison of the Maibach data versus the New York data presented by Dr. Lewis, there are many reactions that one has a difficult time explaining and, on the other hand, there are no reactions. The important finding in the data is the fact that on rechallenge with 15 ppm or 7 ppm, what would be in the marketed products in this country, there were no reactions. With 100 ppm Kathon, which would be an abnormal concentration because no one has been exposed to a marketed product with 100 ppm or even 50 ppm, there was a very low incidence of sensitization.

Dr. McEwen said that if one particularly addresses the concept of "clustering" that Dr. Elder introduced, one should realize that 25 separate formulations are being dealt with, all of which contain the same amount of Kathon. For only two of the formulations, there has been an excess rate of allergenic types of reactions. This suggests that in those two

formulations, something other than Kathon is causing the response. So, it is not a matter of "clustering" meaning a genetic predisposition to Kathon or anything else. It just happens to be that there are two formulations that should not be marketed. Those formulations should be changed.

Dr. Berndt said that it is assumed that those formulations were analyzed for Kathon and that they contained 7.5 ppm and not something else.

Dr. Carlton wanted to know what else it could possibly be, in terms of the biocide, that was a possible sensitizer.

Dr. Crockmall said that some of the products were sunscreens, and particular volunteers who were patch test negative to Kathon turned out to be positive to the final formulations. So, there may have been other ingredients, both active and inactive, that could have been sources of the reaction. The sources of the reaction have not all been pursued. That is why Kathon was not ruled out, but, the worst scenario is to say that Kathon caused the problem. The absolute worst case is what is considered to be a very low incidence of sensitization, compared to other preservatives.

Dr. Schroeter said that although one clusters groups of studies like this, one must realize that there is some scientific error, in that the population groups may be different. That would stem from suggestion of the fact that one of the two studies involved anti-acne preparations, meaning injured skin. In injured skin, the barrier function is altered so that there is greater absorption and, possibly, a better exposure to the human system. He mentioned that the other product was a dry skin lotion/sunscreen and that he did not know what type of population was involved in the testing of this product. It may be very pertinent to the Panel's final conclusion that individuals be warned regarding use of the type of product that contains a biocide which may make them more vulnerable to hypersensitization.

Dr. Bergfeld said that such information should be included in the discussion.

Dr. Schroeter asked that his point be verified in a more detailed way regarding the populations in the two studies.

Dr. Gibson made a point regarding the dry skin lotion with sunscreen: Because we are talking about "clustering", I want to go back to the point that in any range of studies, as we

discussed, out of 220 subjects, one may find a study where there is zero or one may find a study in which there are 10 to 20. I think that it is the 10 to 20 range that is being addressed. I don't think that the term "clustering" has any relevance specifically to Kathon. At this moment, we are talking about a clustered group of positive reactions to which we are not at all sure that they are relevant to Kathon. Therefore, if we are talking about clustering, we should relate it to the subjects that we are specifically addressing. For instance, with regard to that study, 13 subjects experienced reactions that were initially interpreted as possible allergic sensitization. Each of the thirteen was rechallenged. Only two, actually upon that rechallenge, exhibited what one could be led to believe was allergic sensitization. Of the two, only one reaction was due to Kathon. Therefore, "clustering" is a misnomer. It is an aggregation towards a term that I believe is actually quite inappropriate and is leading us in the wrong direction.

Dr. Elder said that he chose to use the term "clustering" rather than the term statistically significant. "I don't think that there is anybody in this room who wants me to say a statistically significant number of positive responders." The term clustering was used to avoid overinterpretation of the data.

Dr. Gibson said that what he had said was not by any means a criticism of the term "clustering".

Dr. Elder said that the numbers are statistically significant, but that he had chosen not to use that terminology.

Dr. Gibson said that Dr. Elder had made a good point.

Dr. Schroeter still wanted to know if the patients in the study had injured skin.

Dr. Berndt said that the formulation was one that would be used in an anti-acne preparation. This does not imply that patients were involved in the study. Normal subjects were involved.

Dr. Crockmall said that the design was the routine inclusion of normal volunteers, as is a standard in industry for patch testing. The products that were applied included a whole range of products, dry skin lotions to acne medications to sunscreens, 25 different types. The only common thread that links all of these products is the fact that they all contain 7.5 ppm

Kathon. That is the reason why they were selected. The large data base of over 2,000 volunteers is a substantial amount of work. The conclusion for all of these volunteers is to try and understand, from a multicenter point of view, the likelihood that these products are going to induce sensitization.

Dr. Berndt said that Dr. Bergfeld had stated motions earlier. The first motion was that the limitation on the rinse-off products not exceed 15 ppm Kathon. Dr. Berndt noted that the original conclusion on rinse-off products was being reaffirmed.

With the exception of Dr. Hoffmann, all Panel members were in favor of the motion.

Dr. Berndt said that the second motion was to say that these chemicals are safe for use in leave-on products at concentrations not to exceed 7.5 ppm. He then asked for any further discussion.

Dr. Schroeter thought that the Panel should reiterate the fact that this is not a scientific decision. This is a purely judgemental decision on the part of the Panel.

Dr. Bergfeld said that the decision is partially scientific.

Dr. Schroeter said that the decision is judgemental based on the scientific data available to the Panel. It is very flawed in terms of other decisions that have been made by the Panel. He had significant reservations regarding the decision.

Dr. Bergfeld had reservations as well regarding setting a limit as to what may be considered safe, realizing that biocides, because of their chemical reactivity, are sensitizers. However, she noted that Kathon falls into the intermediate range of sensitivity in a population. She said that a discussion should be included in the document that will address some of the issues that the Panel had discussed: patch test information and what can be summarized from it; the environmental load, which would include the industrial versus consumer load; the possibility that as antigen load grows sensitization may increase; that there may be a definite population at risk, and, if necessary, a statement indicating that it is a highly active ingredient, even when in the final product, and that it needs to be continually monitored.

Dr. McEwen wanted to know if Dr. Schroeter's concern would be addressed if industry, really the supplier, were willing to report back to the Panel on data acquired yearly, the

ongoing review being done with the North American Contact Dermatitis Group, and on the other multicenter study so that the Panel, on a yearly basis, would have a review of the prevalence of sensitization. He sensed that Dr. Schroeter's concern was that of the possibility of a problem with Kathon in the future.

Dr. Schroeter said that he was sure that the proposal would be accepted by the industry source, Rohm and Haas. But, based on his experience with phase 4 FDA studies, motivation seems to die after the decision to market has been made and the product is safe. Even after the issue of other products that have been brought into question or when they have been put on the market, the company is motivated by legal concern that can drag on and on without a decision. He emphasized that he has significant reservations about the proposal.

Dr. McEwen said that his reason for introducing the proposal is that the Panel would have a chance to file an addendum to the report. The addendum to the report would be issued on the basis of not having received the data that had been promised, if nothing else.

Dr. Berndt thought that Dr. McEwen's proposal was a very good idea. If, as Dr. Schroeter suggests, there is a possibility that the monitoring that is done is flawed, the Panel will probably be able to determine that.

Dr. Bergfeld was also in favor of the proposal. She would also agree if, according to guidelines, the Panel could make a recommendation that information on Kathon be submitted every two years for review.

Dr. Schroeter wanted to discuss qualifiers, because he feels that leave-on products are considerably at more risk than rinse-off products. He noted that Kathon is an unusually active compound that binds to the skin and has an exceedingly longer half life, skin half life, than what he had expected.

Dr. Elder said that the half life is 13.1 days after it is absorbed from the skin.

Dr. Schroeter said that the half-life is exceedingly long, and, therefore, accumulation of bound product is important. He realizes that the studies that had been done to determine sensitization were attempting to dilute that particular fact out. In light of that, he would like to see a caveat included, stating that leave-on products not be used other than for cosmetic

purposes and not on injured skin. He noted that with injured skin, binding is of concern and, also, the percutaneous absorption factor exceeds that of normal skin.

A representative from Rohm and Haas made a statement about binding to the skin and half-life: We searched the literature in order to put this into perspective with other biocides. There is very little, if any, data in the literature about binding at all. We have some of the only data that is available. One piece of data we did find was on formaldehyde, and the half-life was approximately nine days. So, it is not significantly different.

Dr. Schroeter said that he did not think that Kathon could be compared to another ingredient. It has a long binding period and is applied to the skin daily. The cumulative effect is not known.

Dr. Berndt asked if Dr. Schroeter was making a motion to amend.

Dr. Schroeter said that he was not making a motion to amend, but wanted his comments to be included in the discussion.

A representative from Rohm and Haas said that if one looks at the RIPT design, it is probably a worse case scenario than even damaged skin because one is applying something repeatedly and under occlusion, whereas, the products containing Kathon are going to be used under clothing, but not under the tightness and continuous tightness of the bandage that is used in the RIPT. Even with that kind of worst case scenario, one's overall sensitivity is extremely low.

Dr. Schroeter disagreed. He said that the representative was speaking of a small surface area while he was referring to a large surface area of injured skin. He added that products containing Kathon are going to be used on injured skin unless the public is made aware of these products.

Dr. Bergfeld opposed what Dr. Schroeter was saying. She mentioned that the Panel had never stated in any of its conclusions relating to comparable biocides, such as formaldehyde releasers, that the ingredient not be used other than for cosmetic purposes and not on injured skin.

Dr. Schroeter recalled having said that the caveat should be included in the discussion, not in the conclusion. He also said that he did not want to amend the motion.

Dr. Bergfeld said that it should be mentioned in the discussion that it appears that there are certain patient populations at risk, which might include those with dry skin, the atopic, and those with dermatitic skin, etc. A global statement should be made, in the discussion and not in the conclusion, indicating that Kathon is an intermediate range sensitizer and that individuals who have damaged skin might have a higher risk for sensitization.

Dr. McEwen said that he does not think that the Panel would make the blanket statement that there is no formulation that can be used on some "damaged skin", whatever that definition is, that would not be perfectly safe. If the discussion is developed according to Dr. Bergfeld's suggestions, then the formulator will be made aware of the Panel's concerns.

Dr. Elder reminded the Panel that a Tentative Report indicating that Kathon is safe for use in rinse-off products at concentrations not to exceed 15 ppm is on the floor. This decision was discussed at the last Panel meeting. He noted that no data were submitted in response to that decision, and that the decision had been reaffirmed by the Panel. He explained that the Panel had arrived at a second motion and was making a major change, meaning the request for a new discussion on leave-on products. There will now be a new Tentative Final Report because substantial changes will be made in the discussion. The Tentative Final Report will again have to be announced to the public, and there will be a 90-day comment period. One comment on this document was received, and is in agreement with the one submitted previously.

Dr. Berndt said that there had been a motion to approve Kathon at a limit not to exceed 7.5 ppm in leave-on products.

Dr. Schroeter said that the statements made by Drs. Bergfeld and McEwen did not differ greatly from his. He emphasized that he had only extended the explanations that they had given.

Dr. Berndt said that Dr. Schroeter's concerns would be included in the discussion.

Dr. Elder said that there would be two discussions. There are two conclusions. The Panel reaffirmed one and changed the other.

Dr. Berndt said that both issues would be addressed in the discussion. However, whether there is a single discussion or there are two, both issues will be discussed.

Dr. Elder said that he obviously had not made his point. During the review process an analysis of the comments received on an ingredient must be done. The comments received are either accepted or rejected. He also said that the Panel had mentioned a discussion of opinions on the conclusion, and did not know how a discussion on Kathon in leave-on products that does not apply to Kathon in rinse-off products could be written. He then asked if one discussion and one analysis of comments could be written.

Dr. Bergfeld asked if concentration limits could be voted on. She said that after the vote, the Panel could address Dr. Elder's question. There may be some Panel members who feel that the discussion is applicable to both concentrations and both types of products.

The motion is that Methylisothiazolinone and Methylchloroisothiazolinone are safe for use in leave-on products at concentrations not to exceed 7.5 ppm.

With the exception of Dr. Hoffmann, all were in favor of the motion.

Dr. Berndt wanted to know the matters concerning the discussion that should be brought up, and also asked the Panel to give guidance to those who will be writing the discussion.

Dr. Elder said that there is only a partial submission of data on Kathon at 7.5 ppm. As he understood, Dr. Bergfeld wanted the justification for changing from 0 to 7.5 ppm to be based on the following: the new North American Contact Dermatitis data; that the load was increasing, but not the consumer experience; and that there was some data at 7.5 ppm which indicated that there wasn't a problem. These are the three reasons that explain why the Panel changed its mind.

Dr. Berndt said that, in terms of the discussion, there is a section concerning definitions of rinse-offs and leave-ons that can be deleted. The position of the Panel with respect to rinse-off and leave-on products has already been addressed. Therefore, the submission of the definition by Rohm and Haas is no longer relevant or necessary, since the Panel agreed that the information supplied by industry showed that what the Panel has done in the past is consistent with industry's description of a rinse-off product.

Dr. Bergfeld said that the Panel could include definitions of rinse-off and leave-on products in the discussion, if necessary. The Panel might mention limiting concentrations in

rinse-off products, and brief, discontinuous exposure, as was done in the report on Sodium Lauryl Sulfate. Then, the Panel could clarify its position concerning leave-on products as of 1990.

Dr. Berndt said that the material concerning the definitions is not relevant. He agreed that the Panel may include the statement on brief, discontinuous use followed by rinsing as a parenthetical expression after the words rinse-off. This is what the Panel agreed to.

Dr. Elder said that according to the CIR Procedures, CIR has to respond to the comments submitted by Rohm and Haas. The document must inform Rohm and Haas that their comment on leave-on products was received and indicate the Panel's response to the comment

Dr. Berndt asked if the minutes are adequate for doing that.

Dr. Elder said that the analysis of the comments must appear in the report.

Dr. Berndt agreed and then asked if there was any other information that needed to be included in the discussion.

Dr. Hoffmann wanted to know if the fact that the Panel had changed its mind will be included in the document, specifically, that the Panel voted against leave-on products at the last Panel meeting and is now saying that Kathon can be used in leave-on products at concentrations up to 7.5 ppm.

Dr. Bergfeld said that the reasons why the decision was reversed will be included in the discussion.

Dr. Berndt confirmed the fact that the decision was changed and that the reasons, which were stated earlier, will be included in the discussion.

Dr. McEwen wanted to know if when the document is published, whether the different thoughts that were discussed and how the Panel arrived at the final decision would be included.

Dr Elder explained that after announcing a Tentative Final Report, the Panel now has changed its mind. The Panel, after receiving new information and reconsidering various points, changed the conclusion for leave-on products. A new document with these changes will then be announced to the public for a 90-day comment period.

Dr. Bergfeld said that, as recorded in the minutes, the Panel has the understanding that those individuals who are suppliers and users of Kathon will report back to the Panel within a two-year period, monitoring the status of sensitization reactions to Kathon.

Dr. Lewis clarified that the Panel was talking about monitoring prevalence studies and said that he would have no problem doing that.

Dr. McEwen confirmed that he would get a written letter from Dr. Lewis, to the effect that every two years he would be in contact with the Panel.

The Panel concurred with Dr. McEwen's approach.

Dr. Hoffmann confirmed that the fact that the Panel would receive the results from industry every two years would be in the minutes.

Dr. Elder indicated that industry would respond to the Panel in writing.

Dr. Berndt said that Dr. Elder has the material that will be included in the discussion. There will be a mail review of this material, since it is being significantly changed. He then asked if there were any more issues with respect to Kathon.

Dr. Elder said that, as a point of order to the guests, he would estimate that it will take two months to rewrite the document and send it to members of the Panel for their approval. Subsequently, the document will be announced for a 90-day comment period. Industry will be notified when the document has been completed. No one may obtain this document until the Panel agrees that it can be released.

Hydroxybenzomorpholine

Dr. Bergfeld stated that the Panel had requested impurities data concerning the presence of nitrosamines and, also, a 28-day dermal toxicity study. She also noted that the Panel had received percutaneous absorption data and a 28-day oral toxicity study involving rats, but had not received data on impurities. The Bergfeld Team had discussed the two studies received and decided that these studies could provide information that would dissuade the Team from further requesting the 28-day dermal skin test. Additionally, Dr. Bergfeld's Team concluded that at concentrations of use, Hydroxybenzomorpholine is safe.

Amended Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: May 10, 2019
Panel Meeting Date: June 6-7, 2019

The 2019 Cosmetic Ingredient Review Expert Panel 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.; Ronald A. Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

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INTRODUCTION

This safety assessment is on the combination of Methylchloroisothiazolinone (MCI) and Methylisothiazolinone (MI) as used in cosmetics. In 1992, the original report on MCI/MI was published was published by the Cosmetic Ingredient Review (CIR), and the Expert Panel (Panel) concluded that this mixture 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." [1] The stated safe-for-use concentration refers to a mixture containing 76.7% MCI and 23.3% MI (roughly, 3:1). According to its Procedures, the CIR evaluates the conclusions of previously-issued reports. The Panel determined that this safety assessment should be re-opened to reassess the conclusion based on the numerous sensitization studies and reports that have been published since 1992.

While defined as separate ingredients that function as preservatives in cosmetics in the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*),[2] MCI is only known to be used in concert with MI. This safety assessment does not directly address the safety of the cosmetic use of either ingredient alone; however in 2014, the Panel assessed the safety of MI, when formulated without MCI, and concluded that MI is safe for use in rinse-off cosmetic products at concentrations up to 100 ppm and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a QRA.[3]

CHEMISTRY

Definition

Methylchloroisothiazolinone (CAS No. 26172-55-4) is the heterocyclic organic ingredient that conforms to the following structure:[2]

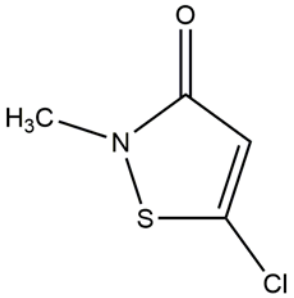


Figure 1. Methylchloroisothiazolinone

Methylisothiazolinone (CAS No. 2682-20-4) is the heterocyclic organic ingredient that conforms to the following structure:[2]

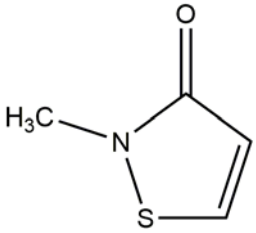


Figure 2. Methylisothiazolinone

Physical Properties

MCI/MI is readily miscible in water, lower alcohols, glycols, and other hydrophilic organic solvents.[1] This mixture is a clear, light amber liquid with a specific gravity of 1.19 (20 ºC), a pH of 3.5 (as supplied), and a freezing point of -18 to -21.5 ºC.

Impurities

Dimethylnitrosamine was reported to be formed as a reaction by-product at very low concentrations.[1] To limit the presence of this impurity, methyl-3-mercaptopropionate is added during production.

USE

Cosmetic

The safety of the cosmetic ingredients included 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 these ingredients 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. Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2019 VCRP survey data, MCI and MI are reported separately and not as a mixture.[4] The total number of uses reported for MCI is 5137; 480 of these are in leave-on products (Table 1). MI has 6037 reported uses; 1042 of these are in leave-on products. The uses have increased significantly since the original report on MCI/MI was published: in 1986, the total number of uses reported for the ingredient mixture was 381.[1] In 2019, the Council reported that MCI/MI (3:1) is used at up to 7.5 ppm in leave-on products and at up to 15 ppm in rinse-off products.[5] In the original report, concentration of use was reported as a range; the concentration of use range for MCI/MI in both leave-on and rinse-off products was reported to be <0.1-1%. (<1000-10,000 ppm)[1]

MCI and MI may be used in products that can be incidentally ingested or come into contact with mucous membranes; for example, there are uses reported in lipsticks (reported in the VCRP only; concentration not reported), bath preparations (0.000019 ppm), and bath soaps and detergents (up to 15 ppm).[4] Additionally, some ingredients have been reported to be used in products that may come into contact with the eyes; for example, these ingredients are reported to be used in eye makeup preparations (reported in the VCRP only; concentration not reported). Moreover, some ingredients have been reported to be used in spray and powder products that could possibly be inhaled; for example, MCI and MI are reported to be used in colognes (0.075 ppm), hair sprays (7.5 ppm), and face powders (reported in the VCRP only; concentration not reported). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm compared with pump spray.[6-9] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[6,7] Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[10-12]

In the European Union, MCI/MI is listed as a preservative in Annex V that is limited to a maximum concentration of 0.0015% (i.e. 15 ppm) in rinse-off products as a 3:1 ratio of MCI:MI.[13] The Scientific Committee on Consumer Safety (SCCS) in 2009 noted that MCI/MI is a well-recognized skin sensitizer at current conditions of use and concentration. The SCCS concluded that MCI/MI in a ratio of 3:1 does not pose a risk to the health of the consumer when used as a preservative at a maximum concentration of 0.0015 % in rinse-off cosmetic products, apart from its sensitizing potential.[14] Induction and elicitation were considered less likely in a rinse-off product than when the same concentration is present in a leave-on product.

Non-Cosmetic

MCI/MI (3:1) has been determined to be safe for use in indirect food additives as adhesive, coating, and paper and paperboard components only as an antimicrobial agent or a slimicide (21CFR §175.105, §175.300, §175.320, §176.170, and §176.300).

MCI/MI is reported to be used in water-based wall paints.[15] Analysis of 60 paint samples found the concentration of MCI to range from 0.5 to 3.5 ppm while the concentration of MI ranged from 1.1 to 142.7 ppm.

TOXICOKINETICS

MCI/MI was absorbed after oral administration and then was excreted in the urine or feces; storage in the tissues was minimal. Up to 62% of a single percutaneous dose was bound to the site of application 24 hours after exposure. The MCI/MI-CG bound to the skin had a 13.1 -day half-life.[1]

In an oral metabolism study in humans, four volunteers received 2 mg of labelled 3-[^{13}C]-MI or 3-[^{3}H]-MCI (16.3 and 13 µMol, respectively) in 200 µL of ethanol in a glass of water separately and at least 2 weeks apart.[16] Over a 48-h period, consecutive and complete urine samples were collected examined for the content of *N*-methylmalonamic acid (NMMA). NMMA represented 23.7% and 13.3% of the dose excreted in urine after exposure of MI and MCI, respectively, with more than 90% excreted within the first 24 h. Excretion of NMMA was rapid with mean half-lives of 6.1 h and 7.6 h for MI and MCI, respectively.

TOXICOLOGICAL STUDIES

MCI/MI was moderately to highly toxic to rats, and highly toxic to rabbits when administered orally, and moderately toxic when applied dermally.[1] No treatment-related effects were observed in rats which received MI/MCI in oral doses up to 24.4 mg/kg/day for 2 weeks. Doses of MI/MCI up to 2.8 mg/kg/day applied dermally to rabbits, 5 days per week for 3 weeks, produced moderate irritation at the application site but no systemic toxicity. Dermal application of MI/MCI at doses up to 0.4 mg/kg/day for 3 months produced no systemic toxicity in rabbits. No toxicologically significant treatment-related effects were observed in rats or dogs at doses up to 30 and 28 mg/kg/day, respectively.

Short Term Toxicity Studies

In a 28-day repeated oral dose study, male and female rats received MCI/MI (1.3%: 0.38%) diluted in corn oil via gavage at 0, 0.26, 0.78, 2.3 and 7.0 mg/kg body weight per day.[17] Water and feed consumption were monitored during the dosing period. At study end, the rats were killed, organs were weighed, and histological examinations were performed. Hematology, serum clinical chemistry, and biomarkers of inflammation were also assessed. No treatment-related effects on weight gains, organ weight, or hematological parameters were observed. A reduction of serum triglyceride levels in males and induction of hepatic phase 1 xenobiotic metabolizing enzymes in females with subtle histological changes in the liver were observed in the 7.0 mg/kg dose group. The authors stated that these changes were likely an adaptive, reversible response. The lowest observed effect level (LOEL) was determined to be 7.0 mg/kg body weight/day.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

MCI/MI administered by gavage to pregnant rabbits (gestation days 6 through 18) at doses up to 13.3 mg/kg/day was toxic to the dam, embryo, and fetus; the compound was not teratogenic.[1] In pregnant rats (gestation days 5 through 15) that received MCI/MI at doses up to 15 mg/kg/day, toxicity was observed in the dams, but no treatment-related effects were noted in any of the reproductive parameters of the surviving dams and fetuses and no teratogenicity was observed.

GENOTOXICITY

The result of genotoxicity testing of MCI/MI varied with the assay used.[1] Mutagenicity was observed without metabolic activation in several Ames tests, but no genotoxicity was observed in several in vitro mammalian cell assays. Results were mixed in a mouse lymphoma cell assay, with genotoxicity observed when there was no metabolic activation.

The mutagenicity of MCI/MI and five cosmetic products that contained MCI/MI was studied in an Ames test using *Salmonella typhimurium* strain TA 100, with and without metabolic activation.[18] The cosmetic products were diluted in distilled deionized water and tested at up to 400 µl/plate; MCI/MI was tested at up to 50 ml/plate. Three of the five products were direct acting mutagens, while the other two were too cytotoxic to determine mutagenicity. Metabolic activation reduced cytotoxicity, but did not eliminate mutagenicity. Mutagenicity was also observed with MCI/MI, with and without metabolic activation, in a dose-dependent manner.

CARCINOGENICITY

Dermal application of 400 ppm of 2.67% MCI/MI in distilled water, 3 times per week for 30 months, had no local or systemic tumorigenic effect in male mice.[1]

DERMAL IRRITATION AND SENSITIZATION

The dermal irritation of MCI/MI was concentration-dependent in rabbits under occlusive patches, with 560 ppm being non-irritating, 2800 ppm being moderately irritating, and 5600 ppm being severely irritating.[1] In humans, MCI/MI was irritating in a dose-dependent manner, with 100 ppm essentially nonirritating, 200 ppm slightly irritating, and 400-800 ppm strongly irritating. MI/MCI is a sensitizer: the concentration of MCI/MI in cosmetic products which produced sensitization varies. The available human sensitization test data at concentrations of 50 ppm and above gave mixed results. MCI/MI was not a sensitizer at a concentration of 15 ppm.

Human

A repeated open application test (ROAT) was performed on 15 patients with known contact allergy to 100 ppm MCI/MI and/or MI (6 patients reacted to MCI/MI only, 6 patients reacted to MI only, and 3 patients reacted to both MCI/MI and MI).[19] Each patient was given two sets of aqueous skin creams. One cream contained MI at 100 ppm while the other contained paraben preservatives. The patients applied the creams twice daily for 2 weeks to the outer aspect of the upper arm on an area of 25 cm^2. The sites were evaluated by dermatologists prior to the ROAT commencement and after 1 and 2 weeks. Eight patients had a positive allergic responses at the test sites that received the MI-containing creams. Of the patients with the known MI allergy, five had positive responses. Of the patients with the known MCI/MI allergy, six had positive responses.

In a human repeated insult patch test (HRIPT) of a hand wash containing 12 ppm MCI/MI in 50 volunteers, no adverse effects were observed during the study and no irritation or sensitization was observed during induction or challenge.[20] The volunteers received 0.2 ml directly on the back as open patches on an area of approximately 2 cm^2.

PHOTOTOXICITY

MCI/MI was not a photosensitizer at a concentration of 15 ppm in human volunteers. [1]

Photopatch tests, baseline series patch tests, and/or photo-tests were performed on a total of 10 patients with suspected photo-aggravated contact dermatitis to MCI/MI or MI.[21] The test concentrations in the baseline series for MCI/MI were 0.01% or 0.02% aq., and for MI was 0.2% aq. Six patients were photopatch tested with cosmetics containing MCI/MI and/or MI (amount of test substance not reported), with one of the two identical patches being irradiated with 5 J/cm^2 long-wave ultraviolet (UVA) light. Photo-tests were performed on two patients with UVA radiation ranging between 290 - 400 nm. Seven patients had positive patch tests to both MCI/MI and MI, and three patients had positive patch tests to only MI. Four patients had transient photosensitivity. Photopatch tests with MCI/MI and/or MI gave stronger reactions than patch tests with these derivatives, indicating photo-aggravation.

OCULAR IRRITATION

MCI/MI in an aqueous solution was not a cumulative ocular irritant when tested at 55 ppm in rabbits; it was corrosive when tested at 1.1% (11,000 ppm and higher.[1]

CLINICAL STUDIES

A sampling of the numerous baseline and retrospective studies, which included testing with MCI/MI, that have become available in the published literature since the original report was issued is presented in Table 2. The results of these studies demonstrate that sensitization to MCI/MI is found world-wide, with rates as low as 0.7% (out of 703 patients; United States) to as high as 15.4% (out of 635 patients; Thailand). [22-56]

Cases studies include reports of MCI/MI sensitization from a wide range of materials, including personal care products, ultrasound gels, paints, glues, cleaners, and industrial biocides. [57-73] Dermal sensitization from paint was theorized to be from airborne exposure in several patients.[62,64,69,70]

RISK ASSESSMENT

A skin sensitization induction risk assessment of MCI/MI was performed with various personal care and cosmetic products.[74] An estimated daily consumer exposure level for rinse-off and leave-on products was calculated using the amount of product applied per application, number of applications per day, a retention factor, the MCI/MI concentration, and body surface area values. The researchers assumed that the products contained the maximum recommended safe concentration of 15 ppm MCI/MI in rinse-off products and 7.5 ppm MCI/MI in leave-on products. The estimated consumer exposure levels (CEL) were compared with the no expected sensitization induction level (NESIL) for MCI/MI of 0.83$\mu g/cm^2$. The sensitization assessment factors were applied to calculate product-specific margins of safety (MOSs). The researchers found that the MOSs for rinse-off products ranged from 5 to 63, whereas the MOSs for leave-on products ranged from 0.03 to 1.49. An MOS of 1 or greater indicates a low likelihood of sensitization induction. The researchers concluded that the results provide evidence that some leave-on products containing the maximum recommended safe concentration of MCI/MI may increase the risk of sensitization induction due to exposure to MCI/MI, while rinse-off products were not associated with a potential increased risk of skin sensitization induction.

In another skin sensitization risk assessment of MCI/MI, the maximum safe concentration of 15 ppm MCI/MI in representative type cosmetics (which included shampoos, conditioners, soap, lotions, hand and face cream, deodorants, wipes, and eye and face makeup) indicated the possibility of skin sensitization when a NESIL of 1.25 $\mu g/cm^2$ was used in the determination.[75] However, there was no potential for skin sensitization at the concentration for just rinse-off products. In this assessment, the MOS was calculated as the acceptable exposure level (AEL)/CEL and was considered safe when the AEL/CEL ratio was 1 or more. The AEL is the NESIL/skin sensitization assessment factor (SAF). For the representative type cosmetics, the SAF was 300, while in rinse-off products in was 100. The MOS for representative type cosmetics was determined to be 0.00538 and the MOS for rinse-off products was 2.14.

Table 3 summarizes the QRA performed by the CIR Science and Support Committee (SSC).[76] A conservative NESIL of 0.83 $\mu g/cm^2$ was derived for MCI/MI based on a weight-of evidence (WoE) evaluation of data from HRIPT data and data from local lymph node assays (LLNA). The NESIL was then used to calculate AELs for the potential for the induction of sensitization from dermal exposure to MCI/MI in cosmetic products, assuming the maximal use concentration of 15 ppm for rinse-off products and 7.5 ppm for leave-on products and product category specific quantitative risk assessment 2 (QRA 2.0) sensitization assessment factors (SAFs). The CEL was then calculated for each of many cosmetic product categories, ranging from baby shampoo (CEL = 0.0024 $\mu g/cm^2$) to skin cleansing products (CEL = 0.0135 $\mu g/cm^2$). The lowest CEL to MCI/MI was 3.8×10^{-9} for bubble baths, and the highest estimated exposure was 0.0315 $\mu g/cm^2$ for permanent

waves. By using the maximum reported MCI/MI concentration of use levels, an adequate MOS for skin sensitization is provided for all reported uses except for permanent waves (using 7.5 ppm MCI/MI) and for skin cleansing products (i.e. cold creams, cleansing lotions, liquids, and pads; using 15 ppm MCI/MI). The maximum supportable concentration of MCI/MI for permanent waves and skin cleansing products are 2 ppm and 9 ppm, respectively. When using the exposure assumptions in this risk assessment on all reported categories of use with the maximum concentrations of use set by the original CIR conclusion of 7.5 ppm in leave-on products and 15 ppm in rinse-off products, adequate MOS could not be assured for baby shampoo (MOS = 0.92), permanent wave (MOS = 0.13), hair tints (MOS = 0.56), skin cleansing products (0.61), or cologne and toiler waters (0.50).

SUMMARY

This safety assessment is on the combination of MCI and MI as used in cosmetics. Each ingredient is reported to function as a preservative in cosmetic products. In 1992, the original report on MCI/MI was published with the Panel's conclusion that this mixture may be "safely used in rinse-off products at a concentration not to exceed 15 ppm and in leave-on cosmetic products at a concentration not to exceed 7.5 ppm." The stated safe-for-use concentration refers to a mixture containing 23.3% MI and 76.7% MCI.

According to 2019 VCRP survey data, MCI and MI are surveyed separately and not as a mixture. The total number of uses reported for MCI are 5137; 480 of these are in leave-on products. MI has 6037 reported uses; 1042 of these are in leave-on products. The uses have increased significantly since the original report on MCI/MI was published; in 1986, the total number of uses for the ingredient mixture was 381. In 2019, the Council reported that MCI/MI (3:1) is used at up to 7.5 ppm in leave-on products and at up to 15 ppm in rinse-off products. In the original report, concentration of use was reported as a range; the concentration of use range both leave-on and rinse-off products was reported to be < 0.1 – 1%.

In the European Union, MCI/MI is listed as a preservative in Annex V; it is limited to a maximum concentration of 0.0015% (i.e. 15 ppm) in rinse-off products as a 3:1 ratio of MCI:MI. The SCCS concluded in 2009 that MCI/MI in a ratio of 3:1 does not pose a risk to the health of the consumer when used as a preservative at a maximum concentration of 0.0015% in rinse-off cosmetic products, apart from its sensitizing potential.

MI and MCI were determined to metabolize into NMMA in humans after oral ingestion. Excretion of the metabolite through urine was rapid.

The LOEL for MCI/MI in a 28-day repeated oral dose study in rats was 7.0 mg/kg body weight/day, the highest dose that was tested. At this dose, a reduction of serum triglyceride levels was observed in males and induction of hepatic phase 1 xenobiotic metabolizing enzymes with subtle histological changes in the liver were observed in females.

Mutagenicity to MCI/MI and cosmetic products containing this mixture was observed in an Ames test, with and without metabolic activation.

Peripheral airway dysfunction was observed in a retrospective assessment of children who were exposed to MCI/MI as a humidifier disinfectant.

Numerous baseline and retrospective studies that included MCI/MI indicate that sensitization to this preservative occurs world-wide. Numerous case studies demonstrate sensitization to MCI/MI resulting from exposure to a wide range of materials, including personal care products, paints, glues, and cleaners.

Skin sensitization induction risk assessments of MCI/MI in multiple personal care and cosmetic products found that some leave-on products with MCI/MI at the recommended safe concentration of 7.5 ppm may increase the risk of sensitization induction. In most rinse-off products, 15 ppm MCI/MI was not associated with a potential increase risk of skin sensitization induction.

TABLES

Table 1. Current (2019) and historical (1986) frequency and concentration of use according to duration and type of exposure for Methylisothiazolinone and Methylchloroisothiazolinone[1,4,5]

	# of Uses (2019)	*# of Uses (2019)*	*Max Conc of Use (2019) (ppm)*	*# of Uses (1986)*	*Max Conc of Use (1986) (%)*
	Methylchloroisothiazolinone*	**Methylisothiazolinone***	**MCI/MI¥**	**MCI/MI**	**MCI/MI‡**
Totals†	**5137**	**6037**	**0.000019-15**	**381**	**≤0.1-1**
Duration of Use					
Leave-On	480	1042	0.021-7.5	137	≤0.1-1
Rinse Off	4521	4849	0.15-15	244	≤0.1-1
Diluted for (Bath) Use	136	146	0.000019	NR	NR
Exposure Type					
Eye Area	32	60	NR	8[d]	≤0.1-1[d]
Incidental Ingestion	NR	1	NR	NR	NR
Incidental Inhalation-Spray	11; 192[a]; 112[b]	14; 470[a]; 286[b]	0.075-7.5; 7.4-7.5[a]	5[a]; 95[b]	≤0.1-1[a,b]
Incidental Inhalation-Powder	1; 112[b]; 2[c]	1; 286[b]; 2[c]	NR	95[b]	≤0.1-1[b]
Dermal Contact	3486	4163	0.000019-15	178[d]	≤0.1-1[d]
Deodorant (underarm)	NR	NR	NR	NR	NR
Hair - Non-Coloring	1567	1780	0.5-15	203[e]	≤0.1-1[e]
Hair-Coloring	68	68	0.15-11	[e]	[e]
Nail	1	4	NR	NR	NR
Mucous Membrane	2981	3099	0.000019-15	8	≤0.1-1
Baby Products	11	16	12	NR	NR

NR = Not reported.

* MCI and MI are reported separately in the VCRP database. While it is likely that all MCI totals are for MCI/MI, there is no way to verify this information.

† 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.

¥ No wipe products were reported.

‡ Concentrations were reported as general ranges in 1986.

[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.

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

[d.] Eye and facial makeup preparations were reported together in the original safety assessment. The reported number was only accounted for in the eye area exposure

[e.] Non-coloring and coloring hair preparations, except for non-coloring shampoos, were reported together in the original safety assessment. The reported number was only accounted for in the non-coloring hair products.

Table 2. Baseline and retrospective studies

Number of Patients	Clinical Testing Type	Country and Time Span	Results	Reference
5899 patients	Swedish baseline patch test series using Finn Chambers secured with Scanpor tape; 15 µl o f 0.02% aq. MCI/MI (200 ppm; 3:1) and serial dilutions of MI alone	Southern Sweden; March 2003-December 2012	184 patients (3.1%) reacted to MCI/MI, with a notable increase in frequency from 4.3% in 2010 to 7.6% in 2012	[22]
141 recently diagnosed with sensitivity to 0.02% aq. MCI/MI	Tested MCI (0.015%) and MI (0.005%) separately with simultaneous application of haptens (0.2 ml); patches were Haye's test chambers with Soffix tape; occluded for 2 days	8 clinics in Italy; January 2016-December 2016	110 patients (78.1%) reacted to MCI, of which 60 (42.6%) reacted only to MCI and 50 (35.5%) reacted to both MCI and MI	[23]
229 children (96 were 7 years old and 133 were 16 years old) identified as having eczema through an allergy screening survey	Patch testing with 10 most common contact sensitizers in children in Europe; MCI/MI tested at 0.01% aq. with Chemotechnique™ IQ Ultra Chambers for 2 days	Poland; 2007	6.3% of 7-year olds and 0.8% of 16-year olds had a positive reaction to MCI/MI	[24]
14,274 work-related contact dermatitis cases	Baseline series of the British Society of Cutaneous Allergy; MCI/MI tested a 0.01% aq. until 2008, then changed to 0.02% aq.	United Kingdom; 1996-2012	4.1% (358) patients per annum had dermatitis attributed to MCI/MI; occupations of affected workers included beauty workers, hairdressers, healthcare workers, cleaners exposed to detergents, painters, manufacturing, and other industrial work.	[25]
3201 with either widespread or localized dermatitis	European baseline series and international standard series along with patients' products; MCI/MI was tested at 0.02% aq.; patches were Finn chambers applied for 2 days	Thailand; January 2005-December 2016	15.4% (98/635) patients with widespread dermatitis and 9.1% (204/2244) patients with localized dermatitis reacted to MCI/MI	[26]
4860 patients	Patch tested with screening series of 70 allergens, including 0.01% MCI/MI aq. and 0.2% MI aq.; patches were Finn chambers on Scanpor tape	13 centers from the North American Contact Dermatitis Group (NACDG); January 2013 to December 2014	6.3% (305) patients had positive reaction to MCI/MI, a significant increase from the previous testing cycle (5.0%; 2011-2012); 10.9% (527) patients had positive reaction to MI	[27,39]
124 patients with long-lasting perianal dermatitis	Patch tests with Spanish research group standard series, and depending on patient clinical history, more specific test series and suspected personal products; patch test were occluded for 2 days; additional diagnostic protocols including biopsies and cultures were performed	Spain; April 2004 to August 2016	13.7% (17/124) of patients reacted to MCI/MI	[28]
2315 patients	Baseline patch tests series with 0.02% MCI/MI aq.	2 centers in the United Kingdom; August 2011 to June 2013	9.4% (217/2315) of patients reacted to MCI/MI	[29]
997 patients	British baseline patch tests series with 0.02% MCI/MI aq.	United Kingdom; January to December 2015	3.9% of patients reacted to MCI/MI, this was a decrease from 7.9% in 2014	[30]
44 patients identified through a survey as having airborne allergic contact dermatitis caused by paint	Tested with 0.02% and /or 0.01% MCI/MI aq. and 0.02%, 0.05%, and 0.2% MI aq.	17 dermatology departments and 2 private offices in France and Belgium; survey occurred May 2015 to May 2016 with patients diagnosed from January 2012 to January 2016	36/44 (82%) patients had positive reactions to MCI/MI and 43/44 had positive reactions to MI	[31]
206 patients	Standard series patch tests (39 allergens); patches were 8 mm Finn chambers on Scanpor tape; results read at 48 and 72 h	Thailand; 2012 to 2015	13.6% (28/206) of patients tested positive to 0.01% MCI/MI	[32]
324 patients	European baseline series with 0.02% MCI/MI aq. and 0.2% MI aq.; patches were IQ Ultra chambers and readings were day 2 and day 4	Turkey; January 2016 to June 2018	6.17% (20/324) of patients tested positive to MCI/MI; 8.02% of patients tested positive to MI	[33]

Table 2. Baseline and retrospective studies

Number of Patients	Clinical Testing Type	Country and Time Span	Results	Reference
1287 patients	Baseline series with 0.02% MCI/MI aq., 0.2% MI aq., 0.1% benzisothiazolinone pet., and 0.1% octylisothiazolinone pet.; the occluded patches were IQ Ultra chambers and readings were on day 2 and day 4	United Kingdom; September 2014 to December 2015	9.2% (118) of patients had positive reactions to any isothiazolinone; cross-sensitization thought to occur between MCI/MI, MI, and octylisothiazolinone	[34]
703 patients	Retrospective review of patients tested with the North American Contact Dermatitis Group standard series; MCI/MI tested at 100 ppm and MI tested at 200 to 2000 ppm	United States; January 1, 2012 to December 30, 2014	0.7% (5) reactions to MCI/MI and 2.4% (17) reactions to both MCI/MI and MI	[35]
2703 patients	Testing in consecutive patients with 0.01% and 0.02% MCI/MI aq.; patches were 8 mm Finn chambers on Scanpor tape	8 centers in 8 countries that included Japan, Germany, Belgium, Sweden, Uruguay, India, Denmark, and Singapore; January 1, 2014 to December 31, 2014	3.7% and 5.6% of patients had reactions to 0.01% and 0.02% MCI/MI, respectively	[36]
2576 patients to MCI/MI and 964 to MI	Patients tested with Mayo Clinic Institution's standard series that included 0.2% MI aq. and 100 ppm MCI/MI aq.; patches were Finn chambers on Scanpor tape	Mayo Clinic; January 1, 2011 to December 31, 2015	5.9% and 13.6% of patients had allergic reactions to MCI/MI and MI, respectively	[37]
1745 patients	Retrospective study of patients tested with a modified Finnish baseline or antimicrobial series; 0.01% and 0.02% aq. MCI/MI, 0.1% and 0.03% aq. MI, 0.05% pet. benzisothiazolinone, and 0.1% pet. octylisothiazolinone; patches were Finn chambers occluded for 48 h	Finland; January 2002 to February 2013	2.6% and 0.2% of patients had allergic reactions to MCI/MI and MI, respectively	[38]
490 patients	Prospective study using the Spanish baseline series; 100 and 200 ppm aq. MCI/MI and 2000 ppm aq. MI; Finn chambers on Scanpor tape and occluded for 2 days	Spain; October 2011 to June 2013	10% and 4.5% of patients had reactions to MCI/MI and MI during the 2-year study; prevalence to MCI/MI allergy increased from 7.8% in 2011 to 14.3% in 2013 while prevalence to MI allergy increased from 1% to 7.7%	[40]
79 patients out of 9037 that had allergic reactions to a wipe	Retrospective analysis of patients patch tested to a screening series of 70 allergens; MCI/MI tested at 0.01% aq. and MI tested at 0.2% aq.	13 centers from NACDG; January 1, 2011 to December 31, 2014	59% of the patients that had reactions to wipes had a reaction to MI while 35.6% had reaction to MCI/MI	[41]
2165 patients	Patients tested with Swedish baseline series; 200 ppm aq. MCI/MI and 2000 ppm aq. MI; 8 mm diameter Finn chambers on Scanpor tape	Sweden; 2012 to 2014	8.1% (175) and 7.1% (153) of patients had reactions to MCI/MI and MI, respectively; 9.5% (206) of patients were found to have allergy to MCI/MI and/or MI	[42]
2028 patients	Patch testing in accordance with European Society of Contact Dermatitis guidelines; 0.2% aq MI and 0.01% aq. MCI/MI	Italy; January 2012 to December 2014	7.5% (152) and 5.2% (106) of patients had reactions to MCI/MI and MI, respectively	[43]
3253 patients	Patients tested with 100 ppm aq. MCI/MI and 2000 ppm aq. MI (only tested in 2014); Finn chambers under occlusive for 2 days	Thailand; January 2009 to June 2014	9.8% of patients had positive patch tests to MCI/MI; 40.7% (22/54) of patients had positive patch test to MI	[44]
80 patients with facial dermatitis	Patients tested with British Society for Cutaneous Allergy (BSCA) standard series and their own cosmetic products; 0.02% MCI/MI and 0.2% MI	Ireland; January 2012 to March 2014	6.3% (5) and 5% (4) of patients tested positive to MCI/MI and MI, respectively	[45]
4094 patients	Patients tested with baseline patch test series; Finn chambers on Scanpor tape; test concentrations not reported	Switzerland; 2000 to 2004	2.1% (88) of patients tested positive to MCI/MI	[46]
964 patients	Retrospective review of patients tested with BSCA standard series and individualized additional test series; IQ ultra chambers for 48 h; 0.01% aq. or 0.02% aq. MCI/MI	Ireland; 2007 to 2010	2.2% (21/964) of patients tested positive to MCI/MI; of these, 1.6% (11/697) were positive to 0.01% and 3.8% (10/267) were positive to 0.02%	[47]

Table 2. Baseline and retrospective studies

Number of Patients	Clinical Testing Type	Country and Time Span	Results	Reference
4227 patients	Patients patch tested with a screening series of 70 allergens; MCI/MI tested at 0.01% aq.; Finn chambers on Scanpor tape	12 centers from NACDG: January 1, 2011 to December 31, 2012	5.0% (213) of patients tested positive to MCI/MI; prevalence of allergy had increased since the previous years (2001-2010)	[48]
219 painters with contact dermatitis	Retrospective study of European baseline series patch test results of all painters registered in the Danish National Database for Contact Allergy; MCI/MI tested at 0.01% aq.; MI, octylisothiazolinone, and benzisothiazolinone were also tested (concentrations not reported); patches occluded for 48 h	Denmark; 2001 to 2010	10% (22/219) of the painters tested positive to MCI/MI; 27% (11/41) tested positive to MI; 25% (5/21) tested positive to octylisothiazolinone; and 19% (7/37) tested positive to benzisothiazolione	[49]
359 patients	Retrospective study of Brazilian standard series results; MCI/MI tested at 0.5% pet.	Brazil; November 2009 to October 2012	11.1% (40/359) of patients tested positive to MCI/MI; increase from previous study period (3.4% from 2006-2009)	[52]
14,693 patients	Retrospective study of European baseline series patch test results; test concentrations not reported	Hungary; 1993 to 2014	Prevalence of MCI/MI hypersensitivity increased from 0.5% (5/1011) of patients in 1993 to 6.0% (23/383) of patients in 2014	[53]
314 patients	Patients prospectively patch tested with 0.01% aq. MCI/MI and 0.2% aq. MI with parallel testing to the European baseline series with 0.01% aq. MCI/MI; IQ chambers and Curatest patches, respectively, and occluded for 48 h	Hungary: February 1, 2014 to January 30, 2015	5.1% (16/314) of patients were positive to MCI/MI and 4.8% (15/314) of patients were positive to MI; no differences between the patch series types	[53]
Up to 6722 patients	Retrospective study of patients consecutively patch tested with 100 ppm aq. or 4 μg/cm^2 (TRUE test) MCI/MI, 200 or 2000 ppm aq. MI, and 500 or 1000 ppm aq. benzisothiazolinone; occluded 48 h	3 centers in Denmark; 2009 to 2012	3.2% (213/6722) of patients tested positive to MCI/MI, 3.2% (170/5290) tested positive to MI, and 0.9% (34/3636) tested positive to benzisothiazolinone	[54]
Up to 48,720 patients	Retrospective study of data collected by the European Surveillance System on Contact Allergies (ESSCA) from European baseline series and other allergen testing; MCI/MI was tested at 0.01% and 0.02% aq. and MI was tested at 0.05%, 0.1%, and 0.2% aq.	54 centers in 12 European countries; January 2009 to December 2012	4.1% of patients were positive to 0.02% MCI/MI and 3.3% were positive to 0.01% MCI/MI; 4.5% of patients were positive to 0.05% MI, 0.21% were positive to 0.1% MI, and 4.9% were positive to 0.2% MI	[55]
45 children with atopic dermatitis (ages 2 months to 17 years)	Patients tested with the TRUE patch test system; patch test plasters applied to upper back for 2 days; concentrations not reported	Turkey; September 2011 to March 2012	20% (9/45) of the patients had a positive reaction to MCI	[51]
24 children with no underlying disease	Retrospective assessment of children with reported high-level inhalation exposure to MCI/MI as a humidifier disinfectant; concentrations not reported	Korea; July 2013 to April 2014, July 2014 to April 2015, and September 2015 to August 2016	Definite or probable irreversible peripheral airway dysfunction was observed in 4 children; exposure to these 4 was high density for up to 6 months and children were initially exposed as infants; one child died at age 4 months after continuous use of the humidifier disinfectant over 3 months (no further details on this death were available)	[56]
7533 out of 20,107 patients	Meta-analysis of 28 studies of the general population from studies written in English available on PubMed; patch tests were conducted with the European baseline series or something similar; concentrations of MCI/MI tested not reported	Various centers from Asia, Europe, North America, and Australia; 2007 to 2017	Prevalence of allergy is 1.5% (95% CI 0.8-2.5) in the general population based on 6 studies	[50]

Table 3. Quantitative risk assessment of MCI/MI at the highest maximum use concentration in cosmetic products[76]

Product Category	Reported Maximum Concentration of Use (ppm)	Product Amount Applied/Day (µg/cm^2)	Sensitization Assessment Factor (SAF)	Acceptable Exposure Level (AEL; µg/cm^2/day)	Consumer Exposure Level (CEL; µg/cm^2/day)	Margin of Safety (AEL/CEL)
Baby shampoo	12	0.83	300	0.0028	0.0027	1.15
Bubble baths	0.000019	0.83	100	0.0083	3.8×10^{-9}	> 2,000,000
Cologne and toilet waters	0.075	0.83	100	0.0083	0.0002	50.07
Hair conditioners	15	0.83	100	0.0083	0.0030	2.77
Hair sprays (aerosol)	7.5	0.83	30	0.0277	0.0104	2.65
Hair sprays (pump)	7.5	0.83	30	0.0277	0.0163	1.70
Permanent waves	7.5	0.83	100	0.0083	0.0315	0.26
Rinses (non-coloring)	11	0.83	300	0.0028	0.0019	1.48
Shampoos (non-coloring)	15	0.83	300	0.0028	0.0026	1.08
Tonics, dressings and other hair grooming aids (rinse-off)	7.5	0.83	100	0.0083	0.0007	11.18
Tonics, dressings and other hair grooming aids (leave-on)	7.4	0.83	100	0.0083	0.0073	1.13
Hair tints	0.4	0.83	100	0.0083	0.0004	20.96
Hair rinses (coloring)	11	0.83	100	0.0083	0.0012	6.92
Hair shampoos (coloring)	6	0.83	300	0.0028	0.0010	2.71
Bath soaps and detergents	15	0.83	300	0.0028	0.0002	18.44
Other personal cleanliness products (liquid hand soap)	15	0.83	100	0.0083	0.0030	2.77
Shaving cream	4.5	0.83	100	0.0083	0.0003	26.350
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	15	0.83	100	0.0083	0.0135	0.61

REFERENCES

1. Elder RL. Final Report on the Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone. *JACT*. 1992;11(1):75-128.

2. Nikitakis J and Kowcz A. wINCI: International Cosmetic Ingredient Dictionary and Handbook. http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC. Last Updated 2019. Date Accessed 2-7-2019.

3. Burnett CL, Boyer IJ, Bergfeld WF, et al. Amended Safety Assessment of Methylisothiazolinone as Used in Cosmetics. Washington, DC: Cosmetic Ingredient Review. 2014. https://online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/FR646.pdf.

4. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. College Park, MD: 2019. Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 3, 2019; received February 13, 2019).

5. Personal Care Products Council. 2019. Concentration of Use by FDA Product Category - MCI/MI (ratio approximately 3:1). Unpublished data submitted by the Personal Care Products Council on May 2, 2019.

6. Bremmer HJ, Prud'homme de Lodder LCH, and Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. Bilthoven, Netherlands: Netherlands National Institute for Public Health and the Environment. 2006. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Date Accessed 8-24-2011. Report No. RIVM 320104001/2006. pp. 1-77.

7. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.

8. Rothe H. Special Aspects of Cosmetic Spray Evaluation. 9-26-2011.

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

10. CIR Science and Support Committee of the Personal Care Products Council (CIR SSC). 11-3-2015. Cosmetic Powder Exposure.

11. Aylott RI, Byrne GA, Middleton J, et al. Normal use levels of respirable cosmetic talc: Preliminary study. *Int J Cosmet Sci*. 1976;1(3):177-186.

12. Russell RS, Merz RD, Sherman WT, et al. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol*. 1979;17(2):117-122.

13. European Union. Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. 2009. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDFDate Accessed 11-9-2017

14. Scientific Committee on Consumer Safety (SCCS). Opinion on the mixture of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one. European Commission. 2009. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_009.pdf. Date Accessed 2-7-2019. Report No. SCCS/1238/09.

15. Thomsen AV, Schwensen JF, Bossi R, et al. Isothiazolinones are still widely used in paints purchased in five European countries: A follow-up study. *Contact Dermatitis*. 2017;78:246-253.

16. Schettgen T and Kraus T. Urinary excretion kinetics of the metabolite N-methylmalonamic acid (NMMA) after oral doseage of chloromethylisothiazolinone and methylisothiazolinone in human volunteers. *Arch Toxicol*. 2017;91:3835-3841.

17. Pelletier G, Valli VE, Rigden M, et al. Effects of a 28-day oral exposure to a 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one biocide formulation in Sprague-Dawley rats. *Drug Chem Toxicol*. 2014;37(2):149-155.

18. Connor TH, Tee PG, Afshar M, et al. Mutagenicity of cosmetic products containing Kathon. *Envrion Mol Mutagen*. 1996;28:127-132.

19. Isaksson M, Gruvberger B, Goncalo M, et al. Repeated open application test with methylisothiazolinone in individuals sensitive to methylchloroisothiazolinone/methylisothiazolinone. *Contact Dermatitis*. 2014;70:244-246.

20. Cantor Research Laboratories,Inc. 2019. 50 Human subject repeat insult patch test skin irritation/sensitization evaluation (open patch; hand wash containing 12 ppm MCI/MI). Unpublished data submitted by the Personal Care Products Council on May 1, 2019.

21. Aerts O, Goossens A, Marguery M-C, et al. Photoaggravated allergic contact dermatitis and transiet photosensitivity caused by methylisothiazolinone. *Contact Dermatitis*. 2017;78:241-245.

22. Isaksson M, Hauksson I, Hindsen M, et al. Methylisothiazolinone contact allergy is rising to alarming heights also in southern Sweden. *Acta Derm Venereol*. 2015;95(1):31-34.

23. Stingeni L, Bianchi L, Foti C, et al. An Italian multicentre study on methylchloroisothiazolinone/methylisothiazolinone contact sensitivity: Understanding the structure-activity relationship. *Contact Dermatitis*. 2018;78(4):297-299.

24. Czarnobilska E, Obtulowicz K, Dyga W, et al. Contact hypersensitivity and allergic contact dermatitis among school children and teenagers with eczema. *Contact Dermatitis*. 2009;60:264-269.

25. Urwin R, Warburton K, Carder M, et al. Methylchloroisothiazolinone and methylisothiazolinone contact allergy: An occupational perspective. *Contact Dermatitis.* 2015;72(6):381-386.

26. Boonchai W, Maneeprasopchoke P, Chaweekulrat P, et al. Associated factors of widespread pattern of dermatitis among patch test population: 12-Year retrospective study. *Australas J Dermatol*. 2018;2018

27. Zirwas MJ, Hamann D, Warshaw EM, et al. Epidemic of isothiazolinone allergy in North America: Prevalence data from the North American Contact Dermatits Group, 2013-2014. *Dermatitis*. 2017;28(3):204-209.

28. Agullo-Perez A-D, Hervella-Garces M, Oscoz-Jaime S, et al. Perianal dermatitis. *Dermatitis*. 2017;28(4):270-275.

29. Ali FR, Shepherd EL, Yell LC, et al. Escalating methylisothiazolinone/methylchloroisothiazolinone allergy probably attributable to methylisothiazolinone in leave-on body cosmetics. *Contact Dermatitis*. 2014;70:316-317.

30. Urwin R, Craig S, Latheef F, et al. Methylisothiazolinone: The epidemic is declining - but not gone. *Contact Dermatitis*. 2017;76:301-302.

31. Amsler E, Aerts O, Raison-Peyron N, et al. Airborne allergic contact dermatitis caused by isothiazolinones in water-based paints: A retrospective study of 44 cases. *Contact Dermatitis.* 2017;77:163-170.

32. Dararattanaroj W, Pootongkam S, Rojanawatsirivej N, et al. Patterns and risk factors of causative contact allergens in Thai adult patients with contact dermatitis. *Asian Pac J Allergy Immunol*. 2017;35:27-32.

33. Salman A. Methylchloroisothiazolinone and methylisothiazolinone contact allergy: A retrospective cohort study from a tertiary dermatology clinic in Turkey. *Contact Dermatitis*. 2019;80:193-194.

34. Craig S, Urwin R, Latheef F, et al. Patch test clinic experience of potential cross-reactivity of isothiazolinones. *Contact Dermatitis*. 2017;76(299):300

35. Yu SH, Sood A, and Taylor JS. Patch testing for methylisothiazolinone and methylchloroisothiazolinone-methylisothiazolinone contact allergy. *JAMA Dermatol*. 2016;152(1):67-72.

36. Engfeldt M, Ale I, Andersen KE, et al. Multicenter patch testing with methylchloroisothiazolinone/methylisothiazolinone in 100 and 200 ppm withint the International Contact Dermatitis Research Group. *Dermatitis.* 2017;28(3):215-218.

37. Veverka KK, Hall MR, Yiannias JA, et al. Trends in patch testing with the Mayo Clinic standard series, 2011-2015. *Dermatitis*. 2018;29(6):310-315.

38. Vauhkala A-R, Pesonen M, Suomela S, et al. Occupational contact allergy to methylchloroisothiazolinone/methylisothiazolinone and methylisothiazolinone. *Contact Dermatitis.* 2015;73:150-156.

39. DeKoven JG, Warshaw EM, Belsito DV, et al. North American Contact Dermatitis Group patch test results 2013-2014. *Dermatitis*. 2017;28(1):33-46.

40. Leiva-Salinas M, Frances L, Main-Cabanas I, et al. Methylchloroisothiazolinone/methylisothaizolinone and methylisothiazolinone allergies can be detected by 200 ppm of methylchloroisiothiazolinone/methylisothiazolinone patch test concentration. *Dermatitis*. 2014;25(3):130-134.

41. Warshaw EM, Aschenbeck KA, Zug KA, et al. Wet wipe allergens: Retrospective analysis from the North American Contact Dermatitis Group 2011-2014. *Dermatitis*. 2017;28(1):64-69.

42. Ponten A, Bruze M, Engfeldt M, et al. Concomitant contact allergies to formaldehyde, methylchloroisothiazolinone/methylisothiazolinone, methylisothiazolinone, and fragrance mixes I and II. *Contact Dermatitis*. 2016;75(5):285-289.

43. Gallo R, Signori A, Gervasio S, et al. Methylisothiazolinone contact allergy - are rinse-off cosmetics and household products relevant sources of exposure? *Contact Dermatitis*. 2016;75(5):319-321.

44. Puangpet P, Chawarug A, and McFadden JP. Methylchloroisothiazolinone/methylisothiazolinone and methylisothiazolinone allergy. *Dermatitis*. 2015;26(2):99-102.

45. Murad A and Marren P. Prevalence of methylchloroisothiazolinone and methylisothiazolinone contact allergy in facial dermatitis: A single centre Irish study. *J Eur Acad Dermatol Venereol*. 2016;30:60-62.

46. Janach M, Kuhne A, Seifert B, et al. Changing delayed-type sensitizations to the baseline series allergens over a decade at the Zurich University Hospital. *Contact Dermatitis*. 2010;63:42-48.

47. Higgins E, Kirby B, Rogers S, et al. Methylchloroisothiazolinone and methylisothiazolinone allergic contact dermamtitis and the effect of patch test concentration. *Dermatitis*. 2013;24(2):73-76.

48. Warshaw EM, Maibach HI, Taylor JS, et al. North American Contact Dermatitis Group patch test results: 2011-2012. *Dermatitis*. 2015;26(1):49-59.

49. Mose AP, Lundov MD, Zachariae C, et al. Occupational contact dermatitis in painters - an analysis of patch test data from the Danish Contact Dermatitis Group. *Contact Dermatitis*. 2012;67(5):293-297.

50. Alinaghi F, Bennike NH, Egeberg A, et al. Prevalence of contact allergy in the general population: A systemic review and meta-analysis. *Contact Dermatitis*. 2019;80:77-85.

51. Akan A, Toyran M, Vezir E, et al. The patterns and clinical relevance of contact allergen sensitization in a pediatric population with atopic dermatitis. *Turk J Med Sci*. 2015;45:1207-1213.

52. Rios Scherrer MA and Rocha VB. Increasing trend of sensitization to methylchloroisothiazolinone/methylisothiazolinone (MCI/MI). *An Bras Dermatol*. 2014;89(3):527-528.

53. Ponyai G, Nemeth I, and Temesvari E. Methylchloroisothiazolinone/methylisothiazolinone and methylisothiazolinone sensitivity in Hungary. *Dermatol Res Pract*. 2016;2016(4579071):1-5.

54. Schwensen JF, Menne T, Andersen KE, et al. Occupations at risk of developing contact allergy to isothiazolinones in Danish contact dermatitis patients: Results from a Danish multicentre study (2009-2012). *Contact Dermatitis*. 2014;71:295-302.

55. Gimenez-Arnau AM, Deza G, Bauer A, et al. Contact allergy to preservatives: ESSCA resutls with the baseline series, 2009-2012. *J Eur Acad Dermatol Venereol*. 2017;31(4):664-671.

56. Cho H-J, Park D-U, Yoon J, et al. Effects of a mixture of chloromethylisothiazolinone and methylisothiazolinone on peripheral airway dysfunction in children. *PLoS ONE*. 2017;12(4):e0176083

57. Wolf R, Orion E, and Matz H. Co-existing sensitivity to metronidazole and isothiazolinone. *Clin Exp Dermatol*. 2003;28(5):506-507.

58. Kujala V and Niinimaki A. Occupational induction of hypersensitivity after an accidental exposure to chloromethylisothiazolinone and methylisothiazolinone (CMI/MI) in an industrial worker. *Occup Med (Lond)*. 1999;49(1):51-53.

59. Cordoba S, Garcia-Donoso C, Villanueva CA, et al. Allergic contact dermatitis from methylisothiazolinone, with acute exanthematous pustulosis-like histopathologic changes. *Dermatitis*. 2011;22(1):60-61.

60. Ducharme O, Labadie M, Briand SM, et al. Allergic contact dermatitis in a child caused by isothiazolinones in a "noise putty". *Contact Dermatitis*. 2018;79(6):393-394.

61. Concha-Garzon MJ, Solano-Lopez G, Montes A, et al. Follicular allergic contact dermatitis due to methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) in a rinse-off soap product. *Clin Exp Dermatol*. 2015;40(6):690-691.

62. Finkbeiner H and Kleinhans D. Airborne allergic contact dermatitis caused by preservatives in home-decorating paints. *Contact Dermatitis*. 1994;31(4):275-276.

63. Hunter KJ, Shelley JC, and Haworth AE. Airborne allergic contact dermatitis to methylchloroisothiazolinone/methylisothiazolinone in ironing water. *Contact Dermatitis*. 2008;58(3):183-184.

64. Jensen J-M, Harde V, and Brasch J. Airborne contact dermatitis to methylchloroisothiazolinone/methylisothiazolinone in a boy. *Contact Dermatitis*. 2006;55:311

65. Corazza M, Amendolagine G, Cristofaro D, et al. Occupational allergic contact dermatitis caused by isothiazolinones in ultrasound gel: 2 cases. *Contact Dermatitis*. 2017;77:325-351.

66. Gardner KH, Davis MDP, Richardson DM, et al. The hazards of moist toilet paper. *Arch Dermatol*. 2010;145(8):886-890.

67. Todberg T, Opstrup MS, Johansen JD, et al. Occupational facial contact dermatitis caused by methylchloroisothiazolinone/methylisothiazolinone in a stainless steel aerosol spray. *Contact Dermatitis*. 2017;77:173-174.

68. Zhang AJ, Boyd AH, Asch S, et al. Allergic contact dermatitis to slime: The epidemic of isothiazolinone allergy encompasses school glue. *Pediatr Dermatol*. 2018;36:e37-e38.

69. Andersson AM, Opstrup MS, Zachariae C, et al. The importance of a complete declaration of isothiazolinones in products beyond cosmetics. *Contact Dermatitis*. 2017;77:171-172.

70. Sechi A, Vincenzi C, Tengattini V, et al. Airborne dermatitis in a child caused by isothiazolinones in a water-based paint in Italy: Call for better regulations. *Contact Dermatitis*. 2018;79:45-46.

71. Vincenzi C, Ravaioli GM, Lambertini M, et al. Allergic contact dermatitis caused by glitter glue used as make-up containing methylchloroisothiazolinone. *Contact Dermatitis*. 2019;80:128-130.

72. Maor D and Nixon R. Allergic contact dermatitis to methylchloroisothiazolinone/methylisothiazolinone in cooling tower technicians. *Dermatitis*. 2015;26(1):62-64.

73. Verdelli A, Francalanci S, and Palleschi GM. Contact allergic dermatitis due to Kathon CG contained in ultrasound gel. *Dermatitis*. 2014;25(1):35-36.

74. Towle KM, Drechsel DA, Warshaw EM, et al. Risk assessment of the skin sensitization induction potential of Kathon CG in risne-off and leave-on personal care and cosmetic products. *Dermatitis*. 2018;29(3):132-138.

75. Kim MK, Kim K-B, Lee JY, et al. Risk assessment of 5-chloro-2-methylisothaizol-3(2H)-one/2-methylisothiazol-3(2H)-One (CMIT/MIT) used as a preservative in cosmetics. *Toxicol Res*. 2019;35(2):103-117.

76. CIR Science and Support Committee of the Personal Care Products Council. 2019. Quantitative Risk Assessment for Allergic Contact Dermatitis: Methylisothaizolinone/Methylchloroisothiazolinone as Used in Cosmetics. Unpublished data submitted by the Personal Care Products Council on May 2, 2019.

JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 11, Number 1, 1992
Mary Ann Liebert, Inc., Publishers

6

Final Report on the Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone

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

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

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

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

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

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

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

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

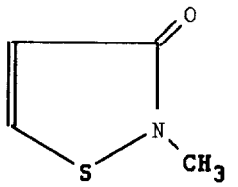
INTRODUCTION

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

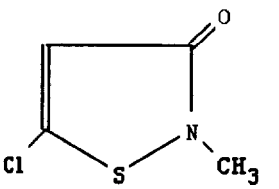
CHEMISTRY

Definition and Structure

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



Methylisothiazolinone



Methylchloroisothiazolinone

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

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

Composition for Cosmetic Use

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

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

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

Properties

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

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

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

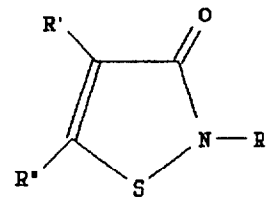
Table 1. Composition, Chemical, and Physical Properties of MI/MCI-CG[a]

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

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

Source: Ref. 3.

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



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

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

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

Source: Ref. 7.

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

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

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

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

Method of Manufacture/Analytical Methods

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

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

Impurities

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

USE

Cosmetic

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

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

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

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

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

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

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

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

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

Source: Ref. (21).

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

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

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

Noncosmetic

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

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

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

BIOLOGY

Fate in the Environment

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

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

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

Antimicrobial Activity

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

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

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

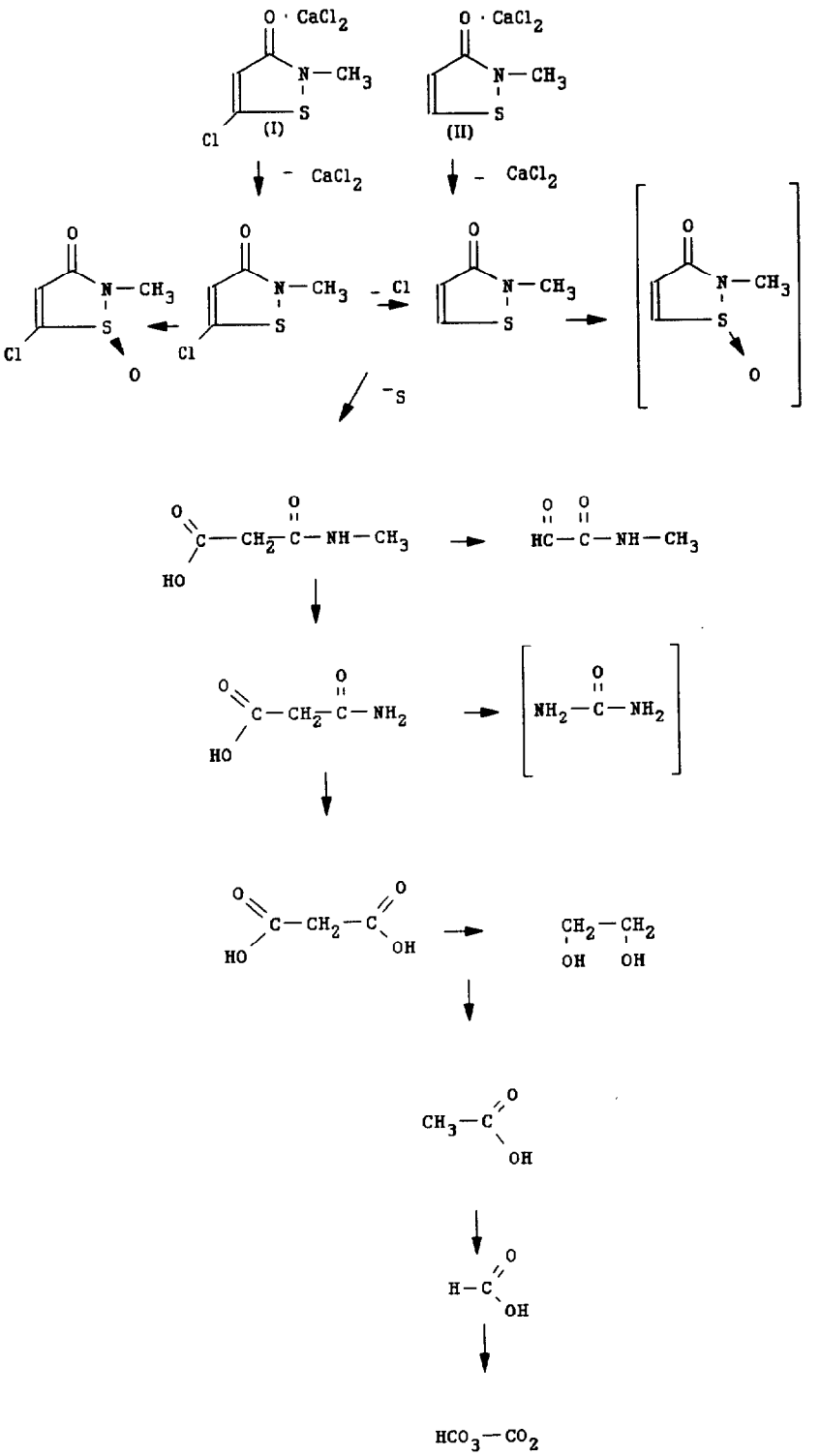

FIG. 1. Major degradative pathway of the calcium chloride salts of Methylchloroisothiazolinone (I) and Methylisothiazolinone (II) (bracketed structures are postulated) Ref. 12.

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

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

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

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

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

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

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

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

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

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

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

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

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

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

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

[c]ND = Not determined.

Source: Ref. 32.

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

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

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

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

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

TOXICOLOGY

Aquatic and Avian Toxicity

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

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

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

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

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

Acute Toxicity

Oral

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

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

Dermal

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

Intraperitoneal

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

Inhalation

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

Irritation

Chorioallantoic Membrane

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

Ocular

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

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

Dermal

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

Short-Term Toxicity

Oral

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

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

Dermal

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

Inhalation

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

Subchronic Toxicity

Oral

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

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

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

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

Dermal

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

Sensitization, Photosensitization, and Phototoxicity

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

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

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

[a]Dosage volume = 0.4 ml/patch.

[b]a.i. = active ingredients.

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

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

Source: Ref. 5.

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

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

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

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

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

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

GENOTOXICITY

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

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

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

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

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

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

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

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

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

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

Table 6. Genotoxicity of Methylisothiazolinone and Methylchloroisothiazolinone

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

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

[a]a.i = active ingredients

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CARCINOGENICITY

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

Teratogenicity and Reproductive Toxicity

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

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

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

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

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation and Sensitization

Predictive Tests

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

TABLE 8. RESULTS OF UNPUBLISHED REPEATED INSULT PATCH TESTS WITH COSMETIC FORMULATIONS, METAL WORKING FLUIDS, AND ACRYLIC EMULSIONS CONTAINING MI/MCI-886/CG (CONTINUED)

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

[a]Study conditions

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Provocative Tests

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Photosensitization and Phototoxicity

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

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

SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

No comments were received during the second public comment period.

CONCLUSION

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

REFERENCES

1. ESTRIN, N.F., CROSLEY, P.A., and HAYNES, C.R. (Editors). (1982). *CTFA Cosmetic Ingredient Dictionary*, Third Edition. Washington, DC: Cosmetic, Toiletry and Fragrance Association, pp. 168–9.
2. BRUZE, M., DAHLQUIST, I., FREGERT, S., GRUVBERGER, B., and PERSSON, K. (1987). Contact allergy to the active ingredients of Kathon-CG. Contact Derm. **16,** 183–8.
3. LAW, A.B., MOSS, J.N., and LASHEN, E.S. (1984). Kathon-CG: A new single-component, broad-spectrum preservative system for cosmetics and toiletries. In: J.J. Karaba (Editor). *Cosmetic and Drug Preservation. Principles and Practice.* New York: Marcel Dekker, pp. 129–41.
4. DeGROOT, A.C., LEIM, D.H., and WEYLAND, J.W. (1985). Kathon-CG: Cosmetic allergy and patch test sensitization. Contact Derm. **12**(2), 76–80.
5. CHAN, P.K., BALDWIN, R.C., PARSONS, R.D., MOSS, J.N., STIRATELLI, R., SMITH, J.M., and HAYES, A.W. (1983). Kathon biocide: Manifestation of delayed contact dermatitis in guinea pigs is dependent on the concentration for induction and challenge. J. Invest. Dermatol. **81**(5), 409–11.
6. PILGER, C., NETHERCOTT, J.R., and WEKSBERG, F. (1986). Allergic contact dermatitis due to a biocide containing 5-chloro-2-methyl-4-isothiazoline-3-one. Contact Derm. **14,** 201–4.
7. LEWIS, S.N., MILLER, G.A., HAUSMAN, M., and SZAMBORSKI, E.C. (1971). Isothiazoles I: 4-isothiazolin-3-ones. A general synthesis from 3,3'-dithiodipropionamides. J. Heterocycl. Chem. **8,** 571–80.
8. WRIGHT, C., GINGOLD, E., VENITT, S., and CROFTON-SLEIGH, C. (1983). Mutagenic activity of Kathon, an industrial biocide and cosmetic preservative containing 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one. Mutat. Res. **119,** 35–43.
9. CROW, W.D. and GOSNEY, I. (1969). Isothiazolone chemistry VI. Reactions of carbanions with *N*-ethyl-3-isothiazolone. Aust. J. Chem. **22,** 765.
10. MONTE, W.C., ASHOOR, S.H., and LEWIS, B.J. (1983). Mutagenicity of two non-formaldehyde-forming antimicrobial agents. Food Chem. Toxicol. **21**(5), 695–6.
11. KRZEMINSKI, S.F., BRACKETT, C.K., and FISHER, J.D. (1975). Fate of microbicidal 3-isothiazolone compounds in the environment: Modes and rates of dissipation. J. Agric. Food Chem. **23**(6), 1060–8.
12. KRZEMINSKI, S.F., BRACKETT, C.K., FISHER, J.D., and SPINNLER, J.F. (1975). Fate of microbicidal 3-isothiazolone compounds in the environment: Products of degradation. J. Agric. Food Chem. **23**(6), 1068–75.
13. DUC, T.L., PAPACONSTANTIN, E., and ETIENNE, J.J. (1983). Concentrated solutions of preservatives: Their technological and analytical advantages in cosmetic products. Int. J. Cosmet. Sci. **5**(2), 29–38.
14. MATISSEK, R., DROSS, A., and HAEUSSLER, M. (1984). Analysis of microbiocidal isothiazolones. II. Identification by thin-layer chromatography. Fresenius' Z. Anal. Chem. **319**(5), 520–3.
15. GRUVBERGER, B., PERSSON, K., BJORKNER, B., BRUZE, M., DAHLQUIST, I., and FREGERT, S. (1986). Demonstration of Kathon-CG in some commercial products. Contact Derm. **15,** 24–7.
16. MATISSEK, R., and WITTKOWSKI, R. (1984). Analysis of microbiocidal isothiazolones. I. Identification by gas chromatography/mass spectrometry. Fresenius' Z. Anal. Chem. **318**(6), 414–8.
17. FEDERAL REGISTER. (February 1, 1985). Indirect food additives; adhesives and components of coatings; paper and paperboard components. United States Food and Drug Administration **50**(22), 4643-5.
18. EUROPEAN ECONOMIC COMMUNITY (EEC) COSMETICS DIRECTIVE. (June 1986). Regulations and recommendations relating to cosmetic products. Directive 76/768/EEC.
19. EEC COSMETICS DIRECTIVE. (February 21, 1989). Regulations and recommendations relating to cosmetic products. Directive 89/174/EEC.
20. RASTOGI, S.C. (1988). Kathon-CG content in cosmetic products. Contact Derm. **19,** 263.
21. FOOD AND DRUG ADMINISTRAITON (FDA). (1986). Cosmetic product formulation data. Washington, DC.
22. CODE OF FEDERAL REGULATIONS. (Revised as of April 1, 1984). Title 21, Part 720.4. Voluntary filing of cosmetic product ingredient and cosmetic raw material composition statements. Information requested about cosmetic products. Government Printing Office, Washington, D.C.

23. MALLAK, F.P., and BRUNKER, R.L. (1984). Determination of the toxicity of selected metal working fluid preservatives by use of the microtox system and *in vitro* enzyme assay. Drug Chem. Toxicol. Toxic. Screening Proc. Using Bact. Syst. **1,** 65–76.
24. CODE OF FEDERAL REGULATIONS. (Revised as of April 1, 1984). Title 21. Part 176.300. Slimicides.
25. CODE OF FEDERAL REGULATIONS. (Revised as of April 1, 1984). Title 21, Part 175.105. Indirect food additives: adhesives and components of coatings. Adhesives. Government Printing Office, Washington, D.C.
26. CODE OF FEDERAL REGULATIONS. (Revised as of April 1, 1984). Title 21, Part 176.170. Indirect food additives: paper and paperboard components. Components of paper and paperboard in contact with aqueous and fatty foods. Government Printing Office, Washington, D.C.
27. VOETS, J.P., PIPYN, P., VAN LANCKER, P., and VERSTRAETE, W. (1976). Degradation of microbiocides (sic) under different environmental conditions. J. Appl. Bacteriol. **40**(1), 67–72.
28. ZEELIE, J.J. and McCARTHY, T.J. (1983). Antioxidants: Multifunctional preservatives for cosmetic and toiletry formulations. Cosmet. Toiletries. **98**(12), 51–5.
29. BECRENS, H., ROMOND, C., and LEPAGE, C. (1980). The synergistic action of isothiazolone E + imidazolidinylurea. Parfums, Cosmet., Aromes. **36,** 81–4, 87. (French.)
30. ROHM AND HAAS COMPANY. (August, 1984). Evaluation of the toxicity of Kathon biocide. Toxicology Dept., Spring House, PA. (Unpublished data.)[1]
31. DEBETHIZY, J.D., LONGACRE, S.L., STEIGERWALT, R.B., DECKERT, F.W., MOSS, J.N., HAYES, A.W., SMITH, J.M., and SCRIBNER, H.E. (1986). Absorption and disposition of ^{14}C-labelled Kathon biocide, a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, following intravenous or dermal administration to male Sprague-Dawley rats. Fd. Chem. Toxicol. **24**(1), 43–9.
32. ROHM AND HAAS COMPANY. (February 16, 1987). Kathon biocide: Dermal/oral absorption study in male rats. Report No. 86R-041. Rohm and Haas Co., Spring House, PA (Unpublished data.).[1]
33. ROHM AND HAAS COMPANY. (December 18, 1987). Kathon biocide: Comparison of ^{14}C-metabolite profiles following oral and dermal dosing in male rats. (Draft Report). Report No. 86R-232. Rohm and Haas Co., Spring House, PA (Unpublished data.)[1]
34. ROHM AND HAAS COMPANY. (December 18, 1987). ^{14}C-Kathon biocide: *In vitro* dermal absorption study in male rat skin. (Draft Report). Report No. 86R-156. Rohm and Haas Co., Spring House, PA (Unpublished data.)[1]
35. HODGE, H.C., and STERNER, J.H. (1949). Tabulation of toxicity classes. Am. Indus. Hyg. A. Quart. **10,** 93–6.
36. KEMPER, F.H. and LUEPKE, N.P. (1986). The HET-CAM test: An alternative to the Draize eye test. Food Chem. Toxicol. 24(6-7):495–96.
37. LUEPKE, N.P. (1985). Hen's egg chorioallantoic membrane test for irritation potential. Food Chem. Toxicol. **23**(2), 287–91.
38. RITZ, H.L., and BUEHLER, E.V. (1970). Planning, conduct, and interpretation of guinea pig sensitization patch tests. In: V.A. Drill and P. Lazar (Editors). *Current Concepts in Cutaneous Toxicity*. New York: Academic Press, pp. 25–40.
39. FINNEY, D.J. (1971). *Probit Analysis*. London: Cambridge University Press.
40. BEUTHE, H.F. (1982). Expert opinion concerning the experimental sensitivity test of Kathon-CG for contact allergy in guinea pigs. Universitaet Hamburg, Universitats-Krankenhaus (University Hospital). Private communication. Unpublished data as cited by Chan et al. (1983).
41. AMES, B.N., McCANN, J., and YAMASAKI, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. **31,** 347–64.
42. VENITT, S. and CROFTON-SLEIGH, C. (1981). Mutagenicity of 42 coded compounds in a bacterial assay using *Escherichia coli* and *Salmonella typhimurium*. In: F.J. de Serres and J. Ashby (Editors). *Progress in Mutation Research*, Vol. 1. *Evaluation of Short-Term Tests for Carcinogens*. Report of the International Collaborative Program. North Holland, Amsterdam: Elsevier, pp. 351–60.
43. GATEHOUSE, D. (1978). Detection of mutagenic derivatives of cyclophosphamide and a variety of other mutagens in a "microtitre" fluctuation test, without microsomal activation. Mutat. Res. 53(3):289–96.
44. ASHBY, J., RICHARDSON, C.R., LeFEVRE, P.A., CALLANDER, R.D., and STYLES, J.A. (1985). Chloroacetamide-*N*-metholol: An example of an *in vitro* and *in vivo* clastogen which is non-mutagenic to *Salmonella*. Mutat. Res. **156**(1–2), 19–32.
45. RICHARDSON, C.R., STYLES, J.A., and BURLINGTON, B. (1983). Evaluation of some formaldehyde-release compounds and other biocides in the mouse micronucleus test. Mutat. Res. **124**(3–4), 241–6.
46. SCRIBNER, H.E., McCARTHY, K.L., MOSS, J.N., HAYES, A.W., SMITH, J.M., CIFONE, M.A., PROBST, G.S., and VALENCIA, R. (1983). The genetic toxicology of Kathon biocide, a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one. Mutat. Res. **118**(3), 129–52.
47. WILLIAMS, G.M. (1977). Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. Cancer Res. 37:1845–51.

[1]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, N.W., Suite 310, Washington, DC 20036.

48. WILLIAMS, G.M. (1978). Further improvements in hepatocyte primary culture DNA repair test for carcinogens: detection of carcinogenic biphenyl derivatives. Cancer Lett. 4:69–75.
49. PROBST, G.S., McMAHON, R.E., THOMPSON, C.Z., EPP, J., and HILL, L.E. (1981). A comparison of DNA repair in cultured rat hepatocytes with bacterial mutagenesis using 216 compounds. Environ. Mutagen. 3:11–32.
50. FEDERAL REGISTER. (September 7, 1988). 2-Methyl-4-Isothiazolin-3-One, 5-Chloro-2-Methyl-4-Isothiazolin-3-One, and Magnesium Nitrate: Exemptions from tolerances. United States Environmental Protection Agency. **53**(173), 34511–2.
51. ENVIRONMENTAL PROTECTION AGENCY SCIENTIFIC ADVISORY COMMITTEE REPORT. (October 25, 1989).Mutagenicity Subcommittee Report.
52. O'HARA, G.P., MATLACK, J.R., MOSS, J.N., DIDONATO, L.J., SMITH, J.M., and HAYES, A.W. (1984). A dermal carcinogenesis study in male mice with Kathon-CG. Toxicologist **4,** 37 (Abstract No. 145).
53. MAIBACH, H.I. (1980). Modified Draize sensitization study—Kathon. Unpublished data. Received September 13, 1989.[1]
54. MAIBACH, H.I. (1985). Diagnostic patch test concentration for Kathon-CG. Contact Derm. **13,** 242–5.
55. CARDIN, C.W., WEAVER, J.E., and BAILEY, P.T. (1986). Dose-response assessments of Kathon biocide. (II) Threshold prophetic patch testing. Cont. Derm. **15,** 10–6.
56. WEAVER, J.E., CARDIN, C.W., and MAIBACH, H.I. (1985). Dose-response assessments of Kathon biocide. (I) Diagnostic use and diagnostic threshold patch testing with sensitized humans. Contact Derm. **12**(3), 141–5.
57. FREGERT, S. (1981). *Manual of Contact Dermatitis*, Second Edition. Copenhagen: Munksgaard. Year Book Medical Publishers, pp. 71–81.
58. SCHWARTZ, S.R., WEISS, S., STERN, E., MORICI, I.J., MOSS, J.N., GOODMAN, J.J., and SCARBOROUGH, N.L. (1987). Human safety study of body lotion containing Kathon-CG. Contact Derm. **16,** 203–7.
59. ROHM AND HAAS COMPANY. (July 1, 1989). Kathon-CG biocide. Draft review of toxicity and carcinogenicity by independent committee(6 pages). Kathon-CG biocide, vehicle effect on dermal absorption in mice (75 pages). Shelanski repeat insult patch testing with generic skin care lotion (50 pages). Study 51 P. (Unpublished data.)[1]
60. ROHM AND HAAS COMPANY. (February 20, 1990). Letter from C. Lawrence to R. Elder. Preservatives used in control lotion in Shelanski repeat insult patch test. (Unpublished data.)[1]
61. ROHM AND HAAS COMPANY. (November 14, 1989). Evaluation of the skin irritating and sensitizing propensities of Lotion TD-86-14 via supervised in-clinic use test in adults. Report #PI-5389-KU. (Unpublished data.)[1]
62. HELENE CURTIS. (November 16, 1989). Repeat insult patch tests of hand and body lotions. Unpublished data.[1]
63. ROHM AND HAAS COMPANY. (May 17, 1990). A double-blind study to determine the topical contact sensitization potential of three test products. Study No. 743 RN 1289. International Research Services, Inc., 52 pp. (Unpublished data.)[1]
64. ROHM AND HAAS COMPANY. (July 18, 1990). Modified Draize skin sensitization study #HIM 89-R-R&H-D-1&2. (Unpublished data.)[1]
65. CIR EXPERT PANEL MEETING MINUTES. April 16–17, 1990.[1]
66. COSMETIC, TOILETRY AND FRAGRANCE ASSOCIATION (CTFA). (1989). Summary of twenty-one RIPT sensitization assays on Kathon-CG. (Unpublished data.)[1]
67. CTFA. (1989). Evaluation of the skin irritating and sensitization propensities of formulation 03046-044 in humans. Unpublished data. Received November 22, 1989.[1]
68. CTFA. (1989). Evaluation of the skin irritating and sensitization propensities of formulation 03046-043 in humans. Unpublished data. Received November 22, 1989.[1]
69. CTFA. (1989). Evaluation of the skin irritating and sensitization propensities of a lotion containing 7.5 ppm Kathon-CG. Unpublished data. Received November 22, 1989.[1]
70. CTFA. (1989). Evaluation of the skin irritating and sensitization propensities of a cream containing 7.5 ppm Kathon-CG. Unpublished data. Received November 22, 1989.[1]
71. CTFA. (1989). Evaluation of potential hazards by dermal contact creams containing 3 ppm Kathon-CG. Unpublished data. Received November 29, 1989.[1]
72. CTFA. (1989). Repeat insult path test on bath gel on 50 subjects containing 15 ppm Kathon-CG. Unpublished data. Received November 22, 1989.[1]
73. CTFA. (1989). Evaluation of the skin irritation and sensitizing propensities of a W/O cream containing 4.5 ppm Kathon-CG. Unpublished data. Received November 22, 1989.[1]
74. CTFA. (1989). Evaluation of the skin irritating and sensitizing propensities of white lotion 108C5 containing 6 ppm Kathon-CG. Unpublished data. Received November 22, 1989.[1]
75. CTFA. (1989). Evaluation to determine potential skin hazards of dermal contact with white lotion Code No. 410 containing 7.5 ppm Kathon-CG. Unpublished data.[1]
76. CTFA. (1989). Repeat insult patch test on 100 subjects—7.5 ppm Kathon-CG. Unpublished data. Received November 22, 1989.[1]
77. CTFA. (1989). Summary of repeat insult patch test data on 2335 subjects with 28 formulations containing 7.5 ppm Kathon-CG. Unpublished data. Received November 16, 1989.[1]
78. MATHIAS, C.G.T., ANDERSEN, K.E., and HAMANN, K. (1983). Allergic contact dermatitis from 2-*n*-octyl-4-isothiazolin-3-one, a paint mildewcide. Contact Derm. **9,** 507–9.

79. THORMANN, J. (1982). Contact dermatitis to a new fungicide, 2-*n*-octyl-4-isothiazolin-3-one. Contact Derm. **8,** 204.
80. FOUSSEREAU, J., BRANDLE, I., and BOUJNAH-KHOUADJA, A. (1984). Allergic contact eczema caused by isothiazolin-3-one derivatives. Dermatosen Beruf. und Umwelt. **32**(6), 208–11. (German.)
81. ROBERTS, D.L., MESSENGER, A.G., and SUMMERLY, R. (1981). Occupational dermatitis due to 1,2-benzisothiazolin-3-one in the pottery industry. Contact Derm. **7,** 145–7.
82. FREEMAN, S. (1984). Allergic contact dermatitis due to 1,2-benzisothiazolin-3-one in gum arabic. Contact Derm. **11,** 146–9.
83. ANDERSEN, K.E., and VEIEN, N.K. (1985). Biocide patch tests. Contact Derm. **12**(2), 99–103.
84. PEDERSON, N.B. (1976). Occupational allergy from 1,2-benzisothiazolin-3-one and other preservatives in plastic emulsions. Contact Derm. **2,** 340–2.
85. BROWN, R. (1979). Concomitant sensitization to additives in a coolant fluid. Contact Derm. **5,** 340–1.
86. DAHLQUIST, I. (1977). Contact allergy to 3-ethylamino-1,2-benzisothiazol-hydrochloride, a veterinary fungicide. Contact Derm. **3,** 277.
87. BJORKNER, B., BRUZE, M., DAHLQUIST, I., FREGERT, S., GRUVBERGER, B., and PERSSON, K. (1986). Contact allergy to the preservative Kathon-CG. Contact Derm. **14,** 85–90.
88. DeGROOT, A.C., LEIM, D.H., NATER, J.P., and VAN KETEL, W.G. (1985). Patch tests with fragrance materials and preservatives. Contact Derm. **12**(2), 87–92.
89. SHUSTER, S., and SPIRO, J. (1987). Measurement of risk of sensitization and its application to Kathon. Contact Derm. **17,** 299–302.
90. HANNUKSELA, M. (1986). Rapid increase in contact allergy to Kathon-CG in Finland. Contact Derm. **15,** 211–4.
91. DeGROOT, A.C., and BRUYNZEEL, D.P. (1987). Letters to the editor, Kathon-CG. Contact Derm. **17,** 189–91.
92. TOSTI, A., MANUZZI, P., and DE PADOVA, M.D. (1986). Contact dermatitis to Kathon-CG. Contact Derm. **14**(5), 326–7.
93. HJORTH, N., and ROED-PETERSEN, J. (1986). Patch test sensitivity to Kathon-CG. Contact Derm. **14,** 155–7.
94. BRUZE, M., FREGERT, S., GRUVBERGER, B., and PERSSON, K. (1987). Contact allergy to the active ingredients of Kathon-CG in the guinea pig. Acta Dermatovenereologica **67,** 315–20.
95. BRUZE, M., DAHLQUIST, I., and GRUVBERGER, B. (1989). Contact allergy to dichlorinated methylisothiazolinone. Contact Derm. **26,** 219–39.
96. DeGROOT, A.C., BRUYNZEEL, D.P., VAN DER SCHROEFF, J.G., and BOS, J.D. (1988). Routine patch testing with the preservative system. Kathon-CG. Int. J. Cosmet. Sci. **10,** 47–51.
97. DeGROOT, A.C., BRUYNZEEL, D.P., BOS, J.D., VAN DER MEEREN, H., VAN JOOST, T., JAGTMAN, B.A., and WEYLAND, W. (1988). The allergens in cosmetics. Arch. Dermatol. **124,** 1525–9.
98. PASCHE, F., and HUNZIKER, N. (1989). Sensitization to Kathon-CG in Geneva and Switzerland. Contact Derm. **20,** 115–9.
99. DeGROOT, A.C., and HERXHEIMER, A. (1989). Isothiazolinone preservative: Cause of a continuing epidemic of cosmetic dermatitis. Lancet, **1,** 314–6.
100. FROSCH, P.J., and SCHULZE-DIRKS. (1987). Kontaktallergie Auf Kathon-CG. Hautarzt **38,** 422–5. (German)
101. FRANSWAY, A.F. (1988). Sensitivity to Kathon-CG; findings in 365 consecutive patients. Contact Derm. **19,** 342–47.
102. FOUSSEREAU, J. (1990). An epidemiological study of contact allergy to 5-chloro-3-methylisothiazolinone in Strasbourg. Contact Derm. **22,** 68–70.
103. RIETSCHEL, R.L., NETHERCOTT, J.R., EMMETT, E.A., MAIBACH, H.I., STORRS, F.J., LARSEN, W.G., ADAMS, R.M., TAYLOR, J.S., MARK, J.G., MITCHELL, J.C., FISHER, A.A., KANOF, N.B., CLEDENNING, W.E., and SCHORR, W.F. (In press). Methylchloroisothiazolinone/Methylisothiazolinone reactions in patients screened for vehicle and preservative hypersensitivity. North American Contact Dermatitis Group. Received 9/15/89.
104. LEWIS, P.G., and MOSS, J.M. (1986). Isothiazolinone preservatives: A toxicological evaluation. Society of the American Academy of Dermatology, 2nd International Meeting on Cosmetic Dermatology.

2019 FDA VCRP RAW DATA

01A - Baby Shampoos	METHYLCHLOROISOTHIAZOLINONE	8
01B - Baby Lotions, Oils, Powders, and Creams	METHYLCHLOROISOTHIAZOLINONE	2
01C - Other Baby Products	METHYLCHLOROISOTHIAZOLINONE	1
02A - Bath Oils, Tablets, and Salts	METHYLCHLOROISOTHIAZOLINONE	6
02B - Bubble Baths	METHYLCHLOROISOTHIAZOLINONE	109
02D - Other Bath Preparations	METHYLCHLOROISOTHIAZOLINONE	21
03A - Eyebrow Pencil	METHYLCHLOROISOTHIAZOLINONE	2
03B - Eyeliner	METHYLCHLOROISOTHIAZOLINONE	3
03D - Eye Lotion	METHYLCHLOROISOTHIAZOLINONE	2
03E - Eye Makeup Remover	METHYLCHLOROISOTHIAZOLINONE	2
03F - Mascara	METHYLCHLOROISOTHIAZOLINONE	13
03G - Other Eye Makeup Preparations	METHYLCHLOROISOTHIAZOLINONE	10
04A - Cologne and Toilet waters	METHYLCHLOROISOTHIAZOLINONE	2
04E - Other Fragrance Preparation	METHYLCHLOROISOTHIAZOLINONE	3
05A - Hair Conditioner	METHYLCHLOROISOTHIAZOLINONE	558
05B - Hair Spray (aerosol fixatives)	METHYLCHLOROISOTHIAZOLINONE	6
05C - Hair Straighteners	METHYLCHLOROISOTHIAZOLINONE	10
05D - Permanent Waves	METHYLCHLOROISOTHIAZOLINONE	1
05E - Rinses (non-coloring)	METHYLCHLOROISOTHIAZOLINONE	4
05F - Shampoos (non-coloring)	METHYLCHLOROISOTHIAZOLINONE	805
05G - Tonics, Dressings, and Other Hair Grooming Aids	METHYLCHLOROISOTHIAZOLINONE	111
05H - Wave Sets	METHYLCHLOROISOTHIAZOLINONE	1
05I - Other Hair Preparations	METHYLCHLOROISOTHIAZOLINONE	63
06C - Hair Rinses (coloring)	METHYLCHLOROISOTHIAZOLINONE	31
06D - Hair Shampoos (coloring)	METHYLCHLOROISOTHIAZOLINONE	32
06H - Other Hair Coloring Preparation	METHYLCHLOROISOTHIAZOLINONE	5
07B - Face Powders	METHYLCHLOROISOTHIAZOLINONE	1
07H - Makeup Fixatives	METHYLCHLOROISOTHIAZOLINONE	1
07I - Other Makeup Preparations	METHYLCHLOROISOTHIAZOLINONE	1
08B - Cuticle Softeners	METHYLCHLOROISOTHIAZOLINONE	1
10A - Bath Soaps and Detergents	METHYLCHLOROISOTHIAZOLINONE	2211
10C - Douches	METHYLCHLOROISOTHIAZOLINONE	2
10E - Other Personal Cleanliness Products	METHYLCHLOROISOTHIAZOLINONE	632
11E - Shaving Cream	METHYLCHLOROISOTHIAZOLINONE	8
11F - Shaving Soap	METHYLCHLOROISOTHIAZOLINONE	1
11G - Other Shaving Preparation Products	METHYLCHLOROISOTHIAZOLINONE	3

12A - Cleansing	METHYLCHLOROISOTHIAZOLINONE	191
12B - Depilatories	METHYLCHLOROISOTHIAZOLINONE	2
12C - Face and Neck (exc shave)	METHYLCHLOROISOTHIAZOLINONE	58
12D - Body and Hand (exc shave)	METHYLCHLOROISOTHIAZOLINONE	54
12F - Moisturizing	METHYLCHLOROISOTHIAZOLINONE	59
12G - Night	METHYLCHLOROISOTHIAZOLINONE	6
12H - Paste Masks (mud packs)	METHYLCHLOROISOTHIAZOLINONE	14
12I - Skin Fresheners	METHYLCHLOROISOTHIAZOLINONE	7
12J - Other Skin Care Preps	METHYLCHLOROISOTHIAZOLINONE	65
13B - Indoor Tanning Preparations	METHYLCHLOROISOTHIAZOLINONE	9
01A - Baby Shampoos	METHYLISOTHIAZOLINONE	10
01B - Baby Lotions, Oils, Powders, and Creams	METHYLISOTHIAZOLINONE	2
01C - Other Baby Products	METHYLISOTHIAZOLINONE	4
02A - Bath Oils, Tablets, and Salts	METHYLISOTHIAZOLINONE	6
02B - Bubble Baths	METHYLISOTHIAZOLINONE	117
02D - Other Bath Preparations	METHYLISOTHIAZOLINONE	23
03A - Eyebrow Pencil	METHYLISOTHIAZOLINONE	2
03B - Eyeliner	METHYLISOTHIAZOLINONE	5
03C - Eye Shadow	METHYLISOTHIAZOLINONE	1
03D - Eye Lotion	METHYLISOTHIAZOLINONE	14
03E - Eye Makeup Remover	METHYLISOTHIAZOLINONE	4
03F - Mascara	METHYLISOTHIAZOLINONE	19
03G - Other Eye Makeup Preparations	METHYLISOTHIAZOLINONE	15
04A - Cologne and Toilet waters	METHYLISOTHIAZOLINONE	2
04E - Other Fragrance Preparation	METHYLISOTHIAZOLINONE	5
05A - Hair Conditioner	METHYLISOTHIAZOLINONE	603
05B - Hair Spray (aerosol fixatives)	METHYLISOTHIAZOLINONE	7
05C - Hair Straighteners	METHYLISOTHIAZOLINONE	10
05D - Permanent Waves	METHYLISOTHIAZOLINONE	1
05E - Rinses (non-coloring)	METHYLISOTHIAZOLINONE	6
05F - Shampoos (non-coloring)	METHYLISOTHIAZOLINONE	842
05G - Tonics, Dressings, and Other Hair Grooming Aids	METHYLISOTHIAZOLINONE	193
05H - Wave Sets	METHYLISOTHIAZOLINONE	4
05I - Other Hair Preparations	METHYLISOTHIAZOLINONE	104
06C - Hair Rinses (coloring)	METHYLISOTHIAZOLINONE	31
06D - Hair Shampoos (coloring)	METHYLISOTHIAZOLINONE	32
06H - Other Hair Coloring Preparation	METHYLISOTHIAZOLINONE	5

07B - Face Powders	METHYLISOTHIAZOLINONE	1
07C - Foundations	METHYLISOTHIAZOLINONE	1
07D - Leg and Body Paints	METHYLISOTHIAZOLINONE	3
07E - Lipstick	METHYLISOTHIAZOLINONE	1
07F - Makeup Bases	METHYLISOTHIAZOLINONE	1
07H - Makeup Fixatives	METHYLISOTHIAZOLINONE	1
07I - Other Makeup Preparations	METHYLISOTHIAZOLINONE	8
08B - Cuticle Softeners	METHYLISOTHIAZOLINONE	1
08C - Nail Creams and Lotions	METHYLISOTHIAZOLINONE	1
08G - Other Manicuring Preparations	METHYLISOTHIAZOLINONE	2
10A - Bath Soaps and Detergents	METHYLISOTHIAZOLINONE	2256
10C - Douches	METHYLISOTHIAZOLINONE	2
10E - Other Personal Cleanliness Products	METHYLISOTHIAZOLINONE	694
11A - Aftershave Lotion	METHYLISOTHIAZOLINONE	2
11B - Beard Softeners	METHYLISOTHIAZOLINONE	1
11D - Preshave Lotions (all types)	METHYLISOTHIAZOLINONE	1
11E - Shaving Cream	METHYLISOTHIAZOLINONE	19
11F - Shaving Soap	METHYLISOTHIAZOLINONE	1
11G - Other Shaving Preparation Products	METHYLISOTHIAZOLINONE	12
12A - Cleansing	METHYLISOTHIAZOLINONE	261
12B - Depilatories	METHYLISOTHIAZOLINONE	1
12C - Face and Neck (exc shave)	METHYLISOTHIAZOLINONE	192
12D - Body and Hand (exc shave)	METHYLISOTHIAZOLINONE	93
12E - Foot Powders and Sprays	METHYLISOTHIAZOLINONE	1
12F - Moisturizing	METHYLISOTHIAZOLINONE	203
12G - Night	METHYLISOTHIAZOLINONE	19
12H - Paste Masks (mud packs)	METHYLISOTHIAZOLINONE	54
12I - Skin Fresheners	METHYLISOTHIAZOLINONE	20
12J - Other Skin Care Preps	METHYLISOTHIAZOLINONE	83
13A - Suntan Gels, Creams, and Liquids	METHYLISOTHIAZOLINONE	5
13B - Indoor Tanning Preparations	METHYLISOTHIAZOLINONE	29
13C - Other Suntan Preparations	METHYLISOTHIAZOLINONE	1

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 1, 2019

SUBJECT: MCI/MI

Cantor Research Laboratories, Inc. 2006. 50 Human subject repeat insult patch test skin irritation/sensitization evaluation (open patch; hand wash containing 12 ppm MCI/MI).

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50 HUMAN SUBJECT REPEAT INSULT OPEN PATCH TEST
SKIN IRRITATION/SENSITIZATION EVALUATION
(OPEN PATCH)

Date: September 14, 2006

CR Ref. No.:

Sponsor:

1.0 Objective: Consumer products or raw materials designed for consistent reapplication to areas of the skin may, under proper conditions, prove to be contact sensitizers or irritants in certain individuals. It is the intention of a Repeat Insult Patch Test (RIPT) to provide a basis for evaluation of this irritation/ sensitization potential if such exists.

2.0 Reference: The method is modified to test 50 panelists and not the 200 cited in the reference Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, published by The Association of Food and Drug Officials of The United States. The method also employs nine inductive patchings and not the ten cited in the reference under open patch conditions.

3.0 Test Material:

3.1 Test Material Description:

On July 19, 2006 one test sample labeled Liquid Hand Soap, Formula No. 1-5-2150 was received signed CR Lab No. D0719-C. (contains 12ppm MCI/

3.2 Handling: Upon arrival at Cantor Research Laboratories, Inc., the test material is assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date and test requested.
Samples are retained for a period of three months beyond submission of final reports unless otherwise specified by the sponsor or, if sample is known to be in support of governmental applications, representative retained samples are kept two years beyond final report submission.

Sample disposition is conducted in compliance with appropriate federal, state and local ordinances.

3.3 Test Material Evaluation Prerequisite:

Prior to induction of a human test panel, animal toxicology, microbiology and other in-vivo or in-vitro performance spectra may be required to assess the feasibility of commencement as dictated by an Institutional Review Board (IRB) described in Section 4.0.

3.3.1 Sponsor purports that prior to sample submission to Cantor Research Laboratories, Inc. the following tests were conducted with no adverse results and that the test data are on file on their premises and have not been made available to Cantor Research Laboratories, Inc. personnel:

- CTFA Preservative Efficacy Test or equivalent
- 90 Day Accelerated Stability and Container Compatibility Study

4.0 Institutional Review Board:

Reference: CFR Title 21 Part 56, Subparts A, B, C, and D. The IRB of Cantor Research Laboratories, Inc., consists of five or more individuals, chosen from within the company for technical expertise and from the local community for lay interaction. The list of IRB members is kept on file at Cantor Research Laboratories, Inc., and is available for inspection during the hours of operation.

5.0 Panel Selection:

5.1 Standards for Inclusion in a Study:

- Individuals who are not currently under a doctor's care.
- Individuals free of any dermatological or systemic disorder which would interfere with the results, at the discretion of the Investigator.
- Individuals free of any acute or chronic disease that might interfere with or increase the risk of study participation.
- Individuals who will complete a preliminary medical history form mandated by Cantor Research Laboratories, Inc. and are in general good health.
- Individuals who will read, understand and sign an informed consent document relating to the specific type

of study they are subscribing. Consent forms are kept on file and are available for examination on the premises of Cantor Research Laboratories, Inc. only.
- Individuals able to cooperate with the investigator and research staff, willing to have test materials applied according to the protocol, and complete the full course of the study.

5.2 Standards for Exclusion from a Study:

- Individuals under 18 years of age.
- Individuals who are under doctor's care.
- Individuals who are currently taking any medication (topical or systemic) that may mask or interfere with the test results.
- Subjects with a history of any acute or chronic disease that might interfere with or increase the risk of study participation.
- Individuals diagnosed with chronic skin allergies.
- Female volunteers who indicate that they are pregnant or nursing.

5.3 Recruitment:

Panel selection is accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

5.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms are available for inspection on the premises of Cantor Research Laboratories, Inc. only. Reference 21 CFR Ch. 1 Part 50, Subpart B.

6.0 Population Demographics:

Number of subjects enrolled		60
Number of subjects completing study		60
Age Range		18 – 68
Sex	Male	23
	Female	37
Race	Caucasian	33
	Hispanic	15
	Asian	0
	African American	12

7.0 Equipment:

- Acculine Surgical Marking Pen (Accu-Line Products, Inc.).
- 1 ml volumetric syringe without a needle.

8.0 Procedure:

- Subjects are requested to bathe or wash as usual before arrival at the facility.
- 0.2 ml or 0.2 g of the test material was dispensed directly onto a designated area of the panelist's back and allowed to air dry. (area ~ 2 cm^2)
- This procedure is repeated until a series of nine consecutive open patch applications have been made for every Monday, Wednesday and Friday for three consecutive weeks. (1.2 MS/cm^2 = dose)
- In the event of an adverse reaction, the area of erythema and edema is measured. The edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin. Reactions are scored just before applications two through nine and the next test date following application nine. Clients are notified immediately in the case of adverse reaction and determination is made as to treatment program if necessary.
- Subjects are then given a 10 - 14 day rest period after which a challenge or retest dose is applied once to a previously unexposed test site. The retest dose is equivalent to any one of the original nine exposures. Reactions are scored 24 and 48 hours after application.
- Comparison is made between the nine inductive responses and the retest dose.

9.0 Results: Please refer to attached Table.

10.0 Observations: No adverse reactions of any kind were noted during the course of this study.

11.0 Archiving: All raw data sheets, technician's notebooks, correspondence files and copies of final reports are maintained on premises of Cantor Research Laboratories, Inc. in limited access storage files marked "Archive" for five years after completion of the study. A duplicate disk copy of final reports is separately archived in a bank safe deposit vault.

12.0 Conclusions: The test material (CR Lab No: D0719-C; Client No: Liquid Hand Soap, Formula I when tested under open patch conditions as described herein, may be considered as a **NON-PRIMARY IRRITANT** and a **NON-PRIMARY SENSITIZER** to the skin according to the reference.

Shyla Cantor, Ph.D.
Study Director

Mellodene Charles, A.A.S.
Technician

Christine M. Bremer, B.S.
Technician

Michelle Peters, B.A.
Quality Assurance Supervisor

9-14-06
Date

TABLE
SUMMARY OF RESULTS
(OPEN PATCH)

CR Lab No.: D0719-C
Client No.: Liquid Hand Soap, F

No.	Subject ID	RACE	SEX	Response									Chall.	
				1	2	3	4	5	6	7	8	9	24 HR	48 HR
1	03-6970	C	M	0	0	0	0	0	0	0	0	0	0	0
2	03-6391	AA	F	0	0	0	0	0	0	0	0	0	0	0
3	03-6755	C	F	0	0	0	0	0	0	0	0	0	0	0
4	03-6994	C	F	0	0	0	0	0	0	0	0	0	0	0
5	03-6153	C	F	0	0	0	0	0	0	0	0	0	0	0
6	03-6975	C	F	0	0	0	0	0	0	0	0	0	0	0
7	03-6668	C	F	0	0	0	0	0	0	0	0	0	0	0
8	03-6065	C	M	0	0	0	0	0	0	0	0	0	0	0
9	03-6871	AA	M	0	0	0	0	0	0	0	0	0	0	0
10	03-6078	C	M	0	0	0	0	0	0	0	0	0	0	0
11	03-6037	C	F	0	0	0	0	0	0	0	0	0	0	0
12	03-6509	AA	F	0	0	0	0	0	0	0	0	0	0	0
13	03-6854	AA	M	0	0	0	0	0	0	0	0	0	0	0
14	03-6986	H	F	0	0	0	0	0	0	0	0	0	0	0
15	03-6081	C	F	0	0	0	0	0	0	0	0	0	0	0
16	03-6889	H	F	0	0	0	0	0	0	0	0	0	0	0
17	03-6076	C	F	0	0	0	0	0	0	0	0	0	0	0
18	03-6933	AA	M	0	0	0	0	0	0	0	0	0	0	0
19	03-6923	H	F	0	0	0	0	0	0	0	0	0	0	0
20	03-6176	AA	F	0	0	0	0	0	0	0	0	0	0	0
21	03-6034	C	F	0	0	0	0	0	0	0	0	0	0	0
22	03-6757	C	F	0	0	0	0	0	0	0	0	0	0	0
23	03-6040	C	F	0	0	0	0	0	0	0	0	0	0	0
24	03-6534	C	F	0	0	0	0	0	0	0	0	0	0	0
25	03-6195	C	F	0	0	0	0	0	0	0	0	0	0	0
26	03-6930	C	F	0	0	0	0	0	0	0	0	0	0	0
27	03-6100	C	F	0	0	0	0	0	0	0	0	0	0	0
28	03-6398	AA	M	0	0	0	0	0	0	0	0	0	0	0
29	03-6161	H	F	0	0	0	0	0	0	0	0	0	0	0
30	03-6920	H	M	0	0	0	0	0	0	0	0	0	0	0
31	03-6907	C	M	0	0	0	0	0	0	0	0	0	0	0
32	03-6287	C	M	0	0	0	0	0	0	0	0	0	0	0

TABLE (CONT'D)
SUMMARY OF RESULTS
(OPEN PATCH)

CR Lab No.: D0719-C
Client No.: Liquid Hand Soap,

No.	Subject ID	RACE	SEX	Response									Chall.	
				1	2	3	4	5	6	7	8	9	24 HR	48 HR
33	03-6251	C	M	0	0	0	0	0	0	0	0	0	0	0
34	03-6822	C	M	0	0	0	0	0	0	0	0	0	0	0
35	03-6431	AA	M	0	0	0	0	0	0	0	0	0	0	0
36	03-6430	AA	F	0	0	0	0	0	0	0	0	0	0	0
37	03-6198	AA	F	0	0	0	0	0	0	0	0	0	0	0
38	03-6162	H	M	0	0	0	0	0	0	0	0	0	0	0
39	03-6247	H	F	0	0	0	0	0	0	0	0	0	0	0
40	03-6264	H	M	0	0	0	0	0	0	0	0	0	0	0
41	03-6184	C	F	0	0	0	0	0	0	0	0	0	0	0
42	03-6316	C	F	0	0	0	0	0	0	0	0	0	0	0
43	03-6376	C	M	0	0	0	0	0	0	0	0	0	0	0
44	03-6375	C	M	0	0	0	0	0	0	0	0	0	0	0
45	03-6911	H	M	0	0	0	0	0	0	0	0	0	0	0
46	03-6921	H	F	0	0	0	0	0	0	0	0	0	0	0
47	03-6496	C	M	0	0	0	0	0	0	0	0	0	0	0
48	03-6882	C	M	0	0	0	0	0	0	0	0	0	0	0
49	03-6030	C	F	0	0	0	0	0	0	0	0	0	0	0
50	03-6372	C	F	0	0	0	0	0	0	0	0	0	0	0
51	03-6929	C	F	0	0	0	0	0	0	0	0	0	0	0
52	03-6354	H	M	0	0	0	0	0	0	0	0	0	0	0
53	03-6635	AA	F	0	0	0	0	0	0	0	0	0	0	0
54	03-6652	C	M	0	0	0	0	0	0	0	0	0	0	0
55	03-6411	C	F	0	0	0	0	0	0	0	0	0	0	0
56	03-6183	H	M	0	0	0	0	0	0	0	0	0	0	0
57	03-6393	AA	F	0	0	0	0	0	0	0	0	0	0	0
58	03-6766	H	F	0	0	0	0	0	0	0	0	0	0	0
59	03-6583	H	F	0	0	0	0	0	0	0	0	0	0	0
60	03-6071	H	F	0	0	0	0	0	0	0	0	0	0	0

Evaluation Period:

This study was conducted from August 9, 2006 through September 13, 2006.

Definition of Symbols Shown In Table:

0 - No evidence of any effect

? - (Barely perceptible) minimal faint (light pink) uniform or spotty erythema

1 - (Mild) pink uniform erythema covering most of contact site

2 - (Moderate) pink\red erythema visibly uniform in entire contact area

3 - (Marked) bright red erythema with accompanying edema, petechiae or papules

4 - (Severe) deep red erythema with vesiculation or weeping with or without edema

D - Patch eliminated due to reaction

Dc - Discontinued due to absence of subject on application date

M - Patch applied to an adjacent site after strong test reaction

S - Skin stained from pigment in product

T - Tan

NOTE: All technical employees of Cantor Research Laboratories, Inc. are required to take and pass a visual discrimination examination conducted by a Board Certified Ophthalmologist using the Farnsworth-Munsell 100 Hue Test as published; which determines a person's ability to discern color against a black background. This test was additionally modified to include a flesh tone background more nearly approaching actual use conditions, wherein erythematous skin is graded according to intensity.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 2, 2019

SUBJECT: Updated Concentration of Use Information: MCI/MI

Concentration of Use by FDA Product Category – MCI/MI (ratio approximately 3:1)

FDA Product Category	Rinse-Off/Leave-On*	Maximum Concentration of Use (ppm MCI/MI)
Baby shampoo	Rinse-off	12
Bubble baths	Rinse-off	0.000019
Colognes and toilet waters	Leave-on	0.075
Hair conditioners	Rinse-off	0.82-15
Hair sprays Aerosol Pump spray	 Leave-on Leave-on	 7.5 7.5
Permanent wave	Rinse-off	7.5
Rinses (non-coloring)	Rinse-off	11
Shampoos (non-coloring)	Rinse-off	0.5-15
Tonics, dressings and other hair grooming aids	Rinse-off Leave-on	7.5 7.4
Other hair preparations	Rinse-off	7.5-12
Hair tints	Rinse-off	0.4
Hair rinses (coloring)	Rinse-off	11
Hair shampoos (coloring)	Rinse-off	0.15-6
Other makeup preparations	Leave-on	0.021
Bath soaps and detergents	Rinse-off	3.4-15
Other personal cleanliness products	Rinse-off hand wash Leave-on	15 7.5
Shaving cream	Rinse-off	0.19-4.5
Other shaving preparations	Rinse-off	14.9
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	Rinse-off	4-15

*For each product category, the survey asked if the product was a rinse-off or leave-on product, or if it was a wipe product. No wipe products were reported.

Information collected 2018-2019

Table prepared March 25, 2019

Updated April 16, 2019: added hand wash to other personal cleanliness products and increased maximum use concentration to 15 ppm

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: CIR Science and Support Committee of the Personal Care Products Council

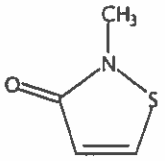
DATE: May 2, 2019

SUBJECT: Quantitative Risk Assessment for Allergic Contact Dermatitis: Methylisothaizolinone/Methylchloroisothiazolinone as Used in Cosmetics

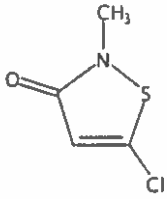
1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

To: Linda Loretz and Carol Eisenmann, Personal Care Products Council
From: CIR Science and Support Committee
Date: May 2, 2019
Subject: Quantitative Risk Assessment for Allergic Contact Dermatitis: Methylisothiazolinone/Methylchloroisothiazolinone as Used in Cosmetics

Purpose: This document provides an exposure-based quantitative risk assessment (QRA) for allergic contact dermatitis to methylisothiazolinone/methylchloroisothiazolinone (MI/MCI) as used in cosmetics. MI/MCI are heterocylic organic compounds that are used in cosmetics as a broad-spectrum preservative system in a mixture of approximately 1 part MI to 3 parts MCI. This preservative is currently under re-review by the Cosmetic Ingredient Review Expert Panel and was last reviewed and published in 1992. The structures of the individual constituents are provided below, along with their individual CAS numbers. When combined in the mixture, the CAS number is given as 55965-84-9.



Methylisothiazolinone (MI; CAS 2682-20-4)



Methylchloroisothiazolinone (MCI; CAS 26172-55-4)

Methods: The approach taken for the QRA is the same as that published for fragrance ingredients (Api et al., 2008). The approach defines an Acceptable Exposure Level (AEL) for daily consumer exposure based on a Weight of Evidence No Expected Sensitization Induction Level (WoE NESIL), to which various Sensitization Assessment Factors (SAFs) are applied, determined by the cosmetic application. The AEL = WoE NESIL/Total SAF. The Consumer Exposure Level (CEL) is determined by the maximum usage levels of MI/MCI recently reported to the Personal Care Products Council (PCPC) by industry members for various cosmetic product types (March 2019). This usage data is coupled with consumer habits and practices data for each cosmetic product type, which typically represents the 90th or 95th percentile cosmetic application rates (as summarized in Api et al., 2008 and the IFRA RIFM QRA Information Booklet Version 7.1, July 2015). Safety assurances for an adequate margin of safety to avoid the induction of allergic contact dermatitis are provided when the AEL/CEL ratio is greater than 1. Aggregate exposure is not considered in this assessment.

Establishment of the WoE NESIL for MI/MCI: The WoE NESIL for MI/MCI is 0.83 μg/cm^2, based primarily on human repeat insult patch test (HRIPT) data (Maibach 1985; Cardin et al., 1986; CIR 1992; SCCNFP/0625/02) and from data from the murine local lymph node assay (LLNA) (Warbrick et al., 1999; Gerberick et al., 2005).

In published studies, no signs of skin sensitization were observed in HRIPTs under patch below 1.04 μg/cm^2. The authors of the study (Cardin et al., 1986) indicated that 1 of 84 subjects tested at 1.04 μg/cm^2 in an aqueous shampoo and 2 of 45 subjects tested at 1.67 μg/cm^2 in an aqueous solution had a reaction suggestive of sensitization late in the induction phase. However, results of rechallenge were inconclusive. A second rechallenge with 8.3 μg/cm^2 confirmed allergy in all 3 subjects. All 3 subjects subsequently participated without incident in provocative use testing (Weaver et al., 1985). Maibach (1985) saw no evidence of sensitization with induction concentrations of 2.5 μg/cm^2 in 96 subjects. At a higher induction concentration of 5.0 μg/cm^2, 2 of 104 subjects responded with reactions to an aqueous solution, while none of 80 subjects responded to the same concentration in a petrolatum vehicle. In an unpublished study (CIR 1992), 184 subjects treated with induction patches at 7.5 ppm MI/MCI-CG (0.75 μg/cm^2) did not respond to challenge at 7.5 ppm, while 1 subjected responded positively to a 100 ppm (10 μg/cm^2) patch challenge. Unpublished data reported in SCCNFP/0625/02 is also included in Table 1.

Table 1: HRIPT data

Test Article	Vehicle, Dose Volume, Patch Size	Induction Concentration (active µg/cm²)	Original Challenge Conc.	Positive responses	Reference
Kathon CG*	Hair conditioner, 10% aq; 0.3 ml / 3.6 cm²	5 ppm (0.42 µg/cm²)	5 ppm	0 / 104	Cardin et al., 1986
Kathon CG	Shampoo, 0.1% aq; 0.3 ml / 3.6 cm²	5 ppm (0.42 µg/cm²)	5 ppm	0 / 197	Cardin et al., 1986
Kathon CG	Liquid soap, 3% aq; 0.3 ml / 3.6 cm²	5 ppm (0.42 µg/cm²)	5 ppm	0 / 115	Cardin et al., 1986
Kathon CG	Shampoo, 0.25% aq; 0.3 ml / 3.6 cm²	6 ppm (0.50 µg/cm²)	6 ppm	0 / 103	Cardin et al., 1986
MCI/MI [3:1]	MI/MCI-CG; unknown volume / HillTop chamber	7.5 ppm (0.75 µg/cm²)	7.5 ppm 100 pm	0 / 184 1 / 184	CIR 1992; SCCNFP/0625/02
Kathon CG	dH_2O; 0.3 ml / 3.6 cm²	10 ppm (0.83 µg/cm²)	10 ppm	0 / 175	Cardin et al., 1986
Kathon CG	Hair conditioner, 3.3% aq; 0.3 ml / 3.6 cm²	10 ppm (0.83 µg/cm²)	10 ppm	0 / 112	Cardin et al., 1986
Kathon CG	Liquid fabric softener, 12.5% aq; 0.3 ml / 3.6 cm²	10 ppm (0.83 µg/cm²)	10 ppm	0 / 163	Cardin et al., 1986
Kathon CG	Body lotion, as is; 0.3 ml / 3.6 cm²	10 ppm (0.83 µg/cm²)	10 ppm	0 / 152	Cardin et al., 1986
Kathon CG	Shampoo, 0.1% aq; 0.3 ml / 3.6 cm²	12.5 ppm (1.04 µg/cm²)	12.5 ppm	1 / 84	Cardin et al., 1986
Kathon CG	Body lotion, as is; 0.3 ml / 3.6 cm²	15 ppm (1.25 µg/cm²)	15 ppm	0 / 200	Cardin et al., 1986
MCI/MI [3:1]	MI/MCI-CG; 0.2 ml / 2.3 cm²	15 ppm (1.34 µg/cm²)	15 ppm 100 ppm	0 / 189 2 / 189	CIR 1992; SCCNFP/0625/02
Kathon CG	dH_2O; 0.3 ml / 3.6 cm²	20 ppm (1.67 µg/cm²)	20 ppm	2 / 45	Cardin et al., 1986
Kathon CG	dH_2O with 2.5% Tween 85; 0.2 ml / 4 cm² (assumed)	50 ppm (2.5 µg/cm²)	50 ppm	0/96	Maibach 1985
MCI/MI [3:1]	Not indicated	50 ppm (2.5 µg/cm²)	50 ppm (assumed)	0 / 109	SCCNFP/0625/02
Kathon CG	dH_2O with 2.5% Tween 85; 0.2 ml / 4 cm² (assumed)	100 ppm (5 µg/cm²)	100 ppm	2/104	Maibach 1985
Kathon CG	petrolatum; 0.2 ml / 4 cm² (assumed)	100 ppm (5 µg/cm²)	100 ppm	0/80	Maibach 1985
MCI/MI [3:1]	Not indicated	100 ppm (5 µg/cm²)	100 ppm (assumed)	5 / 116	SCCNFP/0625/02
MCI/MI [3:1]	Not indicated	150 ppm (7.5 µg/cm²)	150 ppm (assumed)	7 / 196	CIR 1992

*Kathon CG is 1.15% MCI, 0.35% MI, 23.0% magnesium salts and 75.5% water.

Seven LLNA studies were conducted (Warbrick et al., 1999; Gerberick et al., 2005) with the EC3 values ranging from 0.005% to 0.05% (dose per unit area of 1.25 – 12.5 μg/cm²). Based on the criteria provided by ECETOC (2003), this would indicate an extreme sensitizer classification.

Table 2: LLNA data

Test Article	Vehicle	EC3 % (μg/cm²)	Reference
Kathon CG	Acetone: Olive oil, 4:1 (AOO)	0.0049 (1.23)	Warbrick et al., 1999
Kathon CG	Methylethyl ketone (MEK)	0.0068 (1.70)	Warbrick et al., 1999
Kathon CG	Dimethyl formamide (DMF)	0.0075 (1.88)	Warbrick et al., 1999
Kathon CG	Acetone	0.0076 (1.90)	Warbrick et al., 1999
Kathon CG	Dimethylsulfoxide (DMSO)	0.0075 (1.88)	Warbrick et al., 1999
Kathon CG	Propylene glycol (PG)	0.048 (12.0)	Warbrick et al., 1999
MCI/MI	Dimethyl formamide (DMF)	0.009 (2.25)	Gerberick et al., 2005

Guinea pig studies were conducted under the Guinea Pig Maximization Test (GPMT) and Buehler protocols as well as in an open epicutaneous assay (summarized in SCCS/1238/09 and in the previous Cosmetic Ingredient Review (CIR) report of 1992). The guinea pig studies are supportive of the conclusion drawn in this report and the LLNA model is considered more appropriate for quantitative risk assessment since it provides an estimate of potency.

The 1992 review of methylisothiazolinone and methylchloroisothiazolinone by the CIR noted several HRIPTs that were included in the review but were unpublished. The review reflected the general agreement that MI/MCI-CG is a sensitizer. The lowest concentration of MI/MCI-CG in a cosmetic formulation that produced sensitization in a nonclinical population of over 200 subjects was 7.5 ppm. The conclusion of the CIR report was that methylisothiazolinone/methylchloroisothiazolinone may be safely used in rinse-off products at a concentration not to exceed 15 ppm and in leave-on cosmetic products at a concentration not to exceed 7.5 ppm. The present QRA evaluates both the industry currently reported maximum use levels of MI/MCI in cosmetic products (data provided by PCPC) as well as the same products if rinse-off products were formulated to 15 ppm and leave-on products were formulated to 7.5 ppm MI/MCI.

The WoE NESIL considered both the human and mouse data. The lowest reported EC3 value in the LLNA (1.23 μg/cm²) is very close to the no effect level identified in the HRIPTs (0.83 μg/cm² in Cardin et al., 1986 and 2.5 μg/cm² in Maibach, 1985). Taking the weight of evidence approach and favoring the human data over the animal data, a WoE NESIL of 0.83 μg/cm² was chosen. Basketter et al., 2008 published a quantitative risk assessment for preservatives, including MI/MCI with a WoE NESIL of 0.83 μg/cm², consistent with the highest exposure in human patch testing below which no sensitization was reported in the European Union's Scientific Committee on Consumer and Non-Food Products assessment for methylisothiazolinone (SCCNFP/0625/02). The WoE NESIL of 0.83 μg/cm² is also the same as used in a recently published QRA for MI/MCI (Towle et al., 2018).

Derivation of SAFs: The approach to the SAFs initially published in Api et al, 2008 have now been updated as described in Basketter and Safford (2016), informally referred to as "QRA2". The QRA2 terminology for allergic contact dermatitis is a result of the International Dialogue on the Evaluation of Allergens (IDEA) workshops. IDEA was developed with regulators, dermatologists, industry experts and academics in 2013 and is focused on evaluation of fragrance allergens (http://www.ideaproject.info). The SAFs from QRA1 have been updated from a 3-component factor (inter-individual, matrix, and use) to a 6-component factor (inter-individual, site, skin condition, matrix, occlusion, frequency and duration of exposure) in QRA2. The SAFs for QRA2 are not yet published (publication in progress, Anne Marie Api personal communication) but are consistent with those currently used by the RIFM QRA Expert Group for fragrance materials. The International Fragrance Association (IFRA) Standards are currently under consultation regarding QRA2 and the consultation will close on July 21, 2019 with the newly revised standards available around mid-August (https://www.asdsoftware.com/ifra-49th-amendment/). For the present MI/MCI assessment the following product category SAFs have been impacted by QRA2 (Table 3), while the others remain unchanged.

Table 3: Product Category SAFs Impacted by QRA2

Product Type	Total QRA1 SAF	Total QRA2 SAF
Baby shampoo	100	300
Bath soaps and detergents	100	300
Rinses (non-coloring)	100	300
Shampoos (non-coloring)	100	300
Hair shampoos (coloring)	100	300
Shaving cream (aerosol, brushless and lather)	300	100
Cologne and toilet waters	300 (shaved skin) 100 (unshaved skin)	100 100
Hair sprays (aerosol)	100	30
Hair sprays (pump)	100	30

Derivation of CEL: Having an accurate consumer exposure estimate to the chemical of interest is a critical component of the QRA for allergic contact dermatitis. The standard approach is to combine the highest reported use concentrations of the ingredient of interest in various cosmetic product categories with the habits and practices data for that cosmetic product type. For this QRA, the Personal Care Products Council (PCPC) surveyed industry members for their highest use of MI/MCI (active ingredients) in a list of 90 FDA cosmetic product categories, along with whether each cosmetic category was a leave-on, rinse-off, or bath application (March 2019). The exposure to each cosmetic product category where MI/MCI is reportedly used is then determined from published habits and practices data, using either the 90th or 95th percentile daily usage data. The 90-95th percentile data represents the highest usage data for risk assessment purposes. Percentiles greater than this are typically not accurate as they are subject to outliers in the data and may not reflect "real" exposures. The highest reported concentrations of MI/MCI from the industry survey is combined with the highest cosmetic product type usage data to arrive at a maximum applied amount of MI/MCI per product type per day. A retention factor (RF) is applied to the exposure data to reflect the amount of MI/MCI that is available for dermal absorption following leave-on or rinse-off applications to skin or hair. For example, a leave-on cosmetic product such as a facial moisturizer will have a RF of 1.0, while a rinse-off cosmetic product such as a shampoo applied to the hair will have a RF of 0.01 (i.e., 1% of the product applied remains on the skin after rinsing and is considered available for dermal penetration). Lastly, the amount of the ingredient of interest that is available for dermal absorption is divided by the surface area of product exposure to arrive at a dose metric of amount of MI/MCI per unit area of skin. Dose of ingredient per unit of skin is considered the most relevant dose metric for a QRA for allergic contact dermatitis (Kimber et al., 2008).

When conducting a QRA it is important that the assumptions that are being used and the sources of the data are provided so that the reader can re-create the assessment and have a full understanding of the quality of the data. A full QRA for reported uses of MI/MCI from the 90 FDA cosmetic product categories could not be completed for every cosmetic product category. There were 5 product categories with reported uses of MI/MCI where the CEL could not be determined: (1) "other" hair preparations (rinse-off), (2) "other" makeup preparations (leave-on), (3) "other" personal cleanliness products (rinse-off), (4) "other" personal cleanliness products (leave-on), and (5) "other" shaving preparations (rinse-off). It is imperative that industry members conduct a QRA as outlined in this report to assure an adequate margin of safety for products in the "other" categories. Alternatively, members can provide more detail on the "other" cosmetic product applications, along with habits and practices data and surface area exposure data to be included in a subsequent QRA.

Habits and practices data used to estimate cosmetic product type exposure was taken from industry data summarized in Api et al., 2008 or from the IFRA RIFM QRA Information Booklet Version 7.1 (revised July 9, 2015). Exposure to baby shampoo was also supported by Lee et al., 2017 using surface area estimates from the US EPA Child-Specific Exposure Factors Handbook (CSEFH), September 2008.

Results of the MI/MCI QRA:

Table 4: Consumer Exposure Level (CEL) based upon industry reported maximum use levels

Product	Classification	Maximum MI/MCI (ppm)	Product Exposure ($\mu g/cm^2$)	MI/MCI CEL ($\mu g/cm^2$)	Reference
Baby shampoo	Rinse-off	12	200	0.0024	IFRA RIFM QRA Booklet ver. 7.1 Category 9 "baby shampoos".
Bubble baths	Rinse-off	0.000019	200	3.8×10^{-9}	IFRA RIFM QRA Booklet ver. 7.1 Category 9 "other products added to bath water".
Cologne and toilet waters	Leave-on	0.075	2,210	0.0002	Api et al., 2008; Hydroalcoholics for shaved or unshaved skin.
Hair conditioners	Rinse-off	15	200	0.0030	Api et al., 2008; Conditioners, rinse-off.
Hair sprays (aerosol)	Leave-on	7.5	1,390	0.0104	Api et al., 2008; Hair sprays (aerosol).
Hair sprays (pump)	Leave-on	7.5	2,200	0.0163	Api et al., 2008; Hair sprays (pump).
Permanent waves	Rinse-off	7.5	4,200	0.0315	IFRA RIFM QRA Booklet ver. 7.1 Category 5 "hair permanent".
Rinses (non-coloring)	Rinse-off	11	170	0.0019	Api et al., 2008; Shampoos.
Shampoos (non-coloring)	Rinse-off	15	170	0.0026	Api et al., 2008; Shampoos.
Tonics, dressings and other hair grooming aids	Rinse-off	7.5	99	0.0073	Api et al., 2008 Hair styling aids with modified retention factor (0.01) to reflect rinse-off applications.
Tonics, dressings and other hair grooming aids	Leave-on	7.4	990	0.0007	Api et al., 2008; Hair styling aids.
Hair tints	Rinse-off	0.4	990	0.0004	Api et al., 2008; Hair styling aids.
Hair rinses (coloring)	Rinse-off	11	200	0.0012	Api et al., 2008; Hair conditioners.
Hair shampoos (coloring)	Rinse-off	6	170	0.0010	Api et al., 2008; Shampoos.
Bath soaps and detergents	Rinse-off	15	10	0.0002	Api et al., 2008; Bath foams, gels, mousses.
Other personal cleanliness products - Liquid hand soap	Rinse-off	15	200	0.0030	Api et al., 2008; Liquid soap.
Shaving cream (aerosol, brushless and lather)	Rinse-off	4.5	70	0.0003	Api et al., 2008; Shaving cream.
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	Rinse-off	15	900	0.0135	Api et al., 2008; Make-up removers.

Table 5: Margin of safety for skin sensitization (AEL/CEL) based on industry reported maximum use levels

Product	MI/MCI WoE NESIL ($\mu g/cm^2$)	QRA2 SAF	MI/MCI AEL ($\mu g/cm^2$)	MI/MCI CEL ($\mu g/cm^2$)	Margin of Safety (AEL/CEL)
Baby shampoo	0.83	300	0.0028	0.0024	1.15
Bubble baths	0.83	100	0.0083	3.8×10^{-9}	>2,000,000
Cologne and toilet waters	0.83	100	0.0083	0.0002	50.08
Hair conditioners	0.83	100	0.0083	0.0030	2.77
Hair sprays (aerosol)	0.83	30	0.0277	0.0104	2.65
Hair sprays (pump)	0.83	30	0.0277	0.0163	1.70
Permanent waves	0.83	100	0.0083	0.0315	0.26
Rinses (non-coloring)	0.83	300	0.0028	0.0019	1.48
Shampoos (non-coloring)	0.83	300	0.0028	0.0026	1.08
Tonics, dressings and other hair grooming aids leave-on	0.83	100	0.0083	0.0073	1.13
Tonics, dressings and other hair grooming aids rinse-off	0.83	100	0.0083	0.0007	11.18
Hair tints	0.83	100	0.0083	0.0004	20.96
Hair rinses (coloring)	0.83	100	0.0083	0.0012	6.92
Hair shampoos (coloring)	0.83	300	0.0028	0.0010	2.71
Bath soaps and detergents	0.83	300	0.0028	0.0002	18.44
Other personal cleanliness products - Liquid hand soap	0.83	100	0.0083	0.0030	2.77
Shaving cream (aerosol, brushless and lather)	0.83	100	0.0083	0.0003	26.35
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.83	100	0.0083	0.0135	0.61

Table 6: Hypothetical margin of safety for skin sensitization (AEL/CEL) based on 15 ppm MI/MCI in industry reported rinse-off products

Product	MI/MCI WoE NESIL ($\mu g/cm^2$)	QRA2 SAF	MI/MCI AEL ($\mu g/cm^2$)	MI/MCI CEL ($\mu g/cm^2$)	Margin of Safety (AEL/CEL)
Baby shampoo	0.83	300	0.0028	0.0030	0.92
Bath soaps and detergents	0.83	300	0.0028	0.0002	18.44
Bubble baths	0.83	100	0.0083	0.0030	2.77
Hair conditioners	0.83	100	0.0083	0.0030	2.77
Permanent waves	0.83	100	0.0083	0.0630	0.13
Rinses (non-coloring)	0.83	300	0.0028	0.0026	1.08
Shampoos (non-coloring)	0.83	300	0.0028	0.0026	1.08
Tonics, dressings and other hair grooming aids rinse-off	0.83	100	0.0083	0.0015	5.59
Hair tints	0.83	100	0.0083	0.0149	0.56
Hair rinses (coloring)	0.83	100	0.0083	0.0030	2.77
Hair shampoos (coloring)	0.83	300	0.0028	0.0026	1.08
Other personal cleanliness products - Liquid hand soap	0.83	100	0.0083	0.0030	2.77
Shaving cream (aerosol, brushless and lather)	0.83	100	0.0083	0.0011	7.90
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.83	100	0.0083	0.0135	0.61

Table 7: Hypothetical margin of safety for skin sensitization (AEL/CEL) based on 7.5 ppm MI/MCI in industry reported leave-on products

Product	MI/MCI WoE NESIL ($\mu g/cm^2$)	QRA2 SAF	MI/MCI AEL ($\mu g/cm^2$)	MI/MCI CEL ($\mu g/cm^2$)	Margin of Safety (AEL/CEL)
Cologne and toilet waters	0.83	100	0.0083	0.0166	0.50
Hair sprays (aerosol)	0.83	30	0.0277	0.0104	2.65
Hair sprays (pump)	0.83	30	0.0277	0.0165	1.68
Tonics, dressings and other hair grooming aids leave-on	0.83	100	0.0083	0.0074	1.12

Conclusion: A QRA for allergic contact dermatitis was conducted based upon industry reported maximum use levels of MI/MCI in cosmetics. Using the reported cosmetic product types an additional QRA was conducted with the hypothetical maximum use of 15 ppm MI/MCI in rinse-off products and 7.5 ppm in leave-on products. The basis of the exposure assumptions is provided in the report. Using industry maximum reported MI/MCI use levels, an adequate margin of safety for skin sensitization is provided for all reported uses except for permanent waves (using 7.5 ppm MI/MCI) and for skin cleansing (cold creams, cleansing lotions, liquids and pads) products (using 15 ppm MI/MCI). The maximum supportable level of MI/MCI for permanent waves and skin cleansing products are 2 ppm and 9 ppm, respectively. The exposure assumptions for these products should be confirmed by industry members and if confirmed, these products should be reformulated to provide an adequate margin of safety. After evaluating the previous CIR recommendations from 1992, limiting rinse-off products to 15 ppm MI/MCI and leave-on products to 7.5 ppm MI/MCI is no longer enough by itself. Using the exposure assumptions noted in the report, an adequate margin of safety could not be assured for baby shampoo, permanent waves, hair tints, skin cleansing (cold creams, cleansing lotions, liquids and pads), and cologne and toilet waters when blindly applying the previous 1992 maximum limits for rinse-off and leave-on cosmetics. Of course, the QRA for allergic contact dermatitis risk assessment technique did not exist in 1992. The CIR Science and Support Committee now recommends that MI/MCI be deemed safe as used when formulated to be non-sensitizing, which may be supported by a favorable QRA.

References:

Anonymous (1992). Final report on the safety assessment of methylisothiazolinone and methylchloroisothiazolinone. *J Am Coll Toxicol* **11(1)**:75-128.

Api, AM, Basketter, DA, Cadby, PA, Cano, M-F, Ellis, G, Gerberick, GF, Griem, P, McNamee, PM, Ryan, CA, and Safford, R (2008). Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. *Reg Toxicol Pharmacol* **52**:3-23.

Basketter, DA, Clapp, CJ, Safford, BJ, Jowsey, IR, McNamee, P, Ryan, CA and Gerberick, GF (2008). Preservatives and skin sensitization quantitative risk assessment. *Dermatitis* **19(1)**:20-27.

Basketter, D and Safford, R (2016). Skin sensitization quantitative risk assessment: A review of underlying assumptions. *Reg Toxicol Pharmacol* **74**:105-116.

Cardin, CW, Weaver, JE and Bailey, PT (1986). Dose-response assessments of Kathon biocide (II) Threshold prophetic patch testing. *Contact Derm* **15**:10-16.

Cosmetic Ingredient Review: Final report on the safety assessment of methylisothiazolinone and methylchloroisothiazolinone (1992). *J Am Coll Toxicol* **11(1)**:75-128.

European Centre for Ecotoxicology and toxicology of Chemicals (ECETOC). Technical Report No. 87, Contact Sensitisation: Classification According to Potency. Brussels, Belgium, April 2003.

Gerberick, GF, Ryan, CA, Kern, PS, Schlatter, H, Dearman, RJ, Kimber, I, Patlewicz, GY and Basketter, DA (2005). Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. *Dermatitis* **16(4):**157-202.

IFRA RIFM QRA Information Booklet Version 7.1, Revised July 9, 2015.

Kimber, I, Dearman, RJ, Basketter, DA, Ryan, CA, Gerberick, GF, McNamee, PM, Lalko, J and Api, AM (2008). Dose metrics in the acquisition of skin sensitization: Thresholds and importance of dose per unit area. *Reg Toxicol Pharmacol* **52:**39-45.

Lee, E, Yun, J, Ha, J, Park, BC, Park, GH, Kim, HR, Hong, SP, Kim, KB and Kim, MH (2017). Assessment of exposure for baby cosmetic care products in a Korean population. *Food Chem Toxicol* **106:**107-113.

Maibach, H (1985). Diagnostic patch test concentration for Kathon CG. *Contact Derm* **13:**242-245.

Scientific Committee on Consumer Safety (SCCS) 2009. Opinion on the mixture of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one. COLIPA no. P56. SCCS/1238/09.

The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers Opinion Concerning Methylisothiazolinone COLIPA No. P94. Adopted by the SCCNFP during the 23rd plenary meeting of 18 March 2003. SCCNFP/0625/02, final.

Towle, KM, Drechsel, DA, Warshaw, EM, Fung, ES, Novick, RM, Paustenbach, DJ and Monnot, AD (2018). A quantitative risk assessment of the skin sensitization induction potential of the Kathon CG preservative in rinse-off and leave-on personal care and cosmetic products. *Dermatitis* **29(3):**132-138.

United States Environmental Protection Agency Child-Specific Exposure Factors Handbook (CSEFH), September 2008.

Warbrick, EV, Dearman, RJ, Basketter, DA and Kimber, I (1999). Influence of application vehicle on skin sensitization to methylchloroisothiazolinone/methylisothiazolinone: An analysis using the local lymph node assay. *Contact Derm* **41:**325-329.

Weaver, JE, Cardin, CW and Maibach, HI (1985). Dose-response assessments of Kathon biocide. (I) Diagnostic use and diagnostic threshold patch testing with sensitized humans. *Contact Derm* **12:**141-145.

Addendum:

After completion of the QRA for MI/MCI the author became aware of an additional HRIPT that was conducted by Cantor Research Laboratories (report date September 14, 2006). The study involved 60 subjects who were tested with a liquid hand soap containing 12 ppm MI/MCI in an open application test. The liquid hand soap was applied at a volume of 0.2 mL to an area approximately 2 cm^2. Thus, the dose per unit area of MI/MCI is 1.2 $\mu g/cm^2$. None of the 60 subjects completing the study had a positive reaction. These results are consistent with the results presented in the QRA and do not alter the choice of the WoE NESIL.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: March 27, 2019

SUBJECT: Re-review Document: Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone as Used in Cosmetics (draft prepared for the April 8-9, 2019 CIR Expert Panel meeting)

The Personal Care Products Council respectfully submits the following comments on the re-review document, Safety Assessment of Methylisothiazolinone and Methylchloroisothiazolinone as Used in Cosmetics.

Summary of information from the old report still needs to be added to the re-review.

Physical Properties - It is not clear what is meant by "This ingredient". Although MCI/MI is primarily sold as a single trade name mixture, there are other mixtures on the market that contain it.

Cosmetic Use - Although the use information from FDA in the original report was for the MCI/MI mixture, it still would be helpful to discuss the change from the original report.

Non-Cosmetic Use - Based on the case reports, it would be helpful to mention other uses, such as paint and cleaning products in the Non-Cosmetic Use section.

Clinical Studies - In the text, it would be helpful to state that range of sensitization rates reported in patients in the patch test studies. It should include a short description of the population in which the sensitization rate was found.

Risk Assessment - Please state the value of the NESIL used in the risk assessment (reference 45).

Table 2 - Please also state the percentage of patients with positive reactions (reference 27 and 28).

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