BUFF

Memo/Agenda
Minutes
New Data
- Hydroquinone and p-Hydroxyanisole
- Methylisothiazolinone

Re-Review Summaries
- 2-amino-6-chloro-4-nitrophenol
- Phthalates
- Phenylenediamines

CIR EXPERT PANEL MEETING

SEPTEMBER 10-11, 2012

Cosmetic Ingredient Review

MEMORANDUM

To: CIR Expert Panel Members and Liaisons
From: Director, CIR
Subject: 126th Meeting of the CIR Expert Panel — Monday and Tuesday, March 18-19, 2013
Date: February 22, 2013

Enclosed are the agenda and accompanying materials for the 126th CIR Expert Panel Meeting to be held March 18-19, 2013. The location again is the Madison Hotel, 1177 Fifteenth Street, NW, Washington, DC 20005. Phone: (202) 862-1600. Fax: (202) 785-1255. The meeting agenda includes consideration of 13 ingredient groups advancing in the process, 2 re-reviews, 3 re-review summaries, and a discussion of the need for an early re-review of methylisothiazolinone.

We have reserved rooms for the nights of Sunday, March 17 and Monday, March 18 at the Madison. If you encounter any travel problems, please contact me on my cell phone at 301-512-7846.

Because the decision was made to not keep the production copier and because outside printing is prohibitively expensive, we have made the decision that CIR should "go electronic." In fact several Panel members have done this already and the circumstances dictate that we all will have to do this. So we're going to try it for this meeting and see what happens. You should already have received information on just how this will work vis-à-vis providing your comments.

Team meetings

New data - methylisothiazolinone has been selected as "allergen of the year" for 2013. Don Belsito will discuss the implications of that and his recent clinical experience. The question for the Panel will be the need for an early re-review.

Re-reviews - there are 2 safety assessments to re-review and make a determination on the need to reopen to revise the conclusion. Since these are both hair colorants, in neither case does there appear to be an opportunity to reopen to expand the scope.

1. HC Yellow No.4 was reviewed previously (published in 1998) with the conclusion that this hair colorant is safe in the present practices of use. There are fewer uses and lower use concentrations now. Most of the data are unpublished data from a Scientific Committee on Consumer Safety report. Included in this report was the comment that HC yellow no. 4 is a secondary amine and is prone to nitrosation. This was not addressed in the original safety assessment. The Panel should decide if this is an issue to be addressed.

2. HC Orange No.1 reviewed previously (published in 1998) with the conclusion that this hair colorant is safe as used up to 3%, predicated on the level shown not to be sensitizing. Uses have decreased substantially, but the reported maximum use concentration has increased from 0.15% in the original report to 0.55% now. Most of the newly available data are unpublished data from a Scientific Committee on Consumer Safety report.

1101 17th Street, NW, Suite 412, Washington, DC 20036
Ph 202.331.0651 Fax 202.331.0088 Internet www.cir-safety.org Email info@cir-safety.org

Draft reports - there are 4 draft reports for review - “green” is on the cover page.

1. Boron Nitride - This is the first time that the Panel is seeing this document, which addresses this single ingredient. A scientific literature review was issued on November 21, 2012. We addressed technical comments from the Council and added several pieces of unpublished data. Do we need more data or can we proceed to issue a tentative report?

2. Nitrocellulose - This is the first time that the Panel is seeing this document. A scientific literature review was issued on November 21, 2012. The naming of the ingredients in this review (nitrocellulose and collodion) can be a source of confusion. Technical comments from the Council have been addressed and unpublished data were added. Do we need more data or can we proceed to issue a tentative report?

3. Palmitoyl Oligopeptides - This is the first time that the Panel is seeing this report addressing 45 ingredients. A scientific literature review was announced for public comment August 22, 2012. Technical comments from the Council have been addressed and unpublished data were added. Do we need more data or can we proceed to issue a tentative report?

4. Tromethamine - This is the first time that the Panel is seeing this report addressing 45 ingredients. A scientific literature review was announced for public comment November 21, 2012. The CIR Science and Support Committee and Dow Chemical both suggested that we do not use aminomethyl propanol data to read across, and we agree. Technical comments from the Council have been addressed and unpublished data were added. Some of those unpublished data are for chemically-related aminoethyl propanediol and aminomethyl propanediol. Are these data an acceptable basis for read across? Do we need more data or can we proceed to issue a tentative report?

Tentative report – there is a single draft tentative report – “pink” is on the cover page.

1. Animal- and Plant-Derived Amino Acids and Hydrolyzed Proteins – At the December meeting, the Panel combined the source amino acids and hydrolyzed proteins into one report and asked for additional data. We’ve received some new data. We’ve also provided information from Japan about significant concerns there regarding thousands of cases of reactions to a soap containing hydrolyzed wheat protein. If the information is still insufficient, then a tentative conclusion of insufficient data should be issued. If the information now available is sufficient, the Panel should issue a Tentative Report with an appropriate discussion/conclusion.

Final reports - there are 8 final reports for consideration. After reviewing these drafts, especially the rationale in the discussion section, the Panel should issue them as final reports – “blue” is on the cover page.

1. Alkyl Esters - At the December meeting, the Panel issued a tentative amended report with a conclusion of safe in the present practices of use and concentration when formulated to be non-irritating. Technical comments received from the Council were considered. The only new data are the results of the Council’s concentration of use survey on cetyl myristoleate (no use concentrations were reported).

2. Alkyl Ethylhexanoates - At the December meeting, the Panel issued a tentative amended report with a conclusion of safe in the present practices of use and concentration when formulated to be non-irritating. The alkyl ethylhexanoates were created as their own family, rather than included in the review of alkyl esters, because the Panel wanted to focus on the possible reproductive risk associated with 2-ethylhexanoic acid. That information is captured and the issue is addressed in the Discussion of the report. Technical comments received from the Council were considered. No new data were received.

3. 6-Hydroxyanisole - At the December meeting, the Panel issued a tentative report with the conclusion that this ingredient is safe for use in oxidative hair dye formulations. Technical comments from the Council were considered. No new data were received.

4. Hypericum Perforatum-Derived Ingredients - In December, the Panel issued a tentative amended report with a safe in the present practices of use conclusion. Technical comments from the Council were considered. Updated use concentration data have been added.

5. Methyl Glucose Polyethers and Esters – In December, the Panel issued a tentative report with a safe in the present practices of use and concentration conclusion. New use concentration data were reported, including a new use of methyl gluceth-20 in a hair preparation (noncoloring) spray gel at 0.2%. The Council has clarified that the uncertainty about the esters being mono- or diesters largely may be resolved. The intent in the International Cosmetic Ingredient Dictionary and Handbook is that, by convention, esters will be monoesters unless otherwise stated in the definition. An editorial change in the discussion may be appropriate. Take a look.

6. Modified Terephthalate Polymers - At the December meeting, the Panel issued a tentative report with a safe in the present practices of use and concentration conclusion. Comments from the Council were considered. No other comments or data were submitted.

7. Nylon - At the December meeting, the Panel issued a tentative report with the conclusion that these ingredients are safe in the present practices of use and concentration in cosmetics. The Council provided comments on the report, which have been considered. We also received an additional human sensitization study on Nylon-12 at 19.5% in a facial moisturizer (no reactions at induction or challenge).

8. Talc - At the December meeting, while the Panel issued a tentative safety assessment with a safe as used conclusion, the Panel asked industry to submit information on the analytical methods used to ensure that talc is free of asbestiform fibers, as well as information on typical impurity levels in talc. At the time of the preparation of this memo, these data had not been received. The hope is that they will be received in time for the Wave 2 submission to the Panel.

Full Panel Meeting

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

The Panel will consider the 8 reports to be issued as final safety assessments, followed by the rest of the reports advancing in the process, and finish with a discussion of methylisothiazolinone and the need for an early re-review.

Because the bulk of the agenda is the final reports, and there does not appear to be too much controversy (I know, be careful what you say…), it is likely that the full Panel session will conclude before lunch on day 2, so plan your travel accordingly.

Have a safe journey.

Agenda
126th Cosmetic Ingredient Review Expert Panel Meeting
March 18-19, 2013

Monday, March 18			
8:00 am	**CONTINENTAL BREAKFAST**		
8:30 am	**WELCOME TO THE 126th EXPERT PANEL TEAM MEETINGS**		**Drs. Bergfeld/Andersen**
8:45 am	**TEAM MEETINGS**		**Drs. Marks/Belsito**
	Dr. Marks' Team		**Dr. Belsito's Team ***
Blue (WJ)	methyl glucose polyethers & esters	Buff (FAA/IB)	hydroquinone and p-hydroxyanisole – new data
Green (WJ)	palmitoyl oligopeptides	Buff (FAA)	methylisothiazolinone – new data
Blue (CB)	6-hydroxyindole	Buff (FAA)	re-review summaries (3)
Blue (CB)	nylon	Blue (MF)	alkyl esters
Pink (CB)	animal and plant amino acids/hydrolyzed proteins	Blue (MF)	alkyl ethylhexanoates
Blue (LB)	*Hypericum perforatum*-derived ingredients	Blue (MF/IB)	talc
Blue (LB)	modified terephthalate polymers	Green (MF)	boron nitride
Green (LB)	tromethamine	Green (MF)	nitrocellulose
Buff (LB)	HC orange No.1- re-review	Blue (WJ)	methyl glucose polyethers & esters
Buff (LB)	HC yellow No.4 – re-review	Green (WJ)	palmitoyl oligopeptides
Blue (MF)	alkyl esters	Blue (CB)	6-hydroxyindole
Blue (MF)	alkyl ethylhexanoates	Blue (CB)	nylon
Blue (MF/IB)	talc	Pink (CB)	animal and plant amino acids/hydrolyzed proteins
Green (MF)	boron nitride	Blue (LB)	*Hypericum perforatum*-derived ingredients
Green (MF)	nitrocellulose	Blue (LB)	modified terephthalate polymers
Buff (FAA)	methylisothiazolinone – new data	Green (LB)	tromethamine
Buff (FAA/IB)	hydroquinone and p-hydroxyanisole – new data	Buff (LB)	HC orange No.1 – re-review
Buff (FAA)	re-review summaries (3)	Buff (LB)	HC yellow No.4 – re-reviw
Noon	**Lunch for Panel, liaisons, and staff**		
1:00 pm	**Team meetings - continue as needed**		
5:00 pm	**ADJOURN DAY 1 SESSION**		

NOTE: The order of presentation and discussion of each topic will be maintained. However, the scheduled times may be accelerated or delayed depending upon the time required for the Expert Panel to complete its review of each subject.

* Team moves to breakout room.

Tuesday, March 19			
8:00 am	**CONTINENTAL BREAKFAST**		
8:30 am	**WELCOME TO THE 126th FULL CIR EXPERT PANEL MEETING**		
8:45 am	**MINUTES OF THE December 2012 EXPERT PANEL MEETING**		**Dr. Bergfeld**
9:00 am	**DIRECTOR'S REPORT**		**Dr. Andersen**
9:30 am	**FINAL REPORTS, REPORTS ADVANCING TO THE NEXT LEVEL, RE-REVIEWS, and OTHER DISCUSSION ITEMS**		
		Final Reports	
	Blue (WJ)	methyl glucose polyethers & esters - Dr. Marks reports	
	Blue (MF)	alkyl esters - Dr. Belsito reports	
	Blue (MF)	alkyl ethylhexanoates - Dr. Marks reports	
	Blue (MF/IB)	talc - Dr. Belsito reports	
	Blue (LB)	*Hypericum perforatum*-derived ingredients - Dr. Marks reports	
	Blue (LB)	modified terephthalate polymers – Dr. Belsito reports	
	Blue (CB)	nylon - Dr. Marks reports	
	Blue (CB)	6-hydroxyindole - Dr. Belsito reports	
		Reports Advancing	
	Pink (CB)	animal and plant amino acids/hydrolyzed proteins - Dr. Marks reports	
	Green (MF)	boron nitride - Dr. Belsito reports	
	Green (MF)	nitrocellulose - Dr. Marks reports	
	Green (WJ)	palmitoyl oligopeptides - Dr. Belsito reports	
	Green (LB)	tromethamine - Dr. Marks reports	
		Re-reviews	
	Buff (LB)	HC orange no. 1 - Dr. Belsito reports	
	Buff (LB)	HC yellow no. 4 - Dr. Marks reports	
	Buff (FAA)	re-review summaries (3) - Dr. Andersen reports	
		New Data	
	Buff (FAA)	methylisothiazolinone - Dr. Belsito reports	
	Buff (FAA/IB)	hydroquinone and p-hydroxyanisole – Dr. Marks reports	
	ADJOURN - Next meeting *Monday and Tuesday, June 10-11, 2013*		

NOTE: The order of presentation and discussion of each topic will be maintained. However, the scheduled times may be accelerated or delayed depending upon the time required for the Expert Panel to complete its review of each subject.

Cosmetic Ingredient Review
Commitment . . . Credibility
Since 1976

ONE HUNDRED TWENTY-FIFTH MEETING

OF THE

EXPERT PANEL

December 10-11, 2012

The Madison Hotel

Washington, D.C.

Expert Panel Members

Wilma F. Bergfeld, M.D., Chair

Donald V. Belsito, M.D.

Ronald A. Hill, Ph.D.

Curtis D. Klaassen, Ph.D.

Daniel C. Liebler, Ph.D.

James G. Marks, Jr., M.D.

Ronald C. Shank, Ph.D.

Thomas J. Slaga, Ph.D.

Paul W. Snyder, D.V.M., Ph.D.

Liaison Representatives

Consumer

Rachel Weintraub, Esq.

Industry

Halyna Breslawec, Ph.D.

Government

Linda Katz, MD., M.P.H.
(ABSENT)

Adopted (Date)

Wilma F. Bergfeld, M.D.

1101 17th St., NW, Suite 412 Washington, DC 20036-4702
Phone 202.331.0651 FAX 202.331.0088 http://www.cir-safety.org

Others Present at Meeting

F. Alan Andersen	CIR
Jay Ansell	Council
Yutaka Aoki	Kanebo Cosmetics
Robeena Aziz	FDA
Lillian Becker	CIR
Don Bjerke	Procter & Gamble
Ivan Boyer	CIR
Christina Burnett	CIR
Natalie Cook	Avon
Kapal Dewan	FDA
Carol Eisenmann	PCPC
Mark Ellis	IMA-NA
Monice Fiume	CIR
Kevin Fries	CIR
Lillian Gill	CIR
Carsten Goebel	Procter & Gamble
Chris Grissing	IMA-NA
Bart Heldreth	CIR
Carla Jackson	CIR
Wilbur Johnson, Jr.	CIR
William G. Kelly, Jr.	Ctr. For Reg. Effectiveness
Akiho Kinoshita	Shiseido
Dennis Laba	Presperse
Tim McCarthy	J & J
Stanley R. Milstein	FDA
Lauren Nardella	The Rose Sheet
Diego Rua	FDA
Shripal Sharma	Imerys
Noriko Shibuya	Shiseido
David Steinberg	Steinberg & Associates
Lorene Telofiles	J & J

CHAIRMAN'S OPENING REMARKS

The 125th meeting of the CIR Expert Panel was called to order by Dr. Bergfeld at 8:27 a.m. on Tuesday, December 11, 2012, and all attendees were welcomed. She stated that presentations involving infant skin and hair dye self patch testing were made on the preceding day, followed by the review of 13 ingredient reports in Teams. Dr. Bergfeld thanked the CIR staff for producing the excellent documents scheduled for review and the CIR Science and Support Committee for its involvement in the review process. Dr. Bergfeld noted that the use of available ingredient data for read-across, to evaluate the safety of ingredients for which safety test data are not available was also discussed in Teams. It is the Panel's plan to continue to include an explanation relating to the use of read-across in evaluating the safety of cosmetic ingredients in the report discussion.

APPROVAL OF MINUTES

The minutes of the September10-11, 2012 CIR Expert Panel meeting were unanimously approved.

DIRECTOR'S REPORT

Dr. Andersen congratulated the CIR Expert Panel on a productive year in which some 482 individual cosmetic ingredients were reviewed. This brings the total of number ingredients reviewed by CIR to 3156! That total comprises:

Safe in the present practices of use and concentration – 2060
Safe with qualifications – 982
Insufficient data – 7*
Zero use ingredients – 58
Use in cosmetics not supported – 38
Unsafe – 11

He also remarked on the progress being made to improve the functionality of the CIR website. An outside audit of the website structure reported no fundamental flaws and applauded the efficient use of the drupal database structure to deliver the Panel meeting information to users. Areas for improvement in security and the potential benefits of using of more Drupal capabilities were identified and will be addressed as we upgrade the site to accommodate ingredient searching.

Final Safety Assessments

PEGylated Oils

The CIR Expert Panel issued a final amended safety assessment with the conclusion that PEGylated oils are safe in the present practices of use and concentration in cosmetics when formulated to be non-irritating. This conclusion supersedes the earlier conclusion issued by the Expert Panel in 1997 for PEGs castor oils. The 130 ingredients included in this safety assessment are:

PEG-2 castor oil*
PEG-3 castor oil*
PEG-4 castor oil*
PEG-5 castor oil*
PEG-8 castor oil*
PEG-9 castor oil
PEG-10 castor oil*
PEG-11 castor oil*
PEG-15 castor oil*
PEG-16 castor oil*
PEG-20 castor oil*
PEG-25 castor oil
PEG-26 castor oil*
PEG-29 castor oil*
PEG-30 castor oil
PEG-33 castor oil
PEG-35 castor oil
PEG-36 castor oil
PEG-40 castor oil
PEG-44 castor oil*
PEG-50 castor oil
PEG-54 castor oil*
PEG-55 castor oil*
PEG-60 castor oil
PEG-75 castor oil*
PEG-80 castor oil*
PEG-100 castor oil*
PEG-200 castor oil*
PEG-18 castor oil dioleate*
PEG-60 castor oil isostearate*
PEG-2 hydrogenated castor oil
PEG-5 hydrogenated castor oil*
PEG-6 hydrogenated castor oil*
PEG-7 hydrogenated castor oil
PEG-8 hydrogenated castor oil*
hydrogenated castor oil PEG-8 esters*
PEG-10 hydrogenated castor oil
PEG-16 hydrogenated castor oil
PEG-20 hydrogenated castor oil
PEG-25 hydrogenated castor oil
PEG-30 hydrogenated castor oil
PEG-35 hydrogenated castor oil

PEG-40 hydrogenated castor oil
PEG-45 hydrogenated castor oil
PEG-50 hydrogenated castor oil
PEG-54 hydrogenated castor oil*
PEG-55 hydrogenated castor oil*
PEG-60 hydrogenated castor oil
PEG-65 hydrogenated castor oil*
PEG-80 hydrogenated castor oil
PEG-100 hydrogenated castor oil
PEG-200 hydrogenated castor oil*
PEG-5 hydrogenated castor oil isostearate*
PEG-10 hydrogenated castor oil isostearate*
PEG-15 hydrogenated castor oil isostearate*
PEG-20 hydrogenated castor oil isostearate*
PEG-30 hydrogenated castor oil isostearate*
PEG-40 hydrogenated castor oil isostearate*
PEG-50 hydrogenated castor oil isostearate*
PEG-58 hydrogenated castor oil isostearate*
PEG-20 hydrogenated castor oil laurate*
PEG-30 hydrogenated castor oil laurate*
PEG-40 hydrogenated castor oil laurate*
PEG-50 hydrogenated castor oil laurate*
PEG-60 hydrogenated castor oil laurate*
PEG-20 hydrogenated castor oil pca isostearate*
PEG-30 hydrogenated castor oil pca isostearate*
PEG-40 hydrogenated castor oil pca isostearate
PEG-60 hydrogenated castor oil pca isostearate*
PEG-50 hydrogenated castor oil succinate
potassium PEG-50 hydrogenated castor oil succinate*
sodium PEG-50 hydrogenated castor oil succinate*
PEG-5 hydrogenated castor oil triisostearate*
PEG-10 hydrogenated castor oil triisostearate*
PEG-15 hydrogenated castor oil triisostearate*
PEG-20 hydrogenated castor oil triisostearate
PEG-30 hydrogenated castor oil triisostearate*
PEG-40 hydrogenated castor oil triisostearate
PEG-50 hydrogenated castor oil triisostearate*
PEG-60 hydrogenated castor oil triisostearate*
adansonia digitata seed oil PEG-8 esters*
almond oil PEG-6 esters*
almond oil PEG-8 esters *
apricot kernel oil PEG-6 esters
apricot kernel oil PEG-8 esters*
apricot kernel oil PEG-40 esters*
argan oil PEG-8 esters*
avocado oil PEG-8 esters*
avocado oil PEG-11 esters
bertholletia excelsa seed oil PEG-8 esters*
borage seed oil PEG-8 esters*
coconut oil PEG-10 esters
corn oil PEG-6 esters*
corn oil PEG-8 esters*
grape seed oil PEG-8 esters
hazel seed oil PEG-8 esters*
hydrogenated palm/palm kernel oil PEG-6 esters
jojoba oil PEG-8 esters
jojoba oil PEG-150 esters*
linseed oil PEG-8 esters*
macadamia ternifolia seed oil PEG-8 esters*
mango seed oil PEG-70 esters*
mink oil PEG-13 esters*
olive oil PEG-6 esters*
olive oil PEG-7 esters
olive oil PEG-8 esters*
olive oil PEG-10 esters
orbignya oleifera seed oil PEG-8 esters*
palm oil PEG-8 esters*
passiflora edulis seed oils PEG-8 esters*
peanut oil PEG-6 esters*
PEG-75 crambe abyssinica seed oil*
PEG-75 meadowfoam oil
pumpkin seed oil PEG-8 esters*
rapeseed oil PEG-3 esters*
rapeseed oil PEG-20 esters*
raspberry seed oil PEG-8 esters*
safflower seed oil PEG-8 esters*
schinziophyton rautanenii kernel oil PEG-8 esters*
sclerocarya birrea seed oil PEG-8 esters*
sesame seed oil PEG-8 esters*
soybean oil PEG-8 esters*
soybean oil PEG-20 esters*
soybean oil PEG-36 esters*
sunflower seed oil PEG-8 esters*
sunflower seed oil PEG-32 esters*
sweet almond oil PEG-8 esters*
watermelon seed oil PEG-8 esters*
wheat germ oil PEG-40 butyloctanol esters*
wheat germ oil PEG-8 esters*

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

PEGylated Oils is the name CIR devised to describe this large group of cosmetic ingredients. These ingredients are mixtures of the etherification and transesterification products of fatty acid glycerides and fatty acids from plant sources and equivalents of ethylene oxide to produce the desired PEG length. Because of the nature of the process by which these ingredients are produced, PEG compounds unattached to glycerides or fatty acid groups will be present,. Overall, PEGylated oils are complex mixtures of structurally related molecules. The Panel determined that the available data in previous safety assessments of PEGs and of plant-derived fatty acids strongly supported the safety of PEGylated oils. In addition, the Panel considered that the available data on PEGs castor oils and PEGs hydrogenated castor oils could be “read across” to support the safety of the entire group.

The Expert Panel recognized that these ingredients can enhance the penetration of other ingredients through the skin. The Panel cautioned that care should be taken in formulating cosmetic products that may contain these ingredients in combination with any ingredients whose safety was based on their lack of dermal absorption, or when dermal absorption was a concern.

The Expert Panel noted that the earlier safety assessment of PEG castor oils specified safe up to a 50% use concentration. As PEGs castor oils and the rest of the PEGylated oils now are used at concentrations below 50% in leave-on products, the Panel determined that a concentration limit need no longer be specified. Products using these ingredients should be formulated to be non-irritating.

Tin(IV) Oxide

The CIR Expert Panel issued a final safety assessment with the conclusion that tin(IV) oxide is safe in the present practices of use and concentration in cosmetics.

This ingredient is a widely used cosmetic abrasive, bulking, and opacifying agent. Throughout the report, the valence of tin oxide used in studies was specified and, if not available, the absence of this information was noted. The Panel asserted that, while there were no carcinogenicity or reproductive and developmental toxicity data, these endpoints were not of concern because this ingredient is insoluble and would not be absorbed through the skin.

Tentative Safety Assessments

Alkyl Esters

The CIR Expert Panel issued a tentative amended safety assessment for public comment with the conclusion that the 239 alkyl esters listed below are safe in the present practices of use and concentration described in the safety assessment when formulated to be non-irritating.

arachidyl behenate arachidyl erucate*
arachidyl propionate
batyl isostearate*
batyl stearate*
behenyl beeswax
behenyl behenate
behenyl erucate
behenyl isostearate*
behenyl olivate
behenyl/isostearyl beeswax*
butyl avocadate
butyl babassuate*
butyl isostearate*
butyl myristate
butyl oleate*
butyl stearate
butyloctyl beeswax*
butyloctyl behenate*
butyloctyl candelillate*
butyloctyl cetearate*
butyloctyl oleate*
butyloctyl palmitate*
C10-40 isoalkyl acid octyldodecanol esters*
C14-30 alkyl beeswax*
C16-36 alkyl stearate*
C18-38 alkyl beeswax*
C18-38 alkyl c24-54 acid ester*
C20-40 alkyl behenate*
C20-40 alkyl stearate
C30-50 alkyl beeswax*
C30-50 alkyl stearate*
c32-36 isoalkyl stearate*
C40-60 alkyl stearate*
C4-5 isoalkyl cocoate*
caprylyl butyrate*
caprylyl caprylate
caprylyl eicosenoate
cetearyl behenate
cetearyl candelillate
cetearyl isononanoate
cetearyl nonanoate*
cetearyl olivate
cetearyl palmate*
cetearyl palmitate*
cetearyl rice branate*
cetearyl stearate
cetyl babassuate
cetyl behenate*
cetyl caprate
cetyl caprylate
cetyl dimethyloctanoate*
cetyl esters
cetyl isononanoate*
cetyl laurate
cetyl myristate
cetyl myristoleate*
cetyl oleate*
cetyl palmitate
cetyl ricinoleate
cetyl stearate
cetyl tallowate
chimyl isostearate*
chimyl stearate*
coco-caprylate
coco-caprylate/caprate
coco-rapeseedate*
decyl castorate*
decyl cocoate
decyl isostearate*
decyl jojobate*
decyl laurate*
decyl myristate*
decyl oleate
decyl olivate
decyl palmitate*
decyltetradecyl cetearate*
erucyl arachidate*
erucyl erucate*
erucyl oleate*
ethylhexyl adipate/palmitate/stearate*
ethylhexyl C10-40 isoalkyl acidate*
ethylhexyl cocoate
ethylhexyl hydroxystearate
ethylhexyl isononanoate
ethylhexyl isopalmitate
ethylhexyl isostearate
ethylhexyl laurate
ethylhexyl myristate
ethylhexyl neopentanoate*
ethylhexyl oleate*
ethylhexyl olivate
ethylhexyl palmitate
ethylhexyl pelargonate
ethylhexyl stearate
heptyl undecylenate
heptylundecyl hydroxystearate
hexyl isostearate
hexyl laurate
hexyldecyl hexyldecanoate*
hexyldecyl isostearate
hexyldecyl laurate
hexyldecyl oleate*
hexyldecyl palmitate*
hexyldecyl stearate
hexyldodecyl/octyldecyl hydroxystearate*
hydrogenated castor oil behenyl esters*
hydrogenated castor oil cetyl esters *
hydrogenated castor oil stearyl esters*
hydrogenated ethylhexyl olivate
hydrogenated ethylhexyl sesamate*
hydrogenated isocetyl olivate*
hydrogenated isopropyl jojobate*
hydroxycetyl isostearate*
hydroxyoctacosanyl hydroxystearate
isoamyl laurate
isobutyl myristate*
isobutyl palmitate*

isobutyl perlargonate*
isobutyl stearate*
isobutyl tallowate*
isocetyl behenate*
isocetyl isodecanoate*
isocetyl isostearate*
isocetyl laurate*
isocetyl myristate
isocetyl palmitate
isocetyl stearate
isodecyl cocoate
isodecyl hydroxystearate*
isodecyl isononanoate
isodecyl laurate
isodecyl myristate
isodecyl neopentanoate
isodecyl oleate
isodecyl palmitate*
isodecyl stearate*
isohexyl caprate
isohexyl laurate*
isohexyl neopentanoate*
isohexyl palmitate*
isolauryl behenate*
isononyl isononanoate
isooctyl caprylate/caprate*
isooctyl tallate*
isopropyl isostearate
isopropyl arachidate*
isopropyl avocadate*
isopropyl babassuate*
isopropyl behenate*
isopropyl hydroxystearate
isopropyl isostearate
isopropyl jojobate
isopropyl laurate*
isopropyl linoleate
isopropyl myristate
isopropyl oleate*
isopropyl palmitate
isopropyl ricinoleate
isopropyl sorbate*
isopropyl stearate
isopropyl tallowate*
isostearyl avocadate
isostearyl behenate
isostearyl erucate*
isostearyl hydroxystearate
isostearyl isononanoate
isostearyl isostearate
isostearyl laurate
isostearyl linoleate
isostearyl myristate
isostearyl neopentanoate
isostearyl palmitate
isotridecyl isononanoate
isotridecyl laurate*
isotridecyl myristate*
isotridecyl stearate
lauryl behenate*
lauryl cocoate*
lauryl isostearate*
lauryl laurate
lauryl myristate*
lauryl oleate/
lauryl palmitate
lauryl stearate/
lignoceryl erucate*
myristyl isostearate*
myristyl laurate
myristyl myristate
myristyl neopentanoate
myristyl stearate
octyldecyl oleate*
octyldodecyl avocadoate*
octyldodecyl beeswax*
octyldodecyl behenate*
octyldodecyl cocoate*
octyldodecyl erucate
octyldodecyl hydroxystearate*
octyldodecyl isostearate
octyldodecyl meadowfoamate*
octyldodecyl myristate
octyldodecyl neodecanoate*
octyldodecyl neopentanoate
octyldodecyl octyldodecanoate
octyldodecyl oleate*
octyldodecyl olivate
octyldodecyl ricinoleate
octyldodecyl safflowerate*
octyldodecyl stearate
oleyl arachidate*
oleyl erucate
oleyl linoleate
oleyl myristate*
oleyl oleate
oleyl stearate*
propylheptyl caprylate
stearyl beeswax
stearyl behenate*
stearyl caprylate
stearyl erucate*
stearyl heptanoate
stearyl linoleate*
stearyl olivate
stearyl palmitate
stearyl stearate
tetradecyleicosyl stearate*
tetradecyloctadecyl behenate*
tetradecyloctadecyl hexyldecanoate*
tetradecyloctadecyl myristate*
tetradecyloctadecyl stearate
tetradecylpropionates*
tridecyl behenate*
tridecyl cocoate*
tridecyl erucate*
tridecyl isononanoate
tridecyl laurate*
tridecyl myristate*
tridecyl neopentanoate
tridecyl stearate

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

The core relationship between these ingredients is a carboxyl ester functional group flanked on both sides by alkyl chains. These ingredients are reported to function in cosmetics mostly as skin conditioning agents. Although there are data gaps in this report, the relatedness of molecular structures, physicochemical properties, and functions and concentrations in cosmetics allow grouping these ingredients together and interpolating the available toxicological data to support the safety of the entire group. The available data on many of the ingredients, especially the previously reviewed ingredients, and on some of the constituent alcohols and acids, are sufficient, and similar structure-property relationships, biologic characteristics, and cosmetic product usage suggest that the available data may be extrapolated to support the safety of the entire group. For example, a concern was expressed regarding the extent of dermal absorption for certain long-chain, branched alkyl esters because of a lack of information on dermal absorption and metabolism. The consensus of the Panel was that because dermal penetration of long-chain alcohols is likely to be low, and the dermal penetration for alkyl esters is likely to be even lower, inferring safety from ingredients where toxicity data were available was appropriate. Data on previously reviewed ingredients and on some of the constituent alcohols and acids also proved useful in determining the safety of the entire group.

Alkyl Ethylhexanoates

The CIR Expert Panel issued a tentative amended safety assessment for public comment with the conclusion that the 16 alkyl ethylhexanoates listed below are safe in the present practices of use and concentration described in this safety assessment when formulated to be non-irritating.

C12-13 alkyl ethylhexanoate
C12-15 alkyl ethylhexanoate
C14-18 alkyl ethylhexanoate*
cetearyl ethylhexanoate
cetyl ethylhexanoate
decyltetradecyl ethylhexanoate*
ethylhexyl ethylhexanoate
hexyldecyl ethylhexanoate*
isocetyl ethylhexanoate
isodecyl ethylhexanoate*
isostearyl ethylhexanoate*
lauryl ethylhexanoate*
myristyl ethylhexanoate*
octyldodecyl ethylhexanoate*
stearyl ethylhexanoate
tridecyl ethylhexanoate

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

The ingredients in this report are branched alkyl esters that are the result of the esterification of an alkyl alcohol with 2-ethylhexanoic acid or its chloride salt. The core relationship is the same as for the alkyl esters group described above, namely, a carboxyl ester functional group flanked on both sides by alkyl chains. This group was separated from the alkyl esters safety assessment to focus attention on the potential liver and developmental toxicity of 2- ethylhexanoic acid, a possible metabolite of the alkyl ethylhexanoates. It has been postulated that, in animal studies of 2-ethylhexanoic acid, maternal liver toxicity could begin a cascade of effects that includes metallothionein (MT) induction, zinc accumulation in the liver due to MT binding, and a resulting zinc deficiency in the developing embryo. The Panel determined that results of animal tests with di-2-ethylhexyl terephthalate (a 2-ethylhexanoic acid precursor used as a model for exposure without liver toxicity) suggested that the process of metabolic conversion results in a time course that allows clearance of 2-ethylhexanoic acid before sufficient levels can arise to produce toxicity.

The rationale described above applied to the entire group of alkyl ethylhexanoates. Additionally, the similar chemical structures, physicochemical properties, functions, and concentrations in cosmetics allow interpolation of the available toxicological data to support the safety of the entire group.

6-Hydroxyindole

The CIR Expert Panel issued a tentative safety assessment for public comment with a conclusion that 6-hydroxyindole is safe as a hair dye ingredient in the present practices of use and concentration.

The CIR Expert Panel expressed concern that 6-hydroxyindole appears to be a photosensitizer at a concentration of 5%; however, further data did not indicate photosensitization at 2%. The Panel noted that this ingredient has 105 uses in hair dye products at concentrations up to 0.5%. The Expert Panel recognized that 6- hydroxyindole functions as a hair dye ingredient and that hair dyes containing this ingredient, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the Federal Food, Drug, and Cosmetic Act, when the product label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Panel considered the concerns about such self-testing (see discussion under 125th Meeting Notes), but agreed that there was not a sufficient basis for changing this advice to consumers at this time. The Expert Panel continues to expect that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures, but awaits data currently under development by the industry to shed further light on this practice.

Hypericum perforatum-derived ingredients

The CIR Expert Panel issued a tentative amended safety assessment for public comment for the 7 hypericum perforatum-derived ingredients listed below with the conclusion that they are safe in the present practices of use and concentration as described in the safety assessment.

hypericum perforatum extract
hypericum perforatum flower extract
hypericum perforatum flower/leaf extract
hypericum perforatum flower/leaf/stem extract
hypericum perforatum flower/twig extract
hypericum perforatum leaf extract
hypericum perforatum oil

One common name for *Hypericum perforatum* is St. John's wort. These ingredients function in cosmetics as skin-conditioning agents – miscellaneous, skin-conditioning agents – humectants; skin protectants; antioxidants, hair conditioning agents; and antimicrobial agents. Data were submitted to address the insufficient data conclusion of the original report on hypericum perforatum extract and hypericum perforatum oil. The Panel was satisfied that the data address the concentration of use, function, photosensitization/photo-toxicity, reproductive/developmental toxicity, irritation/sensitization, and ocular irritation data needs from that original safety

assessment. The Panel also added the following 5 other ingredients derived from *H. perforatum* to the group: hypericum perforatum flower extract; hypericum perforatum flower/leaf extract; hypericum perforatum flower/leaf/stem extract; hypericum perforatum flower/twig extract; and hypericum perforatum leaf extract.

The Panel also noted that the discussion section of the safety assessment of these ingredients will mention the presence of photoactive constituents of plant extracts, such as hypericin and quercetin, but that the concentrations of such constituents are not at a high level in the Hypericum perforatum-derived ingredients, and that the ingredients themselves are used at low concentrations.

The Panel decided not to add hypericum callus culture extract because it is produced differently (plant cells grown in culture), compared with the other extracts considered, and its composition was uncertain.

Methyl Glucose Polyethers and Esters

The CIR Expert Panel issued a tentative safety assessment for public comment with a conclusion that the 25 methyl glucose polyethers and esters listed below are safe in the present practices of use and concentration.

Esters:	*Polyethers:*	*Esters and polyethers:*
methyl glucose caprylate/caprate*	PPG-10 methyl glucose ether	PEG-120 methyl glucose dioleate
methyl glucose dioleate	PPG-20 methyl glucose ether	PEG-20 methyl glucose distearate
methyl glucose isostearate*	PPG-25 methyl glucose ether*	PEG-80 methyl glucose laurate*
methyl glucose laurate*	PPG-20 methyl glucose ether acetate*	PEG-20 methyl glucose sesquicaprylate/sesquicaprate*
methyl glucose sesquicaprylate/ sesquicaprate*	PPG-20 methyl glucose ether distearate	PEG-20 methyl glucose sesquilaurate*
methyl glucose sesquicocoate*	methyl gluceth-10	PEG-20 methyl glucose sesquistearate
methyl glucose sesquiisostearate	methyl gluceth-20	PEG-120 methyl glucose triisostearate*
methyl glucose sesquilaurate*		PEG-120 methyl glucose trioleate
methyl glucose sesquioleate		
methyl glucose sesquistearate		

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

Ingredients classified as polyethers reportedly function as skin and hair conditioning agents, whereas, the methyl glucose esters function only as skin conditioning agents in cosmetic products.

The Panel noted the absence of dermal penetration, reproductive and developmental toxicity and carcinogenicity data. Limited genotoxicity data and robust dermal irritation and sensitization data were available. After reviewing data on molecular weights, the Panel determined that there likely would be no significant skin penetration of these ingredients. Thus, potential systemic exposure is unlikely and reproductive and developmental toxicity or carciniogenicity data were not necessary to evaluate this group of ingredients.

The Panel discussed the potential effect that methyl glucose would have on glucose metabolism were these ingredients to be absorbed and metabolized. As noted above, however, significant dermal penetration of these ingredients was considered unlikely. While there were no available metabolism data, the complete deesterification of these ingredients to produce methyl glucose was considered highly unlikely. Overall, therefore, any impact of dermal application of these ingredients on glucose metabolism would be very unlikely. The Panel also discussed the apparent uncertainty in the definition of these ingredients as with respect to the extent of esterification. Are they mono-, di-, tri-, or tetra-esters or mixtures thereof? Additional data would be useful to document the extent of esterification that would result from the process of manufacturing these esters.

Modified terephthalate polymers

The CIR Expert Panel issued a tentative safety assessment for public comment for the 6 modified terephthalate polymers listed below with the conclusion that they are safe for use in cosmetics in the present practices of use and concentration.

adipic acid/1,4 butanediol/terephthalate copolymer	polyethylene terephthalate
polybutylene terephthalate	polypentaerythrityl terephthalate
polyethylene isoterephthalate	polypropylene terephthalate.

These reportedly function primarily as exfoliants, bulking agents, hair fixatives, and viscosity increasing agents-nonaqueous. While ethylene/sodium sulfoisophthalate/terephthalate copolymer originally was included in this group, the Panel concluded that this ingredient would have different surface properties than the rest of the ingredients and that it was appropriate to exclude this ingredient from this safety assessment.

Polyethylene terephthalate (PET) is approved for use in medical devices (i.e., surgical sutures, esophageal dilators, and surgical mesh). The Panel considers it likely that cosmetic grade PET would be similar to medical grade PET, in terms of the methods of manufacture, impurities, etc.

There was a concern brought to the Panel's attention that PET, in the form of glitter, could cause physical damage to the cornea if it became imbedded in the eye. In 1985, for example, one company withdrew a glitter product sold as a costume accessory, which may or may not have been intended for use on the face, because of eye injury complaints. However, the available use testing of eye area cosmetic products did not suggest any ocular toxicity and there is a lack of case reports in the literature. Overall, based on the extensive information reviewed by the FDA to support the safety of PET, the Panel concluded that no additional data were needed. In addition, the relatedness of molecular structures, physicochemical properties, and functions and concentrations in cosmetics allow grouping these ingredients together and interpolating the available toxicological data to support the safety of the entire group.

Nylon Polymers

The CIR Expert Panel issued a tentative safety assessment for public comment with the conclusion that the 8 nylon polymers listed below are safe in the present practices of use and concentration in cosmetics.

nylon-6	nylon-10/10	nylon 6/12	nylon-611
nylon-11	nylon-12	nylon-66	nylon-12/6/66

Additional data were submitted that fulfilled data needs concerning irritation and sensitization of nylon ingredients and genotoxicity data on the monomers of nylon ingredients. Concern was expressed that residual monomer data were not available. The Expert Panel reviewed human repeat insult patch test data on nylon-12 at its maximum use concentration of 35%. No sensitization or irritation was observed in this study. From these data, the Panel determined that, whatever residual monomers may be present in nylon-12, were not present at a sufficient level to cause any reactions in test subjects at the maximum use concentration.

Talc

The CIR Expert Panel issued a tentative safety assessment for public comment with the conclusion that talc is safe for use as a cosmetic ingredient in the present practices of use and concentration described in the safety assessment. The Panel did state that talc should not be applied to skin when the epidermal barrier is ulcerated or removed.

The Panel noted that although numerous studies have been performed to examine whether there is a correlation between ovarian cancer and talc, the data do not suggest that application of talc to the perineal area results in migration to the ovaries. Therefore, the Panel did not think there was a causal relationship between

ovarian cancer and the cosmetic use of talc. The Panel also discussed the results of positive findings in inhalation carcinogenicity studies of talc. The Panel agreed that the positive findings in these studies are best interpreted as the result of pulmonary overloading , and not relevant to the exposure levels that can reasonably be expected from the use of cosmetic products containing talc. Additionally, the Panel noted that co-carcinogenicity studies in hamsters in which talc was administered intratracheally with benzo[a]pyrene B[a]P, were not relevant to assessing the safety of talc as used in cosmetics.

The Panel agreed that early analyses of the composition of talc in which asbestiform fibers were detected may not be relevant to the current composition of cosmetic talc, because such information was developed before asbestos-free specifications for talc were developed by the cosmetics industry and unreliable analytical methods may have been used. Limited, recent FDA test data confirmed the absence of such fibers. A talc manufacturer representative reported that adequate analytical methods were in place to determine the presence of asbestiform fibers and that suppliers comply with current talc specifications. The industry representative agreed to submit test protocol information and sample certification sheets.

Finally, the Panel added the caveat regarding use of talc in products that could be applied when the epidermal barrier is ulcerated or removed because of case reports of granuloma formation when talc was applied to areas of the skin where the epidermal barrier was not intact.

Insufficient Data Announcement

Plant and Animal-Derived Amino Acids and Hydrolyzed Proteins

The CIR Expert Panel requested additional data to support the safety of 75 plant- and animal-derived amino acids and hydrolyzed proteins.

The additional data needed are: (1) method of manufacturing data for both plant and animal-derived amino acids and hydrolyzed proteins, especially for hydrolyzed wheat protein; and (2) composition and characterization specifications of plant and animal-derived amino acids and hydrolyzed proteins, including molecular structure and molecular weight ranges from several suppliers to determine if there is a consistency in cosmetic grade plant and animal-derived hydrolyzed proteins, especially hydrolyzed wheat protein.

These ingredients were presented to the Panel in two separate reports, one on source amino acids and one on hydrolyzed source proteins. The Panel decided to combine these 2 reports and title the single report "plant and animal-derived amino acids and hydrolyzed proteins." While data are sought for method(s) of manufacture, it appears that the approaches used to prepare source amino acids and hydrolyzed source proteins would be fundamentally similar, and that the only real difference in the products would be the extent of hydrolysis – either all the way to individual amino acids with potentially some short proteins present, or hydrolysis to short proteins of undetermined or unspecified lengths.
The Panel decided to remove the ingredient hydrolyzed spinal protein from review because spinal-derived ingredients are prohibited by Federal Regulation 21 CFR 700.27.

The 75 ingredients included in this safety assessment are:

Hydrolyzed Proteins:
ammonium hydrolyzed collagen
calcium hydrolyzed collagen
hydrolyzed actin
hydrolyzed albumen
hydrolyzed amaranth protein
hydrolyzed avocado protein
hydrolyzed barley protein
hydrolyzed brazil nut protein
hydrolyzed casein
hydrolyzed conalbumin
hydrolyzed conchiolin protein
hydrolyzed cottonseed protein
hydrolyzed egg protein
hydrolyzed elastin
hydrolyzed extensin
hydrolyzed fibroin
hydrolyzed fibronectin
hydrolyzed gadidae protein
hydrolyzed gelatin
hydrolyzed hair keratin
hydrolyzed hazelnut protein
hydrolyzed hemoglobin
hydrolyzed hemp seed protein
hydrolyzed honey protein
hydrolyzed jojoba protein
hydrolyzed keratin
hydrolyzed lactalbumin
hydrolyzed lupine protein
hydrolyzed maple sycamore protein
hydrolyzed milk protein
hydrolyzed oat protein
hydrolyzed pea protein
hydrolyzed potato protein
hydrolyzed reticulin
hydrolyzed royal jelly protein
hydrolyzed sericin
hydrolyzed serum protein
hydrolyzed sesame protein
hydrolyzed silk
hydrolyzed soy protein
hydrolyzed soymilk protein
hydrolyzed spongin
hydrolyzed sweet almond protein
hydrolyzed vegetable protein
hydrolyzed wheat gluten
hydrolyzed wheat protein
hydrolyzed whey protein
hydrolyzed yeast protein
hydrolyzed yogurt protein
hydrolyzed zein
MEA-hydrolyzed collagen
MEA-hydrolyzed silk
sodium hydrolyzed casein
zinc hydrolyzed collagen
Amino Acids:
apricot kernel amino acids
collagen amino acids
corn gluten amino acids
elastin amino acids
garcinia mangostana amino acids
hair keratin amino acids
jojoba amino acids
keratin amino acids
lupine amino acids
lycium barbarum amino acids
milk amino acids
oat amino acids
rice amino acids
sesame amino acids
silk amino acids
soy amino acids
spirulina amino acids
sweet almond amino acids
vegetable amino acids
wheat amino acids
yeast amino acids

Re-review and New Data

2-Amino-6-Chloro-4-Nitrophenol – not reopened

The CIR Expert Panel reaffirmed the original conclusion that 2-amino-6-chloro-4-nitrophenol and its hydrochloride salt are safe for use in hair dye formulations at concentrations up to 2.0%.

New toxicokinetics, genotoxicity, skin sensitization, and phototoxicity and photoallergenicity studies and a margin of safety calculation were available and presented to the Panel for review, as were updated concentration of data indicating that the maximum use concentration is now 1.5%. The Panel reviewed the new data and determined to not

re-open the safety assessment. The Panel did note that although carcinogenicity data were not available, 2-amino-6-chloro-4-nitrophenol is not significantly absorbed through the skin and is not genotoxic.

m-Phenylenediamine and m-Phenylenediamine Sulfate – not reopened

The CIR Expert Panel reaffirmed the original conclusion that phenylenediamine and m-phenylenediamine sulfate are safe for use in hair dyes at concentrations up to 10%.

According to the European Union Cosmetics Directive, m-phenylenediamine and its salts are among the substances that must not form part of the composition of cosmetic products marketed in the European Union. The Council explained that this language should not be interpreted as a ban, but simply as a natural consequence of an industry decision to not support the safety of phenylenediamine and m-phenylenediamine sulfate as hair dye ingredients in Europe.

The Panel acknowledged that the 10% concentration limit is higher than the maximum use concentrations recently provided by the cosmetics industry from 0.01% to 0.2% or m-phenylenediamine and 1% for m-phenylenediamine sulfate. However, the Expert Panel noted that the 10% limit was based on skin irritation and sensitization test data and does not need to be changed. The CIR Expert Panel determined that there were no new data sufficient to warrant reopening this safety assessment.

PEGs Cocamine - reopened

The CIR Expert Panel reviewed newly provided data and determined to reopen this safety assessment and add 41 ingredients, bringing the total number of ingredients in the report to 47.

In 1999, the CIR Expert Panel concluded that the available data were insufficient to support the safety of PEGs cocamine (PEG-2, -3, -5, -10, -15, and -20 cocamine). The Personal Care Products Council's CIR Science and Support Committee submitted data and analyses relating to these PEGs Cocamine ingredients. This extensive package included: (1) the American Chemistry Council's Fatty Nitrogen Derivatives Panel - Amines Task Group assessment of data availability for the fatty nitrogen derived amines category, including robust summaries for reliable studies; (2) the EPA's human health risk assessment supporting the proposed exemption of alkyl amine polyalkoxylates from the requirement of a tolerance when used as inert ingredients in pesticide formulations; (3) the EPA's human health risk assessment supporting the proposed exemption of *phosphate ester, tallowamine, ethoxylated* from the requirement of a tolerance when used as an inert ingredient in pesticide formulations; (4) a poster presentation on readacross and computer-based analysis to support the safety of PEGs cocamine in cosmetics; and (5) current use concentration data.

There are 3 additional PEGs Cocamine that now are identified as cosmetic ingredients (PEG-4, -8, and -12 Cocamine). Also, other PEG fatty acid amines, which differ from thePEGs Cocamine group only by length of alkyl chain and degree of saturation, may be included. These are:

PEG-2, -7, -11, -15, -20, -22, -25 and -30 tallow amine
PEG-2, -5, -8, -10, -15, -20, -30, -40, and -50 hydrogenated tallow amine
PEG-2 lauramine
PEG-2, -5, -6, -10, -15, -20, -25, and -30 oleamine
PEG-12 palmitamine
PEG-2 rapseedamine
PEG-2, -5, -8, -10, and -15 soyamine
PEG-2, -5, -10, -15, and -50 stearamine

Phthalates – not reopened

The CIR Expert Panel reviewed 3 new studies on phthalates and determined to not reopen the safety assessments of dimethyl, diethyl, or diethyl phthalate, or butyl benzyl phthalate. The conclusion for these ingredients remains that they are safe in cosmetics in the present practices of use and concentration.

Since these original safety assessments were made, the focus of new phthalate studies has been on the potential for endocrine disruption/reproductive and developmental toxicity. The Panel previously reviewed numerous studies, noting that a feeding study using rodents reported a reproductive/developmental toxicity NOAEL of 331 mg/kg/day, but the Panel determined that a reproductive/developmental toxicity NOAEL of 50 mg/kg/day in a rodent gavage study was the worst case NOAEL. To determine exposure, the Panel summed the estimated exposures from all cosmetic product types reported to contain phthalates at specific levels, and determined that exposure to be 9.13 µg/kg/day. Accordingly, a margin of safety of 5,746 was determined.

One new study of children aged 5 to 9, who were part of a Manhattan-Bronx cohort, revealed detectable, although varied, levels of phthalates in the urine of all 244 study participants. Higher levels of both diethyl phthalate and butyl benzyl phthalate were associated with airway inflammation.

Two new studies addressed diabetes and phthalates. Subjects in one study were 1,015 men and women 70 years of age in Uppsala, Sweden. The samples – one sample per subject – were collected in 2001 – 2004 and analyzed 5 – 8 years later. The four phthalates that were the focus of the study included dimethyl phthalate, diethyl phthalate, diisobutyl phthalate, and diethylhexyl phthalate measured in blood and correlated to measures of insulin resistance and poor insulin secretion in non-diabetic subjects.

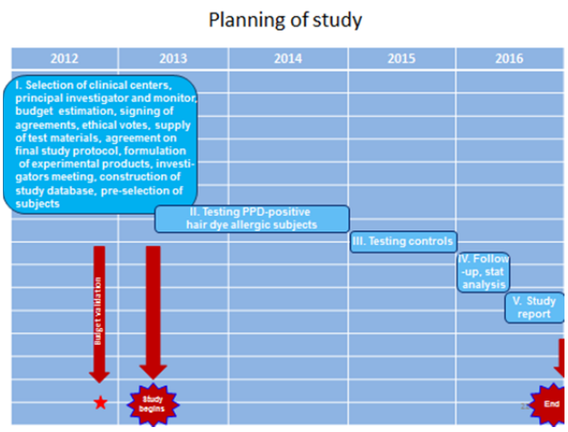
In the second diabetes and phthalates study, urinary concentrations of phthalate metabolites measured by the CDC and self-reported diabetes in 2,350 women ages 20 to <80 participating in the NHANES (2001- 2008) were used. The odds ratio for diabetes in women with higher levels of n-butyl phthalate, isobutyl phthalate, benzyl phthalate, 3- carboxypropyl phthalate, and the sum of diethylhexyl phthalate metabolites was greater than the odds ratio for women with the lowest concentrations of these phthalates.

The Panel noted that all of these studies identified associations between phthalate metabolites and either diabetes or airway inflammation. Such studies did not suggest a causal link between phthalates and any adverse outcome. The possibility that phthalate metabolites may impact peroxisome proliferation pathways was suggested in the diabetes studies, but that mechanism is not established as a mode of action. The Panel agreed that that there is a need for further study of the reported association between phthalates exposures and diabetes and to investigate possible causal links.

125th Meeting Notes

Hair dye self-testing

Dr. Carsten Goebel, Proctor and Gamble, representing the Personal Care Products Council's Hair Coloring Technical Committee (allergy subgroup) reviewed the current status of hair dye self-testing, or, as he termed it, the "allergy alert test." He noted that instructions for such testing are mandatory in the USA, Canada, Japan, Australia, and Brazil, but voluntary in the EU, Latin America, and most Asian countries. Recent reports have suggested that such allergy alert testing may induce allergies to hair dye ingredients.



Dr. Goebel stated that, although it cannot be excluded that an increased application frequency (at a different site) as a result of performing the allergy-alert test may increase the risk of inducing sensitization, the value of the alert test in preventing severe allergic reactions after hair coloring outweighs this potential risk. He asserted that the objective of each allergy-alert test is to prevent severe reactions to an individual hair coloring product in an individual hair-dye user.

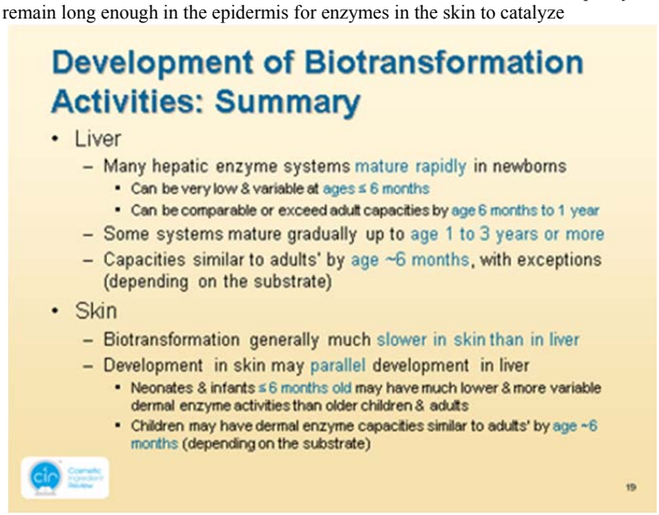
Dr. Goebel described a new effort by the industry to conduct a multicenter proof-of-concept study for the allergy alert test which will address the efficacy of the test under use-like conditions. The study timeline is shown to the left. The study will allow assessment of variations in test parameters, robustness, and independent evaluation by subject/dermatologist.

The CIR Expert Panel noted that hair dyes containing coal tar hair derivatives are exempt from certain adulteration and color additive provisions of the U.S. Federal Food, Drug, and Cosmetic Act, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Panel agreed that there was not a sufficient basis for changing this advice to consumers at this time. The Expert Panel continues to expect that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures, but awaits the data from ongoing investigations by the industry to shed further light on this practice.

Infant skin report

CIR's senior toxicologist, Dr. Ivan Boyer, briefly presented information from a draft overview report on developmental factors that can influence the systemic absorption of topically applied substances through infant skin. CIR staff prepared the draft report as directed by the CIR Expert Panel during their March 2012 meeting.

The draft report addresses two major factors: (1) development of the diffusion barrier of the skin, which is attributed to the stratum corneum; and (2) development of biotransformation enzyme systems in the skin, which can also limit absorption. Dr. Boyer noted that the stratum corneum is an effective semi-permeable barrier at birth, although its effectiveness as a diffusion barrier continues to develop, especially during the first month after birth. He indicated that the skin also has a substantial capacity to metabolize substances that penetrate the stratum corneum, provided that these substances remain long enough in the epidermis for enzymes in the skin to catalyze biotransformation reactions. He noted that there are very little data in the scientific literature specifically addressing the development of biotransformation systems in the skin. However, the information available to characterize development in the liver may be used to support assumptions about the development of biotransformation capacities in the skin.



Dr. Boyer used the slide on the right to emphasize that liver enzyme systems generally develop rapidly after birth, except for enzymes catalyzing glucuronidation reactions. By analogy, the capacities of most biotransformation systems in the skin may be comparable to those in adults by about 6 months of age.

The CIR Expert Panel determined that the draft overview should be developed further as a resource for the Panel and a guide to information that the Panel considers in its safety assessments. They emphasized that a preamble should be included to emphasize that the Panel's purview encompasses cosmetic products intended for use on normal skin, and does not include the use of cosmetic products on preterm infants or infants with skin conditions. They also noted that the normal skin of full-term babies does not appear to have any deficiencies in biotransformation capacities that would warrant concerns that are not already addressed in safety assessments. However, additional information from dermal carcinogenicity animal studies should be incorporated into the document. The Panel also encouraged input from pediatric dermatologists and experts in this field in industry. After receiving comments, the Panel will revisit the overview report.

Report Tabled

Achillea millefolium extracts - The CIR Expert Panel tabled further discussion of re-opening this report on ingredients derived from *Achillea millefolium* (aka yarrow) to give industry the opportunity to submit further available irritation and sensitization data at concentrations of use. This safety assessment will be re-opened if data submitted to the CIR satisfy all the data needs listed in the current insufficient data conclusion. Alternatively, a maximum concentration of use may be stated in the conclusion.

The Panel determined that if the report is re-opened, two achillea millefolium-derived ingredients should be added to this safety assessment, but decided that achillea millefolium oil and achillea millefolium flower water were not appropriate to include because of the different characteristics of the oil and because both of these ingredients function only as fragrance ingredients.

The 3 ingredients that are in this safety assessment include: achillea millefolium extract, achillea millefolium flower extract, and achillea millefolium flower/leaf/stem extract. these ingredients function in cosmetics as skin-conditioning agents – miscellaneous, skin-conditioning agents – humectants; and fragrance ingredients.

The Panel noted that irritation/sensitization data were available to support the use of these ingredients at concentrations up to 0.002%, but was not satisfied that the sensitization data were sufficient to address the reported use of these extracts up to 0.04%. The Panel invited the submission of sensitization data for these ingredients at the use concentration of 0.04%. The Panel also noted that an eventual discussion of these achillea millefolium-derived ingredients will mention the presence of photoactive constituents of plant extracts, such a quercetin, but the concentrations of such constituents are low in the achillea-millefolium-derived ingredients and the ingredients themselves are used at low concentrations.

Cosmetic Ingredient Review
Commitment . . . Credibility
Since 1976

C I R

Memorandum

To: CIR Expert Panel

From: Director, CIR
Senior Toxicologist, CIR

Subject: New information relating to hydroquinone and p-hydroxyanisole

Date February 22, 2013

We have received a request from Dr. David Steinberg (data1 file)asking that the CIR Expert Panel review their conclusions about two cosmetic ingredients that are used as polymerization inhibitors in new nail polishes and other nail products offered for use as "at home" products, including: p-hydroxyanisol (JACT 1985) and hydroquinone (IJT 2010).

The Panel's current conclusions (full safety assessments are included as data2 and data3) about these ingredients are:

- "p-Hydroxyanisole is unsafe for use as a cosmetic ingredient." This conclusion was based primarily on depigmentation of black guinea pig skin in studies in which concentrations as low as 0.25% or less (0.1% in some animals) were applied to the skin daily for 1 or more months (which were close to use concentrations up to 0.1% to 1.0% at the time of the FDA survey in 1981).

- "…hydroxyquinone is safe at concentrations ≤1% in hair dyes" and is "safe for use in nail adhesives in the practices of use and concentration described in this safety assessment," although it "should not be used in other leave-on cosmetics." The Panel noted that, while absorption through the skin could be appreciable in leave-on products, hydroxyquinone in nail adhesives "is oxidized during use and is no longer present in the preparation and minimal dermal exposure and absorption is expected to occur from this application." The use concentration was reported to be 0.5% in nail adhesives at the time of the survey (2008). The Panel's discussion does not explicitly include a warning to avoid skin contact or specify that nail adhesives containing this ingredient should be for professional use only. The cosmetic use section of the CIR safety assessment noted that the EU banned the use of this ingredient in hair dyes in 2008 and approved its use in professional-use-only artificial nail systems up to a maximum of 0.02% after mixing with methacrylate monomers (hydroxyquinone at 0.02% in methacrylate monomer preparations was undetectable in the finished product).

As presented in his letter, he described the new use of these ingredients as (1) using both of these ingredients in products like nail polishes, as opposed to use of one of these ingredients (hydroxyquinone) specifically in nail adhesives, and (2) the sale of such products directly to consumers for at home use, rather than for professional use only.

According to Dr. Steinberg, the maximum use concentration of these ingredients in these products is 0.12% (presumably the maximum sum of the concentrations of these ingredients used singly or combined). He recommends a conclusion of safe as used, with a warning to avoid contact with skin and remove from the skin, if there is inadvertent skin contact, before curing the product on the nail.

Dr. Steinberg notes that such products are "greener" (i.e., release less VOC to ambient air during use), the new products cure under the near visible light (390-420 nm; which probably makes them more amenable to "at home" use), and the cured product is more chip/crack-resistant than older products.

1101 17th St., NW, Suite 412 Washington, DC 20036-4702
Phone 202.331.0651 FAX 202.331.0088 http://www.cir-safety.org

So, Dr. Steinberg is asking the Panel to consider a safe-as-used conclusion of 0.12% in "at-home" use nail polishes and other nail products (with a warning to avoid skin contact), which is:

- About ½ of a concentration of p-hydroxyanisole that consistently depigmented guinea pig skin after subchronic topical application (or about equal to a concentration that depigmented the skin in some of the guinea pigs)

- About ¼ of the use concentration of hydroxyquinone in nail adhesives reported in 2008

The Panel should consider whether or not the safety assessments of these two ingredients should be re-opened to address Dr. Steinberg's proposal.

Steinberg & Associates, Inc.
16 Mershon Lane, Plainsboro, NJ 08536
Ph. (609) 799-1575 Fax (609) 799-5271
Email Davidpreserve@comcast.net

Alan Andersen, Ph.D.

Director

Cosmetic Ingredient Review

1101 17th Street, NW Suite 412

Washington, DC 20036-4702

February 4, 2013

Dear Dr. Andersen,

On behalf of the Nail Manufacturer's Council, part of the Professional Beauty Association, I would like to request that the CIR review their conclusions of two Monographs:

1. Hydroquinone (also known as HQ),"Final Amended Safety Assessment of Hydroquinone as Used in Cosmetics" IJT 29(Supplement) 2745-2875
2. *P*-Hydroxyanisole (also known as MEHQ or Hydryoquinone monomethy ether) "Final Report on the Safety Assessment of p-Hydroxyanisole" JACT, 4(5) 1985

In both reports the CIR acknowledged their use as polymerization inhibitors.

HISTORY of the use of HQ and MEHQ in Monomers

HQ and MEHQ have a long history of use by the chemical industry to inhibit polymerization of certain monomers. The most common are acrylate types, but these also find use in many other monomers such as styrene and vinyls. They are added either alone or in combination, during the manufacturing of monomers to prevent polymerization. This addition of HQ/MEHQ allows the preservation of the integrity of the monomers, as monomers, until they are required to be further used to produce polymers or other derivatives. In order for polymerization to occur, the inhibitors must either be destroyed or made inactive. Oligomers made from these monomers also need inhibitors such as MEHQ/ HQ or they, also, will polymerize further. Oligomers are usually described as dimers, trimers or tetramers of monomers and remain reactive to form higher molecular weight polymers.

The only type of monomer (with HQ and/or MEHQ as inhibitors,) sold as cosmetics until recently, were the cyanoacrylate glues. These are used to glue artificial nail products to the nail plate.

MEHQ was used at one time, as a skin bleaching agent, as was HQ. Both are restricted in this use as these are drugs, not cosmetics. Further HQ is found in certain hair dyes. The FDA reports no products using MEHQ in their VCRP data base. There are 23 listings for HQ which probably reflects its use in hair dyes.

About 20-30 years ago, it was discovered and commercialized to use these monomers inhibited with HQ/MEHQ, in several medical devices, such as bone adhesives, artificial teeth, fillings for natural teeth and hearing aids. In some cases polymerization took place *in situ* and in other cases the polymer was formed into a special mold to yield the desirable fit and application.

It was soon found that this technology could be used to make artificial nail enhancement products. These are sold for "Professional Use Only" and consist of two components: Monomers (having HQ/MEHQ) and polymers containing Benzyl Peroxide (BPO). A small brush is dipped into the liquid and then the powder. The result is the start of polymerization and the nail technicians has a limited time to shape this nail before it becomes a hard plastic. It is then glued unto the consumers nail.

There where kits which were intended for consumer use, but where too difficult for untrained consumers to use. They are rarely found on any market today. The Nail Manufacturers Council did not encourage the use of this technology by consumers. The Retail Nail Association presentations to the CIR showed proven safety for consumer use.

New Technology

The automotive coatings industry has been looking for decades to produce finishes for cars, which used lower levels of VOC's or gave quicker drying times which resulted in less VOC's and "greener" coatings. From this came the type of coatings frequently called UV cures.

Nail products technology based on this chemistry, has advanced in the last few years. The most popular new products are based on the use of almost visible light (390-420 nm) to cure or cause polymerization to occur. Consumer demand for these cured products is significant, especially for the nail polishes. These innovations offer 2 weeks of wear without chipping or cracking and lower VOC's.

Because of their safety and ease of application by consumers, these are now being offered as "at home" consumer products. This results in the new use of a sale of monomers containing MEHQ/HQ as cosmetics to consumers.

The typical formulations of such products consist of monomers and oligomers with an acrylic backbone, and a photo initiator such as Hydroxycyclohexyl Phenyl Ketone forming a viscous shearing thinning gel. The MEHQ/HQ comes with the monomers; it is not added during the manufacturing of the gels.

The maximum level of MEHQ/HQ used does not appear to exceed 1200 ppm (0.12%).

The instructions for use of these nail polishes include:

Washing and sanitize the hands, push the cuticles, clean and detail the nail plates as normal. Gently remove shine from around the cuticles with a special file. Buff the surfaces and shape the edges. Finally dust, cleanse and apply a pH balancing agent and let dry. Apply the nail gel using the brush, being careful to avoid skin contact and apply to within 1/8 of an inch to the cuticle. (if you apply the gel to the cuticle, it will "lift" off after curing.)If any gel is accidentally applied to the skin or cuticle, remove this with the cuticle wooden stick (this is supplied with the kit). The nails are than placed under the UV or LED light. Cure times vary from 1 to 3 minutes.

1. Safety

The safety of MEHQ has been well documented in such resources as the 1985 CIR report, OSHA and the CDC. The 1985 CIR report, which reviewed MEHQ for use in cosmetics, indicated MEHQ was reported to the FDA as performing the function of an antioxidant. This report clearly established that MEHQ is a skin depigmenting agent at levels of 0.25 to 4%. Mequinol is an FDA approved Rx drug for this purpose. MEHQ was used in cosmetics at levels up to 1%, according to that report. MEHQ was a skin sensitizer at 6.2% in animals but not on humans at 2.0%. To put these safety studies in context of the above-noted uses, all were based on leaving the material on the skin during the testing.

However, in nail products, the exposure to MEHQ is minimal as the only contact is with the nail. (What woman wants nail polish on their skin?) Accidental contact with the skin is quickly removed in a manner of minutes, if not less. A 55 patient RIPT using the nail gel (containing both MEHQ and HQ) on the nail resulted in no visible nail or cuticle reaction. The gel was applied by a lab technician (not trained manicurist or nail technician) because the test was intended to include inadvertent application of gels on the cuticles.

The amount of residual MEHQ/HQ on the nail is dependent on types of monomers, levels of MEHQ/HQ in the different monomers, curing time and other variables. Testing of cured nail products after time, shows reduction to levels as low as "not detectable" and as high as 100 ppm. All the MEHQ/HQ is encased in a hard polymer so only by dissolving the polymer, and extraction, is it possible to find the MEHQ.

Publications are available of how difficult it is to get anything to penetrate the nails. In addition, the final product before application is a sheer thinning gel, so any MEHQ/HQ is tightly bound in the gel matrix. This also allows for easy removal if any gel accidentally touches, or is brushed onto, the cuticle or skin.

MEHQ has a water solubility of 40g/L at 25°C. After the gel was mixed in water, no detectable MEHQ was found in the water. Further, cured gel also placed in water, resulted in no detectable material.

The safety of Hydroquinone has been well documented and reviewed by the CIR in 1986, 1994 and 2010. In 1986 they published this conclusion: HQ is safe up to 1% in brief use on the skin which is then removed. It has been reviewed by NTP, EPA, ICSC, and IARC (Category 3). It is currently allowed in the US as an OTC skin bleaching drug at 1.5 to2.0% (and higher levels up to 4% by prescription).

The exposure to the skin and cuticle from HQ is the same as for MEHQ. It is present in the gel and easily removed. Consumers will always remove the gel from the skin to prevent the skin having nail polish on it; and the cuticle, so the nail polish adheres correctly and firmly to the nail.

Conclusions

MEHQ/HQ are not added to nail products but come with the monomers and oligomers as polymerization inhibiting chemicals. The use levels of MEHQ/HQ in the nail gels are to a maximum of 0.12% (1200 ppm). It is requested that the CIR review this new application and that MEHQ/HQ should be permitted in all nail products up to this level, with an appropriate warning: Avoid contact with skin. If contact occurs, remove immediately before curing under the radiation light.

In reviewing with the FDA on serious and non-serious adverse reactions to the entire category of "UV gels" found only 2 reactions from 2005 which where both used to form artificial nail products, not coatings.

Papers Available Upon Request

Cosmetic Ingredient Reviews of Hydroquinone: 1986, 1994, 2010

Cosmetic Ingredient Review of p-Hydroxyanisole (MEHQ) 1985

Expert Risk assessment of MEHQ in Gel Nail Polish by Edward M. Jackson, Ph.D.

Subungual Penetration of Dibutyl Phthalate in Human Fingernails by Edward M. Jackson, Ph.D.

OSHA Report for 4-methoxyphenol (MEHQ)

CDC Report on 4-methoxyphenol (MEHQ)

Report on detect ability in polish of MEHQ at Various curing times

HIRPT of Nail gels-multi tests and products

Consumer instruction sheets for nail gels (3 different companies)

Risk of Skin Cancer Associated with the Use of UV Nail Lamp,by Markowa, Weinstock

Do UV Nail Lamps Emit Unsafe Levels of Ultraviolet Light? By Schoon, Bryson and McConnell

Thank you,

David C. Steinberg, FRAPS

International Journal of Toxicology
29(Supplement 4) 274S-287S

DOI: 10.1177/1091581810385957
http://ijt.sagepub.com
SAGE

Final Amended Safety Assessment of Hydroquinone as Used in Cosmetics

F. Alan Andersen, PhD, Wilma F. Bergfeld, MD, FACP, Donald V. Belsito, MD, Ronald A. Hill, PhD, Curtis D. Klaassen, PhD, Daniel C. Liebler, PhD, James G. Marks, Jr, MD, Ronald C. Shank, PhD, Thomas J. Slaga, PhD, and Paul W. Snyder, DVM, PhD

Abstract

Hydroquinone is an aromatic compound that functions in cosmetics as an antioxidant, fragrance, reducing agent, or polymerization inhibitor. Hydroquinone is also used as a skin bleaching agent. Safety and toxicity information indicate that hydroquinone is dermally absorbed in humans from both aqueous and alcoholic formulations and is excreted mainly as the glucuronide or sulfate conjugates. Hydroquinone is associated with altered immune function in vitro and in vivo in animals and an increased incidence of renal tubule cell tumors and leukemia in F344 rats, but the relevance to humans is uncertain. Quantitatively, however, the use of hydroquinone in cosmetics is unlikely to result in renal neoplasia through this mode of action. Thus, hydroquinone is safe at concentrations of $\leq$1% in hair dyes and is safe for use in nail adhesives. Hydroquinone should not be used in other leave-on cosmetics.

Keywords

cosmetics, hydroquinone, safety

Introduction

The Cosmetic Ingredient Review (CIR) Expert Panel first assessed the safety of hydroquinone (HQ) as a cosmetic ingredient in 1986[1] and concluded that HQ was found to be safe for use at limited concentrations for certain formulations (primarily hair dyes) A subsequent review was conducted following the completion of the National Toxicology Program (NTP) report on HQ. That amended safety assessment,[2] of HQ dealt with the use of HQ in cosmetic leave-on preparations and was published in 1994 with the conclusion from the CIR Expert Panel that HQ "... is safe at concentrations of $\leq$1% for aqueous cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone should not be used in leave-on, nondrug cosmetic products."[2] This amended safety assessment will address the current uses of HQ in cosmetic products and assess information on the safety of HQ in cosmetic products that have become available since the last (1994) published report.

Chemistry

Definition and Structure

Hydroquinone (CAS No 123-31-9) is the aromatic compound that can function as an antioxidant, a fragrance ingredient, a hair colorant, a reducing agent, and a skin bleaching agent.[3] The structure of HQ is presented in Figure 1. Technical names for this ingredient are presented in Table 1.[3]

Physical and Chemical Properties

In its pure form, HQ is a colorless crystalline solid. Commercial preparations of HQ are usually white to off-white crystalline materials. The physical and chemical properties of HQ are presented in Table 2.[4]

Manufacture and Production

There are 5 grades of HQ: photographic, technical, US pharmacopeia (USP), inhibitor, and polyester. Photographic and technical grades are produced in the highest volume, most commonly through either hydroperoxidation of *p*-diisopropylbenzene, hydroxylation of phenol, or oxidation of aniline.[4]

Corresponding Author:
F. Alan Andersen, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA
Email: cirinfo@cir-safety.org

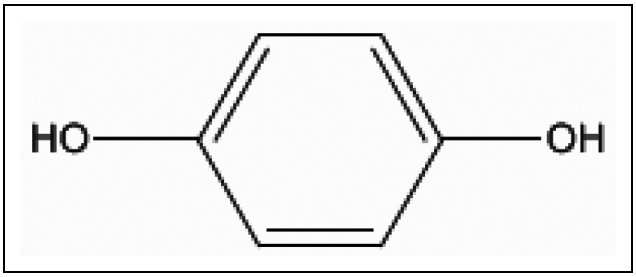


Figure 1. The structure of hydroquinone.

Table 1. Technical Names for Hydroquinone[3]

Ingredient Name	Other Technical Names
Hydroquinone	1,4-Benzenediol 1,4-Dihydroxybenzene p-Dihydroxybenzene 4-Hydroxyphenol p-Hydroxyphenol

Table 2. Physical and Chemical Properties of Hydroquinone[4]

Property	Value
CAS No	123-31-9
Molecular weight	110.11
Color, form	Colorless, crystals
Melting point (°C)	172
Boiling point (°C)	285-287 @760 mm Hg
Vapor pressure @ 25 °C, Pa	2.34×10^{-3}
$LogP_{ow}$	0.50-0.61
Solubility, g per 100 g solvent (30°C)	
Solvent	Value
Ethanol	46.4
Acetone	28.4
Water	8.3

Impurities

Resorcinol (1,3-benzenediol) and catechol can be present in HQ preparations depending on the method of manufacture.[4] In addition, impurities that impart a yellow color to the crystals are often present in technical-grade HQ but can be removed through specialized sublimation processes to produce more refined grades of HQ.

Analytical Methods

Hydroquinone can be analyzed by a number of techniques, including spectroscopic, chromatographic, titrimetric, and electrochemical methods.[4]

Reactions

In solution, HQ is rapidly converted to *p*-benzoquinone via an oxidation reaction upon exposure to air. The rate of this oxidation is increased in alkaline solution.[4] Hydroquinone, which acts as a regulating agent allowing some control of the color-forming coupling reactions, is a "consumable" in the hair dyeing procedure, with its actual concentration decreasing as the color-forming reaction proceeds.[5]

Use

Cosmetic

Hydroquinone is an aromatic compound that is used in cosmetic formulations as an antioxidant, fragrance ingredient, hair colorant, and a reducing agent. Hydroquinone is present in the liquid component of artificial nail systems where it functions to inhibit the polymerization of the methacrylate monomers.[6] According to information supplied to the US Food and Drug Administration (FDA) as part of the Voluntary Cosmetic Registration Program (VCRP), the use of HQ has decreased from 206 uses in 1993 to 151 uses in 2007 to 32 reported uses in 2009 (Table 3).[7,8] A survey of current use concentrations conducted by the Personal Care Products Council reported a use of 0.5% for HQ in other nail care products.[9]

Hydroquinone is added to the methacrylate monomers at a concentration of 0.02% or less than 200 ppm to inhibit their polymerization during transport and storage. In a study to determine the concentration of HQ that remains following the polymerization process, methacrylate polymer was analyzed using the titration method with UV detection (limit of detection [LOD] = 10 ppm) and the concentration of HQ in the finished product was below the LOD.[11]

In 2008, the European Union (EU) banned the use of HQ in hair dyes due to a lack of safety information. They have stated that there is no evidence that HQ as found in hair dye products is safe for human health.[12] Hydroquinone is approved for use in the EU in artificial nail systems, for professional use only, up to a maximum concentration of 0.02% after mixing.[13]

Noncosmetic

Medical. Hydroquinone is used in topical formulations as a skin bleaching and depigmenting agent and is used in the treatment of melasma (chloasma), freckles, senile lentigines, and postinflammatory hyperpigmentation. This use of HQ is considered a drug use that falls under the purview of the FDA. Hydroquinone is available via a prescription and over-the-counter (OTC) products that range in concentration from 0.4% to 5% HQ.[14] These OTC products are used to lighten the skin and are applied all over the face and body with varying use patterns among consumers. It is recommended that exposure to sunlight be avoided when using these products.

The FDA has issued a Federal Register notice on their intent to designate OTC skin bleaching products as no longer generally recognized as safe and effective (GRASE). Any products currently on the market would be considered new drugs that require an approved new drug application (NDA) for continued marketing.[14]

Table 3. Historical and Current Cosmetic Product Uses and Concentrations for Hydroquinone[7-10]

Product Category (FDA 2008)	1993 Uses (FDA 1993)	2007 Uses (FDA 2007)	2009 Uses (FDA 2009)	2009 Concentrations (%; Council 2009)
Hair coloring products				
Dyes and colors	185 (1112)	139 (2481)	13 (2481)	–
Makeup				
Lipstick	2 (937)	1 (1912)	–	–
Nail care products				
Other[a]	–	– (124)	– (124)	0.5
Skin care products				
Cleansing creams, lotions, liquids, and pads	–	1 (1368)	1 (1368)	–
Face and neck creams, lotions, etc	–	– (1195)	2 (1195)	–
Moisturizers	–	1 (2039)	2 (2039)	–
Fresheners	1 (246)	– (285)	2 (285)	–
Other	18 (848)	9 (1244)	12 (1244)	–
Total uses/ranges for hydroquinone	206	151	32	0.5

Abbreviations: FDA, US Food and Drug Administration.
[a] Nail adhesive.

The EU banned the use of HQ in OTC skin lightening products in 2001.[15,16]

Industrial. Hydroquinone is used in the development of black and white film, as an inhibitor of polymerization, as a stabilizer in paints, varnishes, motor fuels and oils, and as an antioxidant for fats and oils.[17]

General Biology

Inhibition of Tyrosinase

The ability of HQ to decrease melanogenesis when applied topically has made this a useful agent for decreasing skin pigmentation. Hydroquinone has been shown to be a weak substrate for the enzyme tyrosinase in kinetic studies but is able to compete with the endogenous substrate, tyrosine in vivo and inhibit the conversion of tyrosine to dopa and dopa to dopaquinone, thus decreasing melanin formation.[18,19]

Absorption, Distribution, Metabolism, and Excretion

Hydroquinone is rapidly absorbed and excreted in urine in rats following oral administration.[2] Absorption from an alcohol vehicle is greater than from an aqueous solution. Hydroquinone in an aqueous solution was absorbed through human skin at a rate of $0.55 \pm 0.13 \mu g/cm^2$ per h

Proposed pathways for the metabolism of HQ in rats and humans are presented in Figure 2. The principal products observed from the metabolic process are the sulfate and glucuronide conjugates. Oxidation to 1,4-benzoquinone results in a reactive metabolite that forms mono- or polyglutathione conjugates.[17] The glutathione conjugates are believed to be the causal agents in rodent nephrotoxicity and renal carcinogenesis.

In vitro. Human skin (500 μm thick) from 6 donors was used to examine the permeation of a 2% [^{14}C]hydroquinone cream alone and following pretreatment with 2% sodium azide.[20] No difference was observed in the permeation of HQ between sodium azide treated (42.7%) and untreated (43.3%) skin. A lag time of 8 hours was noted and believed to be attributed to the time it takes for the HQ cream to penetrate through the skin and into the receiving fluid. Using this model system, 100% of the dose was accounted for.

Metabolism was also examined in this system, with no significant difference in HQ recovery with ($28.1\% \pm 18.4\%$) and without ($28.5\% \pm 12.9\%$) sodium azide treatment. The amount of benzoquinone recovered was significantly decreased, however, from $10.8\% \pm 5.7\%$ without sodium azide pretreatment to $4.1\% \pm 2.0\%$ with sodium azide pretreatment.[20]

The metabolic rate constants for the conversion of HQ to the monoglutathione conjugate (HQ-SG) and subsequently to the mercapturic acid (HQ-Cys) in hepatocyte cultures isolated from F344 rats and humans were measured.[21] The substrate was added to the culture system and the disappearance and subsequent appearance of downstream metabolites were followed using sequential sampling (from 5 to 75 minutes) and high-performance liquid chromatography (HPLC) analysis. In this model system, glucuronidation was the initial favored step in both rat and human hepatocytes, with human hepatocytes having a higher V_{max} and intrinsic clearance (V_{max}/Km) than rat hepatocytes (15.2 vs 3.85 nmol/min per 10^6 cells, respectively). The human hepatocytes also demonstrated a higher capacity for metabolism of the HQ-SG to HQ-Cys than the rat hepatocytes. Acetylation was favored over deacetylation in both species. Overall, the authors concluded that the capacity for the metabolism of HQ and HQ-SG is greater in humans than in rats, suggesting a greater capacity for the detoxification of the glutathione conjugates. These metabolic constants were incorporated into an existing physiologically based pharmacokinetic (PBPK) model, which subsequently predicted that the body burden of these metabolites would be much higher in rats than in humans.[21]

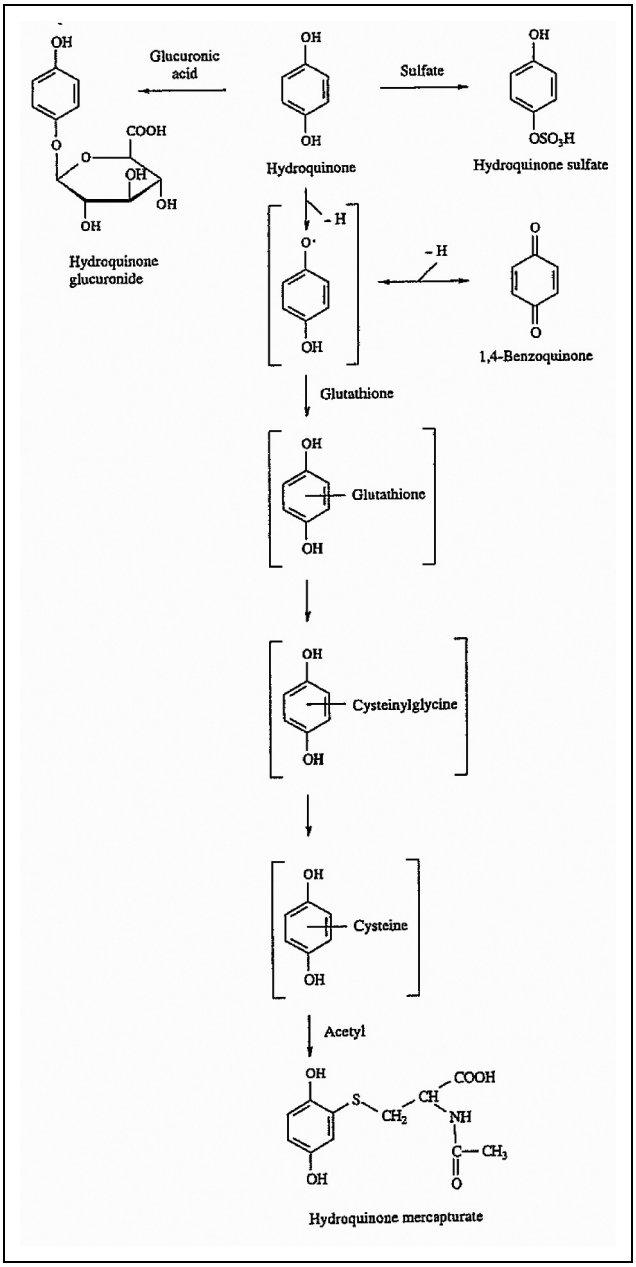


Figure 2. Proposed metabolism of hydroquinone.[17]

In vivo. Male F344 rats (number not specified) were given either a single dose of 1.8 mmol/kg [^{14}C]HQ in corn oil or 14 daily doses of HQ (1.8 mmol/kg in corn oil) followed by a single dose of 1.8 mmol/kg [^{14}C]HQ on day 15 by gavage.[22] Urine was collected for 72 hours and analyzed for total radioactivity. Following a single gavage dose, 21% of the dose was recovered in the 0- to 5-hour urine sample with an additional 35% excreted in the next 19 hours. The major metabolites identified, via HPLC-UVEC, in the 0- to 24-hour urine samples were HQ glucuronide (21%), HQ sulfate (15%), and HQ mercapturate (13%). Following subchronic administration, 46% of the radioactivity was recovered in the 0- to 5-hour urine sample and 31% excreted in the next 19 hours. Subchronic treatment changed the metabolic profile, with an increase in the amount of radioactivity excreted as the glucuronide conjugate (2 fold) and the glutathione conjugate (1.4 fold), while the percentage of the dose that was excreted as the sulfate remained the same. The authors state that these findings lend support to their hypothesis that subchronic administration of HQ increases the rate and extent of HQ metabolism to nephrotoxic glutathione conjugates.[23]

Table 4. Concentrations of Arbutin in Foods Used in the Human Diet Experiments[24]

Food Product	Arbutin Concentration (μg/g)
Tea	0.14 ± 0.02
Coffee	0.31 ± 0.04
Pear (Bosc)	3.84 ± 0.74
Pear (d'Anjou)	15.09 ± 11.69
Wheat cereal	1.04 ± 0.09
Wheat germ	10.65 ± 3.61
Whole wheat bread	2.04 ± 0.35

Hydroquinone occurs as the glucose conjugate, 4-hydroxyphenyl-β-D-glucopyranoside (arbutin), in the leaves of several plants, including cranberries, blueberries, and some varieties of pear. Arbutin is easily hydrolyzed to D-glucose and HQ in hot, dilute acid.[4] To determine the contribution of the diet to total HQ levels in the body, Deisinger et al (1996) measured the concentration of arbutin and HQ in foods and in human plasma and urine samples, respectively, using gas chromatography with an electron capture detector (GC-ECD).[24] They found certain foods to be high in arbutin as shown in Table 4. The researchers measured the concentration of HQ in 2 groups of human volunteers (2/gender per group) who consumed a high- or low-HQ breakfast. Blood samples were collected at 30, 50, and 120 minutes after completion of the meal and urine was collected every 2 hours for 7 to 8 hours after the morning meal. A meal low in HQ was provided for lunch for both the high- and low-HQ breakfast groups. Hydroquinone and phenol concentrations were determined in acid hydrolyzed samples and HQ concentrations were statistically significantly elevated at the 1- and 2-hour time points (exact values not given) in blood plasma, as compared to background concentration. Mean plasma total phenol concentrations did not change with time. A similar pattern was observed for HQ in the urine samples. The concentration of HQ was statistically significantly increased at 2 to 3 hours after the high-HQ meal and decreased slowly after 3 hours. Urinary total phenol excretion rates averaged ~800 μg/h and showed high interparticipant variability but little mean variability over time. Selected, unhydrolyzed blood and urine samples were analyzed and very little free HQ or phenol was found in these samples. It was determined from this that HQ absorbed from these exposures is rapidly conjugated and is not detectable as free HQ or phenol. Plasma and urine levels of HQ and phenol in the low-HQ breakfast group showed a decline (not statistically significant) in their levels over time.

The in vivo bioavailability of HQ was examined in 3 different experiments using a total of 14 healthy males (age

Table 5. Radioactivity Recovery Following Topical Application of 25 μCi of [^{14}C]Hydroquinone to Human Forehead Skin for 24 Hours[20]

	Percentage Administered Dose[a]	
Time (h)	Urine	Skin Surface Wash
24	37.0 ± 9.8	5.2 ± 3.2[b]
48	7.1 ± 2.1	
72	0.9 ± 0.6	
96	0.4 ±0.2	
Total	45.3 ± 11.2	5.2 ± 3.2

[a] Values are mean ± SD (n = 6).
[b] Dosed skin site washed with soap and water after 24-hour dosing period.

Table 6. Radioactivity Recovered Via Tape Stripping, Following Topical Application of 1 μCi of [^{14}C]Hydroquinone to Human Forearm Skin[20]

	Percentage Administered Dose[a]	
Time (h)	Skin Wash Recovery	Skin Tape Strips
0	82.3 ± 8.1	1.2 ± 0.4
1	67.5 ± 25.3	5.4 ±2.5
3	54.8 ± 18.0	8.6 ±4.5
6	53.6 ± 17.3	15.8 ± 4.2
24	15.0 ± 4.5	6.6 ± 2.1

[a] Values are mean ± SD (n = 4).

18-80) following dermal application without occlusion, as the product is normally applied.[20] In group A (n = 6), 25 uCi of ^{14}C-hydroquinone cream (containing 2.5 mg HQ) was applied to a 25 cm^2 area of skin on the forehead. The next day, the area was washed by cotton balls with a 50% soap solution and rinsed with deionized water. The cotton balls were collected and analyzed for radioactivity. Four-day urine samples were collected by the volunteers and submitted to the researchers for analysis. Table 5 provides the results of this study. A total of 45.3% ± 11.2% of the dose was recovered in the urine. 5.2% ± 3.2% was recovered from the cotton ball wash. Thin layer chromatography was used to identify the metabolites in urine and the majority of the recovered dose was excreted as the glucuronide conjugate with lesser amounts of the sulfated conjugate and the parent compound identified.

In group B (n = 4), 5 skin sites (1 cm^2 each) on the ventral forearm were treated with 1 uCi of ^{14}C-hydroquinone cream (containing 0.1 mg HQ). The treated surfaces were washed 5 times and tape stripped 10 times with cellophane tape at time intervals of 0, 1, 3, 6, and 24 hours. The tape strips were analyzed for radioactivity. Table 6 provides the results of this study. The researchers concluded that while HQ can be removed from the skin through washing early after exposure, absorption over time will decrease the amount that can be removed.

In group C (n = 4), 25 uCi of ^{14}C-hydroquinone cream (containing 2.5 mg HQ) was applied to a 25 cm^2 area of skin on the left forearm. Catheters were placed in the vein draining the treated area on the left forearm, the ipsilateral site, and in the same area on the right forearm, the contralateral site. Blood samples (10 mL) were taken from both sites at 0 hour (prior to dosing) and at 0.5, 1, 4, and 8 hours after dosing. The treated skin site was washed by cotton balls with a 50% soap solution and rinsed with deionized water. The cotton balls were not analyzed for radioactivity in this substudy. Urine samples were collected at 24, 48, 72, and 96 hours after dosing, by the volunteers and submitted to the researchers for analysis. The researchers found that initially, the concentration on the ipsilateral side was higher than on the contralateral side; but by 4 hours, the concentration was the same on both sides. The peak plasma concentration of 0.04 ug-Eq/mL occurred at the 4-hour time point. The researchers calculated that 8.0% ± 4.1% of the dose was excreted in the urine for the 8-hour dosing period and that 45.3% of the dose was absorbed. The researchers assumed 100% excretion in making these determinations.

Immunological Effects

Hydroquinone inhibited IL-2-dependent T cell proliferation in primary human T lymphoblasts (HTLs) in vitro.[25] Exposure of HTLs to 50 μmol/L HQ decreased interleukin 2 (IL-2)-dependent proliferation by >90%. Cell viability was not affected by this concentration. Hydroquinone reversibly inhibited DNA synthesis in these cells, but this effect could be reversed up to 6 hours after treatment by removing HQ. No effect on glutathione levels was observed with up to 24 hours of treatment. Hydroquinone did not block binding of 125I-IL-2 to the cells but interfered with the IL-2-dependent progression of the HTLs through S phase of the cell cycle.

Macrophage-mediated immune responses were also inhibited by HQ treatment.[26] Tumor necrosis factor-α (TNF-α) production was inhibited by a 6-hour treatment with 100 μmol/L HQ in mouse RAW264.7 cells. Hydroquinone treatment decreased lipopolysaccharide (LPS)-mediated nitric oxide (NO) production, but HQ alone up to 100 μmol/L had no effect on NO levels. Human U937 cells (a macrophage cell line) and platelets showed a decrease in cell adhesion molecules in response to HQ treatment. The authors believe these effects occur through inhibition of the PI3K/Akt signaling pathway because inhibition of this pathway produced similar effects to those seen with HQ and a synergistic effect was observed with HQ and inhibitors of this pathway.

Animal Toxicology

Oral administration of HQ to rats resulted in dose-dependent mortality, lethargy, tremors, and increased liver and kidney weights.[2] Hydroquinone was found to be cytotoxic to rat hepatoma cells in culture and nephrotoxic in male rats dosed orally by gavage.

Acute Toxicity

Oral. Hydroquinone was found to induce indicators of nephrotoxicity in male and female F344 rats but not in male

Table 7. Functional Observational Battery (FOB) of Sprague-Dawley Rats Treated With 0, 20, 64, or 200 mg/kg per d Hydroquinone 5 d/week for 13 weeks: Statistically Significant Differences[28]

Observation	Change	N	Dose Group	FOB Observation Period
Differences attributed to hydroquinone exposure				
Home-cage activity	Decreased	6/10	200 mg/kg males	6 h
Behavior while removing from cage	Decreased	10/10	200 mg/kg females	1 h
Tremors	Increased	10/10	200 mg/kg females	1 h
Locomotor activity	Decreased	9/10	200 mg/kg males	6 h
Urine stains	Urine discolored papers	10/10	200 mg/kg males	1 and 6 h, days 1, 30, and 60
	under home cages brown	10/10	64 mg/kg males	Days 1, 30, and 60
	when left to stand overnight	10/10	20 mg/kg males	Days 1 and 60
		10/10	20, 64, and 200 mg/kg females	Days 30 and 60
Differences not attributed to hydroquinone exposure				
Urination	Increased	–	200 mg/kg males	1 h
			20 mg/kg males	1 h
Defecation	Increased	–	20 mg/kg males	1 h
	Decreased		200 mg/kg males	1 h
Spontaneous vocalizations	Increased	–	200 mg/kg females	Day 91
Approach response	Increased	–	64 mg/kg females	Day 30
Auditory orientation	Increased	–	200 mg/kg males	Day 60
	Decreased		20 and 200 mg/kg females	Day 91
			64 mg/kg females	Days 30 and 60
Olfactory orientation	Increased	–	64 mg/kg males	Day 7
Visual orientation	Increased	–	64 mg/kg males	Days 30, 60, and 91
Pinna touch response	Decreased	–	200 mg/kg males	Day 14
Tail pinch response	Increased	–	200 mg/kg	Day 30
Grip strength quantitative	Decreased	–	64 mg/kg females	Pre-exposure

or female Sprague-Dawley (SD) rats or B6C3F1 mice.[27] Rats (4-6/group) were gavage dosed with 0, 200, or 400 mg/kg HQ in water. Mice (number not specified) were gavage dosed with 0 or 350 mg/kg HQ in water. Urine and blood samples were collected from all animals. For rats, samples were collected at 0 (prior to dosing), 8, and 24 hours, at 24-hour intervals up to 96 hours after dosing. For mice, urine was collected 16 hours prior to dosing and at 12, 24, and 48 hours after dosing. The samples were examined for markers of kidney toxicity. Female F344 rats were the most sensitive to the indicators examined with statistically significant increases in the urinary activities of the enzymes, alanine aminopeptidase (ALP), alkaline phosphatase (AAP), γ-glutamyl transpeptidase (γ-GT), and *N*-acetyl glucosaminidase (NAG) at the 400 mg/kg dose level 8 hours after dosing. Creatinine (8 hours) and glucose (24 hours) were also statistically significantly increased at the 400 mg/kg dose. Blood urea nitrogen (BUN) was statistically significantly increased at the 400 mg/kg dose at the 48-hour time point. While many of these same parameters were increased in the 200 mg/kg dose group, only the increase in NAG was statistically significant at this dose level.

Male F344 rats had a similar pattern with statistically significant differences at the 400 mg/kg dose, but the change was of a smaller magnitude than that observed with the female F344 rats. At the 8-hour time point, AAP, NAG, and γ-GT activities were statistically significantly increased. Urinary creatinine and BUN were not changed by HQ treatment in male F344 rats. No significant differences were observed at the 200 mg/kg dose level.

Blood urea nitrogen was decreased in female SD rats at the 400 mg/kg dose level. No other changes were observed in the other parameters in male or female SD rats.

Blood urea nitrogen was statistically significantly increased in male and female B6C3F1 mice at the only dose tested (350 mg/kg). The urinary enzyme activities were also increased, but the differences were not statistically significant.

Urinary osmolality was decreased in all groups of rats dosed at the 400 mg/kg level, with statistically significant changes observed in the male and female F344 rats and the female SD rats. Statistically significant changes in diuresis were observed only in female F344 rats at both dose levels. No statistically significant changes in red or white blood cell counts in the urine were observed in any of the treated animals.

Histopathologically, F344 rats of both genders exhibited changes such as cell regeneration, slight mineralization, slight focal necrosis, and the presence of granular casts in the kidneys. The authors described any histopathological changes observed in male SD rats as minimal at either dose level. The kidneys from female SD rats and from mice were not examined.

A range-finding study was conducted in 7 female SD rats (age 10-13 weeks, 1 animal/group) using a single gavage dose of 500, 400, 375, 350, 300, 200, or 100 mg HQ/kg body weight (bw).[28] Table 7 provides the results of this study. Mortality occurred in the 500 and 400 mg/kg bw dose groups. Based on these results, an acute oral study was designed using 7 male

SD rats (age 9 weeks) and 5 female SD rats (age 14-15 weeks). The animals received a single gavage dose at concentrations of 3.75%, 3.45%, 3.15%, and 2.85% in degassed water, which correspond to dose groups of 375, 345, 315, and 285 mg/kg, respectively. Animals were observed 3 times on the day of dosing and once per day on days 1 to 14. Body weight was measured on days 0 (prior to dosing), 7, and 14.

Mortality occurred within 1 hour of dosing in both male (1 of 5) and female (3 of 5) rats at 375 mg/kg and in female rats at 345 (1 of 5) and 285 (1 of 5) mg/kg. The researchers observed mild-to-moderate tremors in all animals after dosing and noted minor convulsions in 1 of 5 males and 3 of 5 females at 375 mg/kg, 1 of 5 males and 1 of 5 females at 345 mg/kg and for 2 of 5 females at 285 mg/kg. With the exception of the male at 345 mg/kg, the animals that convulsed died prior to the 1-hour observation. No signs of tremors or convulsion were observed at the 4-hour observation. Brown discolored urine stains were present in the cages of all surviving animals between day 0 and day 2. From day 3 to the end of the study, all surviving animals appeared to be clinically normal. At necropsy, the researchers noted minor-to-moderate thymus hemorrhage for some of the animals that died on the day of dosing (details not provided) and minor hydronephrosis of the right kidney for a single 345 mg/kg male rat that survived to the end of the study. The hydronephrosis was not considered treatment related, however, as the animal was not in the highest dose group.

Dermal. New Zealand White SPF rabbits (5/gender) were shaved to expose the dorsal skin and a 2000 mg/kg bw limit dose of HQ was applied to the skin, covered and wrapped to secure the treatment in place for 24 hours.[28] The residual HQ was removed with running water after 24 hours. Animals were observed once daily from days 1 to 14 and body weights were measured on days 0 (prior to treatment) 7, and 14.

The researchers observed brown discolored urine stains in the cages of all animals on day 2. There were no adverse dermatological effects at the site of application, no adverse neurobehavioral effects, no changes in weight gain, and no mortalities during the study. No treatment-related findings were observed at necropsy. The dermal LD_{50} was reported as >2000 mg/kg.

Subchronic Toxicity

Dermal. Dermal exposure to a HQ cream did not produce renal toxicity in a subchronic study.[29] F344 rats (20/gender/group) were shaved and treated dermally with 0%, 2%, 3.5%, and 5% HQ in an oil-in-water emulsion cream (1.6 mL/kg bw on a 4 × 6 cm area on the back, with occlusion) 5 days/week for 13 weeks. Body weights and feed and water consumption were monitored and the animals were observed for clinical signs of toxicity and dermal irritation. Urine was collected from all animals to look for markers of cell damage, including NAG, γ-GT, ALP activities, and creatinine concentrations. The authors implanted 5 animals/gender/group with osmotic pumps loaded with 20 mg/mL BrdU (delivery rate 10 μL/h). Animals were then treated dermally with HQ cream as described above and killed 3 days later during the 3rd, 6th, and 13th weeks of treatment to look at renal cell proliferation. No clinical signs of toxicity were observed in any of the treated animals. Erythema was observed in male and female treated animals with increasing concentrations producing increased severity. This finding abated when exposure stopped. In addition, brown discoloration of the skin and scaly skin were observed with higher incidences occurring in the higher treatment groups although no clear dose-related trend was evident. A minimal to minor epidermal hyperplasia was observed at the site of application. No evidence of exogenous ochronosis was observed in this study.

No significant differences in absolute or relative organ weights were observed. Serum levels of protein and alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities were statistically significantly increased as compared to controls in the 5% HQ-treated male rats, but the authors did not feel this represented an adverse effect of HQ treatment because the magnitude seen was not clinically relevant. At 13 weeks, there were no differences in the urine parameters examined in male rats and only the osmolality value was statistically significantly decreased at the highest concentration tested. No histopathological changes and no changes in cell proliferation were observed in males after 13 weeks of treatment. In females, the BrdU labeling index in the 3.5% and 5% treated groups was slightly increased for cells in the outer/inner stripe of the distal tubules. No histopathological changes were observed.

Dermal Irritation and Sensitization

In rats, dermal application produced slight-to-severe irritation.[30] In a guinea pig maximization test, induction with 2% HQ injected intradermally, followed by challenge with 0.5% HQ, showed extreme sensitization. Hydroquinone was classified as an extreme sensitizer in a guinea pig maximization test. Briefly, albino Dunkin-Hartley guinea pigs were given a series of 6 intradermal injections of 2.0% HQ in 0.9% saline in the shoulder region to induce sensitization. After 6 to 8 days, a 48-hour occlusive patch of 1.0% HQ in an acetone−polyethylene glycol 400 vehicle was placed over the injection site. The animals were challenged on a previously untreated area of the flank using a 24-hour occlusive patch of 0.5% HQ, which was the maximum nonirritating concentration. After 14 and/or 48 hours, 100% of the animals had a positive response.

Reproductive/Developmental Toxicity

The literature on Reproductive and Developmental Toxicity of HQ was reviewed in a previous safety assessment and was summarized as follows:

> Oral administration of Hydroquinone did not produce embryotoxic, fetotoxic, or teratogenic effects in rats, nor did it produce significant adverse reproductive effects in a

two-generation study. Using rabbits, various teratogenic/reproductive treatment-related effects were observed at doses of 200-500 mg/kg. All dams dosed with 300-500 mg/kg Hydroquinone died. Some maternal toxicity was observed at a number of dose concentrations.[2]

Growth retardation of offspring was reported at concentrations that also caused maternal toxicity.[1] The overall literature in this area has not shown reproductive or developmental effects even following high-dose HQ exposures.[23]

Genotoxicity

Hydroquinone has been found to be mostly negative for mutagenicity in bacterial assays but positive in many mammalian cell assays in vitro and in vivo including micronuclei formation, sister chromatid exchange (SCE), and chromosomal aberrations.[2]

In Vitro

Blood samples were collected from healthy male volunteers (aged 20-40 years) and used to produce whole blood cultures and isolated lymphocytes for a series of experiments examining the genotoxicity of HQ.[31] Cultures were treated with HQ (0.5-50 μg/mL) for 90 minutes at 37°C. Methylmethanesulfonate (MMS) and hydrogen peroxide (H_2O_2) were used as positive controls. Samples were assayed for DNA damage using the Comet assay. Hydroquinone treatment resulted in dose-dependent increases in tail moment values in isolated leukocytes but had no effect on whole blood samples. Methylmethanesulfonate produced an increase in DNA damage in both types of samples, while H_2O_2 produced damage in only the isolated leukocytes. To investigate whether peroxides may play a role in HQ-induced DNA damage, isolated lymphocytes were treated with HQ in the presence or absence of catalase (250 U/mL). The effects of HQ were almost completely suppressed in the presence of catalase, suggesting that the damage could be due in part to an HQ-generated peroxide or other catalase substrate. Cellular metabolism was not required to generate the causative agent, as treatment of lysed cells also produced the previously observed DNA damage. Finally, the isolated lymphocytes were treated with HQ at 5, 10, and 50 μg/mL and the sample split into 2. One half of the sample was immediately tested in the Comet assay, while the other half was cultured for 66 hours and stimulated by phytohemagglutinin (PHA) to look for micronuclei. The DNA damage previously observed was detected in the freshly analyzed sample, but no significant increase in micronuclei was observed in the cultured cells. These experiments suggest that there are proteins and/or enzymes in whole blood samples that can block the actions of HQ, and it is likely that a self-generated peroxide product is the DNA damaging agent in this system.

The ability of HQ to induce chromosomal aberrations in V79 cells was examined in the presence and absence of S9 mix and antioxidant enzymes.[32] Hydroquinone was found to generate hydroxyl radicals in a time- and pH-dependent manner in the presence of Fe^{3+}/EDTA with more alkaline pH producing higher levels of thiobarbituric acid (TBA) reactive products. The addition of catalase to this system decreased the concentration of TBA-reactive products, suggesting that H_2O_2 is the specific agent generated. The presence of chromosomal aberrations was increased in the presence of 80 μmol/L HQ at pH 7.4 and 8.0, but the increase was not significant as compared to each other, despite the earlier finding that more hydroxyl radicals are produced at pH 8.0. This is believed to be due to a concomitant increase in cytotoxicity as measured by the mitotic index. The addition of S9 mix, superoxide dismutase (SOD), and SOD + catalase all significantly decreased the percentage of cells with chromosomal damage, but catalase alone did not have an effect. The authors suggest that while H_2O_2 generation appears to play a role in the clastogenic activity of HQ, the addition of S9, SOD, or SOD + catalase did not completely abolish the chromosomal aberrations; therefore, other reactive metabolites, such as the semi-quinone radical or the quinone, may play a role.

A total of 27 nonsmoking healthy young caucasian volunteers were enrolled in a study to determine the effect of polymorphisms for the glutathione *S*-transferases (GSTs) on HQ-induced genotoxicity to lymphocytes.[33] Blood samples were collected and genotyped and lymphocytes isolated from the samples were tested for micronuclei induction and SCE in the presence of HQ. For the micronuclei induction experiments, lymphocytes were PHA stimulated and treated with HQ (0, 40, and 80 μmol/L) for 3 hours. Mitomycin C ([MMC] 1.5 μmol/L) was used as a positive control and performed as expected. The number of micronucleated cells was significantly increased at 40 and 80 μmol/L, which were also significantly different from each other. A strong correlation was also observed between the GST phenotype and the frequency of HQ-induced micronuclei, with GSTM1 null lymphocytes exhibiting a significantly higher level with both 40 and 80 μmol/L HQ.

Lymphocyte cultures from these same participants were also PHA stimulated and treated with 0 or 80 μmol/L HQ for 3 hours to test for SCE. The 80 μmol/L HQ treatment induced a significant increase in SCE as compared to controls. No relationship was observed between HQ-induced SCE and the GST polymorphisms examined.

Hydroquinone was tested for induction of DNA damage in HepG2 cells and found to be positive.[34] Cells were exposed to 0, 6.25, 12.5, 25, and 50 μmol/L HQ for 1 hour at 37°C. DNA strand breaks were significantly increased in cells treated with 6.25 up to 25 μmol/L HQ. Cells treated with 50 μmol/L HQ showed a significant increase in DNA strand breaks as compared to controls but were not increased above the 25 μmol/L HQ-treated group. Cell viability was not affected by HQ treatment and no apoptosis was observed (data not shown). The presence of DNA protein cross-links was investigated using a proteinase K posttreatment. Posttreatment with proteinase K did not affect DNA migration in cells exposed to 6.25 to 25 μmol/L HQ; however, cells exposed to 50 μmol/L HQ did

show an increase in DNA migration, suggesting that DNA protein cross-links were present in these cells.

The induction of micronuclei was also examined in HepG2 cells.[34] Cells were treated with 0, 6.25, 12.5, 25, and 50 μmol/L HQ for 24 hours and evaluated for the frequency of micronuclei. Cyclophosphamide (800 μmol/L) was used as a positive control and performed as expected. A dose-dependent increase in micronuclei formation was observed in cells treated with 12.5 to 50 μmol/L HQ. The researchers did further work to look at indicators of oxidative stress. They found that 8-hydroxydeoxyguanosine (8-OHdG) was dose dependently increased in cells treated with 12.5 to 50 μmol/L HQ, reactive oxygen species (ROS) were statistically significantly increased in the 2 highest concentrations, and all treated cells showed statistically significant decreases in glutathione (GSH) content.

In Vivo

Hydroquinone increased the incidence of micronuclei in the bone marrow cells of male Swiss albino mice.[35] Animals were given 1 intraperitoneal (ip) injection of HQ and killed 12, 24, and 36 hours after treatment. The mice (5/dose per time period) were dosed at 0, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 75, and 100 mg/kg body weight. The frequency of micronucleated polychromatic erythrocytes (MPCE) was highest at 24 hours after treatment and was statistically significant as compared to controls at 6.25 mg/kg body weight up to 100 mg/kg body weight for all 3 time points. The frequency of micronucleated normochromatic erythrocytes (MNCE) followed a similar pattern and was highest at 24 hours after treatment and was statistically significantly increased at 6.25 mg/kg body weight up to 100 mg/kg body weight for all 3 time points. The frequency of MNCE reached a plateau at the 75 mg/kg dose. The PCE/NCE ratio decreased in a dose-dependent manner and suggests that HQ treatment resulted in a dose-dependent inhibition of erythropoiesis.

Male Swiss albino mice (4/dose) were given a single dose of HQ (0, 0.5, 1, 2, 4, and 8 mg/kg body weight; ip) per day for 6 days and killed 24 hours after the last treatment.[36] Splenocytes were isolated from these animals, cultured for 72 hours and examined for micronuclei formation. No signs of HQ-induced toxicity were observed over the treatment period. A statistically significant, dose-dependent increase in the number of micronucleated binucleate splenocytes (MNBNS) was observed. The 3 highest doses of HQ also produced increases in the number of BNS.

Carcinogenicity

Hydroquinone administered to rats orally by gavage 5 times/week for up to 103 weeks at doses of 25 or 50 mg/kg resulted in a significant increase of renal adenomas in males at the 50 mg/kg dose and of mononuclear cell leukemia in females with both doses.[37] At doses of 50 or 100 mg/kg on the same schedule, there was a significant increase in hepatocellular adenomas in both male and female mice.[37] Other previously reviewed studies of HQ showed no significant difference in tumors between control and exposed groups, and marginal to no activity as a tumor promoter.[1]

Chronic

Hydroquinone is metabolized to several glutathione conjugates including 2,3,5-tris(glutathione-S-yl)HQ (TGHQ), and this compound was tested for its ability to induce renal cancer in Eker rats.[38] Eker rats are susceptible to renal tumor development because they carry a germline mutation in the tuberous sclerosis 2 (Tsc-2) tumor suppressor gene. Eker rats have 1 mutated copy and 1 wild-type copy and loss of function of the wild-type copy is the rate limiting step for the development of preneoplastic and ultimately neoplastic lesions.

Two groups of male Eker rats (40/group) were given either 0 or 2.5 μmol/kg TGHQ 5 d/week ip in 0.5 mL of saline for 4 months and then 0 or 3.5 μmol/kg TGHQ for an additional 6 months. (These doses represent ~600-800 nmol/rat and are 0.6%-0.8% of the dose of HQ used in the NTP (1989) study.[37] After 4 months, 10 animals/group were killed and examined for cell proliferation in the kidneys.

2,3,5-tris(glutathione-S-yl)HQ treatment for 4 months significantly increased the labeling index of cells in the kidney, as measured by BrdU incorporation, compared with saline controls (133.4 ± 30.9 vs 9.0 ± 1.9 cells/250 μm^2, $P = .016$). Preneoplastic lesions were also observed at this time point, characterized by a peritubular fibrosis surrounding a dilated tubule. Kidney tumors were not observed at this time point.

Following 10 months of treatment, statistically significant increases in basophilic dysplasias, adenomas, and renal cell carcinomas were observed. Most of the renal cell tumors were in the region of TGHQ-induced acute renal injury and using HPLC-based DNA fragment analysis and laser capture microdissection, the researchers found that there was a loss of the wild type *Tsc-2* gene within the preneoplastic lesions.

Renal Tubule Cell Tumor Mode of Action

Hydroquinone treatment has been associated with an increased incidence of renal tubule cell tumors in male F344 rats but not in female F344 rats or B6C3F1 mice. A mode of action (MOA) has been proposed for the HQ-induced renal tubule cell tumors in male F344 rats. This MOA involves HQ or a metabolite interacting with the rat kidney to exacerbate chronic progressive nephropathy (CPN). The combination of CPN, along with HQ-induced cell proliferation in the kidney, promotes neoplasm formation.[39]

Hydroquinone is metabolized in rats to the glucuronide, sulfate, and glutathione conjugates, with increasing levels of the glucuronide and the mercapturic acid measured in the urine following either increasing treatment time or dose.[22,40] Intravenous injection of the glutathione metabolite 2,3,5-(triGSyl)HQ shows that the kidney is a target of this compound, and it produces an increase in cytotoxicity and oxidative DNA damage

as measured by markers of kidney damage in the blood and 8-OHdG in the kidney, respectively.[22] Subchronic treatment (6 weeks) of male F344 rats with 25 and 50 mg/kg HQ (via gavage) produced increased cell proliferation in the kidneys, and dose-dependent and statistically significant increases in the incidence of degenerative and regenerative foci in the tubules.[41] This same treatment regimen using 2.5 mg/kg HQ did not produce these effects in male F344 rats and none of the tested doses (2.5, 25 or 50 mg/kg) produced these effects in female F344 rats or in male SD rats. Glutathione metabolites can be reabsorbed in the proximal tubules of the kidney and metabolized by γ-glutamyl transferase (also γ-GT) where the released compound could generate ROS or covalently bond to cellular targets (ie, proteins or DNA). DNA adducts were not observed in F344 rat kidney following HQ treatment using ^{32}P-postlabeling, suggesting that this is not the target macromolecule.[42] Protein adducts have been identified following gavage and ip treatment with HQ.[43,44]

The localization of a reactive molecule to the kidney along with increased cell proliferation in the kidney seems to also coincide with the occurrence of CPN in these animals.[39,45] Chronic progressive nephropathy is a spontaneous lesion of the kidneys that occurs with age in certain strains of rats, including F344 and SD rats. Male rats are usually more affected than females and the incidence and severity of CPN has been shown to be affected by castration, hormonal status, and caloric intake, with protein overnutrition thought to be a causal factor.[46,47] These lesions are characterized by an increase in basophilic tubules with a thickened basement membrane in the cortex that begin as small discrete areas that expand as the disease progresses. These foci gradually merge into areas of tubule alteration and eventually become areas of frank glomerular pathology with infiltration of inflammatory cells.[48] The impact of CPN on HQ-induced carcinogenicity is unclear, but there does appear to be an association between the severity of CPN and the increased tumor response.[47] The increased proliferation associated with nephrotoxic glutathione conjugates of HQ could leave cells more susceptible to oxidative DNA damage, with subsequent initiation and promotion of neoplasm formation.

The relevance of this process to humans has been questioned on the basis of a proposed lack of a correlative process in humans to the CPN observed in rats. While there does not appear to be a specific human disease that shares all of the features of rodent CPN, humans do experience a glomerular sclerosis of 10% to 30% of nephrons as they age.[46,49,50] In addition, the authors of the NTP analysis cautioned that the presence and severity of CPN in rats did not fully explain the renal tubule cell tumors observed even though a positive correlation between CPN and RTCNs was observed. Their analysis also found that there were many animals with severe CPN that did not develop renal tubule cell neoplasms.[47] Thus, qualitatively, the MOA proposed in animals may have some relevance to humans. Quantitatively, the use of HQ containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

Role of HQ in Benzene Toxicity

Because HQ is a metabolite of benzene, its possible synergistic effect in benzene toxicity has been studied. Possible mechanisms of benzene toxicity have included the consideration of the role of benzene's metabolites in the resulting myelotoxicity. Benzene is a known human carcinogen, inducing leukemia and aplastic anemia. Human bone marrow is a known target, and numerous studies have been conducted to try to determine the mechanism for this effect and the responsible agents. In addition to HQ, benzene metabolites catechol and phenol have also been studied for their effects, both alone and in combination. In the models examined, a synergistic effect does appear to occur depending on the end point under study.[51-55] In examining HQ alone however, there does not appear to be a great deal of similarity in the toxicities observed with the individual compounds.[17,56] Whereas benzene produces leukemia and aplastic anemia in humans, no carcinogenic response has been identified in HQ exposed occupational populations.[17,23] The cancer profiles of the 2 compounds are also different in animals. In NTP studies conducted by the same route of exposure and in the same species of animals, benzene produced neoplasms at multiple sites in all 4 gender-species studies, while HQ induced neoplasia in 1 site in each with the exception of male mice; and all were at different sites.[56] Therefore, it is not clear what role, if any, HQ plays in benzene carcinogenesis. The experimental data suggest that HQ produced at the site of action and in combination with other benzene metabolites probably contributes to the overall toxic profile of benzene, but there are definite differences between species in sensitivity and in the capacity for activating and detoxifying processes that should be considered.

Clinical Assessment of Safety

Exogenous Ochronosis

Use of creams containing at least 1% to 2% HQ has been associated with exogenous ochronosis in people of Asian, Latin American, and African descent.[37,57-59] Ochronosis is the bluish black discoloration of tissues and has been observed in people exposed to several substances in addition to HQ including phenol, trinitrophenol, resorcinol, mercury, picric acid, benzene, and antimalarials. This form of ochronosis has been named exogenous because it does not share any of the systemic complications or the urinary abnormalities observed with the autosomal recessive metabolic disorder alkaptonuric ochronosis even though histologically, the hyperpigmentation in the skin is the same. This condition is associated with prolonged use (>6 months) of HQ containing skin-lightening products and while the mechanism is not understood may involve HQ's effects on tyrosinase, or on homogentistic acid oxidase and resulting deposition of pigment.

Dermal Sensitization

In 80 patients known to be sensitive to aromatic compounds, 0.5% HQ elicited no reactions.[1] Hydroquinone (1%) did not

produce a positive reaction in a cross-reactivity study.[60] Twenty-two volunteers who were classified as sensitized to p-phenylenediamine ([PPD] 9 male; 13 female, 19-72 years old) were patch tested to a serial dilution of benzoquinone (0.1%, 0.2%, 0.5%, and 1.0%) and with other compounds that could be metabolized to benzoquinone including HQ.[30] The control population (n = 8 male and 12 female, 18-77 years old) was tested with the same compounds and concentrations and were selected for no known previous allergy to PPD or other para-benzene derivatives. Among the PPD-sensitized participants, only 1 had reactions to all of the benzoquinone dilutions, while 4 reacted to either 0.2% or 1.0% benzoquinone. Of these participants, 3 presented with erythema, swelling, and papules. In the fourth participant, vesicles were also present

Hair Dye Epidemiology

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes are a preformed color. Hydroquinone functions as a coupler in the hair dye reaction and is a ''consumable'' in the hair dyeing procedure. While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information and have been considered by the CIR Expert Panel.

In 1993, an International Agency for Research on Cancer (IARC) working group evaluated 78 epidemiology literature citations and concluded that ''personal use of hair colorants cannot be evaluated as to its carcinogenicity'' and that ''occupation as a hairdresser or barber entails exposures that are probably carcinogenic.''[61] The IARC report did not distinguish between personal use of oxidative/permanent versus direct hair dyes, or distinguish among the multiple chemical exposures in addition to hair dyes to which a hairdresser or barber might be exposed.

Rollison et al (2006) reviewed the available epidemiology literature published from1992 through February 2005, which includes over 80 citations on personal hair dye use published since the IARC review.[62] The authors found that hair dye exposure assessment ranged from ever/never use to information on type, color, duration, and frequency of use. The authors found insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. The review highlighted well-designed studies with an exposure assessment that included hair dye type, color, and frequency or duration of use, which found associations between personal hair dye use and development of acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin lymphoma. These findings, however, were not consistently observed across studies.

Several studies addressing the possible link between hair dye use and bladder cancer, lymphoma and leukemia, other cancers, reproductive and developmental outcomes, and other end points published since the above review also have been considered.

In February 2008, an IARC Working Group re-evaluated the epidemiology literature including studies considered in the 1993 evaluation as well as all studies subsequently published. For personal use of hair colorants, the Working Group considered the epidemiological evidence inadequate and concluded that personal use of hair colorants is ''not classifiable as to its carcinogenicity to humans.'' The Working Group considered the studies of occupational exposures in hairdressers and barbers as providing limited evidence of carcinogenicity and reaffirmed the previous conclusion made in 1993 regarding occupation as a hairdresser or barber.[63]

A summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

Summary

Hydroquinone is reportedly used in hair dye preparations, skin care products, nail products, and as recently as 2007 in lipstick. Information provided to the FDA through the VCRP indicates that the use of HQ has decreased from 206 uses in 1993 to 151 uses in 2007 to 32 reported uses in 2009. Hydroquinone is a component of artificial nail products because it is added to all types of acrylic monomers to prevent the polymerization of these materials. Upon polymerization of the acrylic monomers, HQ is oxidized and is no longer detectable in the final polymer using analytical techniques for identifying trace amounts in a solid matrix. Any residual HQ is trapped in the polymer and is therefore unavailable and not likely to be absorbed.

While an earlier in vitro study suggested that HQ would be considered a ''slow permeant,'' a more recent in vivo study demonstrated that HQ is in fact rapidly absorbed through the skin from an aqueous preparation. Hydroquinone is metabolized to the sulfate and glucuronide conjugates, with oxidation to 1,4-benzoquinone, resulting in a reactive metabolite that forms mono- or polyglutathione conjugates. The glutathione conjugates are believed to be responsible for the nephrotoxicity observed in rats. In addition to nephrotoxicity, HQ has some immunotoxic effects and has been positive in many mammalian cell assays in vitro and in vivo including micronuclei formation, SCE, and chromosomal aberrations despite being mostly negative in in vitro bacterial mutagenicity assays. The induction of renal cell tubule tumors in male F344 rats has raised concern regarding the nephrocarcinogenicity of HQ and has led to several mechanistic studies which suggest that the male F344 rat is more susceptible to the glutathione conjugates of HQ due to the spontaneous occurrence of CPN which nearly all rats develop as they age. There is no human disease that shares all of the features of rodent CPN, however, there are histopathological similarities between human chronic renal disease and CPN that do not allow the proposed MOA to be ruled out entirely on a qualitative basis. Quantitatively, the use of HQ containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

Hydroquinone has been reported to cause exogenous ochronosis in several ethnic populations following prolonged use (>6 months) of at least a 1% to 2% cream. These effects along

with the NTP cancer study findings have led the FDA to reconsider the GRASE label for HQ in leave-on drug products.

The most recent comprehensive review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

Discussion

The 1994 conclusion of the CIR Expert Panel included the term "aqueous" in discussing the safety of HQ as a cosmetic ingredient. This term was added following discussions of the absorption of HQ from what was termed an alcoholic vehicle (ie, $\sim 70\%$ ethanol), which resulted in estimated HQ absorption of up to 66%. The only other data at the time was an in vitro study that used the stratum corneum of human abdominal skin to examine HQ absorption. The preparation used in that study was described as a 5% aqueous solution of HQ, and the authors calculated a permeability constant of 9.3×10^{-6} which suggested that HQ was a slow permeant with respect to human skin. Since that study, commercial preparations of HQ (commercial cream containing 2.5 mg of HQ) have been shown to be readily absorbed when applied to forehead skin; that is, assuming 100% excretion, 45% of the dose was absorbed. The in vivo studies taken together demonstrate that absorption of HQ will occur if the skin is exposed, and that this absorption occurs from both aqueous and alcohol-based products.

Concerning the use of HQ in hair dye formulations, the question remains regarding the percentage of alcohol in these products. Information provided to the CIR Expert Panel in 1993 suggests that hair dye formulations at the time did contain alcohols but the percentage in the formulation was not provided. Hydroquinone is considered a consumable in the hair dye reaction process and its concentration decreases considerably over time. Therefore, the amount of HQ that may be absorbed during the hair dyeing process is limited by both the decreasing concentration available and by the length of time the hair dye is applied before being rinsed off.

The use of HQ in artificial nail products is considered a safe use because HQ is added to all types of acrylic monomers to prevent the polymerization of these materials. Upon their use, the HQ is oxidized and is no longer present in the preparation and minimal dermal exposure and absorption is expected to occur from this application. Absorption of HQ from other leave-on cosmetic products could be appreciable, and the CIR Expert Panel reiterates that HQ should not be used in these leave-on cosmetics.

The CIR Expert Panel examined the association between oral HQ treatment and the development of renal tubule cell tumors in rats and determined that, while the qualitative relevance of the MOA in humans could not be ruled out, quantitatively, the use of HQ containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

In considering hair dye epidemiology data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other end points, based on lack of strength of the associations and inconsistency of findings.

Amended Conclusion

The CIR Expert Panel concluded that HQ is safe at concentrations of $\leq 1\%$ for cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone is safe for use in nail adhesives in the practices of use and concentration described in this safety assessment. Hydroquinone should not be used in other leave-on cosmetic products.

Authors' Note

The 2010 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, MD, FACP; Donald V. Belsito, MD; Ronald A. Hill, PhD; Curtis D. Klaassen, PhD; Daniel C. Liebler, PhD; James G. Marks Jr, MD, Ronald C. Shank, PhD; Thomas J. Slaga, PhD; and Paul W. Snyder, DVM, PhD. The CIR Director is F. Alan Andersen, PhD.

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

Declaration of Conflicting Interests

The author(s) declared no potential conflict of interest relevant to this article. F. Alan Andersen and Lillian C. Becker are employed by the Cosmetic Ingredient Review.

Funding

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

References

1. Final report on the safety assessment of hydroquinone and pyrocatechol. *J Am Coll Toxicol*. 1986:5(3):123-165.
2. Andersen FA. Addendum to the final report on the safety assessment of hydroquinone. *J Am Coll Toxicol*. 1994;13(3):167-230.
3. Gottschalck TE, Bailey JE. *International Cosmetic Ingredient Dictionary and Handbook*. 12th ed. Washington, DC: CTFA; 2008.
4. Hudnall PM. *Hydroquinone*. 5th ed. New York: Wiley-VCH; 1994:(A13), 499-505.
5. L'Oreal. Rate of disappearance of Hydroquinone during the oxidation of hair dyeing formulation in presence of hair. 1990.
6. Scientific Committee on Cosmetic Products. The Use of Benzoyl Peroxide (BPO), Hydroquinone (HQ), Hydroquinone Methylether (MeHQ) in Artificial Nail Systems. Report No. 0486/01; 2009:1-15.
7. FDA. *Frequency of Use of Cosmetic Ingredients*. Washington DC: FDA; 2007.
8. FDA. *Frequency of Use of Cosmetic Ingredients*. Washington DC: FDA; 2009.
9. Personal Care Products Council. Concentration of Use - Hydroquinone. 2008.

10. FDA. *VCRP Total Number of Products in Each Product Category; March 2008*. Washington, DC: FDA; 2008.
11. Steinberg DC. Submission: Letter from ESSTECH RE: Analysis of HQ/MEHQ content of Finished Nail Matrix, 2000. 2009.
12. EU. Commission directive 2008/88/EC concerning cosmetic products. *Official Journal of the European Union*. 2008; L25612-L25616.
13. EU. Commission directive 2003/83/EC relating to cosmetic products. *Official Journal of the European Union*. 2003; L23823-L23827.
14. FDA. Skin bleaching drug products for over-the-counter human use; Proposed rule. *Federal Register*. 2006;71(167):51146-51155.
15. EU. Commission directive 2000/6/EC relating to cosmetic products. *Official Journal of the European Union*. 2000;L5642-L5646.
16. EU. Council directive 76/768/EEC. *Official Journal of the European Union*. 2008;1-129.
17. IARC. Hydroquinone. *IARC Monogr Eval Carcinog Risks Hum*. 1999;71(pt 2):691-719.
18. Palumbo A, d'Ischia M, Misuraca G, Prota G. Mechanism of inhibition of melanogenesis by hydroquinone. *Biochim Biophys Acta*. 1991;1073(1):85-90.
19. Parvez S, Kang M, Chung HS, et al. Survey and mechanism of skin depigmenting and lightening agents. *Phytother Res*. 2006; 20(11):921-934.
20. Wester RC, Melendres J, Hui X, et al. Human in vivo and in vitro hydroquinone topical bioavailability, metabolism, and disposition. *J Toxicol Environ Health A*. 1998;54(4):301-317.
21. Poet TS, Wu H, English JC, Corley RA. Metabolic rate constants for hydroquinone in F344 rat and human liver isolated hepatocytes: application to a PBPK model. *Toxicol Sci*. 2004;82(1): 9-25.
22. Lau SS, Peters MM, Kleiner HE, Canales PL, Monks TJ. Linking the metabolism of hydroquinone to its nephrotoxicity and nephrocarcinogenicity. *Adv Exp Med Biol*. 1996;387:267-273.
23. DeCaprio AP. The toxicology of hydroquinone—relevance to occupational and environmental exposure. *Crit Rev Toxicol*. 1999;29(3):283-330.
24. Deisinger PJ, Hill TS, English JC. Human exposure to naturally occurring hydroquinone. *J Toxicol Environ Health*. 1996;47(1): 31-46.
25. Li Q, Geiselhart L, Mittler JN, Mudzinski SP, Lawrence DA, Freed BM. Inhibition of human T lymphoblast proliferation by hydroquinone. *Toxicol Appl Pharmacol*. 1996;139(2):317-323.
26. Lee JY, Kim JY, Lee YG, et al. Hydroquinone, a reactive metabolite of benzene, reduces macrophage-mediated immune responses. *Mol Cells*. 2007;23(2):198-206.
27. Boatman RJ, English JC, Perry LG, Bialecki VE. Differences in the nephrotoxicity of hydroquinone among Fischer 344 and Sprague-Dawley rats and B6C3F1 mice. *J Toxicol Environ Health*. 1996;47(2):159-172.
28. Topping DC, Bernard LG, O'Donoghue JL, English JC. Hydroquinone: acute and subchronic toxicity studies with emphasis on neurobehavioral and nephrotoxic effects. *Food Chem Toxicol*. 2007;45(1):70-78.
29. David RM, English JC, Totman LC, Moyer C, O'Donoghue JL. Lack of nephrotoxicity and renal cell proliferation following subchronic dermal application of a hydroquinone cream. *Food Chem Toxicol*. 1998;36(7):609-616.
30. Picardo M, Cannistraci C, Cristaudo A, DeLuca C, Santucci B. Study on cross-reactivity to the para group. *Dermatologica*. 1990;181:104-108.
31. Andreoli C, Rossi S, Leopardi P, Crebelli R. DNA damage by hydroquinone in human white blood cells: analysis by alkaline single-cell gel electrophoresis. *Mutat Res*. 1999;438(1):37-45.
32. do Ceu Silva M, Gaspar J, Duarte Silva I, Leao D, Rueff J. Mechanisms of induction of chromosomal aberrations by hydroquinone in V79 cells. *Mutagenesis*. 2003;18(6):491-496.
33. Silva Mdo C, Gaspar J, Duarte Silva I, Faber A, Rueff J. GSTM1, GSTT1, and GSTP1 genotypes and the genotoxicity of hydroquinone in human lymphocytes. *Environ Mol Mutagen*. 2004;43(4): 258-264.
34. Luo L, Jiang L, Geng C, Cao J, Zhong L. Hydroquinone-induced genotoxicity and oxidative DNA damage in HepG2 cells. *Chem Biol Interact*. 2008;173(1):1-8.
35. Jagetia GC, Aruna R. Hydroquinone increases the frequency of micronuclei in a dose-dependent manner in mouse bone marrow. *Toxicol Lett*. 1997;93(2-3):205-213.
36. Jagetia GC, Menon KS, Jain V. Genotoxic effect of hydroquinone on the cultured mouse spleenocytes. *Toxicol Lett*. 2001;121(1):15-20.
37. Olumide YM, Akinkugbe AO, Altraide D, et al. Complications of chronic use of skin lightening cosmetics. *Int J Dermatol*. 2008; 47(4):344-353.
38. Lau SS, Monks TJ, Everitt JI, Kleymenova E, Walker CL. Carcinogenicity of a nephrotoxic metabolite of the "nongenotoxic" carcinogen hydroquinone. *Chem Res Toxicol*. 2001;14(1):25-33.
39. McGregor D. Hydroquinone: an evaluation of the human risks from its carcinogenic and mutagenic properties. *Crit Rev Toxicol*. 2007;37(10):887-914.
40. English JC, Deisinger PJ. Metabolism and disposition of hydroquinone in Fischer 344 rats after oral or dermal administration. *Food Chem Toxicol*. 2005;43(3):483-493.
41. English JC, Perry LG, Vlaovic M, Moyer C, O'Donoghue JL. Measurement of cell proliferation in the kidneys of Fischer 344 and Sprague-Dawley rats after gavage administration of hydroquinone. *Fundam Appl Toxicol*. 1994;23(3):397-406.
42. English JC, Hill T, O'Donoghue JL, Reddy MV. Measurement of nuclear DNA modification by 32P-postlabeling in the kidneys of male and female Fischer 344 rats after multiple gavage doses of hydroquinone. *Fundam Appl Toxicol*. 1994;23(3):391-396.
43. Boatman RJ, English JC, Perry LG, Fiorica LA. Covalent protein adducts of hydroquinone in tissues from rats: quantitation of sulfhydryl-bound forms following single gavage or intraperitoneal administration or repetitive gavage administration. *Chem Res Toxicol*. 2000;13(9):861-872.
44. Boatman RJ, English JC, Perry LG, Fiorica LA. Covalent protein adducts of hydroquinone in tissues from rats: identification and quantitation of sulfhydryl-bound forms. *Chem Res Toxicol*. 2000;13(9):853-860.
45. Hard GC, Whysner J, English JC, Zang E, Williams GM. Relationship of hydroquinone-associated rat renal tumors with spontaneous chronic progressive nephropathy. *Toxicol Pathol*. 1997; 25(2):132-143.

46. The Kidney. 1998. *Fundamentals of Toxicologic Pathology*. New York, NY: Academic Press.
47. Seely JC, Haseman JK, Nyska A, Wolf DC, Everitt JI, Hailey JR. The effect of chronic progressive nephropathy on the incidence of renal tubule cell neoplasms in control male F344 rats. *Toxicol Pathol*. 2002;30(6):681-686.
48. Hard GC. Mechanisms of chemically induced renal carcinogenesis in the laboratory rodent. *Toxicol Pathol*. 1998;26(1):104-112.
49. Abrass CK. The nature of chronic progressive nephropathy in aging rats. *Adv Ren Replace Ther*. 2000;7(1):4-10.
50. Clark B. Biology of renal aging in humans. *Adv Ren Replace Ther*. 2000;7(1):11-21.
51. Subrahmanyam VV, Doane-Setzer P, Steinmetz KL, Ross D, Smith MT. Phenol-induced stimulation of hydroquinone bioactivation in mouse bone marrow in vivo: possible implications in benzene myelotoxicity. *Toxicology*. 1990;62(1):107-116.
52. Kalf G, Shurina R, Renz J, Schlosser M. The role of hepatic metabolites of benzene in bone marrow peroxidase-mediated myelo- and genotoxicity. *Adv Exp Med Biol*. 1991;283: 443-455.
53. Smith MT, Yager JW, Steinmetz KL, Eastmond DA. Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. *Environ Health Perspect*. 1989;82:23-29.
54. Barale R, Marrazzini A, Betti C, Vangelisti V, Loprieno N, Barrai I. Genotoxicity of two metabolites of benzene: phenol and hydroquinone show strong synergistic effects in vivo. *Mutat Res.* 1990;244(1):15-20.
55. Robertson ML, Eastmond DA, Smith MT. Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes. *Mutat Res*. 1991;249(1):201-209.
56. NTP Toxicology and Carcinogenesis Studies of Hydroquinone (CAS No. 123-31-9) in F344/N Rats and B6C3F1 Mice (Gavage Studies). *Natl Toxicol Program Tech Rep Ser.* 1989;366:1-248.
57. Tan SK, Sim CS, Goh CL. Hydroquinone-induced exogenous ochronosis in Chinese—two case reports and a review. *Int J Dermatol*. 2008;47(6):639-640.
58. Merola JF, Meehan S, Walters RF, Brown L. Exogenous ochronosis. *Dermatol Online J*. 2008;14(10):6.
59. Charlin R, Barcaui CB, Kac BK, Soares DB, Rabello-Fonseca R, Azulay-Abulafia L. Hydroquinone-induced exogenous ochronosis: a report of four cases and usefulness of dermoscopy. *Int J Dermatol*. 2008;47(1):19-23.
60. Lisi P, Hansel K. Is benzoquinone the prohapten in cross-sensitivity among aminobenzene compounds? *Contact Dermatitis*. 1998;39(6):304-306.
61. IARC working group on the evaluation of carcinogenic risks to humans: occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. Proceedings. Lyon, France, 6-13 October 1992. *IARC Monogr Eval Carcinog Risks Hum.* 1993;57:7-398.
62. Rollison DE, Helzlsouer KJ, Pinney SM. Personal hair dye use and cancer: a systematic literature review and evaluation of exposure assessment in studies published since 1992. *J Toxicol Environ Health B Crit Rev*. 2006;9(5):413-439.
63. Baan R, Straif K, Grosse Y, et al. Carcinogenicity of some aromatic amines, organic dyes, and related exposures. *Lancet Oncol*. 2008;9(4):322-323.

JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 4, Number 5, 1985
Mary Ann Liebert, Inc., Publishers

2

Final Report on the Safety Assessment of p-Hydroxyanisole

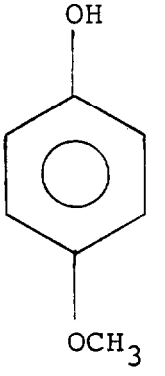
p-Hydroxyanisole is used as an antioxidant in cosmetic products at concentrations of up to 1.0 percent. The acute oral LD_{50} of p-Hydroxyanisole in rats was estimated as 1630 mg/kg. Undiluted p-Hydroxyanisole is a severe skin and ocular irritant in rabbits but produced minimal eye irritation at 0.1 percent and minimal rabbit skin irritation at 5 percent. Skin sensitization to p-Hydroxyanisole occurred when guinea pigs were treated at 0.5 M. p-Hydroxyanisole is a skin-depigmenting agent at concentrations approximating those used in cosmetic products. p-Hydroxyanisole was nonmutagenic in the Ames assay. No local toxic changes or tumors were observed following long-term application of 5 and 10 percent p-Hydroxyanisole. The antioxidant was inactive as a tumor promoter. Solutions of p-Hydroxyanisole produced embryotoxicity but not teratogenicity.

The function of p-Hydroxyanisole in cosmetics is that of an antioxidant; it is not intended for use as a skin lightener or skin-depigmenting agent. Because of the depigmenting action of p-Hydroxyanisole in black guinea pigs at reported concentrations approaching those used in cosmetics, it is concluded that p-Hydroxyanisole is unsafe for use as a cosmetic ingredient.

CHEMISTRY

Definition and Structure

p-Hydroxyanisole (CAS No. 150-76-5) is the substituted phenolic compound with the formula:[1]



p-Hydroxyanisole is also known as Antioxidant 221, 4-hydroxyanisole, p-methoxyphenol, 4-methoxyphenol, and hydroquinone monomethyl ether.[1-4]

Method of Manufacture and Impurities

p-Hydroxyanisole can be made by reaction of hydroquinone with dimethyl ether over a mixture of silica and alumina at 250 to 300°C.[5] The compound is also produced commercially by the methylation of hydroquinone with dimethyl sulfate.[6]

p-Hydroxyanisole used for cosmetic purposes typically has a purity of 99.5 percent. Impurities include an "unidentified compound with a high boiling point" (approximately 0.4 percent) and hydroquinone dimethyl ether (about 0.1 percent). Hydroquinone normally is not detected.[5]

Properties

p-Hydroxyanisole is a white, waxy solid that has an odor of caramel and phenol.[2,4,6] During storage, the compound is quite stable.[6] It is soluble in water, aqueous ethanol, acetone, ether, ethylacetate, and benzene.[2,3] As indicated by its ionization constant (pk) of 10.25, p-Hydroxyanisole has acidic properties characteristic of phenols.[7] Peak absorbance of ultraviolet light occurs at approximately 340 nm.[8] Additional chemical and physical data for p-Hydroxyanisole are presented in Table 1.

p-Hydroxyanisole readily forms hydrogen bonds with itself and with water. Sublimation pressure studies of this antioxidant indicate a high energy of molecular association (6.2 cal/mol), which suggests that the methoxy groups are hydrogen bonded with the hydroxyl groups of adjacent molecules.[14] Because the phenolic moiety of p-Hydroxyanisole also forms hydrogen bonds with water molecules, the acidic properties of this compound are more variable in aqueous systems than such nonaqueous solvents as benzene. The acidic reactivity of p-Hydroxyanisole increases with temperature as a result of the dissociation of these hydrogen bonds.[15] Strong hydrogen bonds are also formed between p-Hydroxyanisole and such proteins as bovine serum albumin and mitochondrial proteins from yeast cells and rat liver. The binding with these proteins is nonspecific.[16,17]

p-Hydroxyanisole is readily oxidized. For example, it is converted by bromine in water to a quinone. This reaction apparently involves the nonionized form of the compound.[18] p-Hydroxyanisole may also undergo a variety of other reactions, including alkylation, halogenation, and other substitutions on the aromatic nucleus. These reactions may occur without loss of the ether group.[9]

Analytical Methods

Analytical methods for the determination of p-Hydroxyanisole include gas and thin-layer chromatography.[5,10,12,19-25] Other reported analytical methods for the determination of p-Hydroxyanisole include gel permeation chromatography,[26,27] a polarographic procedure,[28] and a quantitative oxidimetric procedure.[29]

TABLE 1. Chemical and Physical Data for p-Hydroxyanisole

		References
Appearance	White flakes	9
Formula	$CH_3OC_6H_4OH$	2, 4
Molecular weight	124	4, 6
Boiling point	234°C	2, 3, 6, 10
	243°C	11
	246°C	4
Refractive index	1.5370	10
Melting point	52.5 to 53°C	2, 4, 6, 11, 12
pK (ionization constant)	10.25 at 20°C	7
Heat of vaporization	13.9 Kcal/mole	13
Density	1.413 at 60°C	13
Specific gravity	1.55 at 20/20°C	2, 4
Solidification point	54.0°C	9
Flash point (Cleveland open cup)	132°C (270°F)	9
Fire point	135°C (275°F)	9
Autoignition temperature	421°C (790°F)	9
UV absorbance	340 nm (peak)	8
Solubility at 25°C (g/100 g solvent):		9
Water	4	
10 percent NaOH	> 50	
Acetone	426	
Ethyl alcohol	456	
Ethyl acetate	245	
Benzene	70	
Animal oil (lard)	> 50	
Vegetable oil (cottonseed)	> 50	
Hexane	< 1	

NONCOSMETIC USE

p-Hydroxyanisole has a number of noncosmetic applications, including use as an antioxidant, as a polymerization inhibitor, as a chemical intermediate, and as a stabilizer. It is also used to inhibit the effects of ultraviolet light on the skin.[2,6,9,11]

As an antioxidant, p-Hydroxyanisole is used in concentrations of 0.001 to 0.01 percent to inhibit the development of acidity and discoloration in chlorinated hydrocarbons, aldehydes (such as crotonaldehyde and furfural), and oils of turpentine. It is used also as an antioxidant in synthetic lattices and to inhibit peroxide formation in ethers.[2,9]

As a polymerization inhibitor, p-Hydroxyanisole is employed in combination with hydroquinone or methylhydroquinone to reduce gel time drift in unsaturated polyester resins. The antioxidant is used also to inhibit the polymerization of vinylidene chloride, acrylonitrile, acrylic and methacrylic esters, and various vinyl monomers.[2,9,11]

As a synthetic intermediate, p-Hydroxyanisole is used in the manufacture of dyes, pharmaceuticals, plasticizers, and stabilizers. Butylated hydroxyanisole, which is a food grade antioxidant, is prepared by the alkylation of p-Hydroxyanisole.[2,6,9]

As a stabilizer, p-Hydroxyanisole is used in concentrations of 0.05 to 0.3 percent to inhibit thermal degradation of polyether polyols. The antioxidant can be added at concentrations of 0.5 to 3.0 percent to ethylcellulose in order to maintain the latter compound's viscosity, flexibility, and color. Degradation and formation of aldehydes is retarded by the addition of p-Hydroxyanisole to polyoxyalkylenes. p-Hydroxyanisole is also used as as stabilizer for chlorinated hydrocarbons, textile lubricating oils, and liquid detergent colors.[2,6,9,11]

COSMETIC USE

p-Hydroxyanisole is used in cosmetics as an antioxidant.[5] Data submitted to the Food and Drug Administration (FDA) in 1981 by cosmetic firms participating in the voluntary cosmetic registration program indicated that p-Hydroxyanisole was used that year as an ingredient in 31 cosmetic products (Table 2). Product types in which p-Hydroxyanisole was most frequently used included sachets, makeup bases, and skin care preparations. Cosmetic formulations contained this antioxidant at concentrations of >0.1 to 1.0 percent (8 products) and ≤0.1 percent (23 products).[30,31]

Voluntary filing of product formulation data with the FDA by cosmetic manufacturers and formulators conforms to the prescribed format of preset concentration ranges and product categories as described in Title 21 Part 720.4 of the Code of Federal Regulations.[32] Because data are only submitted within the framework of preset concentration ranges, opportunity exists 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 the possibility of a 2- to 10-fold error in the assumed ingredient concentration.

Cosmetic products containing p-Hydroxyanisole are applied to or have the potential to come in contact with skin and eyes. These products may be used from once a week to several times a day. Many of these products may be expected to remain in contact with the skin for as briefly as a few hours to as long as a few days. Each cosmetic product formulated with p-Hydroxyanisole has the potential for repeated application over the course of several years.

BIOLOGY

Skin Depigmentation

Depigmentation has been observed in guinea pigs and mice following application of p-Hydroxyanisole to the skin. The depigmenting action of this antioxidant has been typically observed at concentrations of 3 to 30 percent. However, Pathak[33] in an unpublished study reported skin depigmentation with this com-

TABLE 2. Product Formulation Data for p-Hydroxyanisole[30,31]

Product Category	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (percent)	
			>0.1–1	≤0.1
Eye shadow	2582	1	–	1
Other eye makeup preparations	230	2	–	2
Colognes and toilet waters	1120	2	1	1
Perfumes	657	1	–	1
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	1	–	1
Sachets	119	7	–	7
Makeup foundations	740	1	–	1
Lipstick	3319	2	–	2
Makeup bases	831	5	–	5
Rouges	211	1	–	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	2	1	1
Face, body and hand skin care preparations (excluding shaving preparations)	832	2	2	–
Moisturizing skin care preparations	747	1	1	–
Night skin care preparations	219	1	1	–
Skin lighteners	44	1	1	–
Suntan gels, creams, and liquids	164	1	1	–
1981 TOTALS		31	8	23

pound at a concentration as low as 0.25 percent (Table 3). The onset of depigmentation at the site of application varies according to concentration, duration of exposure, vehicle, and other test conditions. Results of a number of studies are summarized below.

The subchronic and chronic effects of p-Hydroxyanisole on guinea pig skin were examined in an unpublished study by Pathak.[33] For the subchronic exposure, an unspecifed cream vehicle containing either 0, 0.1, 0.25, 0.5, or 1.0 percent p-Hydroxyanisole was applied daily for 6 weeks (42 days) to the epilated skin of the back of 30 black guinea pigs. The treated and vehicle control groups consisted of 6 animals each. Guinea pigs exposed to 0.25, 0.5, and 1.0 percent p-Hydroxyanisole developed hypopigmentation or depigmentation of the skin and hair at the site of treatment. Only 1 guinea pig of the 0.1 percent group developed depigmentation at the treated site. The skin and hair of all animals of the vehicle control group (0.0 percent p-Hydroxyanisole) had no color change. For the chronic exposure, an unspecified vehicle containing 0.0, 0.5, or 1.0 percent p-Hydroxyanisole was applied daily for 6 months to the ear and epilated backs of

TABLE 3. Skin Depigmentation to p-Hydroxyanisole

Animal	Duration of p-Hydroxyanisole Exposure	p-Hydroxyanisole Concentration at Which Skin Depigmentation Was Observed	Reference
Black guinea pig	6 weeks	0.1,* 0.25, 0.5, 1.0% in unspecified cream	33
Black guinea pig	6 months	0.5 and 1.0% in unspecified cream	33
Guinea pig	Not reported	3%	34
Black guinea pig	1 to 6 months	0.1, 0.25, 0.5 and 1.0 M in acetone, dimethylsulfoxide, or hydrophilic ointment	35
Black guinea pig	18 days	5 to 20% in petroleum jelly	36–38
Black guinea pig	1 to 8 weeks	20% in lanolin	39
Black guinea pig	13 days	20% in dimethylsulfoxide	40
Black guinea pig	7 to 46 days	20% in lanolin	41
Black guinea pig	4 weeks	20% in petroleum jelly	42
BLA mice	4 weeks	20% in petroleum jelly	42
Black guinea pig	30 days	20 and 30% in dimethylsulfoxide	43

*Following exposure to a p-Hydroxyanisole concentration of 0.1 percent, 1 of 6 guinea pigs developed skin depigmentation; the remaining 5 animals had no change in skin or hair color at the site of application.

3 groups of black guinea pigs (12 animals per group). The skin of both treatment groups (0.5 and 1.0 percent) appeared hypomelanotic and amelanotic after 4 months. Animals exposed to 1.0 percent p-Hydroxyanisole developed moderate to severe skin and hair depigmentation at the site of application. Guinea pigs exposed to 0.5 percent of the antioxidant developed either skin depigmentation or skin hypopigmentation. The skin of the vehicle control group (0.0 percent p-Hydroxyanisole) appeared normal.

Pathak et al.[34] examined the depigmenting activity of p-Hydroxyanisole on the epilated skin of the backs and ears of guinea pigs. A 3 percent concentration of the compound "produced marked depigmentation" and had a "selective cytotoxic effect" on melanocytes. Further details have not been published.

Total depigmentation of the back, ear, and nipple was observed in black guinea pigs treated topically with 0.1, 0.25, 0.5, and 1.0 M p-Hydroxyanisole. The antioxidant in either acetone, dimethyl sulfoxide, or hydrophilic ointment was applied in a 0.1 ml dose each weekday for 1 to 6 months. The number of days for complete depigmentation varied with vehicle and p-Hydroxyanisole concentration. No depigmentation was noted on areas distant from the treated site. The histological changes found at the treated sites included reduction or absence of melanin, acanthosis, and an increase in mononuclear–histiocytic cells.[35]

Twenty percent p-Hydroxyanisole was applied topically once daily to the back of the ear of black guinea pigs for periods ranging from 1 to 8 weeks. Depigmentation was observed after 5 to 10 days of treatment. After 5 to 6 weeks of treatment, large areas were completely depigmented. None of the 34 treated animals developed depigmentation extending beyond the area of application. Following the cessation of treatment, depigmented regions slowly became repigmented. Areas of depigmentation persisted for as long as 6 months.[39]

Twenty percent p-Hydroxyanisole in dimethylsulfoxide was applied to the epilated skin of the ear and mammilla of 10 black guinea pigs. Applications were made once daily for 13 days. Depigmentation of the treated areas was observed in 4 animals on the fifth day of antioxidant exposure. Varying degrees of depigmentation and whitening of black hairs was observed in all animals 25 days after the initial exposure. Repigmentation of the treated skin began approximately 1 month after termination of treatment. Microscopic changes in the treated skin included hyperkeratosis, acanthosis, a decrease in the number of DOPA-active melanocytes, and a transposition of melanin granules from the epidermis into the dermis. The amount of melanin granules in the epidermis decreased progressively as the duration of treatment increased.[40]

Riley and Seal[41] applied 20 percent p-Hydroxyanisole in lanolin to the backs of the ears of black guinea pigs. Applications were made daily, 5 days a week for periods ranging from 5 days to 46 days. Skin depigmentation and formation of keratinocyte pseudopods (microinvasion) were observed after 7 days of treatment. Similar treatment of guinea pig ears with 1 percent of the antioxidant in lanolin produced no skin depigmentation or pseudopodial extensions of the basal cell cytoplasm.

The skin-depigmenting property of p-Hydroxyanisole was assessed in black guinea pigs and BLA mice. A single application of 10 percent p-Hydroxyanisole in petroleum jelly was made daily to the ear of each of 5 guinea pigs for 4 weeks; 20 percent p-Hydroxyanisole in petroleum jelly was applied in a similar fashion to a second group of 5 guinea pigs. A 10 percent concentration of the antioxidant in petroleum jelly was applied to the neck of 10 mice daily for 8 weeks, whereas 20 percent p-Hydroxyanisole in petroleum jelly was applied to the neck of 10 mice daily for 4 weeks. Skin irritation (acanthosis) and skin depigmentation were observed in both species with 20 percent p-Hydroxyanisole; 10 percent of the antioxidant produced no observable skin effects.[42]

Twenty and thirty percent p-Hydroxyanisole in dimethylsulfoxide was applied 6 times a week for 30 days to the skin of the right ear and right mammilla of black guinea pigs. The skin of the left ear and left mammilla served as untreated control. After 2 to 4 days of antioxidant exposure, a decrease in DOPA-positive cells of the dermoepidermal junction was observed. A reduction of melanin granules in the epidermis and an increase in acid phosphatase activity in early melanosomes were also noted. Skin depigmentation was visible in treated areas after 5 days. A progressive decrease in melanocytes was observed throughout the course of the study.[43]

Riley[39] and Dumishev[40] reported that leukoderma induced by p-Hydroxyanisole in experimental animals resembles vitiligo in man with respect to the following: (1) loss of skin pigmentation long after termination of treatment with the antioxidant, (2) a tendency for hair follicles to depigment last, (3) repigmentation is initially perifollicular, (4) DOPA-positive epidermal melanocytes are reduced in or absent from the depigmented sections, (5) the degree of depigmentation is inversely proportional to the amount of melanin in the epidermis, (6) grafted pigmented skin remains pigmented in the area of leukoderma, whereas skin depigmented by p-Hydroxyanisole grafted in a normally pigmented zone is slowly repigmented from the periphery, and (7) adenosine triphosphatase-positive basal and dendritic cells are unchanged, whereas the suprabasal cells are damaged in the depigmented zone.

Proposed Mechanism for Skin Depigmentation

The mechanism by which p-Hydroxyanisole causes skin depigmentation in guinea pigs appears to be a result of the preferential destruction of melanocytes. In vitro studies suggest that the melanocytotoxicity of p-Hydroxyanisole is related to its structural similarity to the amino acid tyrosine. As a tyrosine analog, p-Hydroxyanisole is oxidized by tyrosinase and gives rise to cytotoxic, free radical oxidation products. It is hypothesized that these cytotoxic oxidation products damage the cellular membranes of the guinea pig melanocyte by initiating lipid peroxidation.[39,44-47]

Pathak[33] proposed that the cytotoxic effect of p-Hydroxyanisole is related to 3 modes of action: (1) the relative inability of melanocytes to degrade the radical oxides formed as a result of oxidation of p-Hydroxyanisole, (2) the ability of the antioxidant to act as a sulfhydryl scavenger, depriving melanocytes of essential growth factors (e.g., cysteine, methionine), and (3) the ability of p-Hydroxyanisole to inhibit melanocyte growth through the inhibition of DNA synthesis.

Effect on Normal Melanocytes and Keratinocytes

Twenty percent p-Hydroxyanisole in lanolin was applied to the skin of black guinea pigs daily for periods up to 6 months. Microscopic changes in the treated skin included formation of basal keratocyte pseudopodia. The pseudopodia extended from the epidermis through the basal lamina into the underlying dermis. This reaction, termed "microinvasion," was reversed upon termination of antioxidant treatment.[41,48-50] Topical application of 20 percent p-Hydroxyanisole in lanolin to the cheekpouch epithelium of hamsters induced similar extensions of basal cell pseudopodia into the dermis.[51] Grasso and Rostron[52] suggested that both the microinvasion reaction and the epithelial proliferation resulting from topical application of p-Hydroxyanisole are manifestations of an irritant effect on the epidermis and are not indicative of carcinogenic potential.

A cream vehicle containing 0.5 or 1.0 percent p-Hydroxyanisole was applied daily for 6 weeks (42 days) to the left ear and epilated back of black guinea pigs. A selective cytotoxic effect on melanocytes was noted. However, no atypical keratinocytes or melanocytes were observed.[33]

An unspecified vehicle containing either 0.0, 0.5, or 1.0 percent p-Hydroxyanisole was applied daily for 6 months to the left ear and epilated back of black guinea pigs. The antioxidant-treated sites had a number of ultrastructural alterations including a marked decrease in the number of melanized melanosomes, a decrease in the number of actively functioning melanocytes, irregular and disorganized melanosomal lamellae, and swelling and disintegration of outer melanosomal membranes. Melanocytes were observed in which the nuclear envelope and other membranous organelles were vacuolated. The mitochondria of these melanocytes were swollen and in many instances had other changes of degeneration. Degenerative changes in keratinocytes included cytoplasmic vacuolation, swelling and disruption of mitochondria, endoplasmic reticulum and nuclear envelope, dendritic degeneration, intranuclear vacuolization, and convolutions of the nuclear membrane.[33]

The cytotoxic action of p-Hydroxyanisole on guinea pig melanocytes was reported by Riley.[46] Normal guinea pig melanocytes exposed in vitro to the anti-

oxidant selectively incorporated the compound into the melanosome. This selective incorporation was a function of the state of pigmentation of the cells and their tyrosinase activity. Heavily pigmented cells had a greater uptake of p-Hydroxyanisole. The cytotoxic effect on the melanocyte was dependent upon both antioxidant concentration and duration of exposure. At 10^{-3} M, p-Hydroxyanisole was extremely toxic to melanocytes, causing cytoplasmic blebbing and rupture of cell membranes within 30 minutes. The antioxidant had a melanocytotoxic effect at concentrations as low as 10^{-8} and 10^{-9} M, although progressively longer exposure periods (24 to 36 hours) were required. No effects were observed in guinea pig melanocytes after 36 hours of exposure to 10^{-10} M p-Hydroxyanisole.

Electron microscopy was used to assess the effects of p-Hydroxyanisole on normal human melanocytes in both organ culture and disperse tissue culture. In disperse tissue culture, no specific toxic effect on human melanocytes was observed following a 45-minute exposure to either 10^{-2} M or 10^{-3} M p-Hydroxyanisole. Plasma membranes, nucleus, and cytoplasmic organelles, including melanosomes, were unaffected. Keratinocytes likewise had no morphological changes following antioxidant exposure. Whole epidermis exposed to 10^{-1} M p-Hydroxyanisole for 1, 5, and 24 hours had extensive damage to melanocytes and keratinocytes; damage was much less severe, however, at an antioxidant concentration of 10^{-2} M. Melanocytes of PUVA-treated skin exposed up to 24 hours to 10^{-2} M or 10^{-3} M p-Hydroxyanisole had no morphological damage at the ultrastructural level. This study failed to demonstrate a specific toxic effect of p-Hydroxyanisole to normal human melanocytes in dispersed tissue and organ culture.[53]

Human melanocytes and keratinocytes in tissue culture were exposed to tyrosinase (15 μg/ml) and p-Hydroxyanisole (5×10^{-4} M to 5×10^{-2} M) for 1 to 24 hours. No damage was noted in either type of cell exposed below 5×10^{-3} M p-Hydroxyanisole for 6 hours. However, higher concentrations and longer exposures extensively damaged the cells. After 6- and 24-hour exposures to 5×10^{-3} M p-Hydroxyanisole and 15 μg/ml tyrosinase, most melanocytes had less dense cytoplasm, poorly defined cytoplasmic membranes, numerous lipid droplets, fewer mitochondria, and swollen and disrupted mitochondria as compared to control cells. The nuclei of treated melanocytes were morphologically unchanged. Nonkeratinized keratinocytes appeared swollen and had loss of cytoplasmic matrix and filaments, with a virtual absence of mitochondria and an accumulation of electron-dense round bodies (probably of a lipid nature). In many keratinocytes, the nucleus had loss of substance as compared to control cells. One hour exposure to 10^{-2} M p-Hydroxyanisole and tyrosinase (15 μg/ml) produced similar results. A 24-hour exposure of melanocytes and keratocytes to 10^{-2} M p-Hydroxyanisole also produced very severe damage, with loss of practically all cytoplasmic organelles and loss of definition or disruption of the plasma membrane. As with lower concentrations of the antioxidant, the nuclei of melanocytes retained more substance than those of nonkeratinized keratinocytes. Exposure of cells washed free of culture medium to both tyrosinase and 10^{-3} M p-Hydroxyanisole for 1 hour resulted once again in extensive damage. The damage could not be attributed to addition of tyrosinase per se to the medium, since controls with tyrosinase alone had no damage to either cell type. These findings suggested that an early-formed product of the reaction between tyrosinase and

p-Hydroxyanisole was inactivated by constituents of the medium. This was confirmed by liquid chromatography and scanning spectrophotometry. A toxic p-Hydroxyanisole quinone immediately reacted with nucleophilic substances in the medium to form products that, on accumulation, were probably responsible for the damage (6 hours plus) to melanocytes and keratinocytes.[54]

In the previously cited reports of Riley[46] and Breathnach et al.,[53] p-Hydroxyanisole was cytotoxic to guinea pig melanocytes at a concentration of 10^{-3} M but had no toxic effect at 10^{-2} M or 10^{-3} M on human melanocytes. The apparent discrepancy of results may be due to:[53,54] (1) species differences between man and the guinea pig (although the basic structure of the melanocyte and general process of melanogenesis are, as far as is known, identical in the two species), (2) an inability of p-Hydroxyanisole to traverse the plasma membrane and enter the human melanocyte, (3) differences in the reaction between human tyrosinase and p-Hydroxyanisole and in the reaction between the guinea pig enzyme and p-Hydroxyanisole, and (4) the need for human tyrosinase to be present in particularly high concentrations (in Riley's view, the presence of active tyrosinase within the cell is essential for p-Hydroxyanisole to exert its toxic effect).

Effect on Malignant Melanocytes

The selective lethal effect of p-Hydroxyanisole on cell cultures of malignant melanocytes was studied by Bleehan.[55] Two cell lines of human malignant melanoma, as well as Harding-Passey and B16 mouse melanoma cells were exposed in vitro for either 30 or 60 minutes to p-Hydroxyanisole concentrations of 10^{-6} M to 10^{-3} M. A dose-dependent cytotoxic effect was observed. Considerable disruption of cytoplasm, cytoplasmic organelles, and nucleus was noted after 30 minutes of exposure to 10^{-3} M p-Hydroxyanisole. Even at 10^{-5} M, the antioxidant had a marked lethal effect on Harding-Passey and B16 melanoma cells, especially on those cells that were pigmented. No effect was observed on human or mouse fibroblast cultures exposed in vitro to 10^{-3} M p-Hydroxyanisole.

The in vitro effects of p-Hydroxyanisole on mammalian melanocytes were assessed in an unpublished study by Pathak.[33] The study consisted of 3 phases. In the first phase, 2 cell lines of mouse melanoma, S-91A (pigmented) and S-91B (nonpigmented), were exposed for 48 hours to p-Hydroxyanisole concentrations ranging from 10^{-5} M to 10^{-1} M. Concentrations of 10^{-4} M to 10^{-1} M were "clearly cytotoxic," whereas concentrations of 10^{-6} M and 10^{-5} M had a "minimal" cytotoxic effect. Inhibition of thymidine, uridine, and leucine incorporation was also observed (the concentration at which this inhibition was noted was not specified). In the second phase of the study, the same 2 cell lines were exposed to p-Hydroxyanisole concentrations of 10^{-5} M, 10^{-4} M, and 10^{-3} M for 1 hour. The antioxidant had minimal or no effect in either cell line on RNA or protein synthesis at the 3 concentrations evaluated. At 10^{-4} M (0.125 percent), synthesis of DNA was inhibited by 73 percent and 50 percent in S-91A and S-91B cells, respectively. At 10^{-3} M (1.25 percent), the inhibitory effect on DNA synthesis was even greater. No significant effects were observed at 10^{-5} M (0.0125 percent) p-Hydroxyanisole. It was concluded that 10^{-5} M "appeared to be nontoxic" to mouse melanoma cells, whereas 10^{-4} M (0.125 percent) was "definitely cytotoxic." The third phase of the study was conducted to determine the effect of p-Hydroxyani-

sole on the tyrosinase activity of the S-91A cell line. The mouse melanoma cells were incubated with L-tyrosine-3,5-^{3}H and 1×10^{-6} M to 1×10^{-3} M p-Hydroxyanisole in culture medium for an unspecified period of time. p-Hydroxyanisole concentrations of 10^{-6} M and 10^{-5} M did not inhibit tyrosinase activity, whereas 10^{-4} and 10^{-3} M "strongly inhibited" tyrosinase activity. At 10^{-6} M, the antioxidant stimulated tyrosinase hydroxylation.

Dewey et al.[56] reported that p-Hydroxyanisole (0.1 to 0.6 μmol/ml) inhibited the incorporation of ^{3}H-thymidine into Harding-Passey melanoma cells in vitro. Addition of tyrosinase to the culture was associated with increased toxicity of the antioxidant to the melanoma cell. The authors suggested that cells producing the enzyme were preferentially killed by the antioxidant.

Intraperitoneal injection of 2.5 mg p-Hydroxyanisole in saline for periods up to 15 days delayed the appearance of tumors in mice inoculated with B16 melanoma cells.[57] Intratumor injection of 12.5 mg of the antioxidant in saline twice a day for 2 weeks caused increased survival time, reduced tumor size, and in many cases resulted in complete loss of tumor in mice inoculated with Harding-Passey melanoma cells.[56]

Effect on Carcinogen-Induced Tumors

In a series of investigations by Wattenberg et al.,[58-60] p-Hydroxyanisole inhibited both beta-propiolactone- and benzo(a)pyrene-induced neoplasia in the nonglandular area of the stomach of female HCR/Ha mice. Although the mechanism of inhibition was not established, tumor inhibition was observed when the antioxidant was given in the diet or by oral intubation prior to carcinogen administration. When p-Hydroxyanisole was fed to HCR/Ha mice subsequent to benzo(a)pyrene exposure, no significant suppressive effect on gastric neoplasia was observed. In experiments with A/HeJ mice, dietary administration of p-Hydroxyanisole prior to benzo(a)pyrene exposure had no effect on the incidence of carcinogen-induced pulmonary adenoma.

Effect on Enzymes

Marked increases in glutathione S-transferase activity and acid-soluble sulfhydryl concentrations were observed in the esophagus and nonglandular stomach of female HCR/Ha mice fed diets containing p-Hydroxyanisole. The antioxidant was administered daily in the amount of 0.03 nmol/g of food for either 3 or 9 days. Enhancement of glutathione S-transferase activity has been associated with a reduced carcinogenic response in the stomach of mice exposed to benzo(a)pyrene.[61,62]

p-Hydroxyanisole was examined for its ability to induce in vivo changes in hepatic mono-oxygenase and detoxification enzyme activities and to act as a mono-oxygenase inhibitor when added in vitro. Female CD-1 mice were fed a diet containing 42 nmol of p-Hydroxyanisole per kg of food for 12 days. No changes in liver weights were noted. Hepatic microsomal activities of aniline hydroxylase, TMPD:CHP peroxidase, glutathione S-transferase, and epoxide hydratase were increased, whereas the hepatic microsomal activity of aminopyrine N-demethylase was decreased. Dietary administration of p-Hydroxyanisole also caused depressed activities of hepatic cytochrome P450 and a reduced ability of

microsomes to catalyze the binding of benzo(a)pyrene metabolites to DNA. The antioxidant did not inhibit benzo(a)pyrene metabolism or DNA binding when added in vitro to hepatic microsome preparations at concentrations up to 300 μM. Test results were consistent with the hypothesis that inhibition of benzo(a)-pyrene-induced neoplasia by p-Hydroxyanisole was related to inducibility of detoxification enzymes.[63]

The effect of p-Hydroxyanisole on the induction of ornithine decarboxylase (ODC) activity in mouse epidermis by the tumor promoter 12-o-tetradecanoyl-phorbol-13 acetate (TPA) was assessed by Kozumbo et al.[64] Graded doses of p-Hydroxyanisole over a 2-log range were topically applied to the shaved skin of female CD-1 mice 30 minutes prior to skin treatment with TPA. The ID_{50}, the dose that causes 50 percent inhibition of enzyme activity, was determined from the generated dose–response curve. The ID_{50} for p-Hydroxyanisole was >50 μmol, indicating that the antioxidant was relatively ineffective in inhibiting promoter-induced ODC activity.

Induction of microsomal enzymes by p-Hydroxyanisole was measured in livers of female weanling rats. The rats received the antioxidant in daily oral doses of 1.5 mM/kg for 6 days. A weak but significant increase in the activities of hexobarbitone oxidase and aminopyrine demethylase was observed.[65]

Effect on Human Erythrocytes

The in vitro effects of p-Hydroxyanisole on human erythrocytes was examined in several studies. The antioxidant (1 to 20 mM) caused lysis of human erythrocytes in the presence of the enzyme, tyrosinase. A correlation was observed between the in vivo depigmenting action of this compound and the ability of hydroxyanisole isomers to act as substrates for tyrosinase and to cause lysis of erythrocytes in the presence of this enzyme.[45]

In other studies, p-Hydroxyanisole protected human erythrocytes from hypotonic hemolysis in vitro. The high coefficient of correlation (0.963) between the 50 percent antihemolytic concentration of p-Hydroxyanisole (6.0×10^{-3} mol/liter) and the octanol-water partition coefficient suggested a hydrophobic interaction between the compound and the erythrocytic membrane. The antihemolytic effect was associated with membrane expansion.[66,67]

Antimicrobial Activity

p-Hydroxyanisole was bactericidal to *Pseudomonas aeruginosa*[68] and inhibited spore production at a concentration of 10^{-4} M in *Candida (Monilia) fructicola* and *Alternaria oleracca*.[69] The compound produced complete inhibition of *Mycobacterium tuberculosis* growth at a concentration of 25 mg/ml of growth medium,[70] whereas 1.0 mg/ml of the antioxidant was inactive against poliomyelitis virus in tissue culture.[71]

Other Biological Effects

p-Hydroxyanisole (1 mg) was mixed with 100 IU of pregnant mare serum gonadotropin, incubated at 37°C, and injected subcutaneously into 3 immature

female rats. Seventy-two hours after injection, the animals were killed. The average ovarian weight of treated animals was 114 mg, whereas the average ovarian weight of control animals receiving pregnant mare serum gonadotropin alone was 127 mg. These results suggested no in vitro inhibition of gonadotropic activity.[72]

Cultured ascites sarcoma BP8 cells exposed in vitro to 0.01 to 1.0 mM p-Hydroxyanisole had a 15 to 93 percent reduction in cell growth. It was suggested that this inhibition of growth resulted from the interference of the antioxidant with the electron transport function of the sarcoma cell.[73]

Results from an in vitro study by Riley[45] indicated that p-Hydroxyanisole interferes with ribonucleic acid and protein synthesis and with mitochondrial respiration. Addition of 5 mM of the antioxidant to rat liver slices inhibited protein synthesis by 80 percent, whereas addition of 5 mM to HeLa cell cultures caused a 56 percent inhibition of ribonucleic acid production. A 25 to 70 percent inhibition of mitochondrial respiration in isolated rat liver was produced by 0.5 mM p-Hydroxyanisole. The author reported that no direct correlation was observed between these in vitro effects and the in vivo depigmenting action of the compound.

The role of p-Hydroxyanisole as an uncoupling agent on oxidative phosphorylation in isolated rat liver mitochondria was studied by Wynn and Fore.[74] Phosphate and oxygen uptake in mitochondria were measured over a 10-minute period following addition of 5×10^{-5} M p-Hydroxyanisole to the liver preparation. The phosphate:oxygen ratio (P:O ratio) in p-Hydroxyanisole-treated liver was 2.0. Measures of the P:O ratio in nontreated control and positive control liver preparations were 2.5 and 1.2, respectively. The P:O ratio of 1.2 for L-thyroxine (positive control) indicated extensive uncoupling of mitochondrial oxidative phosphorylation.

The ability of p-Hydroxyanisole and other phenols to produce chromosome fragmentation in onion roots (*Allium cepa*) was reported by Levan and Tjio.[75] In a more recent investigation, chromosomal aberrations were observed in barley caryopsis (*Hordeum vulgare*) and onion roots (*A. cepa*) treated with 0.25 mM of the antioxidant.[76]

Denaturation of DNA was observed in T_4 bacteriophage treated in vitro with 0.09 M p-Hydroxyanisole.[77]

ABSORPTION, METABOLISM, AND EXCRETION

The rate of skin absorption of p-Hydroxyanisole was examined in vitro by Riley.[39] A 5×10^{-2} M concentration of the antioxidant was applied to the keratin surface of full-thickness guinea pig skin (0.96 cm^2) excised from the back of the ears. The rate of skin penetration as measured by spectrophotometric analysis was 8.05×10^{-4} mol per cm^2 per minute.

p-Hydroxyanisole was administered by stomach tube to female rabbits weighing 2.5 to 3.5 kg at a dose of 0.7 g as a suspension in water. The compound was excreted mainly as conjugates of glucuronic and sulfuric acids; a small amount was partly demethylated to give hydroquinone. The average percentage

of the dose excreted was 13 percent (range: 10 to 15 percent), 69 percent (range: 65 to 73 percent), and 1 percent (range: 0 to 2 percent) for ethereal sulfate, ether glucuronide, and free phenol, respectively. The average percentage of the single dose accounted for was 82 percent.[78]

The metabolism and excretion of butylated hydroxyanisole and its isomers were determined in the rat. The commercial butylated hydroxyanisole used in the study consisted of a mixture of 15 percent or less of 2-tert-butyl-4-hydroxyanisole (isomer A) and 85 percent or more of 3-tert-butyl-4-hydroxyanisole (isomer B). Chromatographic analysis indicated that isomer A contained 3 to 5 percent p-Hydroxyanisole as an impurity. Isomer A was administered by gastric intubation to 6 rats as a 50 percent (W/V) solution in corn oil. Each rat was given successive daily doses of 0.5 g/kg for 5 doses (total average dose/rat was 0.77 g). For the duration of dosing, urine samples were collected and pooled. The urine was subsequently analyzed for free butylated hydroxyanisole, glucuronide, and ethereal sulfate, which accounted for 5, 25, and 30 percent of the dose fed, respectively. Of isomer A, 0.6 percent of the dose was excreted as the impurity p-Hydroxyanisole and 2.6 percent as its glucuronide, as estimated enzymically and chromatographically. The authors concluded that p-Hydroxyanisole was not demethylated by the rat but that it was largely excreted as a glucuronide.[79]

TOXICOLOGY

Acute Oral Toxicity

Fasted rats weighing 129 to 160 g were given p-Hydroxyanisole by stomach tube as a single oral dose ranging from 150 to 350 mg per rat. Size of the 5 test groups varied from 1 to 10 animals. A dose of 200 mg killed 4 of 10 rats; a dose of 300 mg killed 5 of 6. The acute oral LD_{50} was estimated at 1630 mg/kg.[8]

Corn oil containing 10 percent p-Hydroxyanisole was administered to rats as oral doses of 1000 mg/kg or 2000 mg/kg. Two rats were given each dose (4 total). No deaths resulted from the 1000 mg/kg dose, but "kidney injury" was observed at necropsy. The 2 rats given 2000 mg/kg developed convulsions within 10 minutes after dosing; death ensued within 2 hours.[80]

The acute oral toxicity of 50 percent p-Hydroxyanisole in corn oil was assessed in Sprague-Dawley rats by the methods described by Hagan[81] and Weil.[82] Five to ten animals were used in each test group (number of groups and dose range were not specified). The LD_{50} of the corn oil suspension was 740 mg/kg.[83]

Three cosmetic products containing the antioxidant were also evaluated for acute oral toxicity. Each of the products was tested by the procedures described by Hagan[81] and Weil.[82] Test groups consisted of 5 to 10 rats. However, number of groups and dose range were not reported. Reported acute oral LD_{50} values were as follows:

Test Material	*Oral LD_{50} of Test Material (Rat)*	*Reference*
Moisturizing lotion containing 0.1 percent p-Hydroxyanisole	>21.5 g/kg	84
Moisturizing lotion containing 0.05 p-Hydroxyanisole (product given as a 50 percent suspension in corn oil)	>15.9 g/kg	85
Blusher containing 0.1 percent p-Hydroxyanisole	>15.8 g/kg	86

Intraperitoneal Toxicity

Groups of mice were given various doses of p-Hydroxyanisole by intraperitoneal injection. Mortality was recorded over a 7-day observation period following the single exposure. The LD_{50} was 250 mg/kg.[87]

The acute toxicity of p-Hydroxyanisole following intraperitoneal injection was assessed in mice, rats, and rabbits by Hodge et al.[8] *Mice:* Eight groups of mice (10 to 22 animals/group) weighing 25 to 28 g were given single injections ranging from 4 to 18 mg. A "wobbly gait" immediately following injection was observed. This was followed in a few minutes by paralysis of the hind quarter and spasms. At the "higher doses," the mice developed narcosis 15 minutes after injection. Some mice had "degrees of anesthesia" for as long as 18 hours after the single exposure. No deaths occurred in the group receiving the 4 mg dose; however, all mice in the 18 mg group died. The estimated LD_{50} was 430 mg/kg of body weight. *Rats:* Six groups of albino rats (5 to 15 animals/group) were injected once with 1 of 6 p-Hydroxyanisole doses ranging from 100 to 150 mg per rat. The adult rats ranged in body weight from 150 to 193 g. No deaths were noted at the 100 mg dose, whereas all rats died at the 150 mg dose. The LD_{50} was 730 mg/kg. The acute toxic effects in the rats were similar to those observed in the mice. *Rabbits:* Adult rabbits weighing 2.7 to 4.8 kg of various breeds were given a single intraperitoneal injection ranging from 100 to 3300 mg. Only 1 rabbit was tested at each dose. Doses up to 2800 mg were tolerated without lethal effects; the rabbit treated with 3300 mg died within 24 hours. The LD_{50} was estimated at 720 to 970 mg/kg.

Dental Pulp Irritation

The potential of 100 percent p-Hydroxyanisole to produce dental pulp irritation was assayed in two monkeys (*Macaca fascicularis*). The test material was placed in contact with the pulpal walls of prepared class V cavities in each of 8 quadrants. A piece of gold foil was subsequently placed over the test substance,

and the cavity was then filled with a zinc oxide/eugenol material. Twenty-one days later, the animals were killed, and the teeth were processed for histopathologic evaluation. Pulp irritation was graded on a scale of 0 (no inflammation) to 4 (abscess formation or pulp necrosis). The average pulp score was 0.25 (±0.20), indicating minimal to mild irritation or inflammation. In 1 of the 8 quadrants, there was a single 2.0 response indicative of moderate irritation. Differences in dentin thickness did not lead to differences in pulp response. No abscess formations or "lesions predominating in leukocytes" occurred.[88]

Eye Irritation

The ocular irritating effects of p-Hydroxyanisole were assessed in a 1959 range finding study (unpublished). Both "undiluted" p-Hydroxyanisole and 10 percent p-Hydroxyanisole in propylene glycol were instilled into the eyes of an unspecified number of rabbits. In some rabbits, treated eyes were given a 2 minute water rinse following antioxidant exposure. The propylene glycol solution containing 10 percent p-Hydroxyanisole produced slight conjunctivitis in both the rinsed and unrinsed eye; this irritation dissipated 1 hour after instillation of the test material. No corneal injury was observed. Undiluted p-Hydroxyanisole produced corneal injury, moderate conjunctivitis, and slight iritis in both rinsed and unrinsed eyes. This irritation had "essentially subsided" 1 week after treatment in those rabbits given no water rinse and had "completely subsided" after 1 week in those rabbits given a water rinse.[80] It was not indicated whether or not nontreated or vehicle (propylene glycol) control eyes were used or whether or not treated eyes received single or multiple instillations of the test material.

Undiluted p-Hydroxyanisole and 1.0 percent p-Hydroxyanisole in aqueous solution were evaluated in a second eye irritation study. The procedures used were a modified version of the test methods outlined by Draize.[89] Each test material was instilled into the eyes of 3 to 6 (exact number not specified) New Zealand rabbits. One percent p-Hydroxyanisole in water produced minimal conjunctival irritation 1 hour postinstillation (average score, 2; maximum possible score, 20). The conjunctival irritation dissipated by the 24-hour evaluation. No iridial or corneal lesions were observed. For the undiluted material, average corneal, iridic, and conjunctival irritation scores 1 hour postinstillation were as follows: (1) cornea: score, 20 (max, 80); (2) iris: score, 5 (max, 10); (3) conjunctivae: score, 4 (max, 20). Average ocular irritation scores for the undiluted antioxidant 7 days postinstillation were 80, 10, and 14 for cornea, iris, and conjunctiva, respectively, indicating severe ocular irritation.[83] A test material is considered a severe eye irritant when corneal and iridial lesions have not cleared by the seventh day.[89]

The Draize procedure[89] was used to evaluate the ocular irritation potential of 3 cosmetic products containing p-Hydroxyanisole. The undiluted products were tested on groups of 3 to 6 New Zealand rabbits. A moisturizing lotion and a blusher each containing 0.1 percent p-Hydroxyanisole produced no eye irritation. A moisturizing cream formulated with 0.05 percent of the antioxidant produced minimal conjunctival irritation by the 1-hour evaluation (average score, 4; maximum possible score, 20). This irritation had completely dissipated by the 24-hour evaluation.[84-86]

Skin Irritation

The potential of p-Hydroxyanisole to produce skin irritation in rabbits and guinea pigs was assessed in 3 separate studies.

In a 1959 range finding study (unpublished), p-Hydroxyanisole was tested for skin irritation in 4 separate trials. In the first trial, a single application of undiluted p-Hydroxyanisole was made to the intact skin of an unspecified number of rabbits for either 3 or 7 hours. No irritation was observed on test sites exposed to the antioxidant for 3 hours, whereas treatment sites exposed for 7 hours developed "very slight hyperemia." In the second trial, the undiluted ingredient (0.5 g) was applied for 24 hours under an occlusive patch to the abraded and intact skin of the abdomen of an unreported number of rabbits. "Extensive edema and necrosis" were observed following the single 24-hour exposure. Moderate eschar formation was noted 21 days posttreatment. In the third trial, a single 1 g/kg dose (dose based on solids) of 50 percent p-Hydroxyanisole in dipropylene glycol monomethyl ether was applied to the clipped skin of the torso of 2 rabbits. The single applicatiion was made under an impervious plastic sleeve for 24 hours. "Slight hyperemia" of the skin developed. In the fourth trial, 10 percent p-Hydroxyanisole in dipropylene glycol monomethyl ether was applied to the intact skin of the ear (3 applications), intact skin of the abdomen (3 applications), and abraded skin of the abdomen (3 applications) of 1 rabbit. Applications were made under a cotton pad to each test site daily for 3 consecutive days (total of 9 24-hour applications). No irritation was observed on the intact skin of the ear. However, intact and abraded treatment sites on the abdomen developed "slight hyperemia" and slight to moderate edema.[80]

An abbreviated, unpublished study was conducted in 1950 to evaluate skin irritation and antioxidant absorption following topical application of p-Hydroxyanisole to guinea pig skin. A patch containing 40 percent p-Hydroxyanisole in an acetone/olive oil mixture (92:8) was applied for 24 hours in a single 10 or 20 ml/kg dose to the clipped and depilated skin of 2 guinea pigs (1 animal/dose). Slight to moderate skin irritation was observed.[90]

Five percent p-Hydroxyanisole in sweet almond oil was applied for 24 hours under patch to the clipped skin of 6 New Zealand rabbits. Skin irritation was subsequently assessed according to the evaluation method described by Draize.[89] The irritation index (average score of 6 animals) was 0.3 on a scale of 0 (no irritation) to 8.0 (severe erythema and edema), indicating minimal skin irritation.[83] (It was not specified whether abraded or intact skin was tested).

Skin Sensitization

The skin-sensitizing potential of p-Hydroxyanisole was evaluated by means of the guinea pig maximization test and the Freund's complete adjuvant test. The procedures were those as described by Van de Walle et al.[91] In the guinea pig maximization test, 0.5 M (6.2 percent) p-Hydroxyanisole was given by intradermal injection into the shoulder of 10 guinea pigs on Day 0. On Day 7, a 48-hour induction patch containing 1 M p-Hydroxyanisole was applied to the injection site. Pretreatment with 10 percent sodium lauryl sulfate in petrolatum was performed 24 hours before the patch induction on Day 7 to obtain moderate irrita-

tion. The challenge phase consisted of 2 24-hour patches. One patch was closed and was applied to the shaved skin of the right flank on Day 21. The second challenge patch was open and was applied to the shaved left flank on Day 35. In the Freund's complete adjuvant test, intradermal injections of 0.5 M p-Hydroxyanisole in Freund's adjuvant were made into the shoulder of 8 guinea pigs on Days 0, 2, 4, 7, and 9 (induction phase). Open, 24-hour challenge patches were applied to the shaved right flank on Day 21 and Day 35 (left flank). Challenge concentrations were not specified. p-Hydroxyanisole produced moderate skin sensitization in both tests.[92]

An unpublished study was conducted in 1950 to assess the skin sensitization potential of p-Hydroxyanisole. Seven drops of a "0.1 M solution of the compound in acetone:dioxane:olive oil (1:1:3)" were applied to the clipped backs of each of 5 Hartley strain guinea pigs. The hair "stubble" was removed from the treatment site the following day. Test sites were evaluated for erythema and edema 24 and 48 hours after application. Immediately following the 48-hour evaluation, 10 drops of the test solution were applied to the back. A third induction exposure of 10 drops was applied 48 hours later. After a 3-week nontreatment period, a challenge application of a fresh solution (7 drops) was applied to the clipped right shoulder. The next day, the test area was "depilated." Skin responses were noted 24 and 48 hours following the challenge application. One week later, the challenge procedure was repeated except that the left shoulder was the site of the challenge application. Skin responses to the 2 challenge applications were "not significantly different from the original application," and it was concluded that no sensitization reactions had occurred. Solvent control and positive control (phenylhydrazine) groups were also employed in the study. The positive control group had a "positive response;" however, the response of the solvent control group was not reported. The number of animals in the 2 control groups and the effect of depilation on the treated skin were also not specified.[90]

Concomitant sensitization to p-Hydroxyanisole and various acrylic monomers was observed in monoacrylate sensitized guinea pigs. However, no relation between the antioxidant concentration and the incidence of these reactions could be determined.[91,92] Cross skin sensitization of guinea pigs to hydroquinone (1 M) and p-Hydroxyanisole (3 M) has also been reported.[92]

Photosensitization

The photosensitization potential of 0.1 and 1.0 percent p-Hydroxyanisole in physiological saline and DAE* (20:80) was evaluated in 19 Hartley albino guinea pigs. The phototest consisted of both an induction phase and a challenge phase. During the induction phase, 0.1 ml of 1.0 percent p-Hydroxyanisole was applied 4 consecutive days a week for 3 weeks to the shaved skin of the nuchal area of the back. One hour after application, test sites were irradiated with UV light at 1/2 the minimal erythemic dose. (The minimal erythemic dose, or MED, was determined prior to the study). UVA irradiation was administered during the first

*DAE, 40 percent dimethylacetamide, 30 percent acetone, and 30 percent ethanol.

week of the induction phase, whereas UVB was administered during the second and third week of the induction phase. On the first and third day of the second and third week and before test material application, each guinea pig was given a 0.1 ml intradermal injection of Freund's complete adjuvant in physiological saline (1:1). Adjuvant injections were administered to 4 different areas surrounding the induction site (nuchal area). For the challenge phase, the lumbar area of the shaved back was divided into 6 exposure sites (3 sites on the left of the back, 3 sites on the right). The 3 sites on the left side of the back were treated with 0.1 ml of 1.0 percent p-Hydroxyanisole for 3 consecutive days. One hour after application, these 3 sites were exposed to either no UV light, UVB (1/2 MED), or UVA (1/2 MED). The 3 sites on the right side of the back were treated for 3 consecutive days with 1.0 percent p-Hydroxyanisole and then similarly exposed to UV irradiation as the left side. Each guinea pig was given 3 challenge UV exposures at each of the 2 concentrations (0.1 and 1.0 percent). The time between challenge exposures was not specified. The light source for the second and third week of induction and for challenge consisted of a 150 W Xenon Lamp, which emitted in the UVA (320 to 410 nm), UVB (280 to 320 nm), and visible light (410 nm and greater) range. The same lamp was used for the first week of induction and challenge but was fitted with a WG-345 glass filter to remove UVB waves. Distance between the light source and exposure site was approximately 4.6 cm. Skin reactions were evaluated 24 hours after each UV exposure. A reaction was considered a photocontact sensitization reaction if the skin response was at least 1 grade greater than that observed during the first week of induction. One of 19 guinea pigs reacted to both UVA and UVB irradiation at 0.1 and 1.0 percent. A second animal reacted when challenged at 1.0 percent to UVB irradiation. All skin responses to p-Hydroxyanisole were found after the third consecutive challenge and consisted of minimal erythema. Historical data on 5 percent 6-methylcoumarin (positive control) and undiluted DAE/physiological saline (vehicle control) were positive and negative, respectively, for photosensitization. On the basis of "the limited number of animals responding and the low magnitude of the dermal responses following three consecutive challenge periods," the investigator concluded that the findings with regard to p-Hydroxyanisole were not significant and were not indicative of a photoallergic response. It was also noted that the induction concentration of 1.0 percent p-Hydroxyanisole represented a 10-fold exaggeration of concentration normally used in cosmetics.[93]

Acute Dermal Toxicity

An unpublished study assessed the skin absorption and dermal toxicity of p-Hydroxyanisole. A dipropylene glycol monomethylether solution containing 10 percent p-Hydroxyanisole was applied to the intact skin of the ear (3 applications), intact skin of the abdomen (3 applications), and abraded skin of the abdomen (3 applications) of one rabbit. Applications were made under a cotton pad to each test site daily for 3 consecutive days (total of 9 24-hour applications). The rabbit died within 4 days following the last treatment. It was reported that the cause of death was "possibly from absorption through the abraded skin area."[80]

The skin absorption and acute dermal toxicity of a dipropylene glycol monomethylether solution containing 50 percent p-Hydroxyanisole was assessed in a

second trial by the same investigators. A single 1000 mg/kg application (dose based on solids) was made to the clipped torso of each of 2 rabbits. The test material remained in contact with the skin for 24 hours under an impervious plastic sleeve. No deaths or "untoward reactions" were observed.[80]

An abbreviated, unpublished study was conducted in 1950 to evaluate antioxidant absorption following topical application of p-Hydroxyanisole to guinea pig skin. The study consisted of 2 trials. In the first trial, 20 percent p-Hydroxyanisole in a mixture of acetone and olive oil (92:8) was applied in a single dose to the clipped and depilated skin of 3 guinea pigs. The single dose consisted of a 24-hour patch containing either 5, 10, or 20 ml/kg of body weight of the test solution; 1 animal was tested at each dose. The guinea pig treated with 20 ml/kg (about 4 g of p-Hydroxyanisole/kg of body weight) died during the 14-day observation period following application. In the second trial, a patch containing 40 percent p-Hydroxyanisole in an acetone/olive oil mixture (92:8) was applied for 24 hours as a single 10 or 20 ml/kg dose to the clipped and depilated skin of 2 guinea pigs (1 animal/dose). Both animals survived.[90]

It should be noted that these 3 studies conducted in the 1950s were exploratory or range-finding in nature and were designed primarily to assist in assessing industrial handling hazards and in establishing precautionary measures to be observed for safe manufacturing.

Subchronic Dermal Toxicity

The subchronic dermal toxicity of p-Hydroxyanisole was assessed in guinea pigs, mice, and rabbits.

A water-oil emulsion containing 1.0 percent p-Hydroxyanisole was applied in 0.5 ml doses to the shaved skin of male guinea pigs daily for 30 days. Skin reactions to the emulsion consisted of hyperemia, edema, and slight desquamation. Increased histamine concentrations were also found in the treated skin.[94]

The skin-irritating effects of p-Hydroxyanisole were assessed in both black guinea pigs and BLA mice. A single application of 10 percent p-Hydroxyanisole in petroleum jelly was made daily to the ear of each of 5 guinea pigs for 4 weeks; 20 percent p-Hydroxyanisole in petroleum jelly was applied in a similar fashion to a second group of 5 guinea pigs. A 10 percent concentration of the antioxidant in petroleum jelly was applied to the neck of 10 mice daily for 8 weeks, whereas 20 percent p-Hydroxyanisole in petroleum jelly was applied to the neck of 10 mice daily for 4 weeks. Skin irritation (acanthosis) and skin depigmentation were observed in both species exposed to 20 percent p-Hydroxyanisole. No observable skin effects were produced by 10 percent p-Hydroxyanisole.[42]

A "preliminary" study was conducted to evaluate the dermal toxicity of an alcohol-based suntan lotion containing either 0, 1.0, or 10.0 percent p-Hydroxyanisole. The vehicle control group and the 2 treatment groups each consisted of 6 rabbits (18 total). The suntan lotion was applied in a single 5 ml dose to the clipped and depilated skin of the back and side of each animal. Applications of the product were made daily, 5 days a week for 30 days. Body weights were recorded weekly, and rabbits were killed at the end of the experimental period. Both groups exposed to p-Hydroxyanisole for 30 days had weight losses, but these losses were considered "small." Results of hematological studies and urine

analyses before and during the course of study were normal. Rabbits treated with the lotion containing 0.1 and 10.0 percent p-Hydroxyanisole had a decrease in the average weight of testes and an increase in the average weight of the spleen compared to the vehicle control group. The average weight of heart, lungs, liver, kidneys, brain, and stomach were comparable between control and treatment groups. At necropsy, pitting of the kidney surface (3 rabbits of the 1.0 percent group), an enlarged spleen (1 rabbit of the 1.0 percent group), and an atrophic left testis (1 rabbit of the 10 percent group) were the findings. Principal changes observed in the skin of both control and experimental animals included dryness and crusting of the surface. Microscopic changes ranging from atrophy of the epidermis to ulceration and inflammation were also observed in both control and treatment groups. Rabbits treated with the lotion containing 1.0 and 10.0 percent p-Hydroxyanisole developed "marked irritation" of the skin during the first week of exposure. Control animals treated with the alcohol-based suntan lotion alone had similar but less marked reactions of irritation.[8]

In a follow-up to the previous study, the alcohol-based suntan lotion containing either 0 or 10.0 percent p-Hydroxyanisole was applied to the clipped and depilated skin of the back, sides, and abdomen of each of 12 rabbits (6 rabbits/vehicle control group and 6 rabbits/treatment group). Three hours after the lotion was applied, the treated sites were washed with soap and water, rinsed, and dried. This procedure was repeated daily for 12 days over a 2-week period. The fur was reclipped as required to permit adequate contact of the product with the skin. Vehicle control rabbits (suntan lotion alone) gained weight steadily after an initial small decrease. Rabbits treated with the lotion containing 10 percent p-Hydroxyanisole lost weight during the first week; this weight was largely regained in the succeeding 10 days. None of the weight changes were of "large magnitude." At the conclusion of the 12 days of exposure, skin erythema and escharification were each graded on a scale of 1 (questionable erythema/mild crustiness) to 4 (severe erythema/cracks in the skin). In the vehicle control group, average scores for erythema and escharification were 1.3 and 1.5, respectively, indicating mild skin erythema and mild to moderate skin escharification. In the 10 percent treatment group, average scores for erythema and escharification were 2.1 and 2.3, respectively, indicating moderate skin erythema and moderate skin escharification. Skin from both vehicle control and p-Hydroxyanisole-treated rabbits had "moderate hyperkeratosis" and "slight chronic inflammation." The surface of the skin had red blood cells, necrotic epithelial cells, and keratin. The underlying epithelium was intact in all instances.[8]

Subchronic Oral Toxicity

Subchronic feeding studies were conducted with p-Hydroxyanisole in the rat, rabbit, and dog.

Eighty male and female rats were subdivided into 8 groups (10 rats/group) and then fed diets containing either 0, 0.02, 0.1, 0.5, 2.0, or 5.0 percent p-Hydroxyanisole for 5 to 7 weeks. Two groups of control rats (20 rats total) were fed the diet containing no antioxidant, whereas 2 groups of rats (20 rats total) were maintained on diets treated with 0.5 percent p-Hydroxyanisole. No deaths occurred in any group during the study. A dose-related growth inhibition was ob-

served in males fed 0.1 to 5.0 percent p-Hydroxyanisole and in females fed 0.5 to 5.0 percent of the antioxidant. The inclusion of 2.0 or 5.0 percent p-Hydroxyanisole in the diet produced a marked depression of growth in both sexes. However, it was suggested that the odor and flavor associated with p-Hydroxyanisole at these 2 dietary concentrations may have reduced both the palatability and, thus, the intake of the diet. Qualitative analyses of pooled urine samples collected near the end of the experiment from males revealed slightly elevated concentrations of sugar and p-Hydroxyanisole; protein values were normal. Hematological studies made at various intervals revealed normal red blood cell counts, differential counts, and hemoglobin concentrations. A few high leukocyte counts were observed, but these were likely the result of a mild respiratory infection. Rats of both sexes in the low-dose groups (0.02 to 0.1 percent) had decreased spleen and liver weights; males in these same groups had decreased kidney and testes weights. Organ weights of rats fed 0.5 to 5.0 percent of the antioxidant decreased as the percentage of p-Hydroxyanisole increased. The variations in organ weights (heart, lungs, spleen, liver, kidney, brain, testes) observed at the end of the study period were attributed to general body weight depression. Tissues taken from various organs and the gastrointestinal tract had no changes that could be attributed to dietary administration of p-Hydroxyanisole.[8]

Groups of 6 rabbits were fed diets containing 0, 1.0, 5.0, or 10.0 percent p-Hydroxyanisole for 5 to 9 weeks. Rabbits fed 1.0 and 5.0 percent p-Hydroxyanisole gained weight at the same rate as control animals. However, rabbits fed 10.0 percent had transient weight loss. The "appetite and general condition" of the rabbits fed 1.0 percent p-Hydroxyanisole for 5 weeks was described as "excellent." Urine samples collected at the start and end of the experiment had low concentrations of sugar and protein, but there was "no indication of kidney damage." Blood samples taken midway through the study and at the end of the study generally had normal hemoglobin concentrations and normal white and red cell counts. However, rabbits fed 10.0 percent p-Hydroxyanisole had low red cell counts. Differential leukocyte counts had considerable variation. The average weights of liver and brain were increased in the 10 percent treatment group, whereas average weights were decreased for heart, lungs, spleen, liver, kidney, and testes. These organs and the large and small intestine had no significant pathological changes that could be attributed to the administration of p-Hydroxyanisole.[8]

The subchronic toxicity of p-Hydroxyanisole was assessed in 3 dogs by adding the antioxidant to the diet as follows: (1) dog no. 1 (12.5 kg): 1 g daily for 2 months, (2) dog no. 2 (6.8 kg): 2 g daily for 1 month, then 4 g daily for 6 weeks, (3) dog no. 3 (14.4 kg): 3 g daily for 6 weeks, then 6 g for 1 month, then 12 g for 2 weeks. The body weight of dog no. 1 varied but in general was maintained throughout the study. Dog no. 2 had very little change in body weight. Dog no. 3 fed 3 g daily for 6 weeks lost weight during the first month, regained weight in the next 2 weeks, maintained weight during the period when the dosage was 6 g daily, and then lost weight again when the dosage was increased to 12 g daily. The investigator concluded that up to 6 g of p-Hydroxyanisole daily had no effect on body weight, whereas feeding 12 g a day was associated with body weight loss. The weight loss associated with 12 g a day, however, was not considered excessive. Urine samples examined twice during the study were normal for protein (0.02 to 0.07 percent) and sugar (0.2 to 0.4 percent). Hematological changes in

dog no. 3 included marked decreases in hemoglobin and red blood cell count. The 3 dogs had normal total leukocyte and differential leukocyte counts. Organ weights (heart, lungs, spleen, liver, kidneys, brain, uterus, ovaries) "gave no evidence of toxic effects," and the few pathological changes observed in the lung (diffuse round cell and polymorphonuclear leukocyte infiltration) and spleen (scattered pigment deposit) were not, according to the investigators, attributable to p-Hydroxyanisole.[8]

Other Subchronic Studies

The effects of p-Hydroxyanisole on hamster cheekpouch was assessed by Woods and Smith.[51] A lanolin base containing 20 percent p-Hydroxyanisole was applied to the cheekpouches of golden Syrian hamsters 3 times a week for 45 days. Erythema progressively increased throughout the study. Microscopic examination of treated areas revealed hyperkeratosis, epithelial hyperplasia, formation of small bullae, "disorganization" of basal epithelial cells, "disturbances" at the epithelial-connective tissue junction, "invasion" of basal cell pseudopodia into the lamina densa, and muscle degeneration with scattering of myofilaments.

Chronic Dermal Toxicity

An unspecified vehicle containing either 0.0, 0.5, or 1.0 percent p-Hydroxyanisole was applied daily for 6 months to the left ear and epilated back of 3 groups of black guinea pigs (12 animals per group). The skin of both exposed groups became hypomelanotic and amelanotic after 4 months. Guinea pigs exposed to 1.0 percent p-Hydroxyanisole developed moderate to severe skin and hair depigmentation at the site of application. Animals treated with 0.5 percent of the antioxidant developed either skin depigmentation or skin hypopigmentation. Skin from the vehicle control group (0.0 percent p-Hydroxyanisole) appeared normal when examined grossly and microscopically. At microscopic examination of skin, the treated sites of the two exposure groups had hyperplasia, dyskeratosis, loss of melanin pigment, a decrease in number of DOPA-positive melanocytes, pigment incontinence, decreased pigmentation of melanocytes in the basal layer, focal degeneration of melanocytes, loss of dendritic arborization, presence of melanin-laden macrophages, and loss of pigment in hair follicles. Loss of melanin pigment was more noticeable in skin biopsies of the 0.5 percent exposure group. Maturation disorders of keratinocytes were rare, and the influx of inflammatory cells was minimal suggesting that the p-Hydroxyanisole formulated vehicle was nonirritating and nonsensitizing. There was little or no evidence of atypical cells, vasodilatation, or infiltration of mononuclear cells.[33]

Mutagenicity

p-Hydroxyanisole was nonmutagenic when evaluated in the Ames assay at a concentration of 3 μmol/plate. The qualitative spot test was conducted using 4 histidine-requiring mutants of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537). The antioxidant was tested both with and without metabolic activation using S-9 liver fraction from Aroclor 1254-induced rats.[95]

No mutagenicity was observed in a second assay in which *S. typhimurium* strains TA100 or TA1530 were treated with ≤4 μmol/plate of p-Hydroxyanisole. The S-9 liver fraction of phenobarbitone-induced OF-1 mice provided metabolic activation.[96]

Tumor-Promoting Activity and Carcinogenicity

A benzene solution containing 13.1 percent p-Hydroxyanisole was tested as tumor promoter on the clipped skin of 30 albino mice. The skin was initiated with a single application of 75 μg 7,12-dimethylbenz(a)anthracene (DMBA). A single drop of the benzene solution was applied 1 week later to the DMBA-treated site. Applications of the benzene solution were made twice weekly. After 20 weeks, 1 of the 25 surviving mice developed a benign tumor.[97] Under the experimental conditions, p-Hydroxyanisole was inactive as a tumor promoter.

Acetone solutions of either 5 or 10 percent p-Hydroxyanisole were applied twice a week for life to the dorsal skin of the back of female Swiss albino mice. Each test solution was applied in 0.02 ml doses to a group of 50 animals. The mice developed no local toxic changes or tumors. The incidence of systemic tumors was similar to that of control mice.[98]

The inner surface of the left ear of each of 10 New Zealand rabbits of both sexes was treated twice weekly with 0.02 ml acetone solutions containing either 5 or 10 percent p-Hydroxyanisole. Each test group consisted of 5 rabbits (5 animals per concentration). No local toxic changes or local or distant tumors were observed.[98]

Embryotoxicity and Teratogenicity

A bleach cream containing 5 percent p-Hydroxyanisole and a water-oil emulsion containing 25 percent p-Hydroxyanisole were both assessed for embryotoxicity and teratogenicity. Test animals consisted of groups of 10 to 12 white female rats. Applications of the test materials were made to the skin daily on Days 1 to 20 of pregnancy. Nontreated and cream base control animals were employed. Both test materials produced increased preimplantation mortality of embryos, whereas the bleach cream additionally produced subcutaneous hemorrhages in embryos of treated rats. No significant differences were observed between control and treated groups with respect to skeletal anomalies, postimplantation mortality, craniocaudal dimensions and weight of embryos, or placental weights. To study postnatal development, female rats were also given skin applications of the bleach cream throughout pregnancy; another group of rats served as untreated controls. Newborn rats had subcutaneous hemorrhages, retarded development, and reduced body weight. It was concluded that the bleach cream was embryotoxic but nonteratogenic to rats and that the bleach cream product should not be used by pregnant women.[94]

CLINICAL ASSESSMENT OF SAFETY

Experimental Treatment of Melanoma

A pilot study was conducted to determine the clinical usefulness of p-Hydroxyanisole in treating localized malignant melanomas. Eleven patients with

proven primary or secondary malignant melanomas were given intravenous or intraarterial infusions of p-Hydroxyanisole in physiological saline. Duration of treatment varied from 1 day to 57 days; the total antioxidant dose administered varied from 2 g to 154 g. Evidence of cytotoxic action on melanoma cells and tumor regression was observed in 4 of 5 patients given the antioxidant by the intraarterial route. Intravenous administration of p-Hydroxyanisole had no effect. The investigators noted that the metabolism of the antioxidant and its clearance from the blood were very rapid. No "generalized toxic effects" were observed.[47]

The effect of p-Hydroxyanisole on advanced malignant melonomas was examined in a second clinical study. Twelve of twenty-one cutaneous nodules of malignant melanoma directly injected with 12.5 mg of the antioxidant underwent temporary regression. Intravenous administration of p-Hydroxyanisole was well tolerated in 6 patients up to doses of 1.5 g and total doses of 7.5 g; however, nausea was a frequent complaint. A transient drop in peripheral leukocytes was observed in 1 patient. No other side effects were observed, and 1 patient had a "brief partial response."[57]

Case Reports

A 33-year-old woman developed leukoderma of the face following application of an ointment to a chloasma. The ointment contained an unspecified amount of p-Hydroxyanisole. The duration of application was estimated to have been 2 months. No spontaneous repigmentation of the skin occurred during the 6 months after cessation of ointment usage. PUVA therapy resulted in total repigmentation of the treated skin.[99]

A 56-year-old man developed leukoderma of the hands following occupational exposure to acrylate ester and p-Hydroxyanisole (the latter chemical was referred to by the authors as "methoquinone"). "Plaster experiments" with aqueous solutions containing 10, 5, 2, 1, and 0.5 percent "methoquinone" gave positive results for dermatitis at 48, 72, and 96 hours.[100]

Two of eight workers in a vinylidene chloride plant who had been handling p-Hydroxyanisole for 3 to 3½ years developed depigmentation (occupational leukoderma) of the skin of the forearm and the forehead. In 1 worker, the depigmented areas became erythematous when exposed to sunlight.[101]

The same 2 workers were reexamined by O'Sullivan and Stevenson[102] 8 years later. These 2 authors noted a wider area of vitiligo involvement than initially observed by Chivers.[101] Examination by Wood's light revealed vitiligo of the axillae, groin, feet, shins, and neck. Repigmentation of the previously affected areas was noted in both men. A "blood screen," an "organ-specific antibody screen," and liver function test were normal for both individuals.

A total of 248 men from 3 different factories were screened by Wood's light for vitiligo following occupational exposure to p-Hydroxyanisole. At least 1 of the factories had taken "protective measures" to reduce exposure to the antioxidant. No skin depigmentation was observed.[102]

Skin Depigmentation, Sensitization, and Irritation

The skin depigmentation, sensitization, and irritation potential of both 2.0 percent p-Hydroxyanisole in petrolatum and 2.0 percent p-Hydroxyanisole in

sweet almond oil was examined in 80 male and female test subjects aged 18 to 65. Only individuals with a "moderate degree" of skin pigmentation were tested; fair and dark-skinned individuals were excluded from the study. Each test material was applied to the back under an occlusive patch for 48 hours. Upon removal of the patch, the exposed site was wiped free of excess test material. An identical patch was then applied to the same site and the procedure repeated 3 times weekly for 8 successive weeks. Patches applied on Friday remained in place for 72 hours instead of 48 hours. No patch was applied on the eighth Friday. Seven subjects developed skin reactions to the antioxidant in petrolatum. Two subjects had skin reactions to the antioxidant in sweet almond oil. The majority of these reactions were evaluated as "doubtful;" the few remaining reactions consisted of erythema or erythema plus edema. Most reactions appeared after several weeks of exposure and were transient in nature. Reactions typically lasted for only 2 or 3 successive applications. The investigator considered the 2 test materials as nonirritating and nonsensitizing to the skin. Neither test material caused any observable skin depigmentation.[103,104] The CIR Panel notes that a nontreatment period followed by a challenge is considered a more optimal method for detecting sensitization; such a procedure was not followed in this test. Additionally, it was not reported whether or not Wood's light examination was employed in this study. Screening by Wood's light is often necessary to determine evidence of vitiligo.[102]

A repeated insult patch test was conducted on 102 human panelists to assess the skin sensitization and irritation of 5 percent p-Hydroxyanisole in sweet almond oil. The test material was applied to the skin for 48 hours under an occlusive patch. Patches were reapplied every 48 hours for a total of 10 induction applications. Following a 2-week nontreatment period, a challenge patch was applied. The reported number of patches applied to the 102 subjects totaled 1105. No evidence of skin irritation or sensitization was observed.[83,105]

One hundred human subjects were exposed in a skin irritation study to 5 percent p-Hydroxyanisole in sweet almond oil. The methods employed were those described by Fisher.[106] Occlusive patch test results were reported as "negative." No other details were provided.[83,105]

Three cosmetic products were tested for skin irritation according to the procedures described by Fisher.[106] Occlusive patches containing a moisturizing lotion (formulated with 0.1 percent p-Hydroxyanisole), a blusher (0.1 percent p-Hydroxyanisole), and a moisturizing cream (0.05 percent p-Hydroxyanisole) were applied to groups of 99, 100, and 100 human subjects, respectively. Results were reported as "negative." No other details were available.[84-86,105]

Occupational Exposure to Airborne Concentrations

A "threshold limit value" (TLV) for p-Hydroxyanisole of 5 mg/m^3 is recommended by the American Conference of Governmental Industrial Hygienists (1980). This recommendation is made on the basis of "eye and skin effects and by analogy with hydroquinone." The TLV represents the airborne concentration to which nearly all workers may be repeatedly exposed day after day without adverse effect (assuming an 8-hour workday or 40-hour work week). The TLV serves as a general guide in the control of health hazards in the work environment. As such, it should not be used to differentiate between safe and unsafe airborne concentrations.

SUMMARY

p-Hydroxyanisole is a waxy solid prepared by the reaction of hydroquinone with dimethylether. When used for cosmetic purposes, the compound typically has a purity of 99.5 percent. Impurities consist of hydroquinone dimethylether (about 0.1 percent) and an unidentified compound with a "high boiling point" (about 0.4 percent).

p-Hydroxyanisole has acidic properties characteristic of phenols. It binds by hydrogen bonding to itself, water molecules, and various proteins. The compound is readily oxidized and can undergo a variety of reactions, including alkylation, halogenation, and other substitutions on the aromatic nucleus. Peak absorbance of UV light by p-Hydroxyanisole occurs at about 340 nm.

Noncosmetic uses of p-Hydroxyanisole include applications as an antioxidant, as a polymerization inhibitor, as a chemical intermediate, and as a stabilizer. It is used in cosmetics as an antioxidant.

Data submitted to the FDA by cosmetic firms participating in the voluntary cosmetic registration program indicated that this antioxidant was used in 31 cosmetic products during 1981 at concentrations of >0.1 to 1.0 percent (8 products) and ≤0.1 percent (23 products). Cosmetic formulations containing this compound, such as eye makeup, sachets, makeup bases, and skin care preparations, are normally applied to or have the potential to come in contact with the skin and eyes.

Results of numerous studies indicated that p-Hydroxyanisole is a skin-depigmenting agent. Unpublished data strongly suggested that this cosmetic ingredient was a depigmenter of the skin at concentrations approximating those used in cosmetic products. Skin depigmentation was observed in guinea pigs exposed 6 weeks to 0.25 percent of the antioxidant and in guinea pigs exposed 6 months to 0.5 and 1.0 percent p-Hydroxyanisole. Exposure for 6 weeks to 0.1 percent produced depigmentation at the site of skin application in 1 of 6 guinea pigs. Associated with the skin-depigmenting action of this compound was a selective cytotoxic effect on the melanocyte. The melanocytotoxic effect was dependent upon both antioxidant concentration and duration of exposure. No cytotoxic effects on human melanocytes or morphological changes in human keratinocytes were observed following a 45-minute exposure to either 10^{-2} M or 10^{-3} M p-Hydroxyanisole in disperse tissue culture. However, whole epidermis (human) exposed in vitro to 10^{-1} M for 1, 5, and 24 hours had extensive damage to melanocytes and keratinocytes. Concentrations as low as 10^{-8} and 10^{-9} M were cytotoxic to guinea pig melanocytes in vitro. These latter concentrations are lower than p-Hydroxyanisole concentrations typically used in cosmetics.

p-Hydroxyanisole given orally to rats and mice caused induction and inhibition of various enzymes in the esophagus, nonglandular stomach, and microsomal fraction of the liver. In vitro studies with isolated rat liver suggested that the antioxidant interferes with ribonucleic acid synthesis, protein synthesis, and mitochondrial respiration. The compound inhibited growth or was microcidal in studies with bacteria and fungi. Chromosomal aberrations in plants and denaturation of DNA in bacteriophage were observed following p-Hydroxyanisole exposure.

p-Hydroxyanisole was absorbed by guinea pig skin in vitro. Oral doses of the antioxidant were excreted by rabbits primarily as conjugates of glucuronic and sulfuric acids; small amounts were demethylated and excreted as hydroquinone.

The acute oral LD_{50} of p-Hydroxyanisole in rats was estimated as 1630 mg/kg. The oral LD_{50} in rats of 50 percent p-Hydroxyanisole in corn oil was 740 mg/kg. The acute LD_{50} of the antioxidant when administered by intraperitoneal injection was 250 mg/kg and 430 mg/kg for mice, 730 mg/kg for rats, and 720 to 970 mg/kg for rabbits.

Undiluted p-Hydroxyanisole was a severe skin and ocular irritant in rabbits; a single exposure to the compound produced extensive skin edema and necrosis and corneal injury. Minimal irritation was observed in the eyes of rabbits exposed to a 0.1 percent aqueous solution of the antioxidant and on rabbit skin treated with 5 percent p-Hydroxyanisole in sweet almond oil. Skin sensitization to p-Hydroxyanisole (0.5 M and 1.0 M) was observed in guinea pigs in both the "maximization test" and the "Freund's complete adjuvant test." Cross skin sensitization of guinea pigs to hydroquinone (1 M) and p-Hydroxyanisole (3 M) was also reported. No photosensitization was observed in guinea pigs exposed to both p-Hydroxyanisole (0.1 and 1.0 percent) and UV irradiation.

Application of a water-oil emulsion containing 1.0 percent p-Hydroxyanisole to the skin of guinea pigs for 30 days produced hyperemia, edema, and desquamation. Skin irritation and depigmentation were observed in guinea pigs and mice treated for 4 weeks with 20 percent p-Hydroxyanisole in petroleum jelly and in guinea pigs treated 1 to 6 months with antioxidant concentrations of 0.25 M or 1.0 M in acetone, 0.5 M in dimethylsulfoxide, and 5.0 or 10.0 percent in hydrophilic ointment. Application of 20 percent p-Hydroxyanisole in lanolin base to guinea pig skin for up to 6 months and to hamster cheekpouch 3 times a week for 45 days caused encroachment of basal cell pseudopodia into the dermis. In addition, the hamster cheekpouch had erythema, hyperkeratosis, epithelial hyperplasia, bullae, and muscular degeneration. Rats and rabbits fed diets containing 5 and 10 percent p-Hydroxyanisole and dogs fed up to 12 g daily for 2 weeks had growth inhibition and changes in hematological parameters and organ weights; no other significant toxicological effects were noted.

p-Hydroxyanisole was nonmutagenic in the Ames assay with and without metabolic activation. No local toxic changes or tumors were observed following application of 5 and 10 percent p-Hydroxyanisole in acetone to the skin of mice and rabbits in a lifetime study. The antioxidant (13.1 percent in benzene) was inactive as a tumor promoter when applied for 20 weeks to the DMBA-initiated skin of mice. Application of a bleach cream containing 5 percent p-Hydroxyanisole and a water-oil emulsion containing 25 percent of the antioxidant to the skin of pregnant rats produced embryotoxicity but not teratogenicity.

In clinical studies, p-Hydroxyanisole at a concentration of 2.0 percent in petrolatum and 2.0 percent in sweet almond oil was, at most, minimally irritating to the skin. A 5.0 percent concentration of the antioxidant in sweet almond oil was both nonirritating and nonsensitizing to humans. Several cases were reported in the literature of individuals who developed skin depigmentation following exposure to products containing p-Hydroxyanisole or following occupational exposure to the antioxidant.

DISCUSSION

A few of the available studies on p-Hydroxyanisole were conducted in the 1940s and 1950s and were exploratory in nature. The Panel is aware that many of

these studies do not necessarily reflect current toxicological procedures. In other studies, details of methods, results, and dates of testing were not specified. As a result, the quantity and quality of available information for review was limited. However, it is the Panel's opinion that the sum total of available published and unpublished data support a concern as to the safety of p-Hydroxyanisole as a cosmetic ingredient.

Results of animal studies establish that p-Hydroxyanisole is a skin sensitizer (6.2 percent) and a skin depigmenter (0.25 percent). Results of studies with humans suggest that p-Hydroxyanisole is not a skin sensitizer (2.0 percent). Clinical data on skin depigmentation are inconclusive.

The function of p-Hydroxyanisole in cosmetics is that of an antioxidant. It is not intended for use as a skin lightener or skin-depigmenting agent. Because of the depigmenting action of this compound in black guinea pigs at reported concentrations approaching those used in cosmetics and because of in vitro toxicity to guinea pig melanocytes, p-Hydroxyanisole is an undesirable ingredient in cosmetic products.

CONCLUSION

Based on the available animal data, the CIR Expert Panel concludes that p-Hydroxyanisole is unsafe for use as a cosmetic ingredient.

ACKNOWLEDGMENT

Jonathon T. Busch, Senior Science Analyst and writer, prepared the literature review and technical analysis used by the Expert Panel in developing this report.

REFERENCES

1. ESTRIN, N.F., CROSLEY, P.A., and HAYNES, C.R. (1982). *CTFA Cosmetic Ingredient Dictionary*, 3rd ed. Washington, DC: Cosmetic, Toiletry and Fragrance Association, p. 127.
2. HAWLEY, G.G. (1971). *The Condensed Chemical Dictionary*, 8th ed. New York: Van Nostrand Reinhold.
3. WEAST, R.C. (1978). *CRC Handbook of Chemistry and Physics*, 59th ed. Boca Raton, FL: CRC Press.
4. SAX, N.I. (1979). *Dangerous Properties of Industrial Materials*, 5th ed. New York: Van Nostrand Reinhold, p. 732.
5. COSMETIC, TOILETRY AND FRAGRANCE ASSOCIATION (CTFA). (January 11, 1978). Submission of unpublished data by CTFA. Cosmetic ingredient chemical description: p-Hydroxyanisole.*
6. HAKE, C.L., and ROWE, V.K. (1963). Hydroquinone ethers, in: F.A. Patty (ed.). *Industrial Hygiene and Toxicology*, 2nd ed. New York: Interscience, Vol. 2, pp. 1686–90.
7. DEMERSEMAN, P., PLATZER, N., BACHELET, J.P., CHEUTIN, A., and ROYER, R. (1971). Relations of the structure and the physicochemical properties of alkylated benzene-type phenols. Bull. Soc. Chim. Fr. **1971**(1), 201–10.
8. HODGE, H.C., STERNER, J.H., MAYNARD, E.A., and THOMAS, J. (1949). Short-term toxicity tests on the mono- and di-methyl ethers of hydroquinone. J. Ind. Hyg. Toxicol. **31**(2), 79–92.

*Available upon request: Administrator, Cosmetic Ingredient Review, Suite 810, 1110 Vermont Avenue, N.W., Washington, DC 20005.

9. EASTMAN KODAK. (May, 1977). Industrial Chemicals Publ. No. D-106B, Hydroquinone Monomethyl Ether (HQMME).
10. HABBOUSH, A.E., and AMARIN, A.M. (1974). Separation of monosubstituted phenols by gas-liquid chromatography. Talanta **21**(9), 927–32.
11. AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS, INC. (ACGIH). (1980). *Documentation of the Threshold Limit Values*, 4th ed. Cincinnati, OH, p. 259.
12. VERMA, K.K., TRIPATHI, R.P., BAJAJ, I., PRAKASH, O., and PARIHAR, D.B. (1970). Studies on the synthesis of butylated hydroxyanisole. J. Chromatogr. **52**(3), 507–11.
13. GAKH, I.G., and GAIDAI, N.S. (1970). Determination of the vapor pressures of p-methoxyphenol, butylhydroxyanisole, and 2,5-dibutylhydroxyanisole. Zh. Prikl. Khim. Leningrad **43**(1), 212.
14. AIHARA, A. (1960). Estimation of the energy of hydrogen bonds formed in crystals. II. Phenols. Bull. Chem. Soc. Jpn. **33**(2), 194–200.
15. GHOSH, A., and PALIT, S.R. (1974). Relative activities of phenols in aprotic solvents. Indian J. Chem. **12**(4), 382–6.
16. HANSCH, C., KIEHS, K., and LAWRENCE, G.L. (1965). The role of substituents in the hydrophobic bonding of phenols by serum and mitochondrial proteins. J. Am. Chem. Soc. **87**(24), 5770–3.
17. WADA, S., TOMIOKA, S., and MORIGUCHI, I. (1969). Protein bindings. VI. Binding of phenols to bovine serum albumin. Chem. Pharm. Bull. **17**(2), 320–3.
18. KULIC, J., and VECERA, M. (1974). Kinetics of reaction of p-substituted phenols with bromine in water. Collect. Czech. Chem. Commun. **39**(1), 171–6.
19. ARGAUER, R.J. (1968). Rapid procedure for the chloroacetylation of microgram quantities of phenols and detection by electron-capture gas chromatography. Anal. Chem. **40**(1), 122–4.
20. COPPOLA, G. (1971). Gas-chromatographic method for determining hydroquinone monomethyl ether. Nuova Chim. **47**(1), 49–50.
21. LOUWERSE, H.L. (1968). Gas-liquid chromatographic separation of methyl- and methoxysubstituted benzenes, phenols, phenylacetates, and acetophenols. J. Gas Chromatogr. **6**(11), 563–6.
22. VAHESSAAR, V., KLESMENT, I., and EISEN, O. (1968). Comparative chromatography of phenols and their ethers. Eesti NSV Tead. Akad. Toim. Keem. Geol. **17**(2), 124–32.
23. SOCZEWINSKI, E., and SZUMILO, H. (1973). Investigations on the mechanism and selectivity of chromatography on thin layers of polyamide. I. Systems of the type cyclohexane + polar solvent–polyamide. J. Chromatogr. **81**(1), 99–107.
24. SMITH, R.V., ROSAZZA, J.P., and NELSON, R.A. (1974). Thin-layer chromatographic determination of simple phenols in microbial extracts. J. Chromatogr. **95**(2), j247–9.
25. TOUL, J., and MOUKOVA, N. (1972). Purity control of some o-hydroxy-substituted thiazolylazo dyes by thin-layer chromatography. J. Chromatogr. **67**(2), 355–42.
26. BROOK, A.J.W., and HOUSLEY, S. (1969). The interaction of phenols with Sephadex gels. J. Chromatogr. **41**(2), 200–4.
27. HENDRICKSON, J.G. (1968). Basic gel permeation chromatography studies. II. Benzene results with small molecules. J. Chromatogr. **32**(3), 543–58.
28. LAPITSKAYA, S.K., KOKHANOVSKAYA, M.G., and KHOMYAKOVA, L.K. (1974). Polarographic determination of hydroquinone methyl ethers. Obshch. Prikl. Khim. **6,** 63–4.
29. KARPOV, O.N., GAKH, I.G., and LYSYAK, V.Y. (1967). Quantitative oxidimetric determination of hydroquinone and its monomethyl ether when present together. Zh. Anal. Khim. **22**(3), 472–3.
30. FOOD AND DRUG ADMINISTRATION (FDA). (Dec. 22, 1981). Cosmetic product formulation data: Ingredient used in each product category. Computer printout. Washington, DC.
31. FDA. (Jan. 5, 1982). (a) Frequency of trade name ingredients in the cosmetic product file and (b) frequency of common, usual, or chemical names in the cosmetic product file. Computer printout. Washington, DC.
32. CODE OF FEDERAL REGULATIONS. (Revised as of April 1, 1983). Title 21 Part 720.4. Voluntary Filing of Cosmetic Product Ingredient and Cosmetic Raw Material Composition Statement. Information Requested about Cosmetic Products.
33. PATHAK, M.A. (November 27, 1983). Submission of unpublished data by CTFA. In vitro and in vivo effects of para-hydroxyanisole (PHA): A report on the acute and chronic toxicity studies of PHA on pigmented skin of guinea pigs and on melanocytes in culture.*
34. PATHAK, M.A., WICK, M., FARINELLI, W., and FITZPATRICK, T.B. (1979). Evaluation of azelaic acid on normal skin pigmentation and on the B-16 mouse melanoma. Paper presented at the Society for Dermatological Research Joint Meeting, RAI Congress Centre, Amsterdam, The Netherlands, June 10–14, 1979 (abstr.). J. Invest. Dermatol. **72,** 266–90.

35. GELLIN, G.A., MAIBACH, H.I., MISIASZEK, M.H., and RING, M. (1979). Detection of environmental depigmenting substances. Contact Dermatitis **5**(4), 201–13.
36. BRUN, R. (1959). Contribution a l'etude de la depigmentation experimentale (1st communication). Resultats preliminaries. Dermatologica **118,** 202–7.
37. BRUN, R. (1960). Zur experimentellen depigmentierung. J. Soc. Cosmet. Chem. **11,** 571–80.
38. BRUN, R. (1961). Contribution a l'etude, de la depigmentation experimentale. Bull. Inst. Genevois **61,** 3–89.
39. RILEY, P.A. (1969). Hydroxyanisole depigmentation: In vivo studies. J. Pathol. **97**(2), 185–91.
40. DURMISHEV, A. (1973). Experimental leukoderma induced by the monomethyl ether of hydroquinone. Dermatol. Venerol. (Bulg.) **12**(3), 172–7. English translation.
41. RILEY, P.A., and SEAL, P. (1974). The role of substituted anisoles in epidermal micro-invasion. J. Pathol. **114**(1), 1–7.
42. WOHLRAB, W., and ZAUMSEIL, R.P. (1976). Experimental depigmentation of the skin. Part II. Epidermal reaction by external application of depigmenting substances. Dermatol. Monatsschr. **162**(12), 980–5.
43. DURMISCHEV, A.L., and DAVIDOFF, M.S. (1976). Depigmentation effect of hydroquinone monomethyl ether. Dermatol. Monatsschr. **162**(4), 306–11. English translation.
44. RILEY, P.A., SAWYER, B., and WOLFF, M.A. (1975). The melanocytotoxic action of 4-hydroxyanisole. J. Invest. Dermatol. **64**(2), 86–9.
45. RILEY, P.A. (1969). Hydroxyanisole depigmentation: In vitro studies. J. Pathol. **97**(2), 193–206.
46. RILEY, P.A. (1970). Mechanism of pigment-cell toxicity produced by hydroxyanisole. J. Pathol. **101**(2), 163–9.
47. MORGAN, B.D., O'NEILL, T., DEWEY, D.L., GALPINE, A.R., and RILEY, P.A. (1981). Treatment of malignant melanoma by intravascular 4-hydroxyanisole. Clin. Oncol. **7**(3), 227–34.
48. SEAL, P., RILEY, P.A., and INMAN, D.R. (1969). Basal cell encroachment into the dermis caused by applications of hydroxyanisole. J. Invest. Dermatol. **52**(3), 264–7.
49. RILEY, P.A., and SEAL, P. (1968). Microinvasion of epidermis caused by substituted anisoles. Nature **220**(5170), 922–3.
50. RILEY, P.A., and SEAL, P. (1969). Intranuclear fibers produced by 4-hydroxyanisole. Exp. Mol. Pathol. **10**(1), 63–7.
51. WOODS, D.A., and SMITH, C.J. (1969). The effects of 4-hydroxyanisole on hamster cheek pouch. Exp. Mol. Pathol. **10**(2), 107–14.
52. GRASSO, P., and ROSTRON, C.C. (1970). Microinvasion of epidermis caused by substituted anisoles. Nature **225**(5228), 188–9.
53. BREATHNACH, A.S., DIALA, E.B., GALLAGHER, S.M., PORRO, M.N., and PASSI, S. (1981). Ultrastructural observations on the effect of 4-hydroxyanisole on normal human melanocytes in tissue culture. J. Invest. Dermatol. **77**(3), 292–6.
54. BREATHNACH, A., ROBINS, E., ETHRIDGE, L., BHASIN, Y., GALLAGHER, S., PASSI, S., and NAZZARO-PORRO, M. (1983). Ultrastructural and biochemical observations on the effect of 4-hydroxyanisole plus tyrosinase on normal human melanocytes and keratocytes in tissue culture. Br. J. Cancer **47**(6), 813–22.
55. BLEEHEN, S.S. (1976). Selective lethal effect of substituted phenols on cell cultures of malignant melanocytes. Pigm. Cell **2**(1), 108–15.
56. DEWEY, D.L., BUTCHER, F.W., and GALPINE, A.R. (1977). Hydroxyanisole-induced regression of the Harding-Passey melanoma in mice. J. Pathol. **122**(3), 117–27.
57. WEBSTER, D.J., WHITEHEAD, R.H., and HUGHES, L.E. (1981). Effect of 4HOA on melanoma. Br. J. Cancer **43**(5), 729. Meeting abstract.
58. WATTENBERG, L.W., COCCIA, J.B., and LAM, L.K.T. (1980). Inhibitory effect of phenolic compounds on benzo(a)pyrene-induced neoplasia. Cancer Res. **40** (8 Part 1), 2820–23.
59. WATTENBERG, L.W., BORCHERT, P., and COCCIA, J. (1982). Effects of p-methoxyphenol and dietary alteration on beta-propiolactone (BPL)-induced neoplasia of the mouse forestomach. Proc. Am. Assoc. Cancer Res. **23,** 342.
60. WATTENBERG, L.W., BORCHERT, P., DESTAFNEY, C.M., and COCCIA, J.B. (1983). Effects of p-methoxyphenol and diet on carcinogen-induced neoplasia of the mouse forestomach. Cancer Res. **43**(10), 4747–51.
61. SPARNINS, V.L., and WATTENBERG, L.W. (1981). Enhancement of glutathione S-transferase activity of the mouse forestomach by inhibitors of benzo(a)pyrene-induced neoplasia of the forestomach. J. Natl. Cancer Inst. **66**(4), 769–71.
62. SPARNINS, V.L., CHUAN, J., and WATTENBERG, L.W. (1982). Enhancement of glutathione S-transferase activity of the esophagus by phenols, lactones, and benzyl isothiocyanate. Cancer Res. **42**(4), 1205–7.

63. RAHIMTULA, A.D., JERNSTROM, B., DOCK, L., and MOLDEUS, P. (1982). Effects of dietary and in vitro 2(3)-t-butyl-4-hydroxyanisole and other phenols on hepatic enzyme activities in mice. Br. J. Cancer **45**(6), 935–44.
64. KOZUMBO, W.J., SEED, J.L., and KENSLER, T.W. (1983). Inhibition by 2(3)-tert-butyl-4-hydroxyanisole and other antioxidants of epidermal ornithine decarboxylase activity induced by 12-O-tetradecanoylphorbol-13-acetate. Cancer Res. **43**(6), 2555–9.
65. GILBERT, D., GOLBERG, L., and GANGOLLI, S.D. (1967). Induction of liver microsomal processing enzymes by substituted phenols. Biochem. J. **103**(1), 11P–12P.
66. MACHLEIDT, H., ROTH, S., and SEEMAN, P. (1972). The hydrophobic expansion of erythrocyte membranes by the phenol anesthetics. Biochim. Biophys. Acta **255**(1), 178–89.
67. ROTH, S., and SEEMAN, P. (1972). The membrane concentrations of neutral and positive anesthetics (alcohols, chlorpromazine, morphine) fit the Meyer-Overton rule of anesthesia; negative narcotics do not. Biochim. Biophys. Acta **255**(1), 207–19.
68. BURTON, D.E., CLARKE, K., and GRAY, G.W. (1964). The mechanism of the antibacterial action of phenols and salicylaldehydes. II. Substituted phenols. J. Chem. Soc. **1964,** 1314–8.
69. OWEN, R.G. (1953). Studies on the nature of fungicidal action. II. Chemical constitution of benzenoid and quinonoid compounds in relation to fungitoxicity and inhibition of amino- and sulfhydryl-dependent enzymes. Contrib. Boyce Thompson Inst. **17,** 273–82.
70. GOOTJES, J., ZWART VOORSPUIJ, A.J., and NAUTA, W. TH. (1953). Investigation into the tuberculostatic activity of some aromatic hydroxy compounds. Antonie van Leeuwenhoek J. Microbiol. Serol. **19**(3), 237–44.
71. KRAMER, P.E., ROBBINS, M.L., and SMITH, P.K. (1955). Phenolic compounds as chemotherapeutic agents against poliomyelitis virus tissue culture. J. Pharmacol. Exp. Ther. **113,** 262–71.
72. ROSEN, F., MILLMAN, N., and HOMM, R. (1955). Anti-gonadotropic activities of quinones and related compounds. Endocrinology **57,** 466–71.
73. PILOTTI, A., ANCKER, K., ARRHENIUS, E., and ENZELL, C. (1975). Effects of tobacco and tobacco smoke constituents on cell multiplication in vitro. Toxicology **5**(1), 49–62.
74. WYNN, J., and FORE, W.F. (1965). The effect of hindered phenols on mitochondrial oxidative phosphorylation. J. Biol. Chem. **240**(4), 1766–71.
75. LEVAN, A., and TJIO, J.H. (1948). Induction of chromosome fragmentation by phenols. Hereditas **34,** 453–84.
76. KAUL, B.L. (1979). Cytogenic activity of some common antioxidants and their interaction with x-rays. Mutation Res. **67**(3), 239–48.
77. LEVINE, L., GORDON, J.A., and JENCKS, W.P. (1963). The relationship of structure to the effectiveness of denaturing agents for deoxyribonucleic acid. Biochemistry **2,** 168–75.
78. BRAY, H.G., CRADDOCK, V.M., and THORPE, W.V. (1955). Metabolism of ethers in the rabbit. II. Nuclear-substituted anisoles. Biochem. J. **60,** 225–32.
79. ASTILL, B.D., FASSET, D.W., and ROUDABUSH, R.L. (1960). The metabolism of phenolic antioxidants. 2. The metabolism of butylated hydroxyanisole in the rat. Biochem. J. **75,** 543.
80. DOW CHEMICAL CO. (1959). Letter dated February 14, 1979 from Dr. K.J. Olson (Toxicology Research Laboratory, Midland, MI) to Mr. Ralph C. Wands (Director, Cosmetic Ingredient Review, Washington, DC) regarding studies conducted on p-Hydroxyanisole in 1959.
81. HAGAN, E.C. (1959). Acute toxicity: Appraisal of the safety of chemicals in foods, drugs and cosmetics. Compiled by the staff of the Div. of Pharmacology, Food and Drug Administration–Dept. of Health, Education and Welfare, pp. 17–25.
82. WEIL, C. (1952). Tables for convenient calculation of median-effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics **8**(3), 249.
83. CTFA. (No date). Submission of unpublished data by CTFA. Reference 1. Raw Material FCR/1006A. Acute oral toxicity; acute eye irritation; human patch test; human repeated insult patch test.*
84. CTFA. (No date). Submission of unpublished data by CTFA. Reference 2a. Moisturizing lotion containing 0.1 percent p-Hydroxyanisole. Code No. FCR-5/1006B. Acute oral toxicity; acute eye irritation; animal patch test; human patch test.*
85. CTFA. (No date). Submission of unpublished data by CTFA. Reference 2b. Moisturizing cream containing 0.05 percent p-Hydroxyanisole. Code No. FCR-5/1006E. Acute oral toxicity; acute eye irritation; animal patch test; human patch test.*
86. CTFA. (No date). Submission of unpublished data by CTFA. Reference 2c. Blusher containing 0.1 percent p-Hydroxyanisole. Code No. FCR 6/100F. Acute oral toxicity; acute eye irritation; animal patch test; human patch test.*

87. PLZAK, V., and DOULL, J. (February 1969). A further survey of compounds for radiation protection. USAF School of Aerospace Medicine. Aerospace Medical Division. Brooks Air Force Base, Texas. NTIS Document No. AD691-490.
88. STANLEY, H.R., BOWEN, R.L., and FOLIO, J. (1979). Compatibility of various materials with oral tissues. II. Pulp responses to composite ingredients. J. Dent. Res. **58**(5), 1507–17.
89. DRAIZE, J.H. (1959). Appraisal of the safety of chemicals in foods, drugs and cosmetics. Compiled by the staff of the Div. of Pharmacology, Food and Drug Administration–Dept. of Health, Education and Welfare, pp. 46–59.
90. EASTMAN KODAK. (1950). Letter dated February 13, 1979 from Dr. C.J. Terharr (Eastman Kodak: Toxicology Section, Health Safety and Human Factors Laboratory, Rochester, NY) to Mr. Ralph C. Wands (Director, Cosmetic Ingredient Review, Washington, DC) regarding unpublished studies conducted on p-Hydroxyanisole in 1950.
91. VAN DER WALLE, H.B., KLECAK, G., GELEICK, H., and BENSINK, T. (1982). Sensitizing potential of 14 monomethacrylates in the guinea pig. Contact Dermatitis **8**(4), 223–35.
92. VAN DER WALLE, H.B., DELBRESSINE, L.P.C., and SEUTTER, E. (1982). Concomitant sensitization to hydroquinone and p-methoxyphenol in the guinea pig: inhibitors in acrylic monomers. Contact Dermatitis **8**(3), 147–54.
93. CTFA. (1984). Submission of unpublished data by CTFA. p-Hydroxyanisole: Photocontact sensitization in guinea pigs.*
94. AKHABADZE, A.F., KOROLEVA, N.B., and KOVANOVA, E.K. (1981). Experimental study of substances used in cosmetics containing paragroups, applied to the skin. Vestn. Dermatol. Venerol. **6,** 23–7. English translation.
95. FLORIN, I., RUTBERG, L., CURVALL, M., and ENZELL, C.R. (1980). Screening of tobacco smoke constituents for mutagenicity using the Ames Test. Toxicology **18,** 219–32.
96. BARTSCH, H., MALAVEILLE, C., CAMUS, A.M., MARTEL-PLANCHE, G., BRUN, G., HAUTEFEUILL, A., SABADIE, N., BARBIN, A., KUROKI, T., DREVON, C., PICCOLI, C., and MONTESANO, R. (1980). Validation and comparative studies on 180 chemicals with *S. typhimurium* strains and V79 chinese hamster cells in the presence of various metabolizing systems. Mutation Res. **76,** 1–50.
97. BOUTWELL, R.K., and BOSCH, D.K. (1959). The tumor-promoting action of phenol and related compounds for mouse skin. Cancer Res. **19,** 413–24.
98. STENBACK, F. (1977). Local and systemic effects of commonly used cutaneous agents: Lifetime studies of 16 compounds in mice and rabbits. Acta Pharmacol. Toxicol. **41,** 417–31.
99. COLOMB, D. (1982). Confetti depigmentation following application of Leucodinine B on a chloasma (1982). Ann. Dermatol. Venereol. **109**(10), 899–900. English translation.
100. HIGASHI, N., and MATSUMURA, M. (1982). Three cases of occupational skin hazards. Skin Res. **24**(4), 561–6. English translation.
101. CHIVERS, C.P. (1972). Two cases of occupational leucoderma following contact with hydroquinone monomethyl ether. Br. J. Ind. Med. **29**(1), 105–7.
102. O'SULLIVAN, J.J., and STEVENSON, C.J. (1981). Screening for occupational vitiligo in workers exposed to hydroquinone monomethyl ether and to paratertiary-amyl-phenol. Br. J. Ind. Med. **38**(4), 381–4.
103. CTFA. (October 4, 1979). Submission of unpublished data by CTFA. Eight week progressive exposure for depigmentation. Two percent p-Hydroxyanisole in petrolatum and sweet almond oil. Code Nos. FE 1-67/A and FE 1/67B.*
104. CTFA. (1979). Submission of unpublished data by CTFA. Raw material FCR-6/1007A. Skin depigmentation study on 80 human subjects. Two percent p-Hydroxyanisole in sweet almond oil.*
105. CTFA. (No date). Submission of unpublished data by CTFA. p-Hydroxyanisole: Summary of unpublished safety data.*
106. FISHER, A.A. (1973). *Contact Dermatitis*, 2nd ed. Philadelphia: Lea & Febiger.

Cosmetic Ingredient Review
Commitment . . . Credibility
Since 1976

CIR

Memorandum

To: CIR Expert Panel

From: Director, CIR

Subject: New information relating to Methylisothiazolinone

Date February 22, 2013

It appears as if methyisothiazolinone (MIT) will be named "contact allergan of the year" for 2013. We don't have the article as it appears in *Dermatitis* just yet, but we will by the time of the meeting and will get it to you ASAP.

Our safety assessment of MIT focused on the issue of allergic reactions (see data1). MIT is used as a preservative in cosmetics and personal care products in reported concentrations up to 0.01%. Although recognizing that MIT was a sensitizer in both animal and human studies, the panel concluded that there is a threshold dose response and that cosmetic products formulated to contain concentrations of MIT at 100 ppm (0.0 1%) or less would not be expected to pose a sensitization risk. So, we set a limit of 100 ppm (0.01%) in formulation.

The concerns that prompted MIT's identification as allergan of the year acknowledged that the currently recommended "tray" of allergans for patch testing does currently include methylchloroisothiazolinone and methylisothiazolinone, in combination. Testing with methylchloroisothiazolinone and methylisothiazolinone, in combination, however, may not be adequate to pick up contact allergy to MIT alone.

The contention in the report in Dermatitis is that the combination of the two misses about 40% of allergy to MIT, likely due to the low concentration of MIT in the combination patch test. It further suggested that, in Europe, several groups have documented frequency of allergy to this preservative of about 1.5%.

Dr. Belsito will report on his own experience with MIT patch testing (the short version is that he's seeing more & more relevant +'s). Dr. Marks is not yet testing for MIT alone.

The Panel should examine the implications of these new data/concerns/clinical experience and determine if it is appropriate to begin the process to reopen the safety assessment of MIT.

1101 17th St., NW, Suite 412 Washington, DC 20036-4702
Phone 202.331.0651 FAX 202.331.0088 http://www.cir-safety.org

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

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

Final Report of the Safety Assessment of Methylisothiazolinone

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

Abstract

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

Keywords

methylisothiazolinone, safety, cosmetics

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

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

Chemistry

Definition and Structure

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

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

Physical and Chemical Properties

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

Method of Manufacture

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

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

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

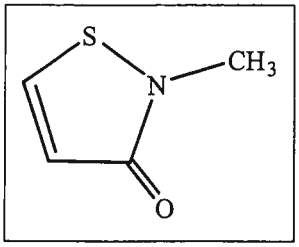


Figure 1. Methylisothiazolinone.

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

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

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

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

Analytical Methods

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

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

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

Impurities

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

Table 3. Composition of MIT Technical Grade[4]

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

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

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

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

Reactions

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

Use

Cosmetic

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

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

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

Table 5. Current Cosmetic Product Uses and Concentrations for Methylisothiazolinone

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

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

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

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

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

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

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

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

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

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

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

Noncosmetic

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

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

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

General Biology

Absorption, Distribution, Metabolism, Excretion

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

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

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

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

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

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

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

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

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

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

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

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

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

Animal Toxicology

Acute Toxicity

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

Acute Oral Toxicity

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

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

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

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

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

Table 6. Acute Toxicity of MIT in Rats and Mice

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Acute Dermal Toxicity

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

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

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

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

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

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

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

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

Acute Inhalation Toxicity

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

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

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

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

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

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

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

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

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

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

Subchronic Oral Toxicity

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Ocular Irritation

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

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

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

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

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

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

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

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

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

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

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

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

Dermal Irritation

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

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

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

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

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

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

Dermal Sensitization

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Local Lymph Node Assay

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

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

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

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

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

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

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

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

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

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

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

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

Phototoxicity

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

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

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

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

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

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

Reproductive and Developmental Toxicity

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Genotoxicity

Bacterial Assays

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

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

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

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

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

Mammalian Cell Assays

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

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

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

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

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

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

Animal Assays

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

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

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

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

Carcinogenicity

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

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

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

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

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

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

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

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

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

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

Neurotoxicity

In Vitro

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

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

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

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

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

In Vivo

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

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

Clinical Assessment of Safety

Dermal Irritation

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

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

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

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

Dermal Sensitization

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Phototoxicity

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

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

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

Case Reports

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

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

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

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

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

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

Margin of Safety

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

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

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

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

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

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

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

Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Discussion

In 1992, the CIR Expert Panel concluded that the mixture MIT/CMIT (23.3% MIT and 76.7% CMIT) may be safely used in rinse-off products at a concentration not to exceed 15 ppm and in leave-on cosmetic products at a concentration not to exceed 7.5 ppm. Currently, MIT is used as a standalone biocide. Accordingly, it was considered necessary to evaluate the safety of MIT alone.

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

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

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

Conclusion

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

Authors' Note

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

Declaration of Conflicting Interests

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

Funding

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

References

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

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

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

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

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

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

Cosmetic Ingredient Review
Commitment . . . Credibility
Since 1976

CIR

Memorandum

To: CIR Expert Panel Members and Liaisons

From: Alan Andersen, Director, CIR

Date: February 22, 2013

Subject: Re-Review Summaries

At the December 2012 meeting, the Panel determined to not re-open the safety assessments of 2-amino-6-chloro-4-nitrophenol, phthalates, and phenylenediamines.

The attached re-review summaries are included for your review and approval.

1101 17th St., NW, Suite 412 Washington, DC 20036-4702
Phone 202.331.0651 FAX 202.331.0088 http://www.cir-safety.org

2-Amino-6-Chloro-2-Nitrophenol

CONCLUSION: In 1997, the Cosmetic Ingredient Review (CIR) Expert Panel concluded that 2-amino-6-chloro-4-nitrophenol and its hydrochloride salt are safe for use in hair dye formulations at concentrations up to 2.0%.[1] The Expert Panel reviewed newly available studies since that assessment, along with updated frequency and concentration of use information.[2-8]

DISCUSSION: The Panel reviewed new toxicokinetics, genotoxicity, skin sensitization, phototoxicity, and photo-allergenicity studies, and a new margin of safety calculation, that were published in the opinion on 2-amino-6-chloro-4-nitrophenol released by the European Commission's Scientific Committee on Consumer Products (SCCP) in 2006.[2] The SCCP concluded that a maximum use concentration of 2% in the finished product does not pose a risk to the health of the consumer, although the SCCP did note that this ingredient is a known sensitizer. According to the European Commission Health and Consumers Cosmetics CosIng database, 2-amino-6-chloro-4-nitro-phenol has a maximum authorized concentration of 2.0% in non-oxidative hair dye products. In oxidative hair dye products, the maximum concentration applied to hair after mixing under oxidative conditions must not exceed 2.0%.[3] Appropriate labeling must be used.

The maximum use concentrations of 1.5% % in hair dyes and colors (all types requiring caution statements and patch tests), and 0.4% in coloring hair rinses, reported in response to a Personal Care Products Council survey[7] are lower than the maximum concentration allowed in the original CIR safety assessment[1] and that allowed by the European Commission.[7] As indicated by Voluntary Cosmetic Registration Program data obtained from the Food and Drug Administration, frequency of use of the hair dye has increased. In 2012, 2-amino-6-chloro-4-nitrophenol was reported to be used in 62 hair dyes and colors requiring caution statements, although no uses were reported for the hydrochloride salt;.[5] the 1997 assessment reported that 2-amino-6-chloro-4-nitrophenol was not reported to be used and 2-amino-6-chloro-4-nitrophenol hydrochloride was used in a total of 15 hair-coloring products.[1]

The Panel did note that although carcinogenicity data were not available, 2-amino-6-chloro-4-nitrophenol is not significantly absorbed through the skin and it is not genotoxic. Therefore, the Panel did not think a request for carcinogenicity data was necessary.

Recent concern has been expressed in Europe regarding the potential induction of sensitization that may result from the currently recommended self-test procedure for hair dyes.[9-11] The CIR Expert Panel noted that hair dyes containing coal tar derivatives are exempt from certain adulteration and color additive provisions of the U.S. Federal Food, Drug, and Cosmetic Act when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Panel agreed that there was not a sufficient basis for changing this advice to consumers at this time.

In considering hair dye epidemiology data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer or other toxicologic endpoints, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, while not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies. A detailed summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/cir-findings.

References

1. Andersen FA (ed). Final report on the safety assessment of 2-amino-6-chloro-4-nitrophenol and 2-amino-6-chloro-4-nitrophenol chloride. *Int J Toxicol*. 1997;16(Suppl 1):131-143.

2. European Commission. Scientific Committee on Consumer Products (SCCP) opinion on 2-Amino-6-chloro-4-nitrophenol. COLIPA Nº B99. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_047.pdf. Date Accessed 8-27-2012.

3. European Commission. European Commission Health and Consumers Cosmetics - CosIng - Database. http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=88714. Date Accessed 10-18-2012.

4. European Commission. 2-Amino-6-chloro-4-nitrophenol (CAS No 6358-09-4) (EC No. 228-762-1). Cosmetic Directive (v.1) 2011/59/EU. Date Accessed 8-27-2012.

5. Food and Drug Administration (FDA). Frequency of use of cosmetic ingredients. *FDA Database.* 2012. Washington, DC: FDA.

6. National Industrial Chemicals Notification and Assessment Scheme (NICNAS). List of chemicals used as dyes in permanent and semi-permanent hair dyes in Australia. http://www.nicnas.gov.au/Publications/Information_Sheets/Existing_Chemical_Information_Sheets/Hair_Dyes_list_Feb_07_PDF.pdf. Date Accessed 8-28-2012.

7. Personal Care Products Council. 2012. Concentration of Use by FDA Product Category: 2-Amino-6-Chloro-4-Nitrophenol.

8. Scientific Committee on Consumer Products (SCCP). Memorandum on hair dye substances and their skin sensitising properties. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_s_05.pdf. Date Accessed 9-27-2012.

9. Goossens A. Self-testing for contact sensitization. *Contact Dermatitis*. 2012;66:299.

10. Thyssen JP, Søsted H, Uter W, Schnuch A, Giménez-Arnau AM, Vigan M, Rustemeyer T, Granum B, McFadden J, White JM, White IR, Goossens A, Menné T, Lidén C, and Johansen JC. Self-testing for contact sensitization to hair dyes - scientific considerations and clinical concerns of an industry-led screening programme. *Contact Dermatitis*. 2012;66:300-311.

11. Yazar K, Boman A, and Liden C. Potent skin sensitizers in oxidative hair dye products on the Swedish market. *Contact Dermatitis*. 2009;61(5):269-275.

m-Phenylenediamine and m-Phenylenediamine Sulfate

CONCLUSION: In the 1997 safety assessment of m-phenylenediamine and m-phenylenediamine sulfate, the Cosmetic Ingredient Review (CIR) Expert Panel stated that these ingredients are safe for use in hair dyes at concentrations up to 10%.[1] The Expert Panel reviewed newly available studies since that assessment along with updated frequency and concentration of use information.[5,8,9,11-28] The Expert Panel determined to not reopen this safety assessment and confirmed the original conclusion of m-phenylenediamine and m-phenylenediamine sulfate.

DISCUSSION: The Expert Panel reviewed mostly genotoxicity data and, also, limited skin sensitization and cross-sensitization data. The Panel noted that, according to the European Union Cosmetics Directive, m-phenylenediamine and its salts are among the substances that must not form part of the composition of cosmetic products marketed in the European Union. The Personal Care Products Council explained that this is a natural consequence of an industry decision to not support the safety of m-phenylenediamine and m-phenylenediamine sulfate as hair dye ingredients in Europe. The Panel acknowledged that the 10% concentration limit is higher than the maximum use concentration recently provided by the cosmetics industry, from 0.01% to 0.2% for m-phenylenediamine and 1% for m-phenylenediamine sulfate. However, the Expert Panel noted that the 10% limit was based on skin irritation and sensitization test data and does not need to be changed.

Table 1. Historic and current uses and concentrations of m-Phenylenediamine and m-Phenylenediamine Sulfate.[1,8,9]

	# of Uses		*Max. Conc. of Use (%)*	
	m-Phenylenediamine and m-Phenylenediamine Sulfate*			
data year	*1995*	*2012*	*1995*	*2012*
Totals	**162(P) 28(S)**	**46(P) 19(S)**	**3(P) 3(S)**	**0.01-0.2(P) 1(S)**
Duration of Use				
Leave-On	*NR*	*NR*	*NR*	*NR*
Rinse Off	*162(P) 28(S)*	*46(P) 19(S)*	*3(P) 3(S)*	*0.01-0.2(P) 1(S)*
Diluted for(bath) use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation - Sprays	NR	NR	NR	NR
Incidental Inhalation – Powders	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR
Hair-Coloring	162(P) 28(S)	46(P) 19(S)	3(P) 3(S)	0.01-0.2(P) 1(S)
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR

*(P) = m-Phenylenediamine; (S) = m-Phenylenediamine Sulfate
NR = Not Reported; Totals = Rinse-off + Leave-on Product Uses
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

References

1. Andersen, F. A. Final report on the safety assessment of m-phenylenediamine and m-phenylenediamine sulfate. *International Journal of Toxicology*. 1997;16(SUPPL):59-115.

2. Thyssen, J. P. Søsted H. Uter W. Schnuch A Giménez-Arnau Vigan M. Rustemeyer T. Granum B. McFadden J. White J. M. White I. R. Goossens A. Menné T. Lidén and Johansen J. D. Self-testing for contact sensitization to hair dyes - scientific considerations and clinical concerns of an industry-led screening programme. *Contact Dermatitis*. 2012;66:300-311.

3. Goossens, A. Editorial: Self-testing for contact sensitization to hair dyes. *Contact Dermatitis*. 2012;66:299.

4. Gottschalck, T. E. and Breslawec, H. P. International Cosmetic Ingredient Dictionary and Handbook. 14 *ed*. Washington, DC: Personal Care Products Council, 2012.

5. Rosenkranz, M., Rosenkranz, H. S., and Klopman, G. Intercellular communication, tumor promotion and non-genotoxic carcinogenesis: relationships based upon structural considerations. *Mutat Res*. 1997;381(2):171-188.

6. Barnowska, I. Pieszko C. Raróg D. and Pielesz A. Analysis of some aromatic amines by means of derivative spectrophotometry. *Journal of Environmental Science and Health*. 2002;A37(10):1841-1848.

7. Liu, Y. Wei Z. and Zhang J. Synthesis of m-phenylenediamine from m-dinitrobenzene over silica-supported nickel catalyst. *Korean J.Chem.Eng.* 2006;23(6):902-907.

8. Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2012. Washington, D.C.: FDA.

9. Personal Care Products Council. Concentration of use by FDA product category. m-Phenylenediamine and m-Phenylenediamine Sulfate. Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 2012.

10. European Union. Consolidated version of Cosmetic Directive 76/768/EEC, as amended, Annexes I through IX. 2010. http://ec.europa.eu/enterprise/sectors/cosmetics/documents/directive/#h2-technical-adaptations-to-be-incorporated-in-the-consolidated-text.Date Accessed 10-15-2010

11. Matsukawa, K., Masuda, K., Kakishima, H., Suzuki, K., Nakagawa, Y., Matsushige, C., Imanishi, Y., Nakamura, T., Mizutani, A., Watanabe, R., Shingai, T., Kaneko, T., Hirose, A., and Ohno, Y. Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (11) EYTEXTM. *Toxicology in Vitro*. 1999;13(1):209-217.

12. Cho, S-A An S. Lee E. Shin K. Cho J-C. and Lee T. R. A new cell-based method for assessing the eye irritation potential of chemicals: An alternative to the Draize test. *Toxicology Letters*. 2012;212(2):198-204.

13. Kalish, R. S. and Wood, J. A. Sensitization of mice to paraphenylenediamine and structurally-related compounds: Adjuvant effects of vitamin a supplementation. *Contact Dermatitis*. 1995;33(6):407-413.

14. Li, Q., Inagaki, H., and Minami, M. Evaluation of cross-sensitization among dye-intermediate agents using a modified lymphocyte transformation test. *Archives of Toxicology*. 1996;70(7):414-419.

15. Ashby, J., Basketter, D. A., Paton, D., and Kimber, I. Structure activity relationships in skin sensitization using the murine local lymph node assay. *Toxicology*. 1995;103(3):177-194.

16. Søsted, H. Basketter D. A. Estrada E. Johansen J. D. and Patlewicz G. Y. Ranking of hair dye substances according to predicted sensitization potency: quantitative structure-activity relationships. *Contact Dermatitis*. 2004;51(5/6):241-254.

17. Roncaglioni, A. Piclin N. Pintore M. and Benfenati E. Binary classification models for endocrine disrupter effects mediated through the estrogen receptor. *SAR and QSAR in Environmental Research*. 2008;19(7-8):697-733.

18. Mouse bone marrow micronucleus assay of meta-phenylenediamine with attachment and cover letter dated 011491. *Epa/Ots.* Doc.

19. Chung, K.-T., Murdock, C. A., Stevens, S. E., Jr., Li, Y.-S., Wei, C.-I., Huang, T.-S., and Chou, M. W. Mutagenicity and toxicity studies of p-phenylenediamine and its derivatives. *Toxicology Letters (Shannon).* 1995;81(1):23-32.

20. Plewa, M. J., Wagner, E. D., Yu, T.-W., and Anderson, D. Genotoxicity of m-Phenylenediamine and 2-aminofluorene in Salmonella typhimurium and human lymphocytes with and without plant activation. *Environmental and Molecular Mutagenesis*. 1995;26(2):171-177.

21. Assmann, N., Emmrich, M., Kampf, G., and Kaiser, M. Genotoxic activity of important nitrobenzenes and nitroanilines in the Ames test and their structure-activity relationship. *Mutation Research*. 1997;395(2-3):139-144.

22. Gichner, T., Stavreva, D. A., Ceãrovskäa, N., Wagner, E. D., and Plewa, M. J. Metabolic activation of m-phenylenediamine to products mutagenic in Salmonella typhimurium by medium isolated from tobacco suspension cell cultures. *Mutat Res*. 1995;331(1):127-132.

23. Wagner, E. D., Smith, S. R., Xin, H., and Plewa, M. J. Comparative mutagenicity of plant-activated aromatic amines using Salmonella strains with different acetyltransferase activities. *Environmental and Molecular Mutagenesis*. 1994;23(1):64-69.

24. Stavreva, D. A., Wagner, E. D., Plewa, M. J., and Gichner, T. Characterization of a macromolecular matrix isolated from tobacco suspension cell cultures and its role in the activation of promutagenic m-phenylenediamine. *Mutation Research*. 1997;379(2):191-199.

25. Plewa, M. J., Wagner, E. D., Stavreva, D. A., and Gichner, T. Plant activation and its role in environmental mutagenesis and antimutagenesis. *Mutation Research*. 1996;350(1):163-171.

26. Vlcek, D., Miadokova, E., Vlckova, V., Slaninova, M., and Podstavkova, S. Metabolic activation of meta-phenylenediamine by the alga Chlamydomonas reinhardtii. *Mutation Research*. 1997;391(3):143-151.

27. Chen, F. Murata M. Hiraku Y. Yamashita N. Oikawa S. and Kawanishi S. DNA damage induced by m-phenylenediamine and its derivative in the presence of copper ion. *Free Radic.Res.* 1998;29(3):197-205.

28. Budunova, I. G. and Williams G. M. Cell culture assays for chemicals with tumor-promoting or tumor-inhibiting activity based on the modulation of intercellular communication. *Cell Biol.Toxicol.* 1994;10:71-116.

29. Mombelli, E. and Devillers J. Evaluation of the OECD (Q) SAR application toolbox and toxtree for predicting and profiling the carcinogenic potential of chemicals. *SAR and QSAR Environmental Research.* 2010;21(7-8):731-752.

Dimethyl, Diethyl, and Diethyl Phthalate, and Butyl Benzyl Phthalate

CONCLUSION: In 1985, the CIR Expert Panel concluded dibutyl, diethyl, and dimethyl phthalate are safe for use in cosmetics in the present practice of use and concentration. An extensive re-review was done in 2005 and the Panel determined to not reopen that report. In 1992, butyl benzyl phthalate was found safe in the present practice of use and concentration. A re-review of that safety assessment done in 2007 confirmed that conclusion. The CIR Expert Panel now has reviewed 3 studies on phthalates published in 2012 and determined to not reopen the safety assessments of dimethyl, diethyl, or diethyl phthalate, and butyl benzyl phthalate. The conclusion for these ingredients remains that these ingredients are safe in cosmetics in the present practices of use and concentration.

DISCUSSION: Since these original safety assessments were made, the focus of new phthalate studies has been on the potential for endocrine disruption/reproductive and developmental toxicity.

One new study of children aged 5 to 9, who were part of a Manhattan-Bronx cohort, revealed detectable, although varied, levels of phthalates in the urine of all 244 study participants.[1] Higher levels of both diethyl phthalate and butyl benzyl phthalate were associated with airway inflammation.

Two new studies addressed diabetes and phthalates. Subjects in one study were 1,015 men and women 70 years of age in Uppsala, Sweden.[2] The samples – one sample per subject – were collected in 2001 – 2004 and analyzed 5 – 8 years later. The four phthalates that were the focus of the study included dimethyl phthalate, diethyl phthalate, diisobutyl phthalate, and diethylhexyl phthalate measured in blood and correlated to measures of insulin resistance and poor insulin secretion in non-diabetic subjects.

In the second diabetes and phthalates study, urinary concentrations of phthalate metabolites measured by the CDC and self-reported diabetes in 2,350 women ages 20 to <80 participating in the NHANES (2001- 2008) were used.[3] The odds ratio for diabetes in women with higher levels of n-butyl phthalate, isobutyl phthalate, benzyl phthalate, 3-carboxypropyl phthalate, and the sum of diethylhexyl phthalate metabolites was greater than the odds ratio for women with the lowest concentrations of these phthalates.

The Panel noted that all of these studies identified associations between phthalate metabolites and either diabetes or airway inflammation. Such studies did not suggest a causal link between phthalates and any adverse outcome. The possibility that phthalate metabolites may impact peroxisome proliferation pathways was suggested in the diabetes studies, but that mechanism is not established as a mode of action. The Panel agreed that that there is a need for further study of the reported association between phthalates exposures and diabetes and to investigate possible causal links.

REFERENCES:

1. Just AC, Whyatt RM, Miller RL, Rundle AG, Chen Q, Calafat AM, Divjan A, Rosa MJ, Zhang H, Perera FP, Goldstein IF, Perzanowski MS. 2012. Children's urinary phthalate metabolites and fractional exhaled nitric oxide in an urban cohort. Am J Respir Crit Care Med [Online 23 August 2012].
2. Lind, P., Zethelius, B., and Lind, L. 2012. Circulating Levels of Phthalate Metabolites Are Associated With Prevalent Diabetes in the Elderly. Diabetes Care [online April 12, 2012].
3. Tamarra James-Todd, Richard Stahlhut, John D. Meeker, Sheena-Gail Powell, Russ Hauser, Tianyi Huang, Janet Rich-Edwards. Urinary Phthalate Metabolite Concentrations and Diabetes among

Women in the National Health and Nutrition Examination Survey (NHANES) 2001-2008. Environmental Health Perspectives [on line July 13, 2012].