Supplement

Achillea

Alkyl Amides

Chamomile

Formaldehyde

Methyl Glucose

PVP

Retinol

Tromethamine

Priorities

CIR EXPERT PANEL MEETING
JUNE 10-11, 2013

Cosmetic Ingredient Review

Commitment & Credibility since 1976

May 31, 2013

MEMORANDOM

To: CIR Expert Panel and Liaisons

From: Lillian C. Becker, M.S.
Scientific Analyst and Writer

Subject: Tentative Amended Safety Assessment of Achillea Millefolium (Yarrow)-Derived Ingredients As Used In Cosmetics

Additional information has been submitted by the Personal Care Products Council. The information is summarized and/or addressed below. Copies of both of the papers discussed will be available at the June Panel meeting.

1) An HRIPT (n = 53) of a body lotion containing achillea millefolium extract (0.04%), it was concluded that the body lotion was neither irritating nor sensitizing. This would appear to suggest that a concentration limit of 0.04% might be established.

2) While there were only limited UV absorption data, no phototoxicity data were available. The Council suggests including additional information from a paper that was included in the 2001 safety assessment of achillea millefolium extract and discusses the potential phototoxicity of material from Compositae species.

3) In the team discussions, the Panel appeared to suggest that LLNA assays are not considered reliable when testing mixtures (as plant extracts are) and can only be used as supporting evidence, not primary. Based on a paper by Lalko and Api (2006), and consultation with Dr. Api, the Council notes that the results of an LLNA of an essential oil “with known composition” containing at least 80% of a single component will “reflect” that component.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

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

DATE: May 21, 2013

SUBJECT: Additional Information *Achillea millefolium*

1. Anonymous. 2007. HRIPT on a body lotion containing 0.04% Achillea Millefolium Extract.

2. Photosensitization: Although the Hausen et al. (1991) paper (attached) was cited in the original CIR report, the CIR report does not include these investigators observation concerning the photosensitization potential of *Achillea millefolium*. In the Discussion of the Hausen et al. (1991) paper it states: "In the past 5 years we have also routinely irradiated the patch test sites of patients suffering from airborne contact dermatitis [including some subjects shown to be sensitive to yarrow] and proven Compositae contact sensitivity with UV light (5-10 J/cm^2, depending on the minimum erythema dose of the individual). During this period we have never seen any exacerbation of the yarrow extract test site." This observation might help alleviate the CIR Expert Panel's concern regarding the photosensitization potential of yarrow.

   Hausen BM, Breuer J, Weglewski J, et al. 1991. α-Peroxyachifolid and other new sensitizing sesquiterpene lactones from yarrow (Achillea millefolium L., Compositae). Contact Dermatitis 24: 274-280.

3. LLNAs for Mixtures: Dr. Anne Marie Api, RIFM was consulted regarding the use of LLNAs for mixtures. She indicated that for essential oils with known composition that contain about 80% of one component, LLNAs will reflect the main individual component. She provided the following paper:

   Lalko J, Api AM. 2006. Investigation of the dermal sensitization potential of various essential oils in the local lymph node assay. Fd Chem Toxicol 44: 739-746.

   The aqueous extract of yarrow that was tested in an LLNA (negative) was composed of 73.5% Water, 20% Butylene Glycol, 5% Pentylene Glycol, 1% Achillea Millefolium Extract and 0.5% Xanthan Gum.

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PRODUCT INTEGRITY USE ONLY
Screened:
To Manufacturer:
Formulated:

CLINICAL STUDY SUMMARY

TEST MATERIAL IDENTIFICATION

No.	ITEM CODE	LAB. FORMULA NUMBER	C No.	PROPOSED DESCRIPTION	QUANTITY	TEST LAB. SAMPLE ID
1	NA	NA	C2267	A body lotion that contains 0.04% Achillea Millefolium Extract	8-oz	na

CONTRACT RESEARCH LABORATORY (CRL) INFORMATION

CRL: Not Applicable

Study/Panel No: (if any) 2240

Completion Date: 14 June 2007

Report Date: 13 July 2007

Prepared by:

Product Integrity & Date

STUDY TYPE	RESULT
STANDARD: Allergenic (HRIPT)	Supported

STUDY PANEL DEMOGRAPHIC SUMMARY

Total Number of Subjects Enrolled: 69

Number of Subjects Completed the Study: 53 (77%)

Panel Gender	Mix Gender	Age Range	18 to 65, Mean 39.8 ±13.3
Skin Sensitivity	Not Specified	Contact Lens Users	Not Applicable

ADVERSE EVENT(S) SUMMARY: NONE

ADDITIONAL INFORMATION: SEE BELOW AND FOLLOWING PAGE(S)

THE STUDY DIRECTOR CONCLUDED THAT UNDER THE CONDITIONS OF A HUMAN REPEATED INSULT PATCH TEST PROCEDURE (MODIFIED DRAIZE; OCCLUSIVE PATCH CONDITIONS), TEST ARTICLE [A BODY LOTION THAT CONTAINS 0.04% OF ACHILLEA MILLEFORLIUM EXTRACT] PRODUCED TRANSIENT, BARELY PERCEPTIBLE (0.5-LEVEL) TO MILD (1-LEVEL) SPECIFIC AND NON-SPECIFIC PATCH TEST RESPONSES ON SIX (6/53) TEST SUBJECTS DURING THE INDUCTION AND/OR CHALLENGE PHASES OF THE STUDY. THE SKIN REACTIVITY OBSERVED WAS CONSIDERED NEITHER EVIDENCE OF CLINICALLY MEANINGFUL IRRITATION NOR ALLERGIC IN NATURE.

Not Applcable

Research & Development Date

Regulatory Affairs Date

[redacted]

[CLINICAL STUDY SUMMARY – ADDITIONAL PAGE(S)]

Data (page 1 of 2):

Subjects' Individual Scores

Subj. No.	Subj. Init.	Subj. Age	Subj. Gender	Induction Exposure No. 1	2	3	4	5	6	7	8	9	Challenge Reading (Hrs) 24	72
1*	SW	44	Female	0	0	0	0	0	0	0	0	0	0	0
3	RH	27	Female	0	0	0	0	0	0	0	0	0	0	0
4	VR	38	Male	0	0	0	0	0	0	0	0	0	0	0
5	JS	35	Male	0	0	0	0	0	0	0	0	0	0	0
6	JJ	38	Female	0	0	0	0	0	0	0	0	0	0	0
7	TG	63	Female	0	0	0	0	0	0	0	0	0	0	0
8	SH	46	Female	0	0	0	0	0	0	0	0	0	0	0
9	DL	42	Female	0	0	0	0	0	0	0	0	0	0	0
10	AT	31	Female	0	0	0	0	0	0	0	0	0	0	0
11	MD	47	Female	0	0	0	0	0	0	0	0	0	0	0
12	VO	46	Male	0	0	0	0	0	0	0	0	0	0	0
13	BJ	43	Male	0	0	0	0	0	0	0	0	0	0	0
14	SS	27	Male	0	0	0	0	0	0	0	0	0	0	0
15	MG	31	Male	0	0	0	0	0	0	0	0	0	0	0
16	JW	65	Female	0	0	0	0	0	0	0	0	0	0	0
17	RB	63	Male	0	0	0	0	0	0	0	0	0	0	0
18	TC	51	Female	0	0	0	0	0	0	0	0	0	0	0
19	OC	50	Female	0	0	0	0	0	0	0	0	0.5	0	0
20	ER	44	Female	0	0	0	0	0	0	0	0	0	0	0
21	CG	25	Female	0	0	0	0	0	0	0	0	0	0	0
22	BB	57	Female	0	0	0	0	0	0	0	0	0	0	0
23	JG	20	Male	Disc										
24	EG	50	Female	0	0	0	0	0	0	0	0	0	0	0
25	JD	44	Female	0	Disc									
26	SV	50	Female	0	0	0	0	0	0	0	0	0	0	0
27	AR	25	Female	0	0	0	0	0	Disc					
28	DR	57	Female	0	0	0	0	0	0	0	0	0	0	0
29	SK	32	Female	0	0	0	0	0	0	0	0	0	0	0
30	RM	52	Female	0	0	0	0	0	0	0	0	0	0	0
31	DF	54	Male	0	0	0	0	0	0	0	0	0	0	0
32	KG	53	Female	0	0	0	0	0	0	0	0	0	0	Disc
33	LD	39	Female	Disc										
34	BM	46	Female	0	0	0	0	0	0	Disc				
35	PG	56	Female	0	0	0	0	Disc						
36	TR	52	Female	0	0	0	0	0	0	0	0	0	0	0

Scale: 0 = No evidence of any effect
0.5 = Barely Perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright-red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

Disc = Discontinued
*Subject No. 2 inadvertently not used.

Data (Page 2 of 2):

Subj. No.	Subj. Init.	Subj. Age	Subj. Gender	Induction Exposure No. 1	2	3	4	5	6	7	8	9	Challenge Reading (Hrs) 24	72
37	JF	20	Male	0	0	0	Disc							
38	CH	47	Female	0	0	0	0	0	0	0	0	0	0	0
39	MS	49	Female	0.5	0.5	0.5	0	0	0	0	0	0	1e	0.5
40**	RW	31	Male	0	0	0	0	0	0	0	0	0	0	0
42	LM	28	Female	0	0	0	0	0	0	0	0	0	0	0
43	LH	19	Female	0	0	0	0	0	0	0	0	0	0	0
44	CM	45	Female	0	0	0	0	0	0	0	0	0	0	0
45	KH	45	Female	0	0	0	0	0	0	0	0	0	0	0
46	VV	25	Female	0	0	0	0	0	0	0	0	0	0	0
47	HC	22	Female	0	0	0	0	0	0	0	0	0	0	0
48	CJ	40	Female	0	0	0	0	0	0	0	0	0	0	0
49	LM	21	Female	0	0	Disc								
50	KP	24	Female	0	0	Disc								
51	RK	20	Male	0	0	0	0.5	Disc						
52	TW	41	Female	0	0	0	0	0	0	0	0	0	0	0
53	CL	56	Female	0	0	0	0	0	0	0	0	0	0.5	0
54	VD	45	Female	0	0	0	0	0	0	0	0	0	0.5	0
55	JR	49	Female	0	0	0	0	0	0.5	0	0	0	0	0
56	OA	45	Female	0	0	0	0	0	0	0	0	0	0	0
57	BG	18	Female	0	0	0	0	0	0	0	0	0	0	0
58	DS	50	Female	0	0	0	0	0	0	0	0	0	0	0
59	JP	24	Female	0	0	Disc								
60	DB	51	Male	0	0	0	0	0	0	0	0	0	0	0
61	RC	18	Female	0	0	0.5	0	0	0	0	0	0	0	0
62	TA	48	Female	0	0	0	0	0	0	0	0	0	0	0
63	KV	55	Female	0	0	0	0	0	0	0	0	0	0	0
64	SR	24	Female	0	Disc									
65	SB	29	Female	0	Disc									
66	NR	19	Male	0	0	0	0	0	0	0	0	0	0	0
67	PW	44	Female	0	0	0	0	0	0	0	0	0	0	0
68	AH	57	Male	0	0	0	0	0	0	0	0	0	0	0
69	HS	19	Female	0	Disc									
70	CM	50	Female	0	0	0	0	0	0	0	0	0	0	0
71	HS	29	Male	0	0	0	Disc							

Scale: 0 = No evidence of any effect
0.5 = Barely Perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright-red erythema with/without petechiae or papules)
4 = Severe (Deep-red erythema with/without vesiculation or weeping)

Disc = Discontinued
e = Mild edema
**Subject No. 41 inadvertently not used.

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett
Scientific Writer/Analyst
Date: May 31, 2013
Subject: Wave 2 for Amino Acid Alkyl Amides

The Council has provided an updated concentration of use survey for Amino Acid Alkyl Amides. The ingredients surveyed (lauroyl proline, palmitoyl proline, potassium caproyl tyrosine, potassium cocoyl rice amino acids, sodium caproyl prolinate, and sodium cocoyl barley amino acids) were not included in the earlier survey. As you can see from the data, only palmitoyl proline has use concentration data.

Additionally, we have received method of manufacturing, impurities, ocular irritation, dermal irritation, and dermal sensitization data on sodium lauroyl silk amino acids. The data have been summarized in the following tables for your convenience.

Table 1. Method of manufacturing and impurities data.

Data	Reference
Method of Manufacturing	
A supplier of sodium lauroyl silk amino acids reports that the material is prepared by acylation of a free amine of silk amino acid obtained by silk protein hydrolysis. The final product is a 20% water solution of sodium lauroyl silk amino acids.	Anonymous, 2013
Impurities	
A supplier of sodium lauroyl silk amino acids reports that the material has heavy metals and arsenic $\leq$ 20 ppm and $\leq$ 2 ppm, respectively.	Anonymous, 2013

Table 2. Irritation and sensitization data.

Concentration	Method	Result	Reference
Ocular Irritation			
2.5% of a 20% solution	Hen's Egg Test (HET0-CAM) for ocular irritancy potential	Slight ocular irritation potential.	Consumer Product Testing Co., 2004
Dermal Irritation			
20% solution, pH 7.2-7.3	4 hour, semi-occluded acute dermal irritation study in New Zealand White rabbits	Mild irritant to rabbit skin according to Draize (PII = 1.8). No corrosive effects noted.	SafePharm Laboratories, 2004
6% active solution	HPT for irritancy in 20 volunteers, patches occluded	Minimally irritating. Cutaneous irritation index after 24h = 10.0, after 48 h = 2.5.	Dermis Research Center Co., 2005
Dermal Sensitization			
25%, 50%, or 100% of a 20% solution in butanone	LLNA	Non-sensitizing. SI at 100% = 2.61	Safe Pharm Laboratories, 2004

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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

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

DATE: May 2, 2013

SUBJECT: Concentration of Use by FDA Product Category: Amino Acid Alkyl Amides, March 2013 Survey

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Concentration of Use by FDA Product Category - Amino Acid Alkyl Amides, March 2013 Survey

Lauroyl Proline
Palmitoyl Proline
Potassium Caproyl Tyrosine
Potassium Cocoyl Rice Amino Acids
Sodium Caproyl Prolinate
Sodium Cocoyl Barley Amino Acids

Ingredient	FDA Code†	Product Category	Maximum Concentration of Use
Palmitoyl Proline	05B	Hair sprays pump spray	0.65%
Palmitoyl Proline	05G	Tonics, dressings and other hair grooming aids	0.42%
Palmitoyl Proline	07B	Face powders	0.3%
Palmitoyl Proline	07C	Foundations	0.0017-0.3%
Palmitoyl Proline	08A	Basecoats and undercoats (manicuring preparations)	0.0065%
Palmitoyl Proline	08G	Other manicuring preparations	0.0055%
Palmitoyl Proline	12D	Body and hand products spray	0.46%
Palmitoyl Proline	12G	Night products not spray	0.3%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.
†Product category codes used by FDA

Information collected in 2013
Table prepared: May 2, 2013

Personal Care Products Council
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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

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

DATE: May 28, 2013

SUBJECT: Information on Sodium Lauroyl Silk Amino Acids

Anonymous. 2013. Summary of information concerning Sodium Lauroyl Silk Amino Acids.

Consumer Product Testing Co. 2004. The hen's egg test - utilizing the chorioallantoic membrane (HET-CAM) of Sodium Lauroyl Silk Amino Acids. Experiment Reference No.: V04-0131-2.

SafePharm Laboratories. 2004. Acute dermal irritation in the rabbit of Sodium Lauroyl Silk Amino Acids. SPL Project Number: 1268/119.

Dermis Research Center Co. 2005. Human patch test under occlusive patch for 48 hours of Sodium Lauroyl Silk Amino Acids.

SafePharm Laboratories. 2004. Local lymph node assay in the mouse Sodium Lauroyl Silk Amino Acids. SPL Project Number 1268/120.

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【Information of Sodium Lauroyl Silk Amino Acids】

(1) Method of manufacturing

Our Sodium Lauroyl Silk Amino Acids is prepared by the acylation of a free amine of silk amino acid obtained by silk protein hydrolysis. The final product is a 20% water solution of Sodium Lauroyl Silk Amino Acids.

(2) Impurities

Our Sodium Lauroyl Silk Amino Acids has heavy metals and arsenic less than or equal to 20 ppm and 2 ppm, respectively.

(3) Safety data (Irritation and Sensitization)

(A) Irritation, Ocular – non human

To determine the degree of eye irritation, an eye irritation study was performed in accordance with HET-CAM method. Our Sodium Lauroyl Silk Amino Acids would have a slight ocular irritation potential. Regarding test report, please see attachment 1.

(B) Irritation, Dermal - non human

The irritation potential of Sodium Lauroyl Silk Amino Acids was investigated in accordance with OECD Guideline for the Testing of Chemicals No. 404 " Acute Dermal Irritation/Corrosion". Our Sodium Lauroyl Silk Amino Acids was classified as a mild irritant to rabbit skin according to the Draize classification scheme. Regarding test report, please see attachment 2.

(C) Irritation, Dermal - human

The irritation potential of Sodium Lauroyl Silk Amino Acids was investigated in a 48h human patch test (occlusive) of 20 subjects. Minimal irritant was observed. Regarding test report, please see attachment 3.

(D) Sensitization, Dermal – non human

The sensitization potential of Sodium Lauroyl Silk Amino Acids was investigated in accordance with OECD Guideline for the Testing of Chemicals No. 429 " Skin Sensitization: Local Lymph Node Assay". The material was considered to be a non-sensitizer under the conditions of the test. Regarding test report, please see attachment 4.

Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT:

SPONSOR: [redacted]

TEST: The Hen's Egg Test - Utilizing the Chorioallantoic Membrane (HET-CAM)

Sodium Lauroyl Silk Amino Acids

TEST ARTICLE: [redacted]

EXPERIMENT REFERENCE NO.: V04-0131-2

Scott 7.1.04

Scott Krupa
Quality Assurance Supervisor

7/1/04

Steven Nitka
Vice President
Laboratory Director

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study No.: V04-0131-2

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance with standard operating procedures and applicable standard protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. The findings of these inspections may have been reported to management and the Study Director.

Date of data inspection: July 1, 2004

Professional personnel involved:

Steven Nitka, B.S.	-	Vice President Laboratory Director (Study Director)
Lillian Deniza, B.S.	-	Laboratory Supervisor
Melissa Pandorf, B.S.	-	Technician
Scott Krupa	-	Quality Assurance Supervisor

The representative signature of the Quality Assurance Unit on the front page signifies that this study has been performed in accordance with standard operating procedures and applicable study protocols.

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Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective:

To evaluate the test article for irritancy potential utilizing the HET-CAM test. The test is a modification of that described by Kemper and Luepke.[1]

Introduction:

The chick embryo has been used extensively in toxicology. "The chorioallantoic membrane (CAM) of the chick embryo is a complete tissue with organoid elements from all germ cell layers. The chorionic epithelium is ectodermal and the allantoic epithelium is endodermal. The mesoderm located between these epithelia is a complete connective tissue including arteries, capillaries, veins and lymphatic vessels. The CAM responds to injury with a complete inflammatory reaction, comparable to that induced in the rabbit eye test. It is technically easy to study, and is without nerves to sense pain."[2]

Test Article: [redacted]

Reference Articles: Johnson's Baby Shampoo
Prell Shampoo Concentrate

[1]Kemper, F.H. & Luepke, N.P., (1986). The HET-CAM Test: An Alternative to the Draize Test. *FD Chem. Toxic.* 24, p. 495 - 496.

[2]Leighton, J., Tchao, R., Verdone, J. & Nassauer, J. Macroscopic Assay of Focal Injury in the Chorioallantoic Membrane. In: *Alternative Methods in Toxicology*, Vol. 3, *In Vitro* Toxicology E2, pp. 357 - 369, Alan M. Goldberg, (ed.), Mary Ann Liebert Publishers, Inc., New York, 1985.

Method:

Fresh, fertile, White Leghorn eggs were obtained from Avian Services in Frenchtown, New Jersey. They were stored at this facility for up to seven (7) days, at 13° C, before being incubated. For incubation the eggs were placed, on their sides, in a Kuhl incubator. The incubator is such that the eggs are automatically rotated once every hour. The temperature was controlled at 99° F (± 1°) with a relative humidity of 60 - 70% for the ten (10) days of incubation. On day eight (8) the eggs were turned so that the acutely angled end faced down.

On day ten (10) each egg was removed from the incubator and placed in a Plexiglas work enclosure. This enclosure had been preheated and humidified so that its environment approached that of the incubator. A cut was made in the larger end of each egg, where the air sack is located. A Dremel® Moto-Flex Tool (model 232-5) equipped with a Dremel® Cut-Off Wheel (No. 409) was used to make each cut. Forceps were then used to remove the shell down to the shell-membrane junction. The inner egg membrane was then hydrated with a warm, physiological saline solution. The saline was removed after a two (2) to five (5) minute exposure. Utilizing pointed forceps, the inner egg membrane was then carefully removed to reveal the CAM.

The test or reference article, at a dosage of three-tenths of one milliliter (0.3 ml) of a liquid was then administered to each of four (4) CAM's. Twenty seconds later, the test or control article was rinsed from each CAM with five (5) milliliters of physiological saline. All CAM's were observed immediately prior to test article administration and at 30 seconds, two (2) and five (5) minutes after exposure to the test article. The reactions of the CAM, the blood vessels, including the capillaries, and the albumin were examined and scored for irritant effects as detailed below:

		Score		
Effect	**Time (min.)**	**0.5**	**2**	**5**
Hyperemia		5	3	1
Minimal Hemorrhage ("Feathering")		7	5	3
Hemorrhage (Obvious Leakage)		9	7	5
Coagulation and/or Thrombosis		11	9	7

The numerical, time dependent scores were totaled for each CAM. Each reaction type can be recorded only once for each CAM, therefore the maximum score per CAM is 32. The mean score was determined for all CAM's similarly tested.

Results:

Test Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
[redacted]	1	0	3	0	3
	2	0	3	3	6
2.5 %	3	5	5	0	10
	4	5 7	0	0	12
				Average:	7.75

Reference Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
Johnson's Baby	1	5 7	0	0	12
Shampoo (50%)	2	5 7	0	0	12
	3	0	3 5	0	8
	4	5 7	0	0	12
				Average:	11.00

Reference Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
Prell Shampoo	1	5 7	7	7	26
Concentrate (50%)	2	5 7	7	7	26
	3	5 7	0	5 7	24
	4	5 7 9	0	0	21
				Average:	24.25

Each article was then classified as indicated in the following:

Mean Score	Irritation Potential
0.0 - 4.9	Practically none
5.0 - 9.9	Slight
10.0 - 14.9	Moderate
15.0 - 32.0	Severe

Discussion:

The sponsor requested the irritation potential of the submitted article, at 2.5%. Previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than is the rabbit eye. Therefore, dilutions of the liquid articles were used.

Historical *In Vivo* Results:

The Johnson's reference product has historically been categorized as being moderately irritating, eliciting scores approaching 10, at 24 hours, when dosed at 100% and tested using the Draize ocular irritation methodologies. The Prell reference product has historically been categorized as being severely irritating, eliciting scores approaching 30, at 24 hours, when dosed at 100% and tested using the Draize ocular irritation methodologies.

Conclusion:

Under the conditions of this test, the results indicate that the sponsor-submitted product ([redacted]) at 2.5%, would have a slight ocular irritation potential *in vivo*.

SafePharm Laboratories

[redacted]

ACUTE DERMAL IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 1268/119

AUTHOR: A Sanders

STUDY SPONSOR:

[redacted]

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD
UK

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	27 May 2003	Standard Test Method Compliance Audit
	06 July 2004	Test Material Preparation
	06, 22 July 2004	Animal Preparation
	06 July 2004	Dosing
	06, 22, 27 July 2004	Assessment of Response
§	19 August 2004	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

J. M. Crowther ..... DATE: 01 OCT 2004 .....

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.

..... DATE: 01 OCT 2004

A Sanders
Study Director

CONTENTS

[redacted]

ACUTE DERMAL IRRITATION IN THE RABBIT

SUMMARY

Introduction. The study was performed to assess the irritancy potential of the test material to the skin of the New Zealand White rabbit. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Commission Directive 92/69/EEC Method B4 Acute Toxicity (Skin Irritation)

Results. A single 4-hour, semi-occluded application of the test material to the intact skin of three rabbits produced very slight to well-defined erythema and very slight oedema. Crust formation and moderate desquamation were also noted. One treated skin site appeared normal at the 72-hour observation and the remaining two treated skin sites appeared normal at the 14-day observation.

Conclusion. The test material produced a primary irritation index of 1.8 and was classified as a mild irritant to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

[redacted]

ACUTE DERMAL IRRITATION IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of the test material following a single, 4-hour, semi-occluded application to the intact rabbit skin. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Commission Directive 92/69/EEC Method B4 Acute Toxicity (Skin Irritation)

The albino rabbit has been shown to be a suitable model for this type of study and is recommended in the test method. The results of the study are believed to be of value in predicting the likely skin irritancy potential of the test material to man.

The study was performed between 29 June 2004 and 13 July 2004.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	[redacted]
Description	:	yellow liquid
Batch number	:	BFA
Date received	:	14 June 2004
Storage conditions	:	approximately 4°C in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

2.2 Preparation of Test Material

For the purpose of the study the test material was used as supplied.

The absorption of the test material was not determined.

2.3 Measurement of pH

The pH of the test material was determined prior to commencement of the study and found to be as follows:

Preparation	pH Measurement
Undiluted as Supplied	7.2
90% v/v aqueous preparation of the test material	7.3

3. METHODS

3.1 Animals and Animal Husbandry

Three New Zealand White rabbits were supplied by David Percival Ltd, Moston, Sandbach, Cheshire, UK. At the start of the study the animals were in the weight range of 2.0 to 3.5 kg and were twelve to twenty weeks old. After an acclimatisation period of at least five days each animal was given a number unique within the study which was written with a black indelible marker-pen on the inner surface of the ear and on the cage label.

The animals were individually housed in suspended metal cages. Free access to mains drinking water and food (Certified Rabbit Diet (Code 5322) supplied by BCM IPS Limited, London, UK) was allowed throughout the study. The diet and drinking water were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 17 to 23°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

On the day before the test each of a group of three rabbits was clipped free of fur from the dorsal/flank area using veterinary clippers. Only animals with a healthy intact epidermis by gross observation were selected for the study.

On the day of the test a suitable test site was selected on the back of each rabbit. A quantity of 0.5 ml of the test material was introduced under a 2.5 cm x 2.5 cm cotton gauze patch and placed in position on the shorn skin. The patch was secured in position with a strip of surgical adhesive tape. To prevent the animals interfering with the patches, the trunk of each rabbit was wrapped in an elasticated corset and the animals were returned to their cages for the duration of the exposure period.

Four hours after application the corset and patches were removed from each animal and any residual test material removed by gentle swabbing with cotton wool soaked in distilled water.

Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the following scale:

EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	**Value**
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

Any other skin reactions, if present, were also recorded.

Additional observations were made on Days 7 and 14 to assess the reversibility of skin reactions.

3.3 Interpretation of Results

Calculation of Primary Irritation Index and Grading of Irritancy Potential Using the Draize Scheme

The scores for erythema and oedema at the 24 and 72-hour readings were totalled for the three test rabbits (12 values) and this total was divided by six to give the primary irritation index of the test material. The test material was classified according to the following scheme devised by Draize J H (1959) "Dermal Toxicity" In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the United States, Austin, Texas, p.47:

Primary Irritation Index	Classification of Irritancy
0	Non-irritant
> 0 to 2	Mild irritant
> 2 to 5	Moderate irritant
> 5 to 8	Severe irritant

If irreversible alteration of the dermal tissue is noted in any rabbit, as judged by the Study Director, which include ulceration and clear necrosis or signs of scar tissue, the test material is classified as corrosive to rabbit skin. Classification according to Draize may, therefore, not be applicable.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

The individual scores for erythema/eschar and oedema, are given in Table 1.

Very slight to well-defined erythema was noted at all treated skin sites one hour after patch removal and at the 24 and 48-hour observations. Very slight erythema was noted at two treated skin sites at the 72-hour observation and persisted at one treated skin site at the 7-day observation.

Very slight oedema was noted at all treated skin sites one hour after patch removal and at the 24 and 48-hour observations and persisted at one treated skin site at the 72-hour observation.

Crust formation was noted at one treated skin site with moderate desquamation noted at one other treated skin site at the 7-day observation.

One treated skin site appeared normal at the 72-hour observation and the remaining two treated skin sites appeared normal at the 14-day observation.

6. CONCLUSION

The test material produced a primary irritation index of 1.8 and was classified as a MILD IRRITANT to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

[redacted] : ACUTE DERMAL IRRITATION IN THE RABBIT

Table 1 Individual Skin Reactions

Skin Reaction	Observation Time	Individual Scores – Rabbit Number and Sex			Total
		54 Male	67 Female	68 Male	
Erythema/Eschar Formation	1 Hour	2	1	2	(5)
	24 Hours	2	1	2	5
	48 Hours	1	1	2	(4)
	72 Hours	1	0	1	2
	7 Days	0 D	0	1 Cf	(1)
	14 Days	0	0	0	(0)
Oedema Formation	1 Hour	1	1	1	(3)
	24 Hours	1	1	1	3
	48 Hours	1	1	1	(3)
	72 Hours	0	0	1	1
	7 Days	0	0	0	(0)
	14 Days	0	0	0	(0)
Sum of 24 and 72-hour Readings (S)		:	11		
Primary Irritation Index (S/6)		:	11/6 = 1.8		
Classification		:	MILD IRRITANT		

( ) = Total values not used for calculation of primary irritation index
D = Moderate desquamation
Cf = Crust formation

Appendix 1 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
Loudon Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

SAFEPHARM LABORATORIES LTD

[redacted]

ACUTE DERMAL IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 1268/119

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

..... DATE: 04 OCT 2004

A Sanders
Study Director

Report Number 050253

9 February 2005

Human Patch Test under occlusive patch for 48 hours

Sodium Lauroyl Silk Amino Acids

[Clinical Research Facility]

Name :DERMIS RESEARCH CENTER Co., Ltd.
Address :KS 7F, 1-12-13, Higashi-temma, Kita-ku, Osaka 530-0044, JAPAN
Phone :+81-6-6882-8201
Facsimile :+81-6-6882-8202

[Study timetable]

Start of the study : 14 December 2004
End of the study : 16 December 2004

[Test method]

1. Volunteers
   The volunteers who accepted to collaborate to this study, after informed consent, were healthy Japanese males and females (the year range 18-60).

2. Number of volunteers
   20 volunteers (18 females and 2 males).

3. Application area
   On the back of volunteers.

4. Patches
   Finn Chamber on Scanpor Tape (produced by Taisho Pharmaceutical Co., Ltd.)

5. Modes of application
   The sample was applied one time under occlusive conditions for 24 hours.

6. Application
   Adequate dose.

7. Dose level and concentration
   Active 6% solution.

8. Negative controls
   White petrolatum (produced by Nikko Rica Corporation)
   Distilled water for injection (produced by Taisho Pharmaceutical Co., Ltd.)
   physiological saline (produced by Otsuka Pharmaceutical Co., Ltd.)

9. Observation
   The cutaneous examinations were performed about 30-60 minutes and 24 hours after removal of the patches.

10. Evaluation of the Cutaneous Irritation index

The possible cutaneous reactions were evaluated, for each volunteer, according to the following numerical scale:

Table 1 Criteria of patch test

Examination	Cutaneous Reaction
-	no reaction
±	slight erythema
+	clear erythema
++	erythema+edema+papulae
+++	erythema+edema+papulae+vesicle
++++	bullous reaction

Table 2 Cutaneous Irritation index

Examination	Cutaneous Reaction	Score
-	no reaction	0
±	slight erythema	0.5
+	clear erythema	1.0
++	erythema+edema+papulae	2.0
+++	erythema+edema+papulae+vesicle	3.0
++++	bullous reaction	4.0

$$\text{Cutaneous Irritation index} = \frac{\text{Sum of the scores at each times point}}{\text{Number of volunteers}} \times 100$$

Table 3 (Reference data) The index of Cutaneous Irritation obtained to classify

Cutaneous Irritation index	Classification (1985)	Classification (1995)
< 5.0	safe	safe
5.0 - 15.0	safe	acceptable
15.0 - 30.0	acceptable	requires Improvement
30.0 - 60.0	requires Improvement	unsafe
> 60.0	unsafe	unsafe

[Results]

The result of this test performed on 20 volunteers is shown in the table elsewhere.
The Cutaneous Irritation index is shown below, along with the grade thus obtained.

After	24 hrs.	48 hrs.
-	16/20	19/20
±	4/20	1/20
+	0/20	0/20
++	0/20	0/20
Cutaneous Irritation index	10.0	2.5

Table 4 The Results of Human Patch Test

No.	Sex	Age	After	Assessment
1	F	35	24 hrs.	-
			48 hrs.	-
2	F	36	24 hrs.	-
			48 hrs.	-
3	F	42	24 hrs.	-
			48 hrs.	-
4	F	34	24 hrs.	-
			48 hrs.	-
5	F	40	24 hrs.	-
			48 hrs.	-
6	F	35	24 hrs.	-
			48 hrs.	-
7	F	35	24 hrs.	±
			48 hrs.	-
8	F	37	24 hrs.	-
			48 hrs.	-
9	F	56	24 hrs.	-
			48 hrs.	-
10	F	29	24 hrs.	±
			48 hrs.	-
11	F	37	24 hrs.	-
			48 hrs.	-
12	F	48	24 hrs.	-
			48 hrs.	-
13	F	34	24 hrs.	-
			48 hrs.	-
14	F	38	24 hrs.	-
			48 hrs.	-
15	F	39	24 hrs.	-
			48 hrs.	-
16	F	45	24 hrs.	-
			48 hrs.	-
17	F	37	24 hrs.	-
			48 hrs.	±
18	F	45	24 hrs.	±
			48 hrs.	-
19	M	25	24 hrs.	±
			48 hrs.	-
20	M	20	24 hrs.	-
			48 hrs.	-

SafePharm Laboratories

[redacted]:

LOCAL LYMPH NODE ASSAY IN THE MOUSE

Sodium Lauroyl Silk Amino Acids

SPL PROJECT NUMBER: 1268/120

AUTHOR: A Sanders

STUDY SPONSOR:

[redacted]

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
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UK

Telephone: +44 (0) 1332 792896

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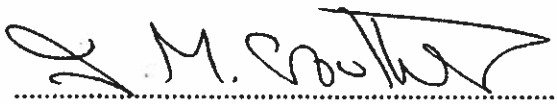
QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	31 January 2003	Standard Test Method Compliance Audit
	05 July 2004	Test Material Preparation
	05 July 2004	Test System Preparation
	09 July 2004	Animal Preparation
	13 July 2004	Dosing
	06 July 2004	Assessment of Response
§	10 August 2004	Draft Report Audit
§	Date of QA Signature	Final Report Audit
§	Evaluation specific to this study	



..... DATE: 20 SEP 2004

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.

..... DATE: 20 SEP 2004

A Sanders
Study Director

CONTENTS

[redacted]:

LOCAL LYMPH NODE ASSAY IN THE MOUSE

SUMMARY

Introduction. A study was performed to assess the skin sensitisation potential of the test material in the CBA/Ca strain mouse following topical application to the dorsal surface of the ear. The method was designed to meet the requirements of the following:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)

Methods. Following a preliminary screening test, three groups, each of four animals, were treated with 50 µl (25 µl per ear) of the undiluted test material or the test material as a solution in butanone at concentrations of 25% or 50% v/v. A further group of four animals was treated with butanone alone.

Results. The Stimulation Index (SI) expressed as the mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group are as follows:

Concentration (% v/v) in butanone	Stimulation Index (SI)	Result
25	1.44	Negative
50	1.78	Negative
100	2.61	Negative

Conclusion. The test material was considered to be a non-sensitiser under the conditions of the test.

[redacted]:

LOCAL LYMPH NODE ASSAY IN THE MOUSE

1. INTRODUCTION

A study was performed to assess the skin sensitisation potential of the test material in the CBA/Ca strain mouse following topical application to the dorsal surface of the ear. The method was designed to meet the requirements of the following:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)

The assay has undergone extensive inter-laboratory validation and has been shown to reliably detect test materials that are moderate to strong sensitisers.

The strain of mouse used in these laboratories has been shown to produce satisfactory responses using known sensitisers and non-sensitisers during the in-house validation. The results of routine positive control studies are shown in Appendix 1 and Appendix 2. The results of the study are believed to be of value in predicting the sensitisation potential of the test material to man.

The study was performed between 30 June 2004 and 04 August 2004.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	[redacted]
Description	:	yellow liquid
Batch number	:	BFA
Date received	:	14 June 2004
Storage conditions	:	approximately 4°C in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

2.2 Preparation of Test Material

For the purpose of the study the test material was used undiluted and freshly prepared in butanone. This vehicle was chosen as it produced the most suitable formulation at the required concentration. The concentrations used are given in the procedure section.

Determination, by analysis, of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female CBA/Ca (CBA/CaBkl) strain mice were supplied by B & K Universal Ltd, Hull, UK. On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were in the weight range of 15 to 23 g, and were eight to twelve weeks old.

The animals were individually housed in suspended solid-floor polypropylene cages furnished with softwood woodflakes. Free access to mains tap water and food (Certified Rat and Mouse Diet (Code 5LF2) supplied by BCM IPS Limited, London, UK) was allowed throughout the study.

The temperature and relative humidity were controlled to remain within target ranges of 19 to 25°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was approximately fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06.00 to 18.00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

3.2.1 Preliminary Screening Test

As no toxicological information was available regarding the systemic toxicity/irritancy potential of the test material a preliminary screening test was performed using one mouse. The mouse was treated by daily application of 25 µl of the undiluted test material to the dorsal surface of each ear for three consecutive days (Days 1, 2, 3). The mouse was observed twice daily on Days 1, 2 and 3 and once daily on Days 4, 5 and 6. Any signs of toxicity or excessive local irritation noted during this period were recorded. The bodyweight of the mouse was recorded on Day 1 (prior to dosing) and on Day 6.

3.2.2 Main Test

3.2.2.1 Test Material Administration

Groups of four mice were treated with the undiluted test material or the test material at concentrations of 25% or 50% v/v in butanone. The preliminary screening test suggested that the test material would not produce systemic toxicity or excessive local irritation at the highest suitable concentration. The mice were treated by daily application of 25 µl of the appropriate concentration of the test material to the dorsal surface of each ear for three consecutive days (Days 1, 2, 3). The test material formulation was administered using an automatic micropipette and spread over the dorsal surface of the ear using the tip of the pipette.

A further group of four mice received the vehicle alone in the same manner.

3.2.2.2 ^{3}H-Methyl Thymidine Administration

Five days following the first topical application of the test material (Day 6) all mice were injected via the tail vein with 250 µl of phosphate buffered saline (PBS) containing ^{3}H-methyl thymidine (^{3}HTdR: 80 µCi/ml, specific activity 2.0 Ci/mmol, Amersham Biosciences UK Ltd) giving a total of 20 µCi to each mouse.

3.2.2.3 Observations

Clinical Observations: All animals were observed twice daily on Days 1, 2 and 3 and on a daily basis on Days 4, 5 and 6. Any signs of toxicity or signs of ill health during the study were recorded.

Bodyweights: The bodyweight of each mouse was recorded on Day 1 (prior to dosing) and Day 6 (prior to termination).

3.2.2.4 Terminal Procedures

Termination: Five hours following the administration of ^{3}HTdR all mice were killed by carbon dioxide asphyxiation. The draining auricular lymph nodes from the four mice were excised and pooled for each experimental group. For each group 1 ml of PBS was added to the pooled lymph nodes.

Preparation of Single Cell Suspension: A single cell suspension of pooled lymph node cells was prepared by gentle mechanical disaggregation through a 200-mesh stainless steel gauze. The lymph node cells were rinsed through the gauze with 4 ml of PBS into a petri dish labelled with the project number and dose concentration. The lymph node cell suspension was transferred to a 10 ml centrifuge tube. The petri dish was washed with an additional 5 ml of PBS to remove all remaining lymph node cells and these were added to the centrifuge tube. The pooled lymph node cells were pelleted at 1400 rpm (approximately 190 *g*) for ten minutes. The pellet was resuspended in 10 ml of PBS and re-pelleted. To precipitate out the radioactive material, the pellet was resuspended in 3 ml of 5% Trichloroacetic acid (TCA).

Determination of ^{3}HTdR Incorporation: After overnight incubation at 4°C, the precipitates were recovered by centrifugation at 2100 rpm (approximately 450 *g*) for ten minutes, resuspended in 1 ml of TCA and transferred to 10 ml of scintillation fluid (Optiphase 'Trisafe'). ^{3}HTdR incorporation was measured by β-scintillation counting. The "Poly Q™" vials containing the samples and scintillation fluid were placed in the sample changer of the scintillator and left for approximately twenty minutes. The purpose of this period of time in darkness was to reduce the risk of luminescence, which has been shown to affect the reliability of the results. After approximately twenty minutes, the vials were shaken vigorously. The number of radioactive disintegrations per minute was then measured using the Beckman LS6500 scintillation system (Beckman Instruments Inc, Fullerton, CA).

3.3 Interpretation of Results

The proliferation response of lymph node cells was expressed as the number of radioactive disintegrations per minute per lymph node (dpm/node) and as the ratio of ^{3}HTdR incorporation into lymph node cells of test nodes relative to that recorded for the control nodes (Stimulation Index).

The test material will be regarded as a sensitiser if at least one concentration of the test material results in a threefold or greater increase in ^{3}HTdR incorporation compared to control values. Any test material failing to produce a threefold or greater increase in ^{3}HTdR incorporation will be classified as a "non-sensitiser".

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Preliminary Screening Test

Clinical observations, bodyweight and mortality data are given in Table 1.

No signs of systemic toxicity were noted.

Based on this information the dose levels selected for the main test were 25%, 50% v/v in butanone and 100%.

5.2 Main Test

5.2.1 Estimation of the Proliferative Response of Lymph Node Cells

The radioactive disintegrations per minute (dpm) per lymph node and the stimulation index (SI) are given in Table 2.

A stimulation index of less than 3 was recorded for the three concentrations of the test material (25%, 50% v/v in butanone and 100%).

5.2.2 Clinical Observations and Mortality Data

Individual clinical observations and mortality data for test and control animals are given in Table 3.

There were no deaths. No signs of systemic toxicity were noted in the test or control animals during the study.

5.2.3 Bodyweight

Individual bodyweights and bodyweight changes for test and control animals are given in Table 4.

Bodyweight changes of the test animals between Day 1 and Day 6 were comparable to those observed in the corresponding control group animals over the same period.

6. CONCLUSION

The test material was considered to be a non-sensitiser under the conditions of the test.

[redacted]: LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 1 Clinical Observations, Bodyweight and Mortality Data – Preliminary Screening Test

Concentration (%)	Animal Number	Bodyweight (g)		Day								
				1		2		3		4	5	6
		Day 1	Day 6	Pre-Dose	1 Hr Post Dose	Pre-Dose	1 Hr Post Dose	Pre-Dose	1 Hr Post Dose			
100	S-1	21	20	0	0	0	0	0	0	0	0	0

Hr = Hour
0 = No signs of systemic toxicity

[redacted]: LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 2 Dpm, Dpm/Node and Stimulation Index (SI)

Concentration (% v/v) in butanone	Dpm	Dpm/Node [a]	Stimulation Index (SI) [b]	Result
Vehicle	3581.90	447.74	N/A	N/A
25	5174.28	646.79	1.44	Negative
50	6368.95	796.12	1.78	Negative
100	9333.31	1166.66	2.61	Negative

a = Dpm/node obtained by dividing the Dpm value by 8 (total number of lymph nodes)
b = Stimulation Index of 3.0 or greater indicates a positive result
N/A = Not applicable

[redacted]: LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 3 Individual Clinical Observations and Mortality Data

Concentration (% v/v) in butanone	Animal Number	Day 1		Day 2		Day 3		Day 4	Day 5	Day 6
		Pre-Dose	1-Hr Post Dose	Pre-Dose	1-Hr Post Dose	Pre-Dose	1-Hr Post Dose			
Vehicle	1-1	0	0	0	0	0	0	0	0	0
	1-2	0	0	0	0	0	0	0	0	0
	1-3	0	0	0	0	0	0	0	0	0
	1-4	0	0	0	0	0	0	0	0	0
25	2-1	0	0	0	0	0	0	0	0	0
	2-2	0	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0	0
	2-4	0	0	0	0	0	0	0	0	0
50	3-1	0	0	0	0	0	0	0	0	0
	3-2	0	0	0	0	0	0	0	0	0
	3-3	0	0	0	0	0	0	0	0	0
	3-4	0	0	0	0	0	0	0	0	0
100	4-1	0	0	0	0	0	0	0	0	0
	4-2	0	0	0	0	0	0	0	0	0
	4-3	0	0	0	0	0	0	0	0	0
	4-4	0	0	0	0	0	0	0	0	0

Hr = Hour
0 = No signs of systemic toxicity

[redacted]: LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 4 Individual Bodyweights and Bodyweight Changes

Concentration (% v/v) in butanone	Animal Number	Bodyweight (g)		Bodyweight Change (g)
		Day 1	Day 6	
Vehicle	1-1	20	19	-1
	1-2	22	22	0
	1-3	21	22	1
	1-4	21	22	1
25	2-1	20	21	1
	2-2	21	21	0
	2-3	19	20	1
	2-4	23	23	0
50	3-1	19	20	1
	3-2	21	21	0
	3-3	21	19	-2
	3-4	20	19	-1
100	4-1	21	21	0
	4-2	20	21	1
	4-3	17	18	1
	4-4	20	19	-1

[redacted]: LOCAL LYMPH NODE ASSAY IN THE MOUSE

Appendix 1 Latest Positive Control Study for the Local Lymph Node Assay

Introduction. A study was performed to assess the sensitivity of the strain of mouse used at these laboratories to a known sensitiser. The method was designed to meet the requirements of the following:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)

Test Material:	α-HEXYLCINNAMALDEHYDE
SPL Project number:	039/688
Study dates:	23 April 2004 to 29 April 2004

Methods. Three groups, each of four animals, were treated with 50 µl (25 µl per ear) of α-HEXYLCINNAMALDEHYDE as a solution in acetone/olive oil 4:1 at concentrations of 5%, 10% and 25% v/v. A further control group of four animals was treated with acetone/olive oil 4:1 alone.

Results. The Stimulation Index (SI) expressed as the mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group are as follows:

Concentration (% v/v) in Acetone/Olive Oil 4:1	Stimulation Index (SI)	Result
5	1.74	Negative
10	2.20	Negative
25	8.89	Positive

Conclusion. α-HEXYLCINNAMALDEHYDE was considered to be a sensitiser under the conditions of the test.

Appendix 2 Summary of Positive Control Data for the Local Lymph Node Assay

Project Number	Start Date	Finish Date	Test Material	Concentration	Vehicle	Stimulation Index[a]	Classification [b]
039/586*	13/08/02	19/08/02	α-Hexylcinnamaldehyde	5, 10, 50% w/v	4:1 acetone/olive oil	5.7, 5.5, 33.5	Positive
039/629*	13/03/03	19/03/03	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	2.8, 2.3, 5.5	Positive
039/630•	13/03/03	19/03/03	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	2.0, 1.9, 6.8	Positive
039/656*	10/10/03	16/10/03	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	1.76, 2.78, 5.06	Positive
039/658•	16/10/03	22/10/03	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	1.49, 1.73, 5.26	Positive
039/687•	29/04/04	05/05/04	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	1.40, 2.23, 6.09	Positive
039/688*	23/04/04	29/04/04	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	1.74, 2.20, 8.89	Positive

a = Ratio of test to control lymphocyte proliferation
b = Stimulation index greater than 3.0 indicates a positive result
* = Standard Test Method 595 ('Pooled' nodes)
• = Standard Test Method 599 ('Individual' nodes)

Appendix 3 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

SAFEPHARM LABORATORIES LTD

[redacted]:

LOCAL LYMPH NODE ASSAY IN THE MOUSE

SPL PROJECT NUMBER: 1268/120

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

..... DATE: 22 SEP 2004

A Sanders
Study Director

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: May 31, 2013
Subject: Wave 2 Data on Chamomile Ingredients

Updated use concentration data (chamom062013data8 pdf file) received from the Council on May 10 are attached. These data are more recent than the use concentration data (chamom062013data7 pdf file) that were submitted to the Panel on May 17, and the new maximum use concentrations/use concentration ranges identified were:

- Chamomilla recutita (matricaria) flower extract: 0.000025-0.12% (hair conditioners)
- Chamomilla recutita (matricaria) flower extract: 0.00001-0.0003% (aerosol hair sprays)
- Chamomilla recutita (matricaria) flower extract: 0.0007% (hair straighteners)
- Chamomilla recutita (matricaria) flower extract: 0.00001-0.0075% (tonics, dressings and other hair grooming aids)
- Chamomilla recutita (matricaria) flower extract: 0.0004% (feminine hygiene deodorants [aerosol])
- Chamomilla recutita (matricaria) flower extract: 0.0002-0.02% (skin cleansing [cold creams, cleansing lotions, liquids, and pads])
- Chamomilla recutita (matricaria) flower extract: 0.0002-0.02% (body and hand products [spray])

The draft report will be revised to include these data after the Panel meeting.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

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

DATE: May 10, 2013

SUBJECT: Concentration of Use by FDA Product Category: Updated information on Chamomile-derived ingredients

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Concentration of Use by FDA Product Category - Chamomile-Derived Ingredients

Chamomilla Recutita (Matricaria) Extract
Chamomilla Recutita (Matricaria) Flower
Chamomilla Recutita (Matricaria) Flower Extract (alternate VCRP name Matricaria Chamomilla Flower Extract)
Chamomilla Recutita (Matricaria) Flower/Leaf Extract
Chamomilla Recutita (Matricaria) Flower/Leaf/Stem Extract
Chamomilla Recutita (Matricaria) Flower/Leaf/Stem Water
Chamomilla Recutita (Matricaria) Flower Oil
Chamomilla Recutita (Matricaria) Flower Powder
Chamomilla Recutita (Matricaria) Flower Water
Chamomilla Recutita (Matricaria) Leaf Extract
Chamomilla Recutita (Matricaria) Oil
Anthemis Nobilis Flower Extract
Anthemis Nobilis Flower Oil
Anthemis Nobilis Flower Powder
Anthemis Nobilis Flower Water

Ingredient	FDA Code†	Product Category	Maximum Concentration of Use
Chamomilla Recutita (Matricaria) Extract	03A	Eyebrow pencil	0.0001%
Chamomilla Recutita (Matricaria) Extract	03B	Eye liner	0.071%
Chamomilla Recutita (Matricaria) Extract	03C	Eye shadow	0.02%
Chamomilla Recutita (Matricaria) Extract	03D	Eye lotion	0.4%
Chamomilla Recutita (Matricaria) Extract	07B	Face powders	0.0004%
Chamomilla Recutita (Matricaria) Extract	07C	Foundations	0.002-0.4%
Chamomilla Recutita (Matricaria) Extract	07E	Lipstick	0.002%
Chamomilla Recutita (Matricaria) Extract	10A	Bath soaps and detergents	0.61%

Chamomilla Recutita (Matricaria) Extract	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.01-0.1%
Chamomilla Recutita (Matricaria) Extract	12C	Face and neck products not spray spray	 0.0025-0.13% 0.1%
Chamomilla Recutita (Matricaria) Extract	12D	Body and hand products not spray	 0.0009-0.13%
Chamomilla Recutita (Matricaria) Extract	12F	Moisturizing products not spray	 0.002%
Chamomilla Recutita (Matricaria) Extract	13A	Suntan products not spray	 0.13%
Chamomilla Recutita (Matricaria) Flower	05D	Permanent waves	0.5%
Chamomilla Recutita (Matricaria) Flower	05E	Rinses (noncoloring)	0.02%
Chamomilla Recutita (Matricaria) Flower	05G	Tonics, dressings and other hair grooming aids	1.2%
Chamomilla Recutita (Matricaria) Flower	06A	Hair dyes and colors (all types requiring caution statement and patch test)	0.3%
Chamomilla Recutita (Matricaria) Flower	06E	Hair color sprays aerosol	 0.02%
Chamomilla Recutita (Matricaria) Flower Extract	01A	Baby shampoos	0.0097%
Chamomilla Recutita (Matricaria) Flower Extract	02A	Bath oils, tablets and salts	0.00051%
Chamomilla Recutita (Matricaria) Flower Extract	02B	Bubble baths	0.00051%
Chamomilla Recutita (Matricaria) Flower Extract	03B	Eye liner	0.064-0.2%
Chamomilla Recutita (Matricaria) Flower Extract	03C	Eye shadow	0.0001-0.2%

Chamomilla Recutita (Matricaria) Flower Extract	03D	Eye lotion	0.02%
Chamomilla Recutita (Matricaria) Flower Extract	03E	Eye makeup remover	0.02%
Chamomilla Recutita (Matricaria) Flower Extract	03F	Mascara	0.005-0.02%
Chamomilla Recutita (Matricaria) Flower Extract	04A	Colognes and toilet waters	0.01%
Chamomilla Recutita (Matricaria) Flower Extract	04E	Other fragrance preparations not spray	 0.02%
Chamomilla Recutita (Matricaria) Flower Extract	05A	Hair conditioners	0.000025-0.12%
Chamomilla Recutita (Matricaria) Flower Extract	05B	Hair sprays aerosol pump sprays	 0.00001-0.0003% 0.0001-0.01%
Chamomilla Recutita (Matricaria) Flower Extract	05C	Hair straighteners	0.0007%
Chamomilla Recutita (Matricaria) Flower Extract	05D	Permanent waves	0.00001%
Chamomilla Recutita (Matricaria) Flower Extract	05E	Rinses (noncoloring)	0.00004%
Chamomilla Recutita (Matricaria) Flower Extract	05E	Shampoos (noncoloring)	0.00006-1%
Chamomilla Recutita (Matricaria) Flower Extract	05G	Tonics, dressings and other hair grooming aids spray	0.00001-0.0075% 0.0002-0.002%
Chamomilla Recutita (Matricaria) Flower Extract	06A	Hair dyes and colors (all types requiring caution statement and patch test)	0.0005-0.005%
Chamomilla Recutita (Matricaria) Flower Extract	06C	Hair rinses (coloring)	0.00001%
Chamomilla Recutita (Matricaria) Flower Extract	06F	Hair lighteners with color	0.00005-0.02%
Chamomilla Recutita (Matricaria) Flower Extract	06G	Hair bleaches	0.02%
Chamomilla Recutita (Matricaria) Flower Extract	07A	Blushers (all types)	0.00032-0.02%

Chamomilla Recutita (Matricaria) Flower Extract	07C	Foundations	0.003-0.025%
Chamomilla Recutita (Matricaria) Flower Extract	07E	Lipstick	0.0002-0.5%
Chamomilla Recutita (Matricaria) Flower Extract	07F	Makeup bases	0.2%
Chamomilla Recutita (Matricaria) Flower Extract	07H	Makeup fixatives	0.0005-0.1%
Chamomilla Recutita (Matricaria) Flower Extract	07I	Other makeup preparations	0.00032-0.086%
Chamomilla Recutita (Matricaria) Flower Extract	08B	Cuticle softeners	0.01-0.3%
Chamomilla Recutita (Matricaria) Flower Extract	08F	Nail polish and enamel removers	0.002%
Chamomilla Recutita (Matricaria) Flower Extract	08G	Other manicuring preprations	0.3%
Chamomilla Recutita (Matricaria) Flower Extract	09A	Dentifrices (aerosol, liquid, pastes and powders)	0.002%
Chamomilla Recutita (Matricaria) Flower Extract	09B	Mouthwashes and breath fresheners (liquids and sprays)	0.002%
Chamomilla Recutita (Matricaria) Flower Extract	10A	Bath soaps and detergents	0.0001-0.034%
Chamomilla Recutita (Matricaria) Flower Extract	10D	Feminine hygiene deodorants aerosol	0.0004%
Chamomilla Recutita (Matricaria) Flower Extract	10E	Other personal cleanliness products	0.000025-0.02%
Chamomilla Recutita (Matricaria) Flower Extract	11A	Aftershave lotions	0.009-0.2%
Chamomilla Recutita (Matricaria) Flower Extract	11E	Shaving cream (aerosol, brushless and lather)	0.00017-0.00019%
Chamomilla Recutita (Matricaria) Flower Extract	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0002-0.02%
Chamomilla Recutita (Matricaria) Flower Extract	12B	Depilatories	0.0075-0.2%
Chamomilla Recutita (Matricaria) Flower Extract	12C	Face and neck products	

		not spray	0.002-0.088%
Chamomilla Recutita (Matricaria) Flower Extract	12D	Body and hand products not spray spray	 0.0002-0.02% 0.01%
Chamomilla Recutita (Matricaria) Flower Extract	12F	Moisturizing products not spray	 0.01-0.1%
Chamomilla Recutita (Matricaria) Flower Extract	12G	Night products not spray	 0.002-0.05%
Chamomilla Recutita (Matricaria) Flower Extract	12H	Paste masks and mud packs	0.0075-0.038%
Chamomilla Recutita (Matricaria) Flower Extract	12J	Other skin care preparations	0.005-0.15%
Chamomilla Recutita (Matricaria) Flower Extract	13A	Suntan products not spray	 0.2%
Chamomilla Recutita (Matricaria) Flower/Leaf Extract	05G	Tonics, dressings and other hair grooming aids spray	 0.0001%
Chamomilla Recutita (Matricaria) Flower/Leaf Extract	07B	Face powders	0.002%
Chamomilla Recutita (Matricaria) Flower/Leaf Extract	07C	Foundations	0.02%
Chamomilla Recutita (Matricaria) Flower/Leaf Extract	07E	Lipstick	0.01%
Chamomilla Recutita (Matricaria) Flower/Leaf Extract	08B	Cuticle softeners	0.01%
Chamomilla Recutita (Matricaria) Flower Oil	03E	Eye makeup remover	0.001%
Chamomilla Recutita (Matricaria) Flower Oil	05B	Hair sprays	

		aerosol	0.007%
Chamomilla Recutita (Matricaria) Flower Oil	05G	Tonics, dressings and other hair grooming aids	0.1%
Chamomilla Recutita (Matricaria) Flower Oil	06A	Hair dyes and colors (all types requiring caution statement and patch test)	0.06%
Chamomilla Recutita (Matricaria) Flower Oil	07E	Lipstick	0.03%
Chamomilla Recutita (Matricaria) Flower Oil	10A	Bath soaps and detergents hand soap	 0.0001%
Chamomilla Recutita (Matricaria) Flower Oil	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.012%
Chamomilla Recutita (Matricaria) Flower Oil	12C	Face and neck products not spray	 0.001%
Chamomilla Recutita (Matricaria) Flower Oil	12D	Body and hand products not spray spray	 0.2% 0.066%
Chamomilla Recutita (Matricaria) Flower Powder	12H	Paste masks and mud packs	1%
Anthemis Nobilis Flower Extract	03B	Eye liner	0.001-0.025%
Anthemis Nobilis Flower Extract	03D	Eye lotion	0.02%
Anthemis Nobilis Flower Extract	03E	Eye makeup remover	0.003%
Anthemis Nobilis Flower Extract	05A	Hair conditioners	0.000025-0.001%
Anthemis Nobilis Flower Extract	05F	Shampoos (noncoloring)	0.000025-0.1%
Anthemis Nobilis Flower Extract	05G	Tonics, dressings and other hair grooming aids spray	0.0002% 0.0004%

Anthemis Nobilis Flower Extract	10A	Bath soaps and detergents	0003%
Anthemis Nobilis Flower Extract	10E	Other personal cleanliness products	0.002-0.01%
Anthemis Nobilis Flower Extract	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.002-0.0075%
Anthemis Nobilis Flower Extract	12C	Face and neck products not spray	0.01%
Anthemis Nobilis Flower Extract	12D	Body and hand products not spray	0.00004%
Anthemis Nobilis Flower Extract	12E	Foot powders and spray spray	0.03%
Anthemis Nobilis Flower Extract	12F	Moisturizing products not spray	0.0028-0.03%
Anthemis Nobilis Flower Extract	12H	Paste masks and mud packs	0.000001%
Anthemis Nobilis Flower Extract	12J	Other skin care preparations	0.007-0.5%
Anthemis Nobilis Flower Oil	02A	Bath oils, tablets and salts	0.007%
Anthemis Nobilis Flower Oil	03D	Eye lotion	0.000057-0.01%
Anthemis Nobilis Flower Oil	04B	Perfumes	2.8%
Anthemis Nobilis Flower Oil	04E	Hair conditioners	0.000039%
Anthemis Nobilis Flower Oil	05F	Shampoos (noncoloring)	0.00033-0.004%
Anthemis Nobilis Flower Oil	05G	Tonics, dressings and other hair grooming aids spray	0.0006-0.01% 0.006%
Anthemis Nobilis Flower Oil	07C	Foundations	0.02%

Anthemis Nobilis Flower Oil	10A	Bath soaps and detergents	0.00077%
Anthemis Nobilis Flower Oil	11E	Shaving cream (aerosol, brushless and lather)	0.0002%
Anthemis Nobilis Flower Oil	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.001-0.0063%
Anthemis Nobilis Flower Oil	12C	Face and neck products not spray	 0.0063-0.5%
Anthemis Nobilis Flower Oil	12D	Body and hand products not spray spray	 0.0063-0.5% 0.006-0.37%
Anthemis Nobilis Flower Oil	12J	Other skin care preparations	0.0063%
Anthemis Nobilis Flower Oil	12G	Night products not spray	 0.5%
Anthemis Nobilis Flower Oil	12H	Paste masks and mud packs	0.05%
Anthemis Nobilis Flower Water	03B	Eye liner	1%
Anthemis Nobilis Flower Water	07C	Foundations	4%
Anthemis Nobilis Flower Water	11A	Aftershave lotions	2%
Anthemis Nobilis Flower Water	11E	Shaving cream (aerosol, brushless and lather)	2%
Anthemis Nobilis Flower Water	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	10%
Anthemis Nobilis Flower Water	12C	Face and neck products not spray	 3%
Anthemis Nobilis Flower Water	12F	Moisturizing products not spray	 1%

Anthemis Nobilis Flower Water	12J	Other skin care preparations rinse-off	3%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

†Product category codes used by FDA

Information collected in 2013

Table prepared: May 2, 2013

Updated May 10, 2013: Chamomilla Recutita (Matricaria) Flower Extract: body and hand products: changed high concentration from 0.5 to 0.02%; skin cleansing: changed 0.001-2% to 0.0002-0.02%; hair conditioners: changed high concentration from 1% to 0.12%; aerosol hair spray: changed 0.00003-10% to 0.00001-0.00003%; hair straighteners: changed 7% to 0.0007%; hair grooming aids: changed 0.00024-10% to 0.00001-0.0075%; feminine hygiene deodorants: changed 0.4% to 0.0004%; shampoo changed 0.00006-1% to 0.00001-0.25%

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Ivan J. Boyer, Ph.D., D.A.B.T.
Senior Toxicologist
Date: May 20, 2013
Subject: Professional Keratin Smoothing Council (PKSC) 5 May 2013 Submission on Methylene Glycol/Formaldehyde Exposures from the Use of Hair Smoothing Products

At the September 2011CIR Expert Panel meeting, the Panel issued a final amended safety assessment concluding as follows:

> "Formaldehyde and methylene glycol are safe for use in cosmetics when formulated to ensure use at the minimal effective concentration, but in no case should the formalin[1] concentration exceed 0.2% (w/w), which would be 0.074% (w/w) calculated as formaldehyde or 0.118% (w/w) calculated as methylene glycol. Additionally, formaldehyde and methylene glycol are safe in the present practices of use and concentration in nail hardening products. However, formaldehyde and methylene glycol are unsafe in the present practices of use and concentration in hair smoothing products."

The Professional Keratin Smoothing Council (PKSC) submitted a letter report to the Cosmetic Ingredient Review (CIR) on 5 May 2013, which presents their observations, explanations and recommendations for the use of hair smoothing (a.k.a. hair straightening) products containing methylene glycol/formaldehyde. There are three attachments to this PKSC report, including:

- Cover letter dated 4 March 2011 from Exponent, which summarizes the results of Exponent's study measuring airborne concentrations of formaldehyde during the use of a keratin hair smoothing product by a stylist on a model in a salon; the Exponent report, in its entirety, was previously submitted to the CIR on 5 May 2011, and was reviewed at the 27-28 June 2011 Panel meeting
- Analytical chemistry report (Analytical Sciences, 14 September 2011) of the results of analyzing 250-ml air samples, each collected 2 inches from a hot flat iron (440 ^{o}F) after applying one of two hair smoothing products or formalin (amounts not specified) directly to the flat iron; the glass tubes used to collect the air samples contained N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA), rather than dinitrophenyl hydrazine (DNPH), as the derivatizing agent
- Analytical chemistry report (Process NMR Associates, 16 May 2012) of the results of analyzing the concentrations of "free formaldehyde" and other components in formalin samples, using a quantitative ^{1}H NMR method; the report indicates that formalin contained 0.0002% to 0.0061% "free formaldehyde

[1] Formalin is an aqueous solution wherein formaldehyde (gas) has been added to water to a saturation point, which is typically 37% formaldehyde (w/w). Because of the equilibrium between formaldehyde and methylene glycol in aqueous solution, formalin contains both formaldehyde and methylene glycol.

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(as methanal)," depending on whether an acidifying internal standard was added to the sample before analysis

PKSC's 17 May 2013 letter report re-visits several issues that have been reviewed in the past, including their position that methylene glycol is not chemically or toxicologically equivalent to formaldehyde and that proper training, product application procedures, ventilation, and attention to avoiding sensory irritation can ensure that exposures associated with professional-use only keratin smoothing products do not exceed OSHA standards and American Conference of Government Industrial Hygienists (ACGIH) recommendations for occupational exposures to formaldehyde.

In addition, the letter report notes the results of a study in which formalin samples (amounts not specified) were vaporized at 400 ^{o}F in either a tightly sealed dry box or a small sealed room, and air samples were collected 6 and 18 inches from the vaporization point in the box and 6 and 36 inches from that point in the room, using DNPH or 2-hydroxymethyl pyridine (2-HMP) as the derivatizing agent in the sampling tubes; a fan was used in the testing chamber during sampling to ensure mixing. The results from 2-HMP were not reported because of a sampling preparation error. The results from air samples collected using DNPH as the derivatizing agent indicated that 44% to 47% of the methylene glycol in the formalin samples vaporized to release "formaldehyde gas and methylene glycol vapour" in the chambers. No other information was provided about this study.

PKSC's recommendations for ensuring that OSHA standards and ACGIH recommended occupational exposure limits are met include instructing stylists to ask clients about their previous history of sensitivity to formaldehyde or formalin and setting blow dryers to cooler temperatures after applying a smoothing product to the hair. PKSC also recommends that keratin smoothing products contain no more than "3% methylene glycol or 0.2% dissolved formaldehyde gas." The stated basis for this recommendation is "all of the information and data presented to the CIR by the PKSC in this and previous reports." In addition, PKSC recommends that all products of this nature carry a warning that heating may release formaldehyde gas, which can irritate the eyes, nose and throat, and safe use and handling requires adequate ventilation and adherence to the manufacturer's directions.

After reviewing the PKSC submittal, the CIR Expert Panel should determine whether the recommendations and discussion presented in the submittal warrants re-opening the formaldehyde and methylene glycol safety assessment.

May 17, 2013

Alan Andersen, PhD
Director, Cosmetic Ingredient Review
1101 17th St., N.W., Ste. 412
Washington, D.C. 20036-4702

Dear Dr. Andersen:

The Professional Keratin Smoothing Council (PKSC) is a beauty industry association that represents manufacturers of professional-use only keratin smoothing products across the country. The members of the PKSC represent a select few members of the industry producing these products. The principles that bring the members of the PKSC together include corporate responsibility, utmost care and concern for the safety of salon professionals and consumers, and a commitment to producing the most effective products with the lowest level of active ingredients necessary to achieve desired keratin smoothing results.

In 2011, the CIR Expert Panel reached a conclusion concerning the use of methylene glycol and/or releasers of formaldehyde as ingredients used in professional-use only keratin smoothing products for the purposes of performing keratin smoothing treatments in salons. During their deliberations, the Expert Panel recognized the evidence demonstrating that professional use keratin smoothing products were being used safely in many salons. This included the vast majority of salons where stylists did not report symptoms of formaldehyde-related sensory irritation. This was supported by salon air monitoring studies conducted by the Oregon Occupational Safety and Health Administration (OSHA) as well as the independent study by Exponent, LLC, all demonstrating that formaldehyde exposures were below both the OSHA permissible exposure limits (PEL) as well as the more conservative threshold limit value (TLV) established by the American Council of Government Industrial Hygienist (ACGIH), i.e.,0.75 ppm and 0.3 ppm, respectively, as 8 hour time weight averages (TWA). These studies demonstrate that a variety of methylene glycol-containing keratin smoothing products can be safely used. Indeed, it is noteworthy that during recent meetings, the CIR has debated the issue of formaldehyde-induced sensory irritation. In this regard it is of interest that on two separate occasions, half of the CIR Expert Panel members expressed the opinion that methylene glycol-containing products are safe to use if they do not cause sensory irritation. This conclusion was based on the fact that the eyes are the most sensitive biological indicator of excessive formaldehyde exposure. Therefore, when no ocular sensory irritation occurs, by definition, the conditions of use are protective against overexposure including any long or short term adverse effects. In fact, this view was so strongly held that half of the CIR's expert panel members abstained from voting on the motion to conclude that formaldehyde/methylene glycol were

unsafe in the present practices of use and concentration in keratin smoothing products during the full CIR (2011) meeting stating "*So, our team doesn't second it.* ***We still like the biologic endpoint****, we use that for leave-on products, non-irritating, so we think aerosolized, if it causes irritation in whatever the conditions are, it shouldn't be used. So, that's where we come down on it."* (Formaldehyde CIR 2011) [emphasis added].These same statements were also made at the CIR meeting in March 2012, before the full panel finally agreed upon their latest conclusion. Clearly, the notion that sensory irritation is the most accurate measure of potentially excessive exposure to formaldehyde is accepted by many authoritative experts. The PKSC agrees that keratin smoothing procedures with products containing methylene glycol as the active ingredient should be held to this high standard, e.g., eye irritation should not occur under any circumstances.

This report continues to address these issues and presents new data and other information which demonstrates and/or recommends:

1. Methylene glycol is not chemically equivalent to formaldehyde.
2. Methylene glycol is not toxicologically equivalent to formaldehyde.
3. Steps to be taken by users to prevent formaldehyde-related sensory irritation.
4. Proper product application procedures and ventilation to ensure that any formaldehyde emissions are below established safe levels of exposure.
5. Safe concentrations for methylene glycol in keratin smoothing products.

A. Introduction

Keratin smoothing products typically contain 2-5% of 37% formalin, timonacic acid or other formaldehyde-releasing agents as the ingredients primarily responsible for the keratin smoothing effect. Recently, much attention about the use of such products has properly focused on potential formaldehyde exposure and sensory irritation of the eyes, nose and throat to either cosmetologists or their customers. Sporadic reports of sensory irritation occurring in conjunction with the use of keratin smoothing products have been attributed solely to the presence of airborne formaldehyde gas emitted when these products are heated as part of the keratin smoothing service.

In 2011, the CIR concluded that formaldehyde and methylene glycol should be treated as though they were essentially "equivalent", due to the belief that because of the equilibrium which exists between these two chemicals, any concentration of methylene glycol is essentially equivalent to formaldehyde, e.g., "formaldehyde equivalents". Since methylene glycol (or other formaldehyde releasers) is the key functional ingredient in most keratin smoothing products, the practical implications of this conclusion is that whatever concentration of methylene glycol is formulated into a product, that same concentration of anhydrous formaldehyde gas would also be available for release upon heating during normal use. It is important to note that the concept of "formaldehyde equivalents" was not based on any empirical data, but rather was a precautionary assumption arising from a concern that methylene glycol, under the conditions of use in keratin smoothing products, could release the same concentration of formaldehyde into the air, e.g., any concentration of methylene glycol in a product could be 100% converted to formaldehyde. If true, the inescapable conclusion of the assumption of chemical equivalence would be that these two, distinctly different chemicals would be toxicologically equivalent as well. If not true, this would demonstrate

that methylene glycol and formaldehyde are not "equivalent" and that their potential toxicity should be considered individually.

This report demonstrates that methylene glycol and formaldehyde are NOT equivalent by addressing several key misunderstandings concerning the equilibrium between formaldehyde and methylene glycol and by correcting some factual errors related to the supposed equivalency between these two chemicals. In addition, this report also addresses the comparative toxicity of methylene glycol and formaldehyde. New data generated to empirically test the assumption of equivalence, used standardized formalin alone to avoid potential confounders from other ingredients which may hinder the formation of formaldehyde from methylene glycol at the temperatures used for keratin smoothing procedures. This new information helps to better understand the actual potential for formaldehyde exposure during use of keratin smoothing products containing methylene glycol (or other formaldehyde donors) as the functional component. Particularly as they pertain to the issue of chemical equivalence, the new empirical data summarized below, demonstrate that formaldehyde and methylene glycol are NOT chemically equivalent (even at 400^{o}F) which is consistent with the well-established equilibrium kinetics of these distinctly different chemical substances.

B. Chemical Equivalence Question: Methylene glycol is not chemically equivalent to formaldehyde

By definition, when equilibrium exists between two <u>different</u> chemical species, a shift in the equilibrium results in a change of concentration between these <u>different</u> chemical species. Ionic chemical equilibriums demonstrate this phenomenon quite well. For example, an ionic equilibrium exists between hydrochloric acid (HCl) and hydrogen ions (H^{+}) and chloride anions (Cl^{-}) and it is clearly scientifically incorrect and misleading to claim that hydrogen cations or chloride anions are equivalent to hydrochloric acid, when these are two distinctly different chemical ionic species. It does not matter that the reverse reaction proceeds quickly and easily; neither of these ions are equivalent to HCl and the reversibility of this reaction does not establish that these are chemical equivalents.

The claim that methylene glycol is equivalent to formaldehyde does not fulfill the simplest chemical definition of "equivalent" as "*having the same combining or reacting value*" [1]. Furthermore, according to the "*Compendium of Analytical Nomenclature*" published by the International Union of Pure and Applied Chemistry (IUPAC) Analytical Chemistry Division, which is considered the most authoritative source on chemical definitions and nomenclature, the concept of "*chemical equivalence*" does not include equilibrium reactions between two species and therefore use of this terminology is inappropriate from a chemical point of view. The IUPAC definitively states, that the term "equivalence" applies only when, "*The equivalent of a species X is that entity in which a specific reaction would combine with or be in any other appropriate way of equivalent in: a. An acid-base reaction to one entity of type tradable hydrogen ions, H+, OR b. A redox reaction to one entity of electrons,e-".* Neither of these conditions are met by the equilibrium between formaldehyde and methylene glycol. The IUPAC further states, "*the concepts of equivalence and the use of the term equivalent is well-established in the studies of ion-exchange phenomena and in electroanalytical chemistry (notably in electogravimetry and couloimetric procedures). Thus any proposal made for standardization of terminology in titrimetric analysis [for which this concept was primarily developed] must be equally applicable to*

[1] Webster's New International Dictionary, Unabridged, 2nd Ed. 1950

these and relevant fields." None of the above conditions are met by the equilibrium between formaldehyde and methylene glycol. Therefore, for these reasons and others presented later in this report, it is scientifically inappropriate by internationally accepted convention to refer to methylene glycol and formaldehyde as "equivalent".

C. Formaldehyde and Methylene Glycol in Air

It is generally not appreciated that when either methylene glycol-containing products or formalin are heated to 400^0F, both formaldehyde and methylene glycol are vaporized in the process along with water. Two different researchers (Little 1999 and Utterback et al. 1985) have independently reported capturing, identifying and measuring methylene glycol in the air by utilizing chemical dervatization methods and GCMS techniques, thus conclusively demonstrating that methylene glycol is stable and can exist in the vapor phase. Using a chemical specific derivatizing agent (N,O-bis-(trimethylsilyl) trifluoroacetamide; BSTFA), methylene glycol in the air can be identified by GCMS. It is important to note that the derivatizing agent most commonly used to measure formaldehyde, e.g. 2,4-dinitrophenylhydrazine (DNPH), is specific only to formaldehyde (i.e., an aldehyde) and will not directly derivatize methylene glycol (i.e., an alcohol), which serves as further indirect evidence that these two molecules are not chemically equivalent.

To confirm the above reports in 2011 the PKSC tested two methylene glycol-containing keratin smoothing products and a sample of 37% formalin by individually vaporizing each and testing as described above. The resultant vapors were drawn through a tube containing BSTFA/glass wool. Methylene glycol and several of its short chain oligomers were readily identified in the vapors (Analytical Sciences 2011; attached). These results are compelling because formaldehyde is a highly reactive, anhydrous gas that reacts in milliseconds in the presence of water to form methylene glycol. Since the vapor phase consists largely of warm water vapor, it is unlikely that anhydrous formaldehyde gas can exist for very long. For this reason, under these conditions, air concentrations of formaldehyde are expected to diminish quickly. While methylene glycol clearly exists in equilibrium with formaldehyde, the driving force "strongly" favors methylene glycol formation and only "weakly" favors formaldehyde formation, particularly in situations involving heating in which only small amounts of formaldehyde are present along with substantial amounts of water vapor.

D. Empirical Testing of the Chemical Equivalence Assumption: Measuring the degree of conversion of methylene glycol to formaldehyde.

Many regulators[2] erroneously assume, <u>without providing any empirical proof,</u> that methylene glycol is equivalent to formaldehyde which can only mean that it can/would be 100% converted to formaldehyde gas when heated under the conditions that keratin smoothing products are used. To test the equivalence hypothesis as to whether methylene glycol does or does not undergo complete conversion to formaldehyde at elevated temperatures, the PKSC

[2] "….*the formation of methylene glycol or the release of gaseous formaldehyde occurs extremely quickly. Via this dynamic equilibrium in aqueous solution, formaldehyde and methylene glycol are mutually converted and hence inherently linked with each other due to low energy barriers of formation and degradation of methylene glycol*" (SCCS 2012) and "*It is proposed that 'free formaldehyde' could be defined as 'all hydrated or non-hydrated formaldehyde present in aqueous solution, including methylene glycol'* (ACCC 2012).

commissioned several independent studies to determine what percentage of a standardized formalin solution, when rapidly heated to force complete vaporization, will convert into formaldehyde gas. This can be considered as a best case scenario for producing the maximum concentration of formaldehyde that could be formed, due to the lack of any other potential chemical confounders that might hinder its formation. Using two different derivitizing agents to measure formaldehyde by different test methods, one using HPLC and the other GCMS, provides compelling and relevant data for empirically testing the hypothesis of chemical equivalence between formaldehyde and methylene glycol.

Table 1 summarizes the PKSC-sponsored research showing the specific test methods, the analytical laboratories performing the testing and the specific purpose of each test.

Table 1. PKSC Testing In-Progress

Test Method	Company	Purpose
13C-NMR	Process NMR, Assoc.	Directly measure methylene glycol in aqueous solutions without any chemical derivitzation or potential modifications that force methylene glycol to degrade.
1H-NMR (Proton NMR)	Process NMR Assoc.	Directly measure the total amount of releaseable formaldehyde gas.
DNPH/HPLC (Dinitrophenyl hydrazine)	Analytical Sciences	Indirectly measure the percentage of methylene glycol converted to formaldehyde gas when a known amount of standardized formalin is completely vaporized in a closed space of known volume.
2-HMP/GCMS (2-hydroxymethly pyridine)	Analytical Sciences	Indirectly measure the percentage of methylene glycol converted to formaldehyde gas when a known amount of standardized formalin is completely vaporized in a closed space of known volume.
BSTFA (N,O-bis-(trimethylsilyl) trifluoroacetamide /GCMS	Analytical Sciences	Derivatizing agent used to detect methylene glycol in air.

To measure the actual percent conversion of methylene glycol to formaldehyde, the precise concentrations of methylene glycol, total releasable formaldehyde, methanol, formic acid, as well as dimers, trimers and longer chain oligomers were measured using 13C-NMR, 1H-NMR, as well as titration with standardized sodium formate. This standardized solution of a commercial 37% formalin solution was determined to contain 36.26% total releasable formaldehyde (i.e., as methylene glycol and polymers) (Process NMR Associates 2013).

A precisely measured amount of the standardized formalin solution was then vaporized in a tightly sealed laboratory dry box with a known volume. Two different derivatizing agents, widely used by both OSHA and NIOSH, were placed into separate air sampling tubes and after 15 minutes of air sampling, were tested via the methods shown in Table 1. A fan was used in used in each test chamber to ensure that adequate mixing occurred. The dinitrophenyl hydrazine (DNPH) sample tubes were measured via HPLC while the 2-hydroxymethly pyridine (2-HMP) air sampling tubes were measured using GCMS. A total of four measurements were made, two with each derivatizing agent placed at different distances (i.e., 6 and 18 inches) from the 400^{o}F source point where vaporization occurred. This dry box experiment was repeated to ensure repeatability and reproducibility of the results. This was followed by the same test protocol conducted in a small sealed room with a known volume to determine any potential differences due to the size of the containment area with the derivitizing agents placed at 6 and 36 inches from the source point where vaporization occurred. The results obtained from each run using DNPH are presented in Table 2. The results from the 2-HMP derivitizing agent were not reported due to a sample preparation error, which lead to the loss of any useful data. However, the methodology works well and will likely be utilized in future salon air monitoring studies.

As shown in Table 2, in none of the test situations was the vaporized volume of 36.4% formalin solution converted completely into formaldehyde. Because methylene glycol was not 100% converted to formaldehyde, these results clearly confirm that methylene glycol should NOT be considered to be equivalent to formaldehyde even at 400^{0}F.

Table 2. Measured Conversion Rates of Methylene Glycol into Formaldehyde

Test Method	**Container/ Collection Distance**	**Container Volume**	**% Conversion of liquid Methylene Glycol to Formaldehyde gas & Methylene Glycol vapour**
DNPH/ HPLC	Dry Box at 6 inches	0.2724 m^3	44.3
DNPH/ HPLC	Dry Box at 18 inches	0.2724 m^3	47.3
DNPH/ HPLC	Small Room at 6 inches	15.18 m^3	46.5
DNPH/ HPLC	Small Room at 36 inches	15.18 m^3	47.3

As discussed above, methylene glycol vapors readily exist in ambient air particularly following vaporization at 400^{o}F. Therefore, when these vapors are drawn into the air monitoring tubes containing derivatizing agents, such as DNPH or 2-HMP, the captured methylene glycol will be forced to convert to formaldehyde gas to maintain the equilibrium as the derivatizing agent removes formaldehyde from the system thereby creating an artificial equilibrium shift. As a result, methylene glycol is thus consumed and reported as “formaldehyde”, even though it did NOT exist as formaldehyde in the air. This substantially skews air monitoring results to over report formaldehyde concentrations in air samples.

Consequently, the results in Table 2 represent the conversion of liquid methylene glycol into both formaldehyde gas and methylene glycol vapour. These results indicate that

when formalin is heated, only about 47% of the expected formaldehyde gas and methylene glycol vapors are produced and measured. It is not yet known, how much of this 47% comes from methylene glycol vapors and how much is attributed to formaldehyde gas. While the PKSC is striving to determine this ratio in air, the Winkelman equilibrium constant can be used to provide insights into the likely behavior of methylene glycol when it is heated. According to equilibrium calculations[3] the expected concentration of formaldehyde gas that can be produced when 37% formalin is heated to 400-425^{0}F should be less than 12.0%, which suggests that the contribution of methylene glycol to the values reported in Table 2 is approximately 36% (i.e., these values would be reduced by ≈ 65%).

The PKSC's next steps will be to test the vaporization of keratin smoothing products to determine what percentage of methylene glycol is converted into airborne formaldehyde gas. Initial evaluations indicate that the presence of other ingredients, such as keratin extracts and botanicals, could lower the conversion of methylene glycol into anhydrous formaldehyde gas and methylene glycol vapors upon vaporization into the air. Consequently, it is expected that substantially lower amounts of methylene glycol liquid are vaporized into formaldehyde gas and methylene glycol vapor as compared to the approximately 47% converson upon vaporization of the 36.23% formalin standard.

1. Salon Implications

The implications of the above discussion for the salon setting are substantial. Methylene glycol vapors are created either by direct vaporization when keratin smoothing products are rapidly heated with a blow dryer or electric flat iron or when formaldehyde gas emitted simultaneously reacts with water in the vapor cloud created when these products are heated. This is likely true for any high humidity situation. As expected and indicated in Table 2, any released formaldehyde gas rapidly diminishes by reacting with water vapor thereby further reducing any potential for formaldehyde gas exposure in the salon. This likely explains why, given the tens of thousands of keratin smoothing procedures that have been performed, reports of sensory irritation are relatively rare.

PKSC's air monitoring studies in salon settings where keratin smoothing products are used have aided in determining which work practices and ventilation methods minimize potential formaldehyde exposures. The data described above demonstrate that cosmetologists are exposed to much less formaldehyde than predicted or expected, and more methylene glycol than previously suspected. This then raises another relevant question, i.e., What is the potential toxicity of methylene glycol, if any?

[3]The Arrhenius equation, kh=e –Ea/(RT),demonstrates the effect of temperature on the equilibrium between methylene glycol and formaldehyde. When formalin is heated to 400^{0}F (477^{0}K), kh=e((3769/T)-5.494) 0.078248 1/kh x 100= 9.03% is the amount of available methylene glycol expected to convert to formaldehyde gas. At 425^{0}F, the conversion rate to formaldehyde gas is expected to be 11.31%.

E. Toxicological Equivalence Question: Methylene glycol is not toxicologically equivalent to formaldehyde.

While the health effects of anhydrous formaldehyde gas are well understood and extensively characterized, those of methylene glycol are not. This is due primarily to the fact that properly designed experimental studies (in both animals and humans) can be conducted on anhydrous formaldehyde gas alone with no potential confounding methylene glycol. However, it is essentially impossible to study methylene glycol as a stand-alone chemical since, by definition it is always in equilibrium with trace (e.g., biological systems) to larger amounts (e.g., formalin) of formaldehyde.

Consequently, the only data available from which to assess or deduce potential health effects from methylene glycol following ingestion exposures are case reports and/or studies in which 37% formalin (59% methylene glycol/0.05% dissolved formaldehyde in an aqueous solution) is used by humans for suicidal purposes or administered to animals under controlled conditions. Inhalation exposure studies include those on embalmers or anatomists or students in gross anatomy dissection labs where formalin preservatives are frequently utilized. Both types of data can be used to assess the potential irritant and/or systemic effects of methylene glycol with the oral exposure studies being more informative since larger exposure dosages can be achieved. Furthermore, as discussed below, the results from the formalin suicide case reports are augmented by additional case studies in which formic acid, the direct metabolite of formaldehyde, are also used for the purpose of suicide. Such case studies are not confounded by simultaneous exposure to methylene glycol.

However, in assessing the above types of studies there is an obligatory need to separate any potential adverse effects caused by methylene glycol from those that are properly attributable to formaldehyde. Because the effects of formaldehyde, whether inhaled or ingested, have been extensively characterized and reported, it is possible to “subtract” such effects from the totality of reported effects and by deductive reasoning determine the potential effects of methylene glycol, if any. If all reported effects can be reliably attributed to formaldehyde, this would provide corroborating evidence that methylene glycol was without observable toxicity from such exposures. Conversely, if there were reported effects “not accounted for” or not reliably attributable to formaldehyde, this would constitute presumptive evidence that such effects may be due to methylene glycol exposure.

1. Oral Exposure to Formalin

There are two types of studies and/or data in which oral exposure to methylene glycol can be reliably documented; (1) human case reports in which formalin was either used in an intentional suicide attempt or accidentally ingested and (2) animal studies where the effects of formaldehyde are systematically investigated following gavage or drinking water/dietary exposures with formalin. These are briefly summarized below with a description of the reported effects and an evaluation of whether all such effects can be reliably attributed to formaldehyde or conversely, if some effects are plausibly due to methylene glycol. .

a. Human Case Reports

Despite its pungent disagreeable odor and highly corrosive properties, there are a sufficient number of case reports that can be used to assess the acute oral toxicity of formalin.

Depending on the dose of 37% formalin ingested, all case reports describe varying degrees of severe corrosive injuries of the esophagus and stomach similar to those produced by strong acids. Additionally, since the formaldehyde component of formalin at these concentrations is readily absorbed into the circulation from the gastrointestinal tract and rapidly metabolized to formic acid in the liver and red blood cells, metabolic acidosis is reported in virtually all cases. This is a typical consequence following suicide attempts due to the inability of the body to rapidly accommodate the sudden large quantities of formic acid in the circulation. Because formic acid-induced metabolic acidosis is typically severe (and difficult if not impossible to control), this results in numerous other life-threatening signs and symptoms including chest pain, heart palpitations, arrhythmias (ventricular tachycardia) and/or low blood pressure, nausea, vomiting, abdominal pain, breathing abnormalities, neurological symptoms (e.g., lethargy, stupor, coma, seizures) and death (Isselbacher et al. 1994, Pandey et al. 2000).

For example, as reported by Koppel et al. (1990) in two separate suicide attempts, a 55-year-old woman and a 34-year-old man ingested an unknown amount of formalin. The female patient was found in a coma, in shock (blood pressure 50 mm Hg), respiratory insufficiency, and metabolic acidosis. Similarly, the male patient also exhibited shock (blood pressure 60 mm Hg), respiratory insufficiency, and metabolic acidosis. Analysis of the formalin samples ingested by both patients failed to detect methanol, even though this was expected and no other drugs or methanol were found in their systems. Both individuals died from cardiac failure. The lack of methanol in the formalin ingested by the two subjects as confirmed by no detection of elevated methanol in their bodies suggests that this component of formalin plays an inconsequential role in its ultimate toxicity profile. Since methanol (which is present in most formalin solutions) is also metabolized to formic acid (EPA 2009), its role in systemic toxicity would be indistinguishable from that produced by formaldehyde.

In another suicide attempt, a 41-year-old woman swallowed 120 mL formalin (37% formalin solution; and was brought to the hospital within 30 minutes after complaining of abdominal pain and subsequently lost consciousness (Eells et al. 1981). Upon admission, the patient was cyanotic, apneic, and hypotensive with laboratory tests showing severe metabolic acidosis. Despite intubation, initiation of ventilation, administration of IV fluids and gastric lavage the patient died 28 hours after admission.

With minor and inconsequential variations on the above reported cases, depending on the dose ingested and the extent to which clinical chemistry was considered, described and/or assessed, none of the other numerous reported cases of ingested formalin report any symptoms that are not a direct result of formaldehyde, e.g., corrosive injuries of the esophagus and stomach and severe metabolic acidosis, or sequelae from these initial symptoms (Hawley and Harsch 1999, Yanagawa et al. 2007, Nishi et al. 1990, Burkhart et al. 1990, Spellman 1983, Bartone et al. 1968, Spellman 1983, Eells et al. 1981, Watt 1912, Kochhar et al. 1986, Heffernon and Hajjar 1964, Roy et al. 1962).

2. Oral Exposure to Formic Acid

Further confirmation that formaldehyde alone, with no potential contribution from methylene glycol, is responsible for all reported symptoms resulting from formalin ingestion is provided by the numerous case studies and reports of formic acid ingestion, solely for suicidal purposes. Formic acid is the principal metabolite of absorbed formaldehyde and responsible for all metabolic effects following formalin ingestion. One particularly relevant report by Rajan et al.

(1985) documents the clinical sequelae in 53 cases following intentional ingestion of formic acid. Of the 53, 38 survived and 15 died; the age range was 16-46 years. Symptoms reported included adverse effects on the respiratory system due to inhalation pneumonitis and the cardiovascular system with both increased and decreased heart, arrthythmias and vascular hypotension. Adverse effects were also observed in the renal system (e.g., hematuria, tubular necrosis and renal failure), all symptoms consistent with severe metabolic acidosis identical with those reported following formalin ingestion.

In a 2-year retrospective analysis Dalus et al. (2012) reviewed the medical charts of 302 patients with acute intentional ingestion of formic acid to determine patterns of initial presentation of symptoms as well as to find predictors of mortality which occurred in 35% of the population. Symptoms associated with 100% mortality included bowel perforation, shock, and tracheoesophageal fistula all due to the corrosive properties of formic acid. Symptoms significantly associated with severe morbidity could be roughly divided into those secondary to the corrosive properties of formic acid, i.e., quantity of formaldehyde consumed ($p<0.001$), consuming undiluted formic acid ($p< 0.001$), severe degree of burns ($p=0.020$), hematemesis ($p=0.024$) and those likely related to metabolic acidosis ($p < 0.001$) such as respiratory distress (p, 0.001).

Other case reports of ingestion of formic acid (Sigurdsson et al. 1983) or of formic acid-containing agents (Naik et al. 1980) also document the key etiological role played by metabolic acidosis in the pattern of symptomatology leading to severe morbidity or death. The data on formic acid ingestion, comprising fatal and non-fatal outcomes in more than 350 individuals who intentionally ingested this liquid for suicidal purposes demonstrate that all symptomology can be reliably attributed solely to its corrosive or metabolic acidosis-inducing properties.

Therefore, it can be concluded that following human ingestion of formalin solutions or formic acid itself, all reported symptoms can be reliably attributed to formic acid the sole formaldehyde metabolite, with no evidence or suggestion that methylene glycol played an etiological role. Based on these data, it is reasonable to conclude that methylene glycol is not toxic, particularly in comparison with formaldehyde. In a very real sense, the numerous case studies, whether with formalin or formic acid, are approximations of LD_{50} studies in which lethal doses of chemicals are tested. As such, they convincingly demonstrate that the toxicity of formalin is due solely to the formaldehyde component with no demonstrable toxicity from methylene glycol. This is confirmed by the animal studies briefly summarized below in which far less concentrated formalin solutions are administered with no indication of the severe metabolic acidosis produced in the formalin (or formic acid) ingestion suicide case reports.

3. Animal studies

Numerous high quality oral exposure studies in rodents have been conducted to investigate the potential effects of aqueous solutions of methylene glycol and formaldehyde. These studies use either para-formaldehyde suspensions in water or formalin solutions which are then diluted with water to achieve the desired test concentrations. Such aqueous solutions of formaldehyde are actually predominantly (i.e., 99.94%) methylene glycol, although most such studies are incorrectly referred to as formaldehyde studies. Animals are then exposed via drinking water to various doses of diluted formalin (i.e., methylene glycol) solutions.

For example, Tobe et al. (1989) dissolved para-formaldehyde in distilled water followed by mixing for 5 hours at 80 ^{0}C. Wistar rats were then exposed via drinking water at formalin concentrations of 0, 0.02, 0.10, and 0.50% equivalent to doses of 0, 10, 50 and 100 mg/kg/day, respectively. The primary target for formaldehyde toxicity was the stomach with gastric mucosal lesions of various types noted at doses of 150 or 100 mg/kg/day. The no observed effect level (NOEL) was 0.02% (i.e., 10 mg/kg/day = 9.96 mg/kg methylene glycol and 0.04 on mg/kg formaldehyde). Because this study demonstrated no effects other than those clearly attributable to formaldehyde, it can be reasonably concluded that the methylene glycol component of formalin played no etiological role in the reported effects. Similar drinking water studies in dogs for 90 days (Johannsen et al. 1986), rats for 4 weeks (Til et al. 1988) or two years (Til et al. 1989) report a similar lack of effects attributable to methylene glycol. Rather all reported effects (e.g., hyperkeratosis or focal gastritis) could be reliably attributed to the corrosive properties of formaldehyde on the gastrointestinal mucosa.

4. Inhalation Exposure To Formalin

There are numerous studies involving workers with high potential for exposure, such as those working as embalmers and/or in gross anatomy/dissection labs where 37% formalin is typically used. Due to its volatility, formaldehyde from these concentrations readily escapes as attested by the numerous studies in which sensory irritation is reported in conjunction with these occupations. However, the significantly lower vapor pressure of methylene glycol suggests a much lower potential for volatilization at ambient temperatures. Despite the diversity of populations in these studies it is notable that other than reports of sensory irritation of the eyes, nose and throat, as well as occasional nausea, headache, transient breathing difficulties and dermal sensitization in approximately 4% of the populations, no other adverse effects are reported consistent with formaldehyde as the sole causative agent (Holness and Nethercott 1989, Chia et al. 1992, Mirabelli et al. 2012, Takahashi et al. 2007, Ward et al. 1984, Khaliq and Tripathi 2009).

5. Conclusions on Methylene Glycol Toxicity

Taken together, the high dose human case reports and controlled animal studies on formalin (i.e., methylene glycol), the human case reports on formic acid ingestion and the inhalation exposure studies in embalmers and similar workers demonstrate that all reported adverse effects are attributable solely to formaldehyde alone with no evidence that co-exposure to methylene glycol played any contributory role. Therefore it can be concluded that methylene glycol does not have any toxicity. This is not surprising since, in biological systems, it appears that methylene glycol serves no role other than as a biological reservoir for metabolically-produced formaldehyde. Consequently it can be reliably concluded that formaldehyde and methylene glycol are not toxicologically equivalent.

F. Recommended Steps to Prevent Excessive Exposure to Formaldehyde when Using Keratin Smoothing Products

The chart and italicized quotations below highlight key conclusions and observations from the attached Exponent study, *"Formaldehyde Exposure Assessment during the Application of Keratin Hair Smoothing Products,"* which reports the results obtained from 74 air samples collected while monitoring six keratin smoothing treatments performed in six separate salons."

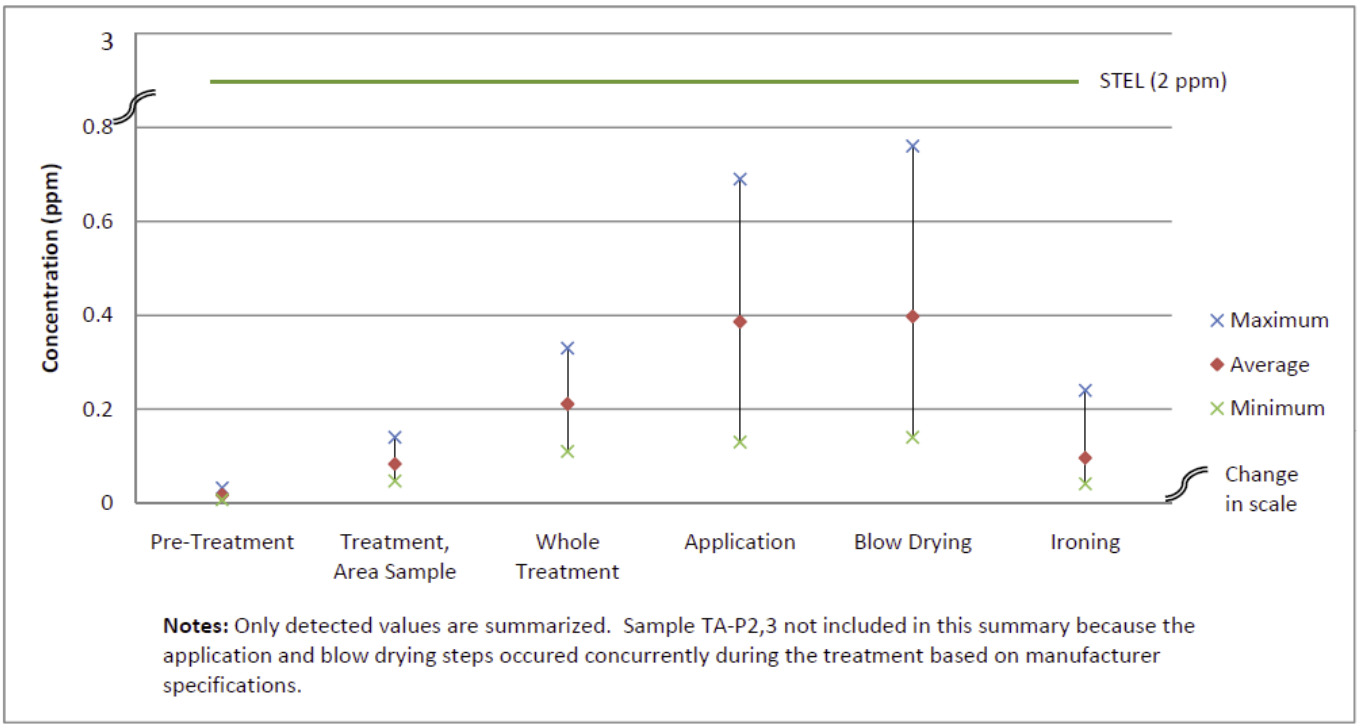
As shown in Figure 1, and summarized in this report, *"From a regulatory perspective, the exposure levels of formaldehyde associated with use of these products in one or two hair treatments ranges were well below the 8 hour TWA of 0.75 ppm and ranged from 0.04 to 0.08 ppm. No concentrations exceeded the OSHA STEL of 2 ppm… The tasks associated with the highest exposure level were blow drying, followed closely by product application. However, these short-term concentrations were not sustained." "Several factors potentially contribute to the measured formaldehyde exposures. A general trend was observed between measured exposure levels and the amount of product and manner in which the product was applied by the stylist*." (Page 7, Exponent Report).

Notwithstanding the erroneous contribution of methylene glycol to the above reported formaldehyde air concentrations, there is a reported potential that short term spikes above 0.3 ppm of formaldehyde can occasionally occur during the blow drying phase. In these instances, although the likelihood of sensory irritation is increased, the PKSC believes that warnings and task training will help cosmetologists to avoid even short-term spikes, thereby further reducing the potential for sensory irritating effect. Exposures can be minimized in three ways that have been demonstrated to decrease vaporization and reduce vapor exposure in the salon settings:

1. Use of a fine-tooth comb will prevent over application of keratin smoothing product to client's hair. Excessive amounts of product do not improve the quality of service and should be avoided. The PKSC believes that if the importance of portion control is stressed this will ensure that salon air quality is maintained at healthy levels.
2. Prevention of formaldehyde spikes above the ACGIH ceiling of 0.3 ppm can be achieved during the hair blow drying phase if cosmetologists are advised to dry the hair with a cooler setting on the blow dryer. The largest potential for over exposure spikes is during blow drying, as indicated in Figure. 1. Instructing cosmetologists to perform this step using a blow dryer set on a medium setting, rather than hot, will minimize the potential for these spikes.
3. Separating the head of hair in smaller sections before using the flat iron also minimizes the potential for spikes in formaldehyde concentration

Evidence of the effectiveness of these methods can be seen in the Exponent report. This report also demonstrates that even when keratin smoothing products containing higher than average levels of methylene glycol (as measured by 13C-NMR) are used, they can be applied and services performed in such a way as to produce very low levels of formaldehyde exposure. When the techniques above were utilized by the cosmetologist, the 8 hour TWA exposures were 0.05 ppm and no spikes above 0.16 ppm were reported. These data clearly show that through proper training and application, salon professionals using PKSC members company's products can significantly reduce potential formaldehyde exposures to prevent sensory irritation. These techniques have been and will continue to be a part of the PKSC-endorsed training program, and will be taught as best practices for minimizing the potential for exposure. .

Figure 1. Task-Specific Formaldehyde Air Concentrations

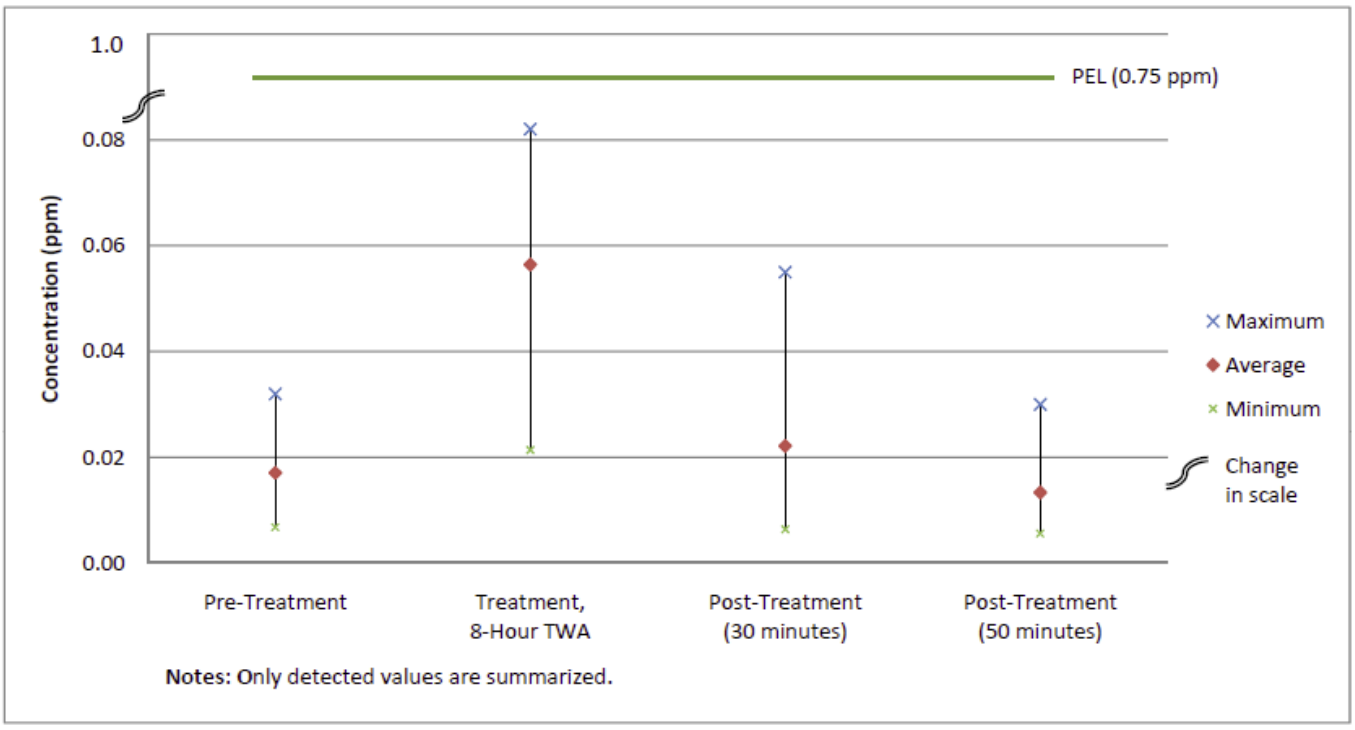


1. Safe product use and proper handling procedures to help ensure exposure is well below maximum safe levels of exposures.

The PKSC notes that the reported formaldehyde exposure values presented in Tables 3-5 of the Exponent report are similar to the results of air monitoring studies provided in our initial CIR submission. This information supports the clear conclusion that no Formaldehyde exposures in salon settings are above applicable regulatory limits.

In addition, the Exponent data have shown that even when salons perform two services consecutively, exposures are similar for both services, the combined exposures never exceed regulatory limits, and there is no "build-up" of formaldehyde in the salon air. The Exponent study (see Figure 2) further demonstrates that formaldehyde air concentrations are quickly reduced to below 0.06 ppm within 30 minutes and below 0.03 ppm within 90 minutes, even without using specialized ventilation systems designed to further reduce air concentrations. However, when a local source capture ventilation system was utilized, exposures were reduced to 0.019 ppm within 30 minutes and 0.0081 ppm within 90 minutes to provide a 31% and 27% greater reduction in formaldehyde at 30 and 90 minutes, respectively, even though 40% more product was applied when the local source capture ventilation system was utilized.

Figure 2. Pre-Treatment, Treatment (8-hour TWA) and Post-Treatment Formaldehyde Air Concentrations



Notably, previously reported air monitoring test results from Oregon OSHA and PKSC members are in agreement with the results of this Exponent study. These results show that many (and perhaps even most) salons using our member's products to perform these services remain well below both the OSHA and ACGIH 8 hour TWAs. These salons aren't often heard from because they don't report sensory irritation resulting from their services. As confirmed by the latest Exponent data, the success of these salons can be attributed to their utilization of proper techniques to control and minimize vapors.

The PKSC will be conducting additional salon testing using a range of products in different salons to measure the effects of ventilation on further reducing potential formaldehyde concentrations in the air.

2. Recommended best practices and techniques

1. Avoid using keratin smoothing products under conditions which cause cosmetologist/clients to experience sensory irritation of the eyes, nose or throat and explain that these symptoms are indicators of safe vs. unsafe use practices.
2. Cosmetologists should be warned about the importance of using correct product portion control.
3. Cosmetologists should wear nitrile disposable gloves and protective eyewear.
4. Proper care should be taken to avoid skin/scalp contact with keratin smoothing products.
5. Do not applying keratin smoothing lotion closer than ¼ inch from the scalp to avoid skin irritation or allergic reactions.

6. Cosmetologists should ask clients about pervious history of formaldehyde or formalin sensitivity and avoid performing services on clients with known or suspected skin sensitivity.
7. Cosmetologists should be warned about the importance of setting blow dryers to cooler temperatures to help reduce any potential for exposure to excessive amounts formaldehyde.
8. Cosmetologist should be warned to use proper and appropriate salon ventilation to ensure safe handling and that the purpose of this ventilation is to help prevent cosmetologist/client sensory irritation and overexposure.

3. Recommended Safe Levels of Methylene Glycol in Keratin Smoothing Products

Based on salon air monitoring by Oregon OSHA, Exponent and others, the results demonstrate that when the products of PKSC members are used with the recommended best practices described above, formaldehyde levels have repeatedly been shown to be well below the OSHA standards, as well as the more stringent level recommended by ACGIH.

Based on all of the information and data presented to the CIR by the PKSC in this and previous reports, sufficient evidence exists to demonstrate the safety of keritan smoothing producs that are formulated with concentrations <3% methylene glycol and < 0.2% dissolved formaldehyde gas. The PKSC recommends that such concentrations should be considered safe when used according to manufacturer's directions with all recommended warnings being heeded. Warnings should include explicit cautionary labeling language to warn cosmologists to work in a manner that minimizes formation and inhalation of irritating vapors and also that any signs of sensory irritation are an unmistakable indication of inadequate ventilation and/or improper use. All keritan smoothing products should carry the following warning; "CAUTION: Upon heating this product may release low levels of formaldehyde gas which could irritate the eyes, nose, and throat. Safe use and handling requires the use of proper and appropriate ventilation to eliminat eye, nose or skin irritation while services are performed and in accordance with manufacturer's directions."

The PKSC research program will continue to perform studies with the aim of better understand about how much methylene glycol vapors and formaldehyde gas are vaporized into air, as well as, how to minimize exposure to formaldehyde gas when keratin smooth products in salons.

G. Conclusions and Recommendations

The long standing confusion over the terms formaldehyde, formalin and methylene glycol, in conjunction with the wide spread use of inaccurate test methods have permitted the dissemination of inaccurate and misleading information concerning the use of keratin smoothing products. This in turn has resulted in unwarranted fear and needless concern that the use of keratin smoothing products can lead to formaldehyde exposures far greater than actually exist. However, the tens' thousands of keratin smoothing procedures performed with a variety of different products that do not lead to sensory irritation of the eyes, nose or throat attests to the

fact that these products can be safely used by trained professionals. The following salient points summarize the key issues addressed in this document:

- The terms methylene glycol and formaldehyde should neither be used interchangeably nor are they synonymous.
- Methylene glycol is naturally present and produced in all living organisms, is regularly ingested in foods, and is non-toxic.
- Upon rapid vaporization of formalin at 400^{o}F, because less than half of the available methylene glycol is converted to formaldehyde gas these two different chemicals cannot be considered as equivalent.
- Current methods for measuring formaldehyde concentrations in air samples following heating also include methylene glycol vapors which are misreported as formaldehyde.
- No type of keratin smoothing product should be used in a manner that causes cosmetologists or clients to develop sensory irritation of the eyes, nose or throat as these are considered signs of inappropriate conditions of use.
- Keratin smoothing products should be restricted for use by trained and licensed professionals who are required to undergo additional training and certification on proper use, safe handling and a better understanding of ventilation issues.
- Cosmetologists should be advised to use a fine tooth comb to remove excess keratin smoothing product before any heating occurs.
- Use of best practices including proper amount of product applied, use of appropriate ventilation including source capture devices, all with the goal of ensuring cosmetologists and clients do not experience sensory irritation of the eyes, nose, or throat.
- Recommend a safe use concentration of not more than 3% methylene glycol and less than 0.2% dissolved formaldehyde gas as a “safe” concentration in keratin smoothing products when used according to manufacturer’s directions with all warnings heeded.
- Recommend explicit cautionary labeling language that warns cosmologists to work in a manner that minimizes formation and inhalation of irritating vapors and also that any signs of sensory irritation is a warning sign of poor ventilation and/or improper use, e.g. "CAUTION: Upon heating this product may releases low levels of formaldehyde gas which could irritate the eyes, nose, and throat. Safe use and handling requires the use of proper and appropriate ventilation to eliminate eye, nose or skin irritation while services are performed and in accordance with manufacturer's directions."

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Date: May 16, 2013

To: Larry Soloman,
Professional Keratin Smoothing Council

From: Dr. John Edwards
Process NMR Associates

Re: Quantitative ^{1}H NMR Analysis Formalin – Observation of Free Formaldehyde as Methanal

It has been requested to pursue the identification and quantification of free formaldehyde (methanol) in 37.4% Formalin solution provided by Analytical Science, Petaluma, CA – ID 2106-01. The sample had been previously analyzed by Analytical Science and found to be 37.4% formaldehyde. The sample was analyzed using several quantitative approaches to determine if formaldehyde (methanal) could be observed.

Materials Used:
Maleic Acid (Internal Standard) – Aldrich HPLC Grade 99.0% LotSLBC1970V
Formic Acid (Internal Standard) – Aldrich Reagent Grade >95% Lot SHBC8396V
Dimethyl sulfoxide – d6 – Cambridge Isotope Laboratories, Lot Lot 12L-181
Formalin – Analytical Science, Petaluma, CA - 37.4% by HPLC – Lot 2106-01

Sample Preparation:
Samples were prepared several different ways:

1) Sample was prepared by placing a few drops of CD_3OD into pure formalin in a 5 mm NMR tube
2) Sample was prepared by addition of formic acid to pure formalin sample
3) Sample was prepared by placing a known weight of formalin (582.9mg) and maleic acid internal standard (150.3 mg) in a 5 mm NMR tube with 2 drops of DMSO-d6.
4) Sample was pure formalin with 2 drops of DMSO-d6 in a 5 mm NMR tube
5) Sample was prepared by placing a known weight of formalin (695.9mg) and maleic acid internal standard (18.3 mg) in a 5 mm NMR tube with 2 drops of DMSO-d6.

Experimental:

Experiments were performed on a Varian Mercury 300 spectrometer operating at a resonance frequency of 299.94 MHz, with a 2.224 second acquisition time, a relaxation delay of 2 seconds, performing a 12 degree tip angle pulse (required due to the high proton containing sample content, and accumulating approximately 1024-2048 transients. The probe was a Varian 5mm 4-Nucleus probe.

Calculations:

The following Equation was used to calculate the concentration of the various components present in the formalin sample.

Component Wt% = 100 * $(I_{Comp} / I_{std}) * W_{Std}) * (MW_{Comp}/MW_{std}) / W_{Sample}$

Where, I_{Comp} = mole adjusted integral of the component of interest, I_{std} = mole adjusted integral of standard, W_{std} = weight of standard, W_{Sample} = weight of sample, MW_{Comp} = molecular weight of the component of interest, MW_{std}= molecular weight of maleic acid standard (116.07 amu)

For formaldehyde the MW_{Comp} = 30.02 amu
For methylene glycol the MW_{Comp} = 48.04 amu
For methanol the MW_{Comp} = 32.02 amu
For formic acid the MW_{Comp} = 46.03 amu

For two of the samples that did not contain internal standards it was decided to calculate the formic acid and methanol concentrations (and in one case the methylene glycol concentration) from the methanol/hemiformal CH3-O signal that can act as a constant internal standard of the formalin once the concentration of methanol is known.

Results and Discussion:

As there are no references referring to the chemical shift of pure methanol we decided to predict the ^1H NMR spectrum of methanol from first principles utilizing MestreLabs MNova software. It predicted that methanal would yield a peak at around 9.6 ppm. The result is shown in Figure 1.

When we ran formalin with a few drops of CD_3OD to lock the signal we observed a peak at 9.5 ppm which is assigned to methanal and another peak at 8.04 ppm which we assigned to formic acid. The result is shown in Figure 2.

We then spiked the formalin sample with formic acid and observed that resulting large formic acid peak coincided with the resonance at 8.04 ppm showing that we had correctly assigned that peak (see Figure 3).

We then analyzed the first quantitation experiment which compared the signal intensities of the various formalin components with an internal standard (maleic acid – added at a known concentration). The methylene glycol (MG) content was observed in the experiment but in order to determine the strength of the formalin solution we back calculated from the methylene glycol content what amount of formaldehyde gas would have to react to form that concentration. In this case we had issues with the large water resonance that partially overlaps the MG signals and so we feel that though the calculated value of 36.3 wt% is close to the 37.4 wt% obtained by HPLC we are probably underestimating the content by NMR due to the baseline corrections required to remove the water peak from the analysis. The fact that we have a value close to the expected value makes us confident in the magnitude of the concentrations calculated for methanol, formic acid and free formaldehyde (methanal). Table I shows the values obtained on the first quantitation analysis. The data for this analysis is shown in Figure 4.

Table I: ^{1}H qNMR Analysis of Formalin

	Wt (mg)	Wt%	ppm
Formalin	582.9	-	-
Maleic Acid	150.3	-	-
Formaldehyde (as MG and Polymers)		36.26	-
Methanol		12.42	-
Formic Acid		0.0128	128
Methanal (free formaldehyde)		0.0002	2

We then approached the result obtained in Figure 3 which was simply formalin spiked with formic acid. The addition of formic acid caused an advantageous pH shift in the water resonance which then allowed better integration of the MG and MG polymers/hemiformal signal. A calculation of the formaldehyde required to create the observed MG and MG polymer/hemiformal signals was based on the fact that we now know that there is 12.42 wt% methanol in the sample. Using the methanol as an internal standard we calculated from the data in Figure 3 that the formaldehyde content is 37.2 wt% a number more closely aligned with the HPLC result and confirming that addition of acidic internal standards may allow better calculation of MG and added formaldehyde values.

Utilizing this approach again (using the methanol in the formalin as the standard by which to calculate the concentrations of methanal and formic acid), we calculated the concentration of these components in a sample comprised of pure formalin with only 2 drops of DMSO-d6 (no acidic, pH changing internal standards were added). The result for this analysis is shown in Figure 5 and Table II shows the calculated concentrations of free formaldehyde (methanal) and formic acid.

Table II:^{1}H qNMR Analysis of Formalin (no acidifying internal standards added) – methanol concentration from previous analysis utilized to calculate methanal and formic acid concentrations.

Component	Wt%	ppm
Formaldehyde (as MG and MG polymers)	Not Calculated	-
Methanol (used as internal standard)	12.42	-
Formic	0.0143	143
Free Formaldehyde (as methanal)	0.0061	61

Of concern is the large increase in the observed concentration of methanal. In utilizing different internal standards that are acidic in nature (maleic acid, formic acid) and changing the lock solvent (CD_3OD versus DMSO-D6) the observed methanal and formic acid signals appear to vary on a fairly large scale. This is a worrying aspect and a more defined and repeatable methodology should be determined.

Finally, another qNMR sample was weighed out this time with a lower concentration of maleic acid which should induce a lower pH change in the sample. The results for this analysis are shown in Figure 6 and Table III.

Table III: ^{1}H NMR of Formalin (Lower Internal Standard Concentration).

	Wt (mg)	Wt%	ppm
Formalin	695.9	-	-
Maleic Acid	18.3	-	-
Formaldehyde (as MG and Polymers)		Not Calculated	-
Methanol		11.96	-
Formic Acid		0.0126	126
Methanal (free formaldehyde)		0.0020	20

Limit of Detection Estimation

Looking at the signal-to-noise obtained on the methanal resonance in the various experiments we have estimated that the limit of detection (sample concentration that results in a 3:1 signal-to-noise (S/N) ratio) is 2 ppm. This is based on the fact, for example, that at 61 ppm the S/N observed for methanal in Figure 5 was 90:1 (based on Varian spectrometer algorithm calculation). As the signal observed is directly proportional to the concentration of the sample this means that to obtain a 3:1 ratio one would require 1/30 th of the concentration of methanal to be present. Thus 61 ppm/30 = 2 ppm. This description is given because we did not do a series of dilutions of a known concentration of formalin to obtain this value. It is a single experiment approximation. The limit of quantification (concentration that results in a 10:1 S/N ratio) would therefore be approximately 6-7 ppm.

Conclusion

It appears from the analyses performed that methanal can be observed in Formalin (37.4%) by ^{1}H NMR analysis. The results vary widely depending on the choice of locking solvent and internal standard chemistry. One experiment that was not tried was to simply run the experiment unlocked on pure formalin with nothing added and see what result would be obtained. NMR experiments utilizing an electronic reference signal may also allow less perturbation of the sample and yield more repeatable calculated values.

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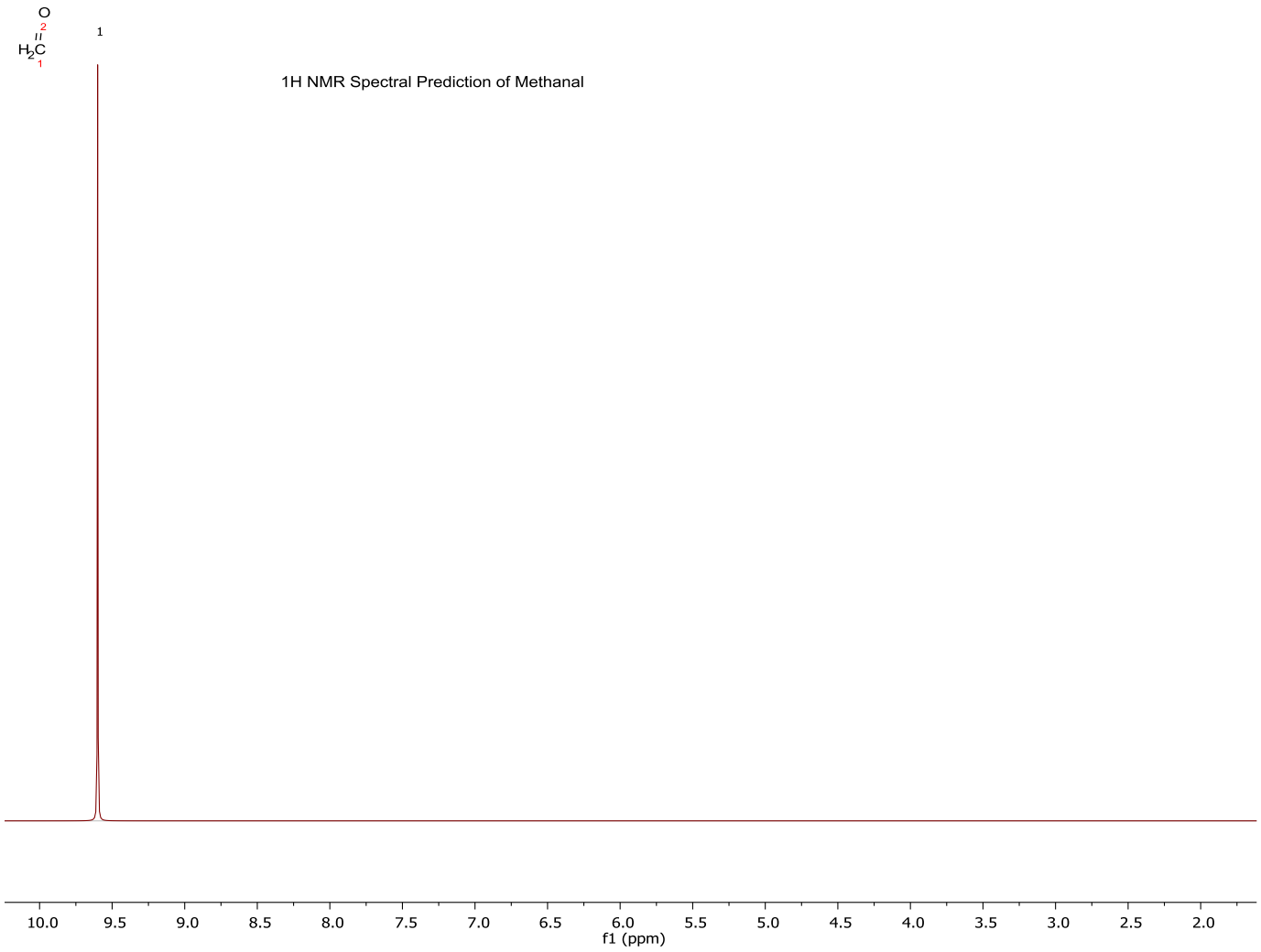

Figure 1: ^{1}H NMR spectral prediction for methanol by Mestrelab MNova NMR software version 8.1.2.

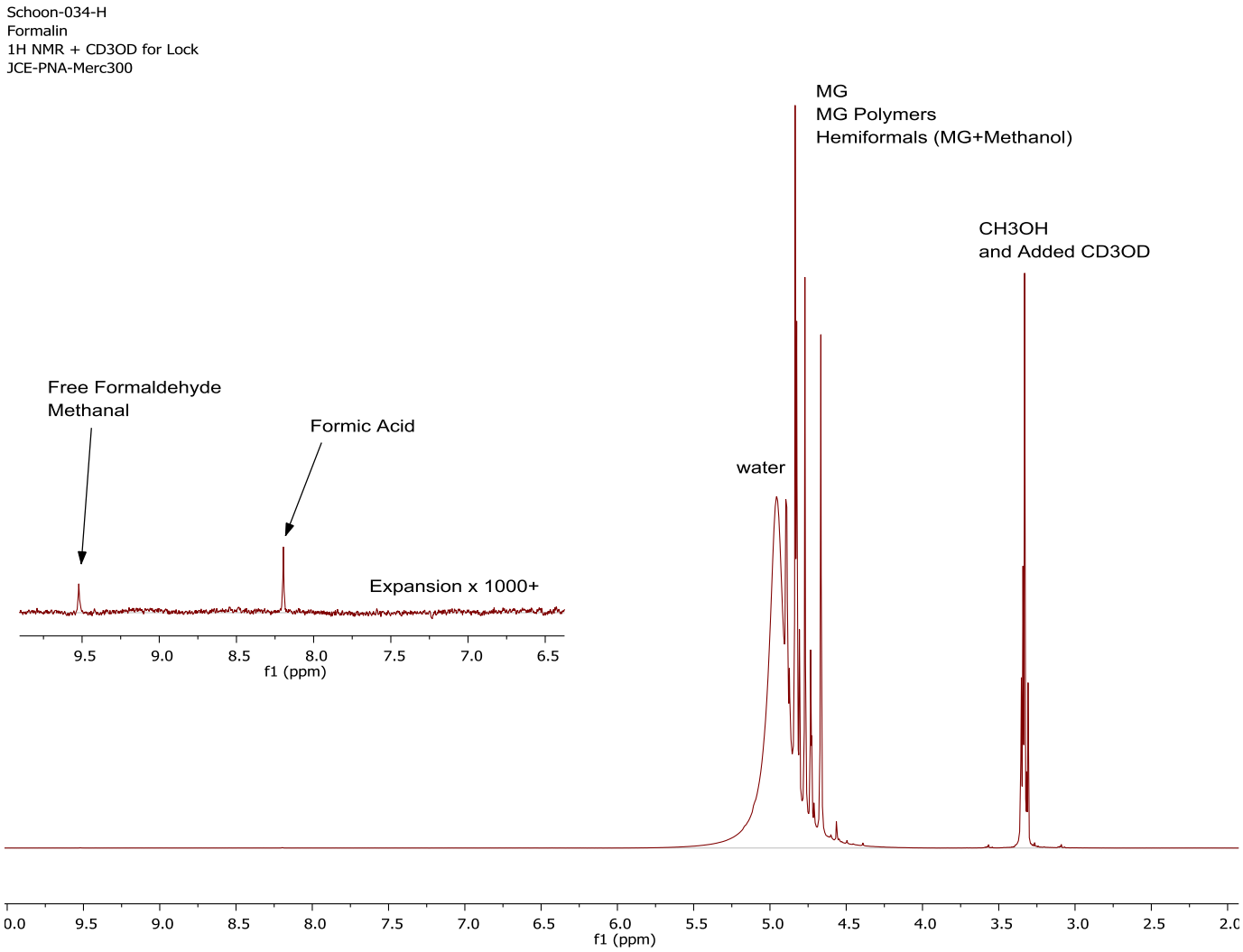

Figure 2: ^{1}H NMR of Formalin sample with assignments of methylene glycol based polymers, methanol, hemiformals, formic acid and free formaldehyde (methanal). Peak at 9.5 ppm is in agreement with spectral prediction to be methanal.

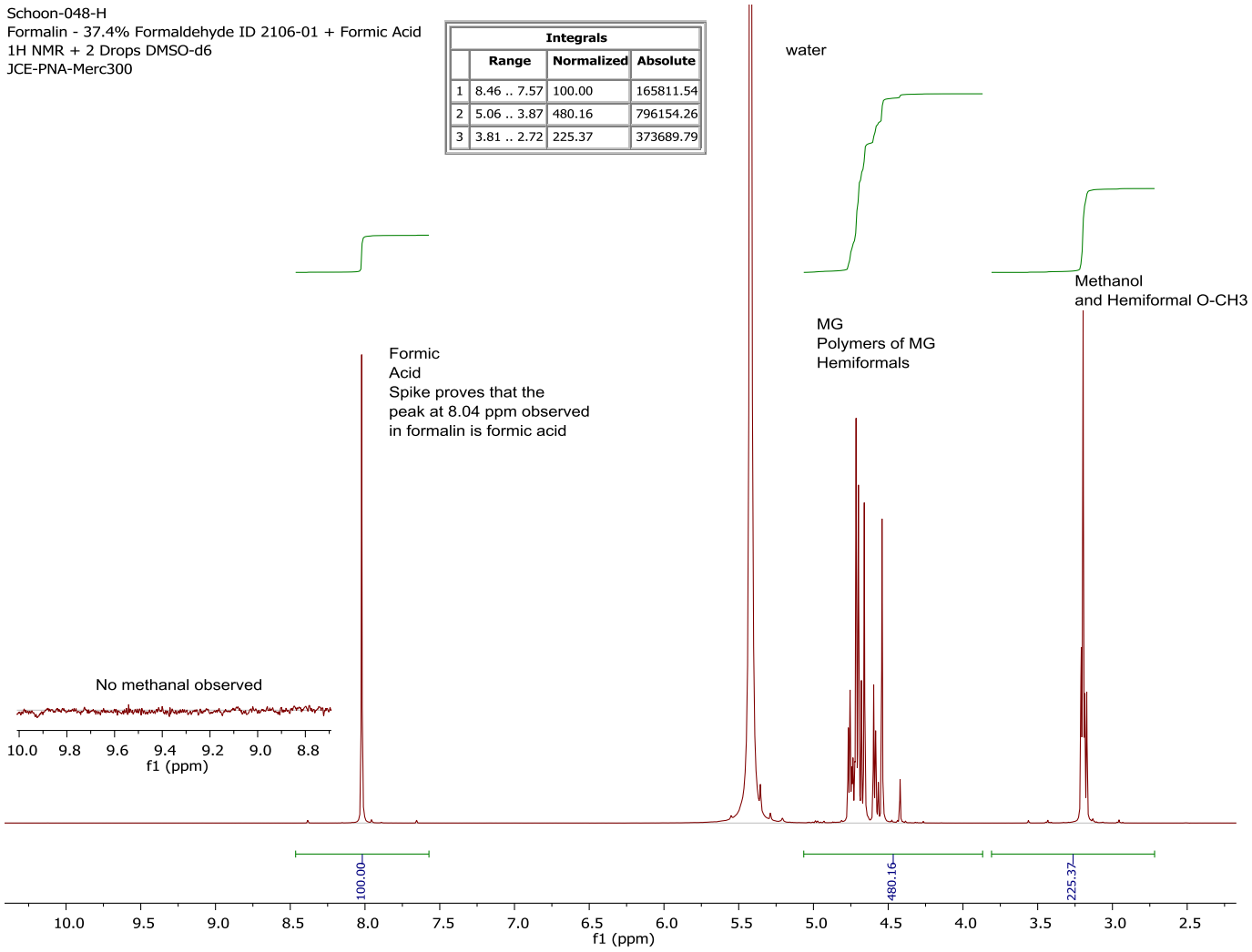

Figure 3: ^{1}H NMR of Formalin spiked with formic acid to determine the presence of formic acid in formalin. The resonance appeared at the same shift as the small peak assigned initially to formic acid thus confirming the assignment. Note: no methanal resonance is observed.

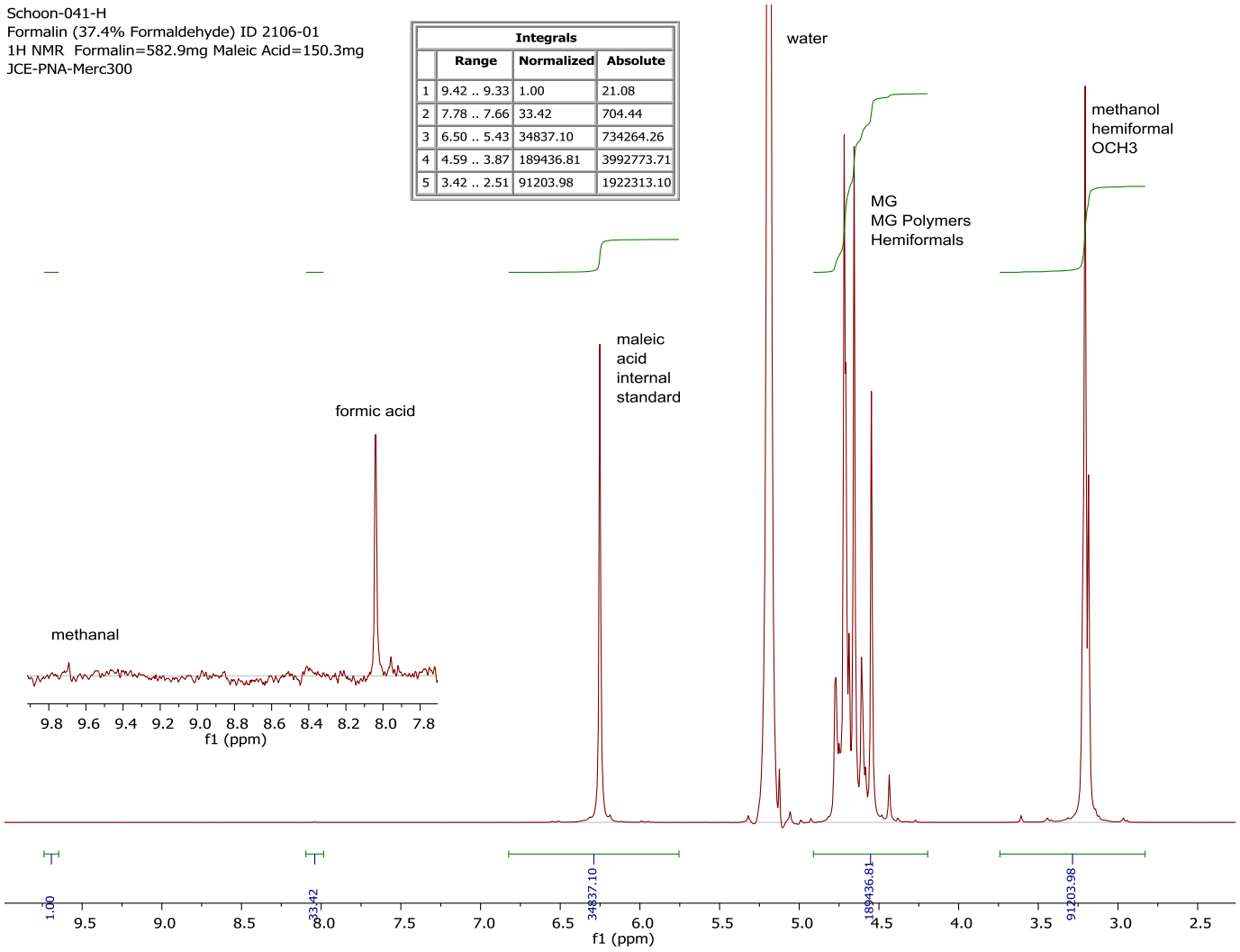

Figure 4: ^{1}H qNMR spectrum of formalin (582.9 mg) and maleic acid internal standard (150.3 mg) with a drop of DMSO-d6 to lock the experiment.

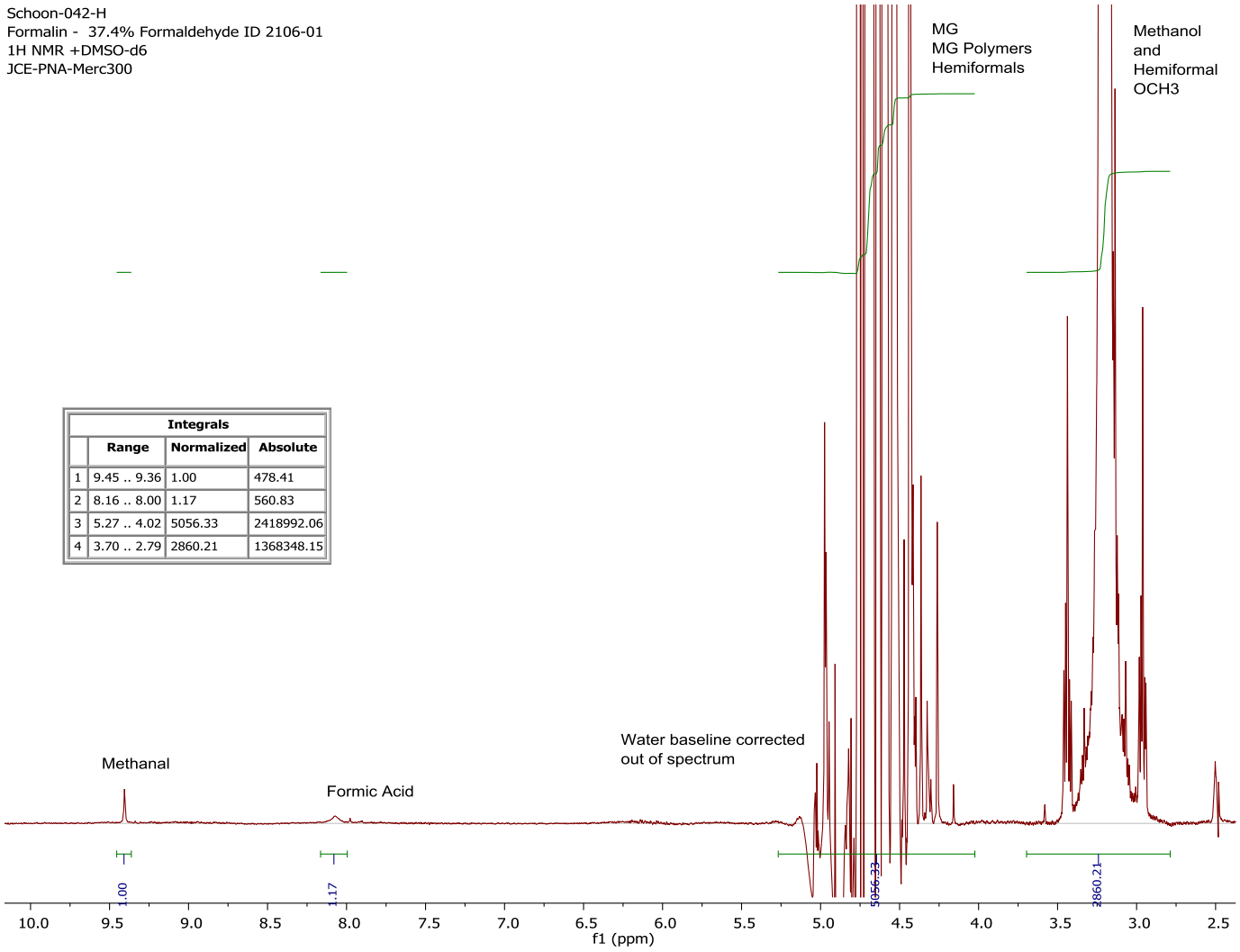

Figure 5: ^{1}H NMR of pure formalin with two drops of DMSO-d6 to lock the experiment.

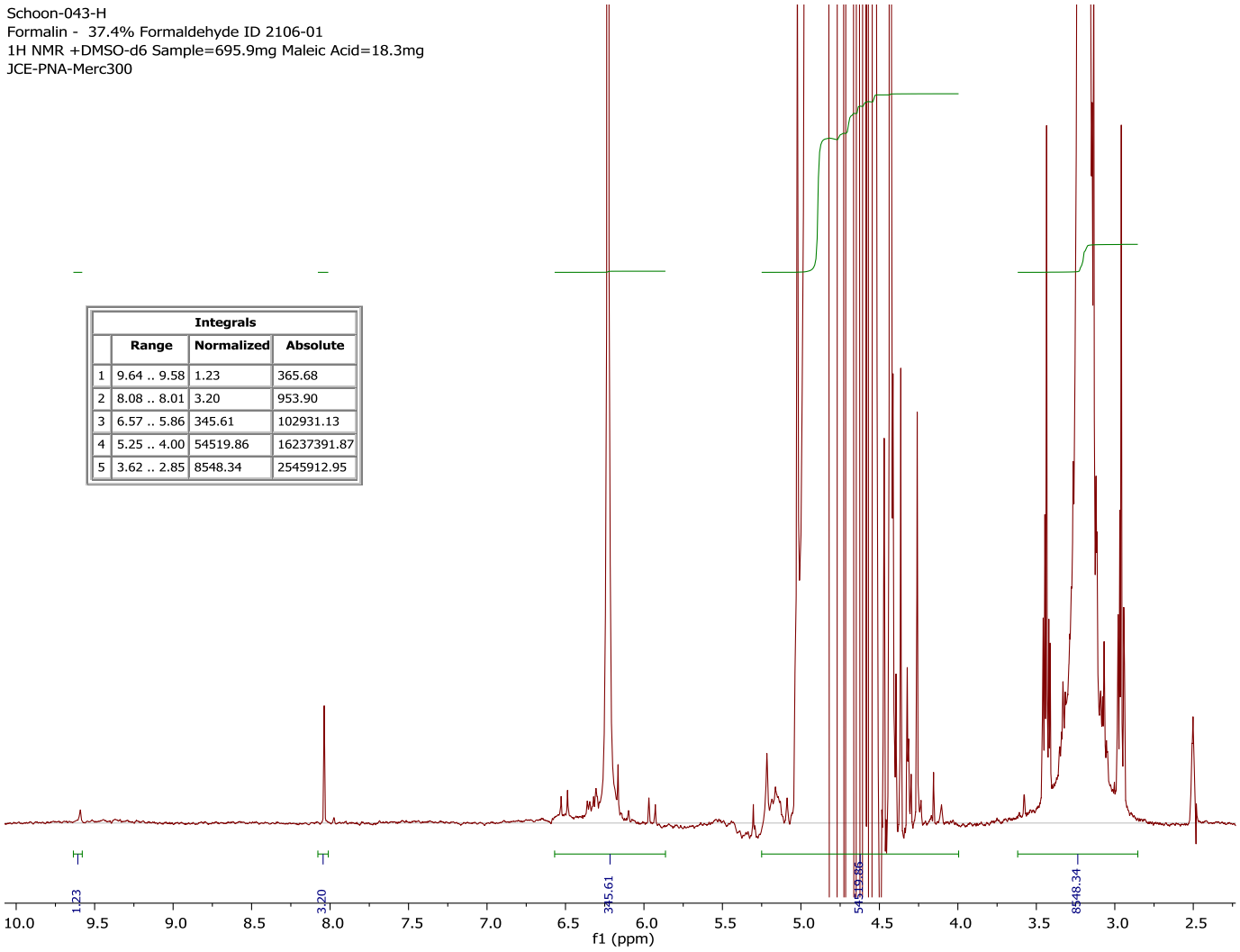

Figure 6: ^{1}H NMR of formalin (695.9 mg) and maleic acid internal standard (18.3 mg) with 2 drops of DMSO-d6 used to lock the NMR experiment.

Analytical Sciences

September 14, 2011

Ms. Patty Schmucker
Executive Director
Professional Keratin Smoothing Council
2761 Plaza del Amo #904
Torrance, CA 90503

Dear Ms. Schmucker,

Analytical Sciences executed an experiment involving the application of hair products to a hot (440 degree F) flat iron and then capturing the vapors emitted using a low flow personal air sampling pump connected to a glass tube containing N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) as a derivatizing agent. As general references, the following two published papers were used as guidance: D. Utterbeck et al, Advances in Chemistry, American Chemical Society, Chap. 5, 1985 and J.G.M Winkelman, Chemical Engineering Sciences, 57 (2002) 4067-4076. The goal was to determine the exact nature of the volatile products produced with a specific focus on formaldehyde (CH_2O) and methylene glycol ($CH_2(OH_2)$). The ultimate goal was to obtain a quantitative ratio of methylene glycol to formaldehyde in the hot vapors arising from the heated flat iron.

The tube used to perform the sampling was prepared by Analytical Sciences. The straight glass tube was one-quarter inch in diameter and four inches long with both ends open. A small amount of new glass wool was inserted into the tube carefully. The tube was connected to a low flow air sampling pump. The pump and tube arrangement allowed air to be inhaled through the open end of the tube at 50 milliliters per minute. The tube was mounted two inches away from the hot flat iron (440 degrees F). Just prior to obtaining a sample, 50 microliters of a concentrated fresh derivatizing agent (BSTFA) was injected onto the glass wool in the sampling tube. With the tube in place two inches away from the heated flat iron the pump was turned on and the hair product being tested was applied directly to the flat iron using glass pipette. Application of the hair product was continuously made directly to the hot flat iron during the timed five minute sampling interval (250 milliliters of air collected). The two hair products were Keratin Complex Smoothing Therapy and Marcia Extreme De-Frizzing Treatment. Immediately after the vapor was collected from each sampling the glass wool in the collection tube was expelled into a small vial to which exactly one milliliter of clean hexane was added. The two hair products were sampled in this manner as well as a room air blank and a sample where 37.4% formalin was applied directly to the hot flat iron. The expectation was that formaldehyde and methylene glycol emitted into the air from the heated flat iron surface would be derivatized in the sampling tube by the BSTFA silylation agent to form two unique products that could be analyzed uniquely

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using a gas chromatograph connected to a mass spectrometer. In all samples 250 milliliters of air was drawn over the glass wool containing the BSTFA derivatizing agent.

A portion of the one milliliter hexane extract of the glass wool from each air sample was placed into an injection vial and analyzed using a gas chromatograph connected to a mass spectrometer. The four chromatograms obtained are attached (see Figure 1a, b, c, d, e). The figure 1a chromatogram depicts the room air sample. The figure 1b chromatogram depicts the 37.4% formalin sample. Figure 1c and 1d chromatograms present the chromatograms for the Keratin Complex Smoothing Therapy and Marcia Extreme De-Frizzing Treatment products respectively. All four samples chromatogram appear in Figure 1e in a time overlaid format.

The first observation to note when looking at the chromatograms is the number of chromatographic peaks observed. From the mass spectral data associated with each peak it appears that the larger number of peaks observed (than expected) are resulting because multiple organic chemicals are being derivatized in addition to the formaldehyde and methylene glycol. Also it should be recognized that organic components coming from the hair products may have been trapped on the glass wool and are analyzed as underivatized chemicals.

The focus initially was to understand the formalin sample since it would be expected that both formaldehyde and methylene glycol chromatographic peaks would be evident since both chemicals would be derivatized to form two separate products. The large, primary chromatographic peak in Figure 1b has a retention time of 9.845 minutes. This peak has a unique mass spectra that was not able to be readily identified by the computerized National Bureau of Standards Mass Spectral database. The mass spectrum of the large peak at 9.845 minutes is displayed in Figure 2. In looking at several articles available through the Internet it was apparent that this unique mass spectral pattern has been observed in the analysis of BSTFA derivatized formalin before (James L. Little, “Artifacts in trimethylsilyl derivatization reactions and ways to avoid them” journal of Chromatography A, 844 (1999) 1-22). The study of the reaction products of BSTFA and formalin by Little clearly reveals the same mass spectrum as Analytical Sciences has observed. The mass assignments are specific to fragments actually identified in the Little review paper and displayed in Figure 3. The chemistry depicted in Figure 4 from the Little review article illustrates how a reaction of BSTFA with methylene glycol (formaldehyde hydrate) produces a final product which yields the mass spectrum observed by both Analytical Sciences and James Little. The molecular formula of the expected BSTFA derivative of formaldehyde would have a molecular weight of 277. No chromatographic peak in the formalin sample or either of the two hair product samples suggests the presence of a BSTFA derivatize formaldehyde component. This conclusion is reached knowing the molecular weight of the derivatized formaldehyde and the expected atomic weights of the potential fragmentation products. The laboratory also knows where to look in the chromatogram for the potential formaldehyde derivatization product (see notation on Figure 1B). It appears clear that formaldehyde (CH_2O) is not being detected in vapors emitted from the hot flat iron when

either formalin or either hair products are placed on the hot flat iron. The estimate for the limit of detection for formaldehyde would be approximately 1 mg/m^3. The laboratory is convinced that were formaldehyde released, commingled with methylene glycol, from the hot flat iron that it would have been observed in the gas chromatographic analysis using the mass spectrometer detector. It is possible that the formaldehyde may not be present due to the relative large amount of water in the hot vapors heavily favoring the presence of the hydrated methylene glycol over free formaldehyde.

Based on the work done and the facts observed it is clear that formaldehyde represents an insignificant or undetectable portion of the emitted gases from the heated flat iron to which either the Keratin Complex Smoothing Therapy or Marcia Extreme De-Frizzing Treatment is applied. The primary component observed in the formalin produced vapors is methylene glycol which is detectable in both the air samples collected from the hair products. No specific formaldehyde derivative was detected in any sample collected in vapors emitted from the heated flat iron surface. Should you or others have any specific questions regarding the details of this letter report please contact me at your convenience.

Sincerely,

Mark A. Valentini

Mark A. Valentini, Ph.D.
Laboratory Director

Figure 1a

MB

```
File       : D:\HPCHEM\1\DATA\DATA11\090901\090901.D
Operator   : MAV
Acquired   :  9 Sep 2011    4:07 pm using AcqMethod 11MY8270
Instrument :   FS8270
Sample Name: MB 250ml room air (1ml Hexane)
Misc Info  : 50ul BSTFA / TCMS derivatized
Vial Number: 1
```

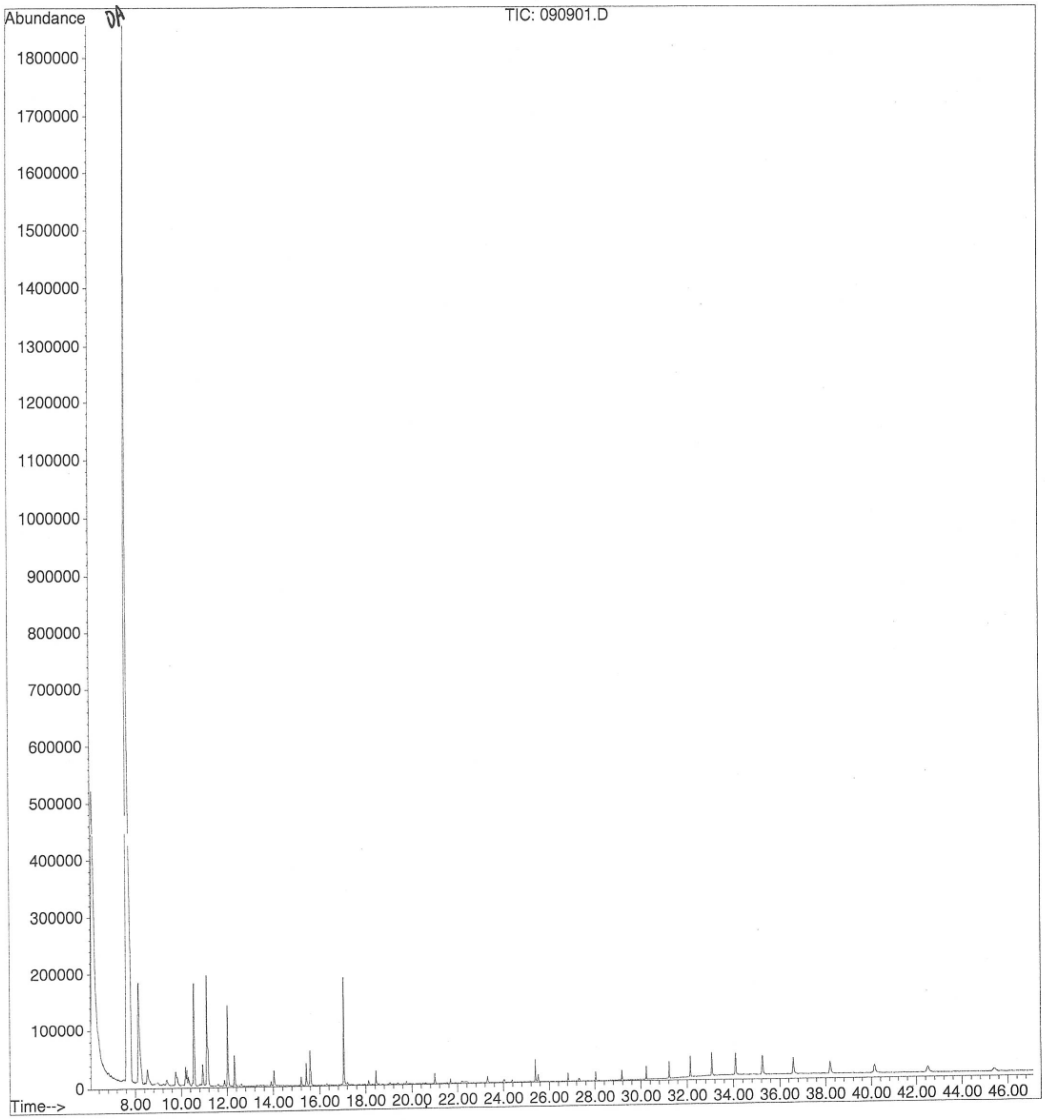

Figure 16

```
File       : D:\HPCHEM\1\DATA\DATA11\090901\090902.D
Operator   : MAV
Acquired   :  9 Sep 2011    5:04 pm using AcqMethod 11MY8270
Instrument :   FS8270
Sample Name: 37.4% Formalin 250ml room air (1ml Hexane)
Misc Info  : 50ul BSTFA / TCMS derivatized
Vial Number: 2
```

Formalin 37.4%

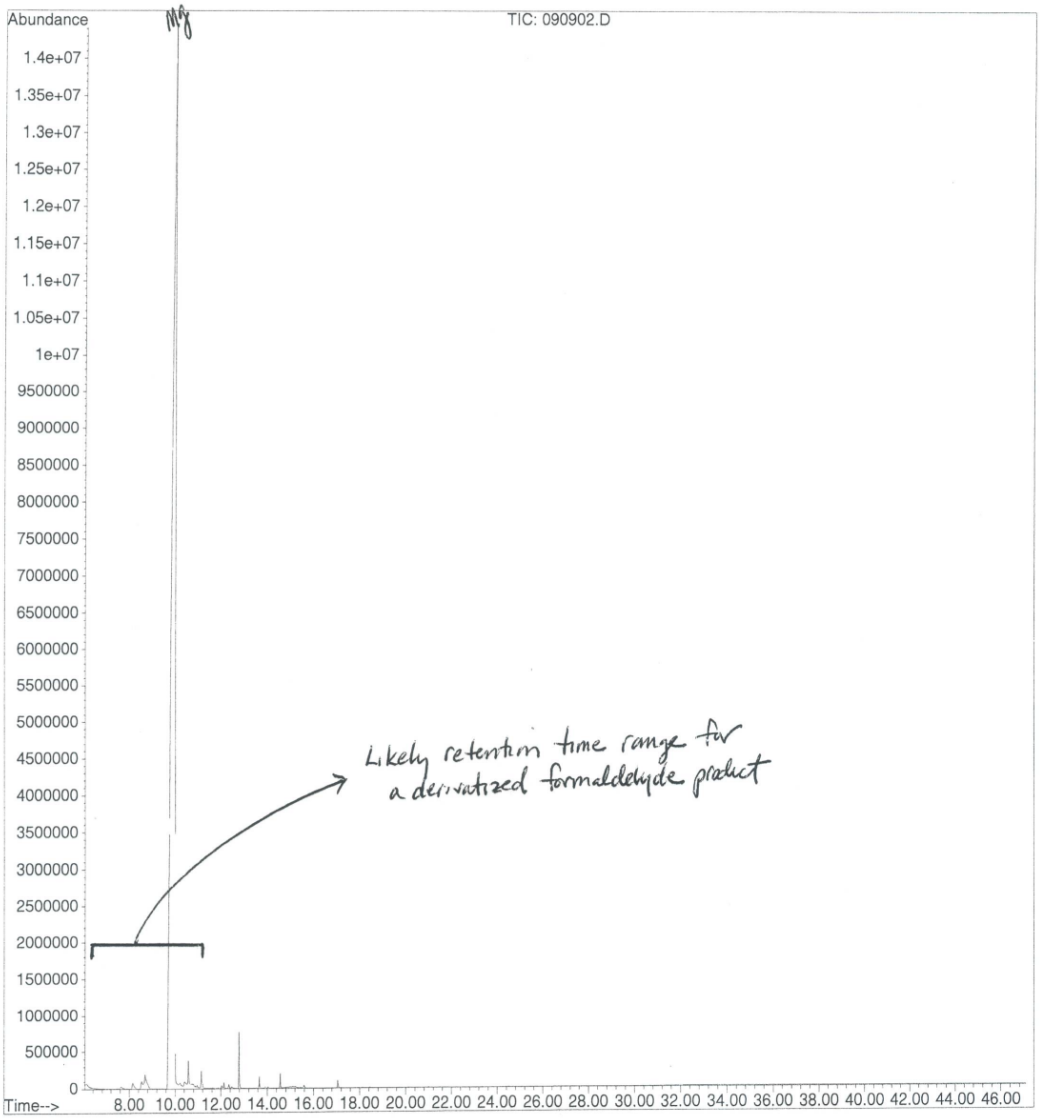

Figure 1c

Keratin
Natural
Keratin
Smoothing
Therapy

```
File        : D:\HPCHEM\1\DATA\DATA11\090901\090903.D
Operator    : MAV
Acquired    :  9 Sep 2011    6:01 pm using AcqMethod 11MY8270
Instrument :    FS8270
Sample Name: Ker product #1 250ml room air (1ml Hexane)
Misc Info   :  Natural Keratin  50ul BSTFA / TCMS
Vial Number: 3
```

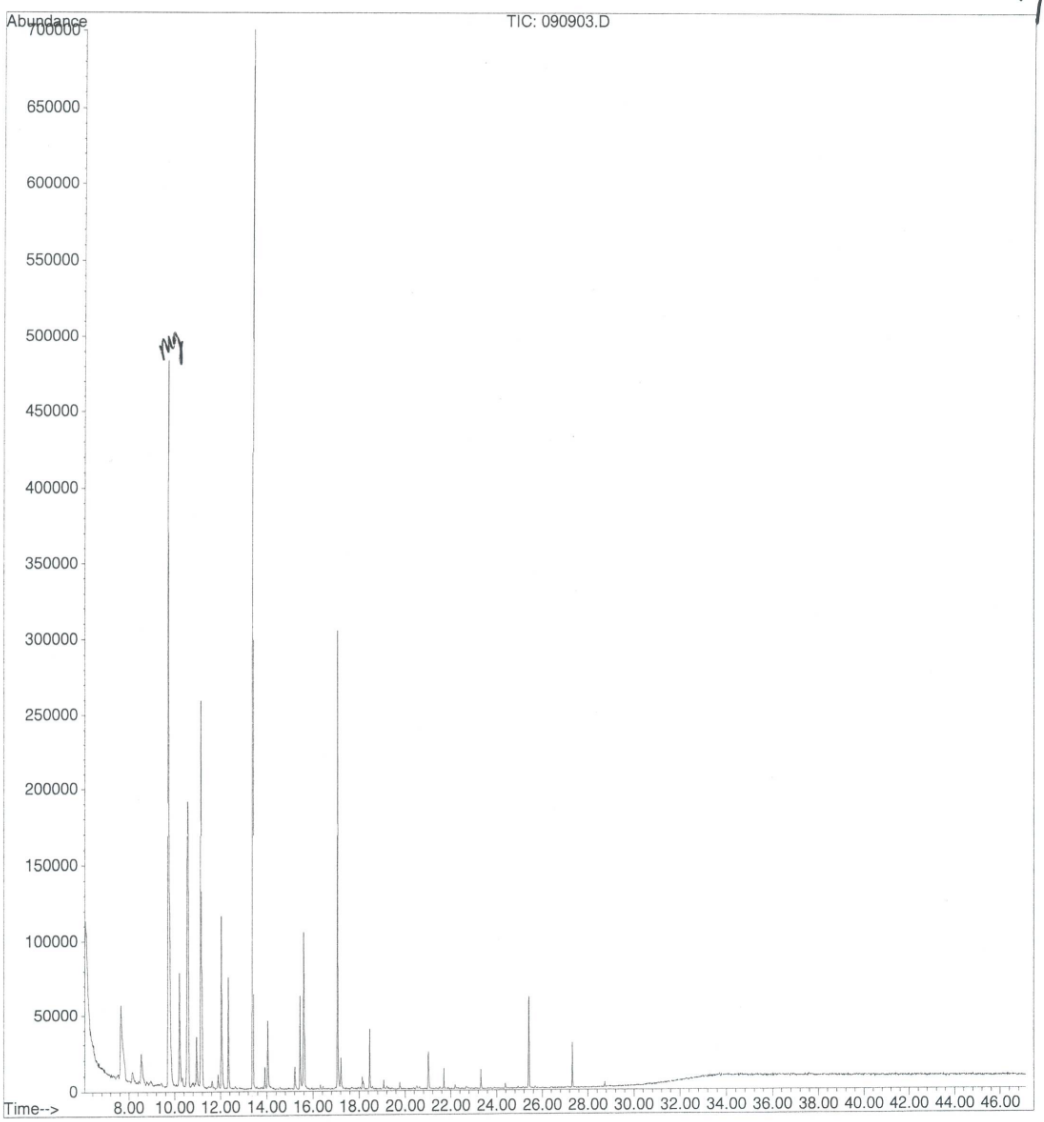

Figure 1d

Marcia Extreme De-Frizzing Treatment

```
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Operator   : MAV
Acquired   :  9 Sep 2011    6:58 pm using AcqMethod 11MY8270
Instrument :   FS8270
Sample Name: Marcia product #2 250ml room air (1ml Hexane)
Misc Info  : Friz Treatment  50ul BSTFA / TCMS
Vial Number: 4
```

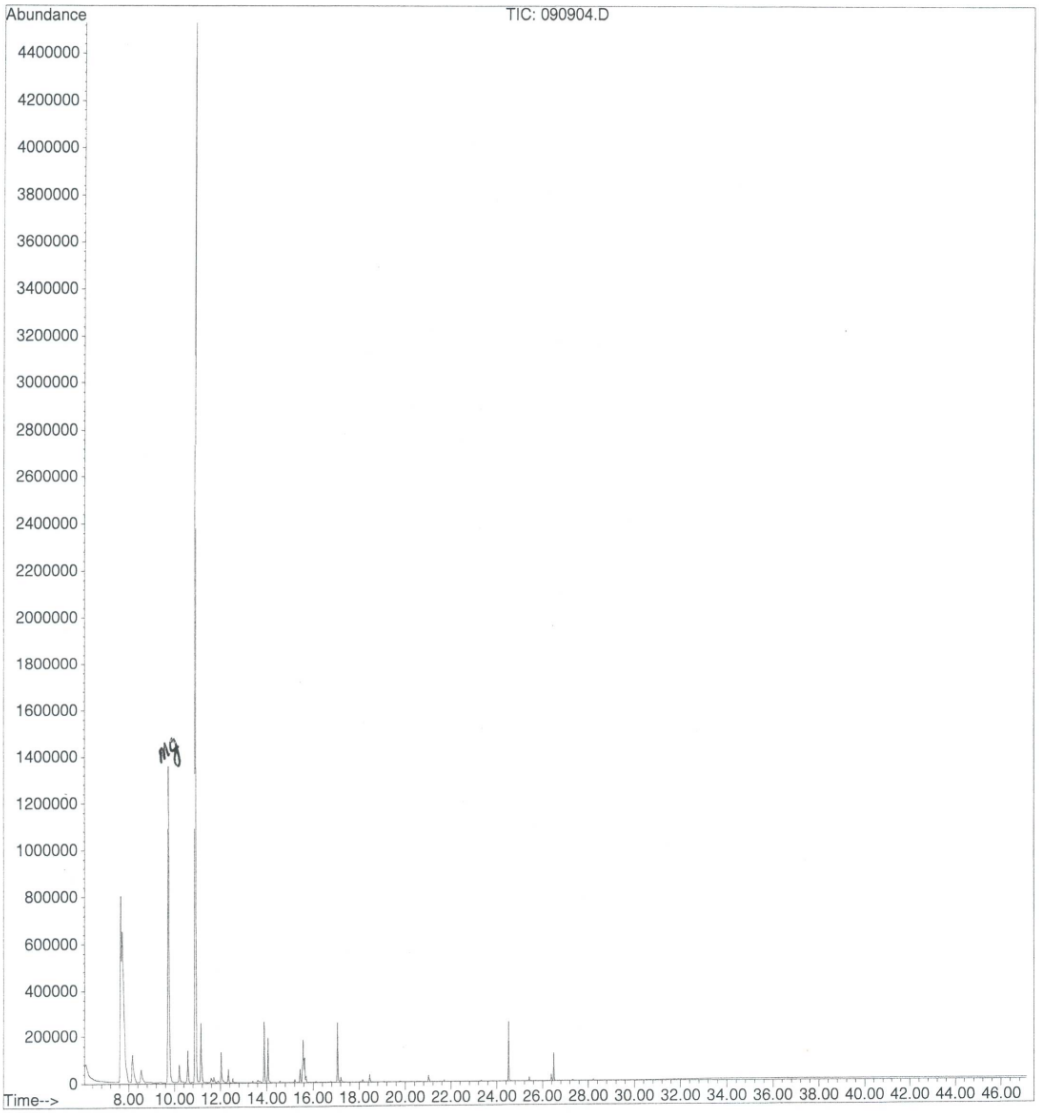

Figure 1e

```
File       : D:\HPCHEM\1\DATA\DATA11\090901\090901.D
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Instrument :   FS8270
Sample Name: MB 250ml room air (1ml Hexane)
Misc Info  : 50ul BSTFA / TCMS derivatized
Vial Number: 1
```

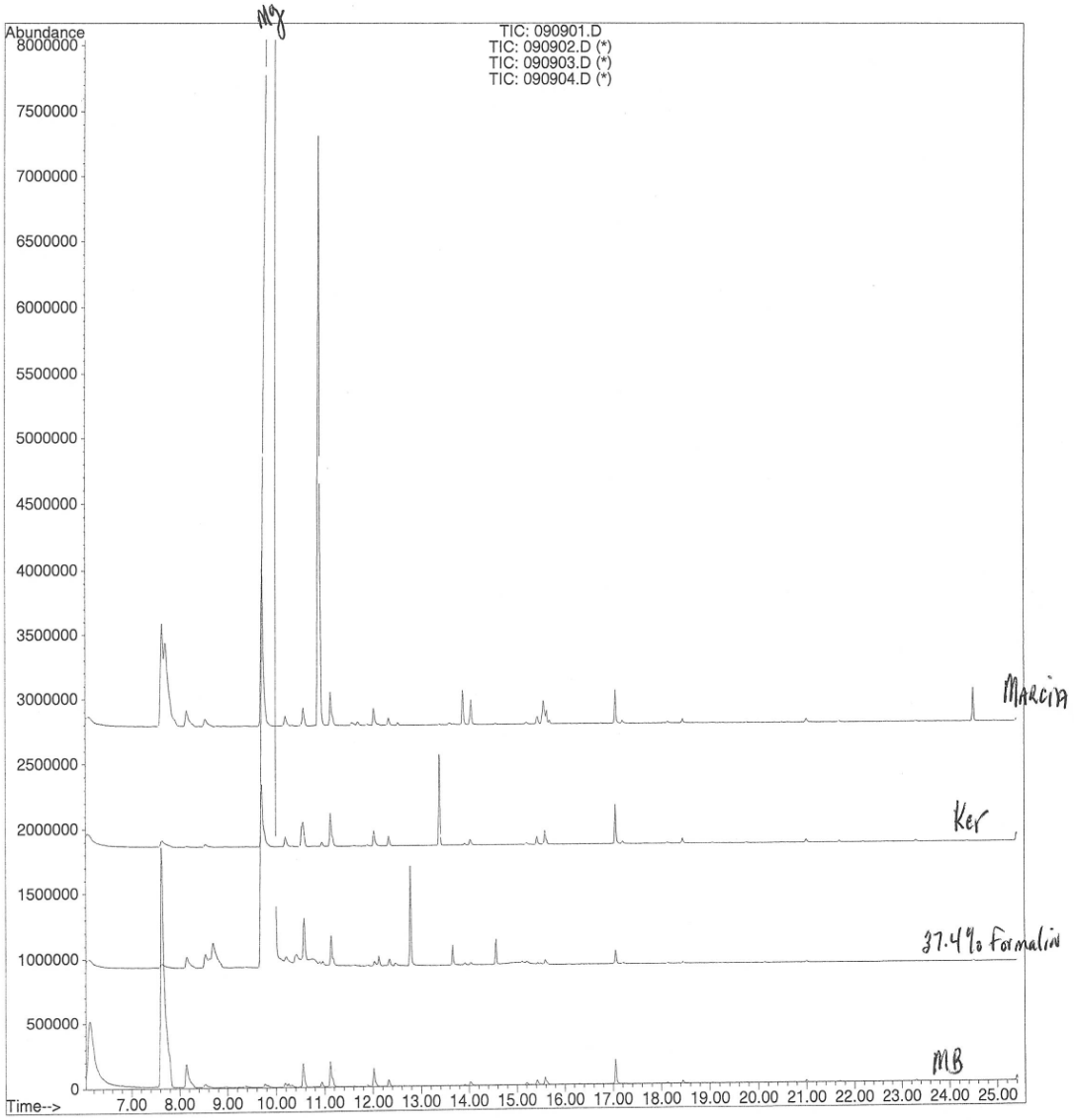


Figure 2

```
File       : D:\HPCHEM\1\DATA\DATA11\090901\090902.D
Operator   : MAV
Acquired   :  9 Sep 2011    5:04 pm using AcqMethod 11MY8270
Instrument :   FS8270
Sample Name: 37.4% Formalin 250ml room air (1ml Hexane)
Misc Info  : 50ul BSTFA / TCMS derivatized
Vial Number: 2
```

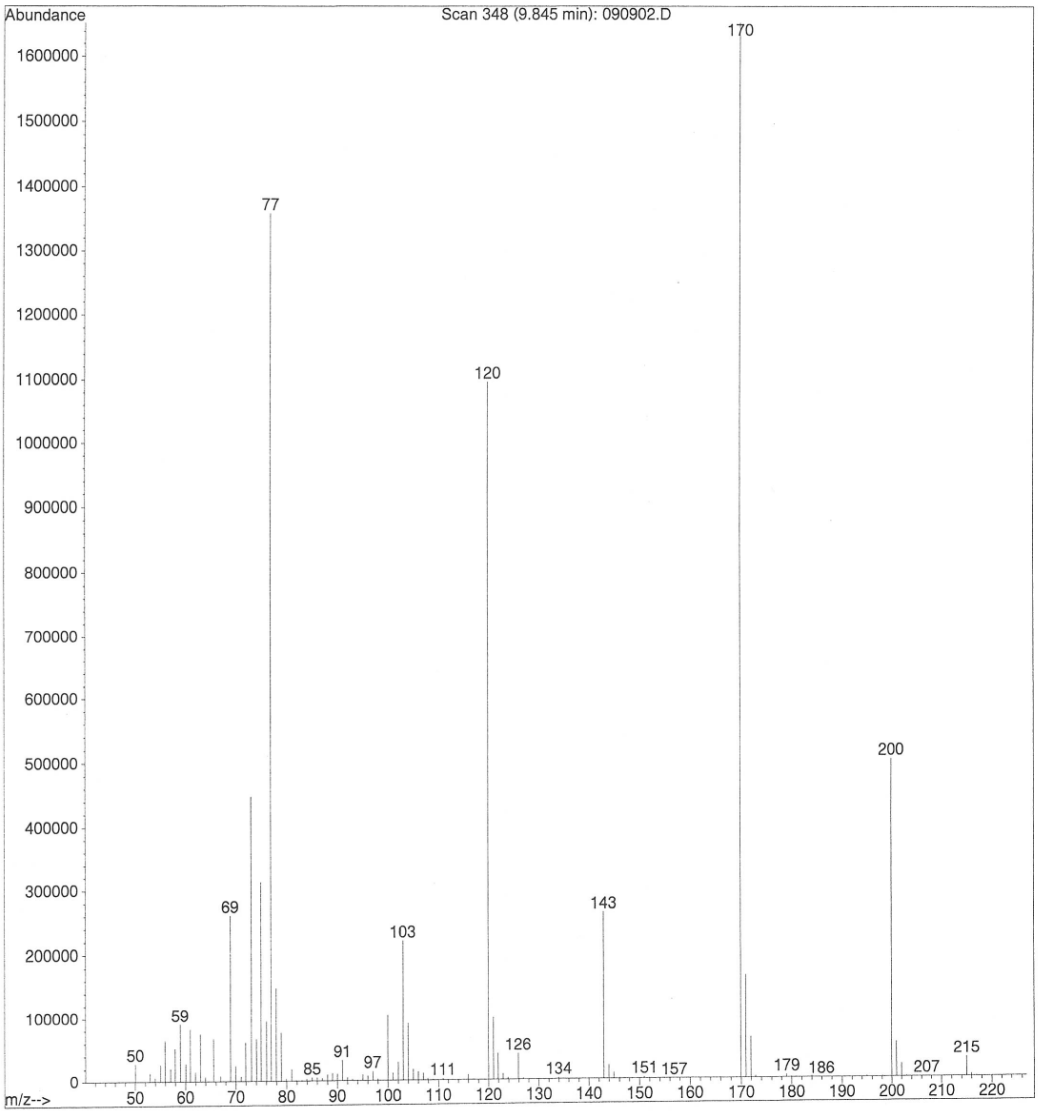
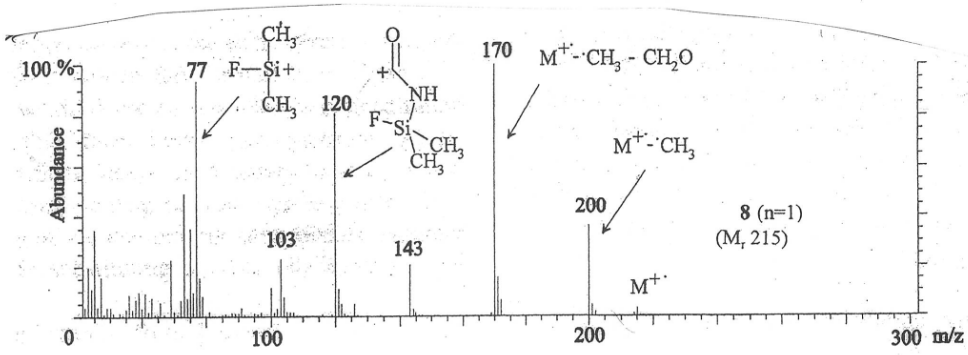
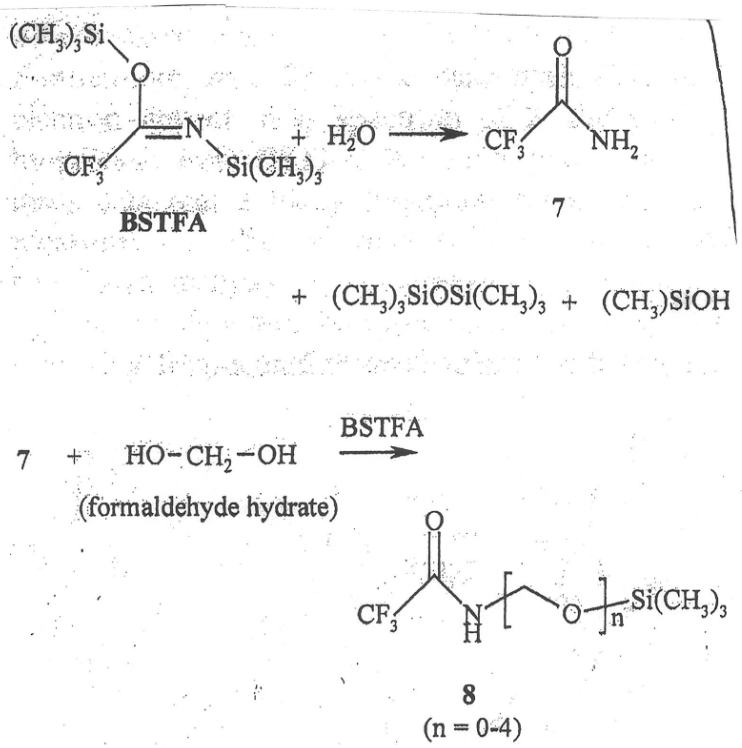


Figure 3



J.L. Little / J Chromatogr. A 844 (1999) 1–22

Figure 4



J. L. Little / J. Chromatogr. A 844 (1999) 1-22

Exponent
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Oakland, CA 94607

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March 4, 2011

[redacted]
Keratin Complex
7700 Congress Avenue, Suite 2201
Boca Raton, Florida 33487

Subject: Formaldehyde Exposure Assessment of Keratin Hair Smoothing Treatment Product
Exponent Project: 1008216.001

Dear Ms. Franklin:

Potential exposure to formaldehyde associated with use of the Keratin Complex Natural Keratin Smoothing Treatment product (Batch 0061KT- Lot 011711) in a salon was assessed for compliance with occupational criteria and other guidance. The key components of this evaluation are as follows:

- Exposure simulation — Description of methods used and airborne concentrations of formaldehyde measured during use of hair product.
- Exposure assessment — Description of relevant exposure scenarios and comparison of airborne concentrations to acceptable occupational exposure levels. In addition, a calculation of an inhalation dose associated with potential lifetime exposure to formaldehyde from typical use of the hair product is presented.
- Conclusion — Summary of assessment and conclusions with regard to potential exposure to formaldehyde associated with the hair product.

Exposure Simulation

Methods

The simulation took place in a commercial hair salon located in Pleasant Hill, California and consisted of a hair stylist applying the Natural Keratin Smoothing Treatment product to a model's hair. No central air conditioning or heating was used in the salon during the day of simulations. According to the hair stylist, when it became warm in the room the standard practice was to open a window. The simulation was the only service performed in the salon that day. Prior to the simulation, two background air samples were obtained over a 30-minute period to ensure that the test samples reflect potential formaldehyde exposure from product use

only, as some potential sources of formaldehyde emissions such furniture, as well as other hair products were present in the simulation area.

The stylist was experienced and trained by Keratin Complex in the proper application of this treatment. She conducted the treatment using the following general techniques:

The stylist shampooed and towel-dried the model's hair. She poured 2 ¼ ounces of the Keratin product into a glass bowl. The stylist spent approximately 20 minutes applying the product to the hair and combing it in. She then blow dried the hair for approximately 15-20 minutes. She used a flat iron set at 450ºF and passed the iron through small sections of hair approximately three times or until the hair appeared to stop "smoking." The flat ironing process took approximately 52 minutes. A window was opened during the process; the stylist indicated that she typically did this during the ironing, as the room became warm.

Samples were collected in the breathing zones of the stylist and model, and in the area where the process was conducted. Air sampling commenced after the stylist opened the bottle of product and poured it into a bowl. The overall application process took 142 minutes. Several photos of the simulation depict the process and placement of air-sampling devices on the stylist and model (Attachment A). The following six samples were collected as follows:

- Sample S-1 was collected in the breathing zone of the stylist during the treatment process.
- Sample S-2 was a duplicate of sample S-1.
- Sample M-1 was collected in the breathing zone of the model during the treatment process.
- Sample M-2 was a duplicate of sample M-1.
- Sample A-1 was collected in the area of the process within 3 feet of the models (located at the station).
- Sample A-1 was collected in the area of the process within 3 feet of the model (located at the window and then moved to the station after the window was opened).

Air samples were collected using EPA Method TO-11 for airborne formaldehyde analysis by high-performance liquid chromatography (HPLC). Samples were submitted under appropriate chain-of-custody procedures to Berkeley Analytical Laboratories, in Richmond, California, for analysis. An unexposed field blank was also submitted to the laboratory for analysis.

Results

The results of the simulation are presented in Table 1, and the analytical report produced by the laboratory is included as Attachment B. The two background air samples were measured to contain 29.8 and 30.6 micrograms per cubic meter (µg/m^3) formaldehyde.[1] The average of these values (30.2 µg/m^3) was subtracted from the raw results, to provide an adjusted concentration that accurately represents formaldehyde exposures from the product. No formaldehyde was detected in the blank sample.

Table 1. Simulation results

Sample ID	Sample description	Duration (min)	Volume (L)	Raw Concentration (µg/m^3)	Adjusted Concentration (µg/m^3)
S-1	Personal sample - stylist	142	27	322	292
S-2	Personal sample - stylist	130	26	263	233
				Average stylist	**262**
M-1	Personal sample - model	141	27	209	179
M-2	Personal sample - model	142	27	225	195
				Average model	**187**
A-1	Area sample - station	95	62	331	301
A-2	Area sample - window/station	139	31	268	238

[1] The measured background formaldehyde concentration is typical of indoor environments. CPSC reports that levels less than 30 ppb (36 µg/m^3) are considered normal in both indoor and outdoor air; residences and offices containing products that release formaldehyde may have concentrations greater than 30 ppb (36 µg/m^3) (U.S. CPSC 1997). The International Agency for Research on Cancer (IARC) indicates that levels of formaldehyde in indoor air are often higher by one order of magnitude or more than those outdoors (IARC 2006). Several studies of formaldehyde levels in indoor air in the U.S. indicate that mean concentrations range from approximately 11 to over 400 ppb (12–491 µg/m^3), with most studies reporting concentrations less than 50 ppb (61 µg/m^3) (IARC 2006). One study specific to the San Francisco Bay Area reported mean concentrations of 41 and 36 ppb (50 and 44 µg/m^3) in homes.

Exposure Assessment

Time-Weighted Average 8-hour Occupational Exposures (Stylist)

A time-weighted average (TWA) exposure for the stylist was calculated to represent the stylist's potential exposure throughout an 8-hour work day. Using the higher of the two personal samples for the stylist (322 µg/m^3) for 142 minutes and the background level of 30.2 µg/m^3 for the balance of eight hours results in an 8-hr TWA exposure of 117 µg/m^3.

The Time Weighted Average Permissible Exposure Limit (TWA-PEL) established for formaldehyde by the Occupational Safety and Health Administration (OSHA) is 0.75 ppm (923 µg/m^3) over an 8-hour time period to protect workers from adverse health effects and 2 ppm (2,460 µg/m^3) over a short-term 15-minute time period. OSHA PELs represent concentrations that cannot be exceeded during any 8-hour work-shift of a 40-hour workweek.

Based on this simulation, the estimated TWA for stylists providing one Keratin Complex treatment per day is 117 µg/m^3, an exposure that is almost eight times lower than the PEL. In addition, the American Conference of Governmental Industrial Hygienists (ACGIH) recommends a Threshold Limit Value (TLV) of 0.3 ppm (369 µg/m^3) that should not be exceeded at any time over the working period; no formaldehyde airborne concentrations were detected above this level.

Lifetime Average Daily Dose (Stylist and Model)

Another method that can be used to evaluate the resulting airborne formaldehyde concentrations requires calculating an average daily dose of formaldehyde that a stylist or a model may be exposed to during typical use of the product.

The lifetime average daily dose form inhalation associated with potential airborne exposure during use of the Keratin Product was calculated using the following equation:

$$Dose = \frac{AC \times BR \times ET \times EF \times ED}{AT}$$

The parameters for estimating exposure for the stylist and model, and the rationale for the selection of each, are summarized in Table 2.

Air Concentration: Dose estimates were calculated using the average detected formaldehyde air concentration of 262 µg/m^3 for the stylist and 187 µg/m^3 for the model to represent concentrations available to be inhaled while receiving the Keratin product hair treatment.

Exposure Time: Based on experience from the simulation, the treatment process time period of 140 minutes (2.3 hours) was used.

Exposure Frequency: Based on information provided by the stylist, the average user would receive this treatment twice per year to maintain straight hair. Based on information provided by Keratin a stylist is not likely to perform the straightening process more than once per week to various clients.

Exposure Duration: Based on U.S. EPA's default exposure assumption for exposure to contaminants, a conservative estimate of 30 years of continuous use was used for the model. Based on U.S. EPA's on occupational tenure, the median years of occupational tenure for hairdressers and cosmetologists is 8.9 and this value was used in the assessment.

The resultant estimated inhalation dose is approximately 10 μg/day for a hair stylist and 1.1 ug/day for the model. Both of these doses are below the California Proposition 65 warning criteria of 40 μg/day. The default exposure assumptions and other factors used in this analysis provide an evaluation of potential exposure that is intended to be a conservative estimate of the average exposure during use of the Keratin product.

Table 2. Exposure parameters

Symbol	Parameter	Value	Units	Rationale
AC	Air concentration	262	µg/m^3	Average personal concentration for stylist from study data
		187	µg/m^3	Average personal concentration for model from study data
BR	Breathing rate	1	m^3/hr	Short-term adult breathing rate (U.S. EPA 1997)
ET	Exposure time	2.3	hr/treatment	140 minutes, based on simulation
EF	Exposure frequency	2	treatments/year	for model
		50	Treatments/year	For hair stylist – assuming one treatment per week per information provided by Keratin
ED	Exposure duration	8.9	years	U.S. EPA 1997; median years for hair dressers and cosmetologists
		30	years	U.S. EPA 1997; default residential assumption
AT	Averaging time for carcinogens	25,550	days	Default value (365 days×70 years)

Conclusions

This assessment evaluated potential exposure to formaldehyde associated with the use of Keratin Complex Natural Keratin Smoothing Treatment product. Based on this analysis, the potential exposure of a hair stylist is estimated to be 117 µg/m^3 over an 8-hour work day; a level nearly eight times below the OSHA permissible exposure limit of 923 µg/m^3 (0.75 ppm). No concentrations were detected above the OSHA short-term exposure limit of 2 ppm (2,460 µg/m^3) or above the ACGIH recommended ceiling concentration of 0.3 ppm (369 µg/m^3).

The potential dose of formaldehyde to a hair stylist was estimated to be 10 μg/day while the dose was estimated to be 1.1 μg/day for the model. Both of these doses are below the California Proposition 65 warning level of 40 μg/day.

Thank you for providing Exponent the opportunity to assist with this exposure assessment. Please contact us if you have any questions regarding this evaluation.

Sincerely,

Renee Kalmes, CIH
Senior Managing Scientist

Emily Goswami, CIH
Managing Scientist

Attachments (2)

References

CCR. 2001. California Code of Regulations Title 22, Chapter 3: Safe Drinking Water and Toxic Enforcement Act of 1986.

IARC. 2006. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 88. International Agency for Research on Cancer, Lyon, France, pp. 39–32. Available at: http://monographs.iarc.fr/ENG/Monographs/vol88/mono88-6.pdf .

U.S. CPSC. 1997. Update on formaldehyde, 1997 revision. U.S. Consumer Product Safety Commission. Available at: http://www.cpsc.gov/CPSCPUB/PUBS/725.pdf.

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Attachment A

Photos of Simulation

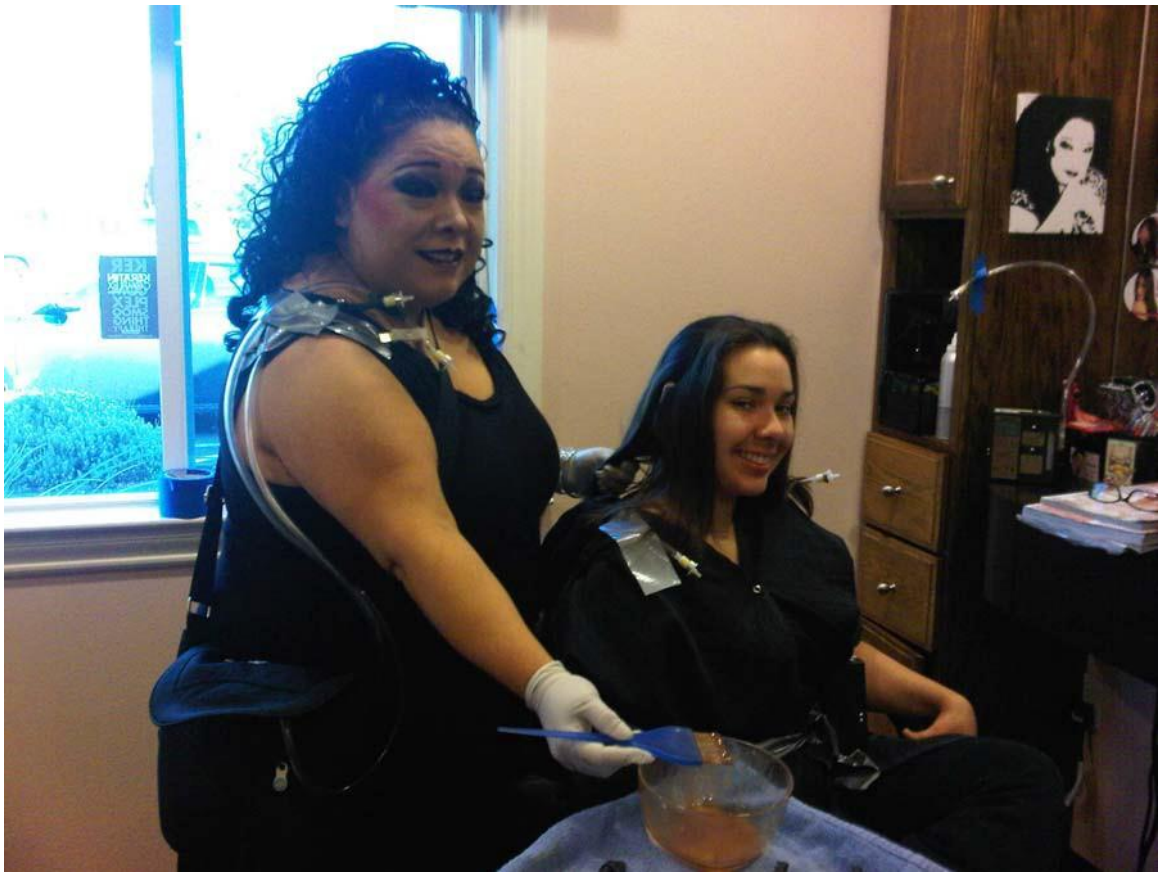

Photo of stylist and model showing sampler placement.

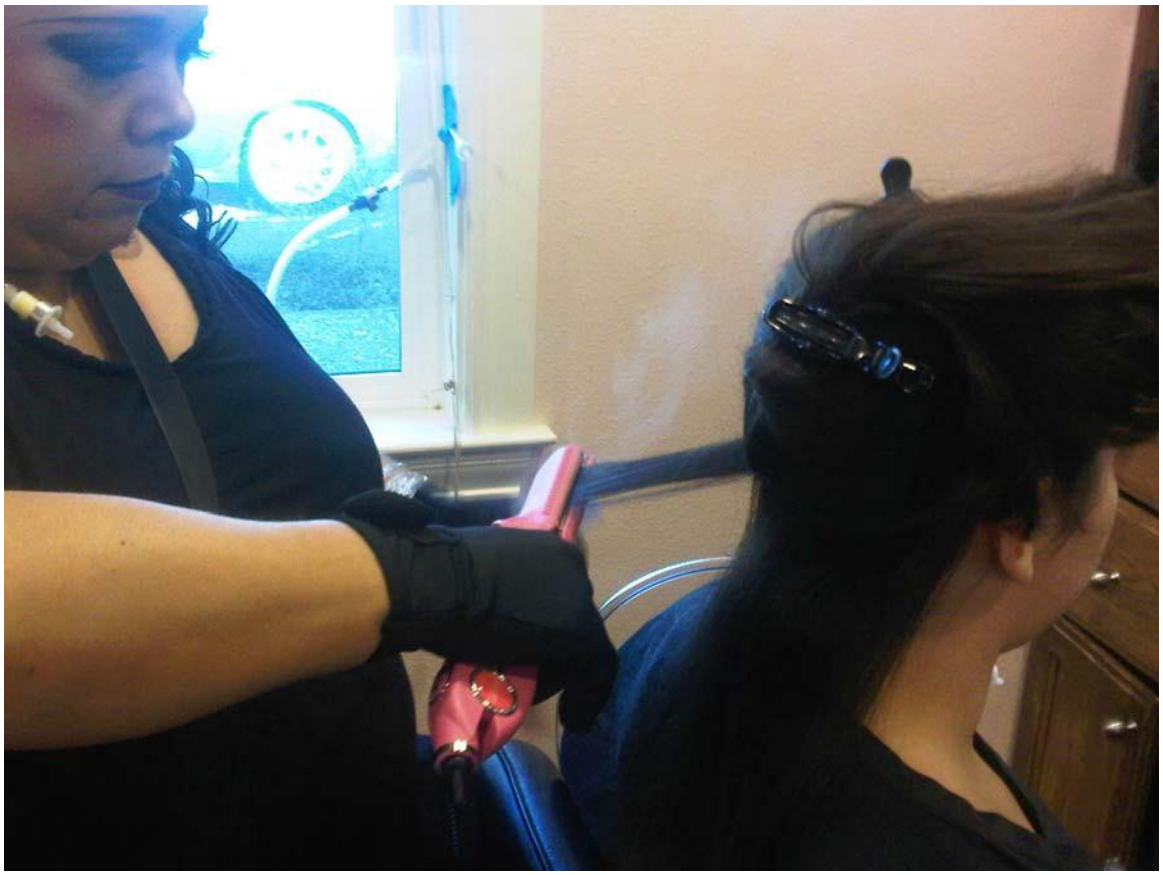

Photo of flat-ironing process.

Attachment B

Laboratory Report

BERKELEY ANALYTICAL
815 Harbour Way South, Suite 6
Richmond, CA 94804-3612
Ph. 510-236-2325; Fax 510-236-2335
E-mail baalab@berkeleyanalytical.com

Analysis of Field-Collected Air Samples

'Customen ana Projet Jriformation

Report Certification	
Report number	096-029-IH-Feb1611
Report date	February 16, 2011
Certified by (Name/Title)	Alfred T. Hodgson, Research Director
Signature	
Date	*February 16, 2011*

Methods	
ASTM D 5197-09	Std. Test Method for Determination of Formaldehyde...

Customer Information	
Customer:	Exponent Failure Analysis Associates
City/State/Country	Oakland, CA, USA
Contact name/Title	Renee Kalmes
Phone number	510-268-5007

Project Information	
Project number	1008216.001
Project name	KC-2
Project location	Not given
Project date	February 13, 2011

Laboratory Receiving Information	
Date samples received by lab	February 15, 2011
Condition of samples	No observed problems
Lab tracking numbers	096-029-01 to 096-029-09

Sample No.	Sample Description	Sampler No.	Date Collected	Volume (L)	Analysis Type	Specified Method
BK-1	Background	Waters Cart	Feb, 13, 2011	22	Formaldehyde	D 5197-09
BK-2	Background	Waters Cart	Feb, 13, 2011	21	Formaldehyde	D 5197-09
S-1	Personal Stylist	Waters Cart	Feb, 13, 2011	27	Formaldehyde	D 5197-09
S-2	Personal Stylist	Waters Cart	Feb, 13, 2011	26	Formaldehyde	D 5197-09
M-1	Personal Model	Waters Cart	Feb, 13, 2011	27	Formaldehyde	D 5197-09
M-2	Personal Model	Waters Cart	Feb, 13, 2011	27	Formaldehyde	D 5197-09
A-1	Area	Waters Cart	Feb, 13, 2011	62	Formaldehyde	D 5197-09
A-2	Area	Waters Cart	Feb, 13, 2011	31	Formaldehyde	D 5197-09
Blank	None	Waters Cart	Feb, 13, 2011	--	Formaldehyde	D 5197-09

Sample No.	Lab Track. No.	Method	Date Analyzed	Analyst	Data File
BK-1	096-029-01	D 5197-09	Feb 15, 2011	R. Gill	110215_A\002-0201
BK-2	096-029-02	D 5197-09	Feb 15, 2011	R. Gill	110215_A\003-0301
S-1	096-029-03	D 5197-09	Feb 15, 2011	R. Gill	110215_A\004-0401
S-2	096-029-04	D 5197-09	Feb 15, 2011	R. Gill	110215_A\005-0501
M-1	096-029-05	D 5197-09	Feb 15, 2011	R. Gill	110215_A\006-0601
M-2	096-029-06	D 5197-09	Feb 15, 2011	R. Gill	110215_A\007-0701
A-1	096-029-07	D 5197-09	Feb 16, 2011	R. Gill	110216_A\002-0201
A-2	096-029-08	D 5197-09	Feb 15, 2011	R. Gill	110215_A\009-0901
Blank	096-029-09	D 5197-09	Feb 15, 2011	R. Gill	110215_A\010-1001

Rrqject'Specific Information

Air samples for the analysis of formaldehyde were received by the laboratory on February 15, 2011. There were nine DNPH cartridges for the analysis of formaldehyde. The analytical results are reported in Table 1. All laboratory data, including but not limited to raw instrument files, calibration files, and quality control checks used to generate the results will be made available to the customer upon request.

berkeley analytical

ACCREDITED

Table 1. Quantitative analysis of formaldehyde

Sample No.	Sample Name	Volume (L)	Mass (ng)	Conc ($\mu g/m^3$)	Conc* (ppb)
BK-1	Background	22	657	29.8	24.3
BK-2	Background	21	643	30.6	25.0
S-1	Personal Stylist	27	8,710	322	263
S-2	Personal Stylist	26	6,830	263	214
M-1	Personal Model	27	5,630	209	170
M-2	Personal Model	27	6,080	225	184
A-1	Area	62	20,500	331	270
A-2	Area	31	8,310	268	218
Blank	None	--	--	--	--

Concentration in ppb calculated assuming standard conditions of 25° C and 101.3 kPa.

Berkeley Analytical Associates, LLC
815 Harbour Way South, Sune 6
Richmond, CA 94804-3612
Phone: 510-236-2325 Fax: 510-236-2335
e-mail: baalab@berkeleyanalytical.com

CHAIN-OF-CUSTODY RECORD
for ANALYTICAL SERVICES
2009Update

Client Information*			
Company:	Exponent		
Street Address:	1500 12th Street, Suite 200		
City/State/Zip Code:	Oakland CA		
Country:	Contact (for reporting):	Renee Kachmar	
Project/Job Name:	VC-2		
Project/Job #:	1008216.001	P.O.#:	
Phone:	510-268-5007	Fax:	
E-mail address:	rkachmar@exponent.com		

Multisorbent tube samplers	D
Aldehyde cartridge samplers	bl'.
Samplina pump(S)_	n
Other materials (sgecify):	D

BAA Analysis Code	
Total VOCs by GC/MS total-ion-current method (TIC)	a
Qualitative GC/MS of individual VOCs	b
Qualitative GC/MS plus quantitation of up to 15 individual VOCs	c
Quantitative GC/MS analysis of target list of compounds	d
Quantitative GC/MS Total VOCs plus 4-PCH	e
Quantitative HPLC analysis of formaldehyde	f
Quantitative HPLC analysis of formaldehyde & acetaldehyde	g
Quantitative HPLC analysis of formaldehyde & C2-C9 carbonyls	h
Quantitative HPLC analysis of glutaraldehyde	i
	k
Other	z

LspeciM

/JO<I.vfl 7 -<) v""'-""-A ·

Air Sampling Record

Tube/Cartridge Sampler No.	Collected Sample ID	Date Collected	Collection Minutes	Collected Volume (L)	Sample Location / Description / Remarks	nalysis Code	BAA Remarks
Lot WAT	BK-1	12/13/11	29	22	Background	f	
047205	BK-2	"	29	21	Background	"	
00283030918	S-1		142	27	personal stylist		
	S-2		130	26	personal stylist		
	M-1		141	27	personal model		
	M-2		142	27	personal model #1		
	A-1		95	62	Area		
	A-2	↓	139	31	Area	↓	
	Blank	↓	N/A	N/A			

Sample(s) Handling

Relinquished By	Received By	Signature	Date	Company
		Renee Kachmar	12/14/11	Exponent
	RGill		12/15/11	BAA

Final Amended Report

Formaldehyde and Methylene Glycol

December 7, 2011

The 2011 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; 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.; and Paul W. Snyder, D.V.M., Ph.D. This report was prepared by Ivan J. Boyer, Ph.D., D.A.B.T, and Bart A. Heldreth, Ph.D.

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CONTENTS

ABSTRACT

Methylene glycol is continuously converted to formaldehyde, and vice versa, even at equilibrium, which can be easily shifted by heating, drying, and other conditions to increase the amount of formaldehyde. This rapid, reversible formaldehyde/methylene glycol equilibrium is distinguished from the slow, irreversible release of formaldehyde resulting from so-called formaldehyde releaser preservatives, which are not addressed in this safety assessment (formaldehyde releasers may continue to be safely used in cosmetics at the levels established in their individual CIR safety assessments).

Formaldehyde and methylene glycol may be used safely in cosmetics if established limits are not exceeded, and are safe for use in nail hardeners in the present practices of use and concentration, which include instructions to avoid skin contact. In the present practices of use and concentration (on the order of 10% formaldehyde/methylene glycol, blow drying and heating, inadequate ventilation, resulting in many reports of adverse effects), hair smoothing products containing formaldehyde and methylene glycol are unsafe.

INTRODUCTION

In 1984, CIR published its original safety assessment of formaldehyde,[1] concluding that this ingredient is safe for use in cosmetics applied to the skin if free formaldehyde was minimized, but in no case > 0.2%. This conclusion was based on data from numerous human skin irritation and sensitization tests (number of subjects ranging from 8 to 204) of cosmetic products (skin cleansers and moisturizers and a hair rinse) containing 0.2% formalin (37% w/w aqueous formaldehyde solution). Except for a few mild, equivocal, or inconsistent reactions, the results of these tests showed that such products have little potential to irritate or sensitize the skin. The Panel also said that it cannot be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized.

The Panel re-reviewed the safety assessment of formaldehyde and confirmed the original conclusion in 2003.[2]

Since that re-review, methylene glycol has been listed as a cosmetic ingredient and CIR has become aware of increasing uses of formaldehyde/methylene glycol in hair smoothing products intended to be heated. In addition to the issues related to increasing uses and identification of methylene glycol as a cosmetic ingredient, the U.S. EPA National Center for Environmental Assessment (NCEA) released a draft toxicological review of formaldehyde for external review on 2 June 2010, including interagency comments on an earlier draft of the document.[3] The NCEA Risk Assessment provides a comprehensive summary of the toxicological literature, including both human and animal studies and all of the major exposure routes of concern (inhalation, ingestion, and skin contact). The U.S. National Research Council (NRC) has released their review of the draft assessment.[4] Much of the significant new toxicology data are related to genotoxicity, carcinogenicity, and reproductive and developmental toxicity.

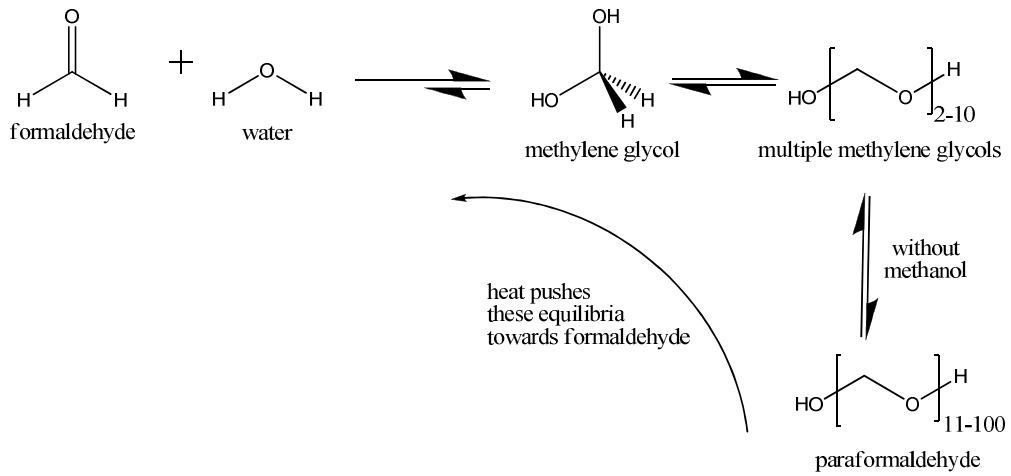
Data and analysis were provided by the Nail Manufacturer's Council (NMC) the Professional Keratin Smoothing Council (PKSC), the Personal Care Products Council, and the American Chemistry Council. Additional data from the U.S. Food and Drug Administration's (FDA's) adverse event reporting system and results of FDA laboratory product analyses are included.

CHEMISTRY

Formaldehyde – Formalin –Methylene Glycol

Formaldehyde, a gas, is not used in cosmetics in its pure, anhydrous form, but is instead most commonly produced as an aqueous solution called formalin.[5] Formalin is industrially produced from methanol. First, a mixture of vaporized methanol and steam is passed over a catalyst bed, where the methanol is oxidized to formaldehyde gas. Since this reaction is highly exothermic, the gas stream is cooled directly after passing over the catalyst to prevent thermal decomposition. Next, the formaldehyde reacts with water in an absorption column, because formaldehyde in its pure, gaseous form is highly unstable. Formaldehyde quickly reacts with water to produce methylene glycol and, without a polymerization inhibitor (eg, methanol), polymethylene glycols via a series of reversible reactions (Scheme 1). In the absence of methanol, these reactions proceed to form a mixture of long chain polymethylene glycols, which are referred to as paraformaldehyde.

Scheme 1 – Equilibria in aqueous formaldehyde solutions such as formalin



Methylene glycol, as a pure and separate substance, is not commercially available, but is instead produced as an aqueous solution called formalin, as denoted above for formaldehyde. Methylene glycol is a *geminal* (*gem*) diol, or a diol with both hydroxyl groups on the same carbon. *Gem* diols are typically unstable compounds. Indeed, methylene glycol exists only in aqueous solution, where it is stabilized by hydrogen bonding with water molecules. Thus, the high solubility of formaldehyde in water is due to the rapid hydration of formaldehyde to methylene glycol and the capacity of the aqueous solution to stabilize methylene glycol and small polymethylene glycols (ie, two to ten methylene glycol units long).[6] The rate of the hydration reaction is very fast (the half-life of formaldehyde in water is 70 milliseconds) and the equilibrium between methylene glycol and formaldehyde strongly favors methylene glycol at room temperature and neutral pH.[7] The equilibrium is dependent on temperature, solution density, pH, and the presence of other solutes. Increased temperature favors formation of formaldehyde. While the concentration of methylene glycol in formalin is much greater than formaldehyde, at room temperature, neutral pH stasis, this says nothing about the reversibility of this equilibrium shift or about the rate of dehydration when this stasis is disrupted (eg, formalin is exposed to air or a formulation containing formalin is heated). This reaction is reversible. The dehydration of methylene glycol to formaldehyde happens rapidly and can be catalyzed by lower pH.[8]

The formation of the higher polymethylene glycols is much slower than the rates of hydration and dehydration, and can be inhibited by methanol. Accordingly, a typical solution of formalin consists of water (~40-60%), methylene glycol (~40%), methanol (~1-10%), small methylene glycols (eg, dimers and trimers; ~1%), and a very small amount of formaldehyde (~0.02-0.1%). The multiple equilibria between these components favor methylene glycol at room temperature.[9] However, removal of water, increase in solution density, heating, reduction of pH, and/or the reaction of the small amount of free formaldehyde in the solution will drive the equilibrium back toward formaldehyde.[10] Moreover, a product formulated with either of the ingredients methylene glycol or formaldehyde actually contains an equilibrium mixture of the components: methylene glycol, polymethylene glycols and formaldehyde. While it can be pointed out that formaldehyde and methylene glycol are different and distinct molecules, the ever present equilibrium between the two makes this distinction of virtually no relevance to ingredient safety.[11] Due to the equilibria demonstrated above, any aqueous formulation that reportedly contains formalin, formaldehyde, or methylene glycol, actually contains both formaldehyde and methylene glycol. Accordingly, the ingredients formaldehyde and methylene glycol can be referred to as formaldehyde equivalents. Under any normal conditions of cosmetic use, including at room temperature and above, methylene glycol is not stable in the gas phase and very rapidly dehydrates to formaldehyde and water.[12] Accordingly, heating of a formulation containing formaldehyde or methylene glycol will primarily off-gas formaldehyde. For this reason, the hazards of formaldehyde equivalents in a heated solution are the same as the hazards of gaseous formaldehyde, since the solution so readily releases gaseous formaldehyde.

Formaldehyde Equivalents

Formalin, as recited above, is an aqueous solution of formaldehyde, methylene glycol and polymethylene glycols, all in equilibria and often stabilized with methanol. Formalin, *per se*, is not listed as an ingredient in the International Cosmetic Ingredient Dictionary and Handbook (INCI Dictionary) but is often recited herein as the material tested (therefore representing formaldehyde/methylene glycol). Of special importance is an understanding of the meaning of percent formalin. "100% formalin" means an aqueous solution wherein formaldehyde has been added to water to the saturation point of these equilibria, which is typically 37% (by weight) formaldehyde equivalents in water. Accordingly, a 10% formalin solution contains approximately 3.7% formaldehyde equivalents. More specifically, an aqueous solution which is 3.7% of formaldehyde (by weight) relates directly to a solution which is 5.9% methylene glycol (because the molecular weight of formaldehyde is 30 g/mol and the molecular weight of methylene glycol is 48 g/mol).

All of the toxicity studies relied upon for determining the current 0.2% limitation in cosmetic products are based on the idea of "free formaldehyde," what we now are calling formaldehyde equivalents. However, it seems quite probable that this number actually meant 0.2% formalin. Accordingly, based on the average formalin solution being 37% formaldehyde equivalents, this represents a true limit of 0.074% formaldehyde equivalents.

Moreover, the ingredients in this review are not to be confused with "formaldehyde releasers," which are not analogous to formaldehyde or methylene glycol, but release small amounts of formaldehyde over considerable intervals (eg, Diazolidinyl Urea), acting as preservatives.

Analytical Methods

Most commonly used analytical methods for qualitative and quantitative detection of formaldehyde are non-specific to non-hydrated formaldehyde, but can accurately describe formaldehyde equivalent presence and quantity. A typical method, for example the method used by the Oregon OSHA Laboratory, can detect formaldehyde equivalents present in a formulation, or released into the air, via a two stage process: 1) derivatization of a sample with a hydrazine (which reacts with formaldehyde or methylene glycol, in a formulation sample or in an air sample), and 2) detection of the resultant hydrazone (ie, the reaction product of the hydrazine and formaldehyde) with a diode array, after separation on a column (eg, high performance liquid chromatography (HPLC) separation followed by ultraviolet/visible light (UV/Vis) detection).[11] Accordingly, published values for "formaldehyde" levels should be taken to mean formaldehyde equivalents.

While other formaldehyde/methylene detection techniques are known, the methods used by OSHA are the most common methods and are what current regulations, globally, have been based on. These techniques would find that a typical formalin solution contains approximately 37% formaldehyde equivalents. Some may argue that using nuclear magnetic resonance (NMR) spectrometry techniques would demonstrate that this same formalin solution is only 0.037% formaldehyde.[13] This is a technically correct interpretation of the amount of non-hydrated formaldehyde molecules present in the static environment of an NMR sample tube. This scenario, however, exists only in the highly controlled experimental system where the conditions (room temperature, neutral pH, closed NMR tube) maintain an artificially constant level of non-hydrated formaldehyde. This does not represent the conditions under which formaldehyde or methylene glycol are used in hair smoothing products, and as such, drastically underestimates the exposure risk. In use, hair smoothing treatments containing formaldehyde or methylene glycol involve elevated temperatures (eg, 450 degrees F) and reduced pH formulations (eg, as low as pH = 4).[13] Further, the solutions are used in a system where the bottle is opened, the solution is poured, applied, and allowed to partially evaporate/off gas. Focusing on the equilibrium between formaldehyde and methylene glycol in a closed system that artificially favors a liquid state is not representative of the conditions of use of these ingredients in hair smoothing products.

An alternative technique has also been proposed for specifically addressing the vapor/gas present in the headspace above an aqueous formaldehyde/methylene glycol solution, which involves trimethylsilyl (TMS) derivatization of those moieties present, followed by detection of the resultant derivatives.[13] However, the chemical specificity for this method is not conclusively defined. The resultant derivatives detected could have arisen from a variety of constituents present in the headspace. Furthermore, no standards were recited which validate this method's ability to detect non-hydrated formaldehyde.

COSMETIC USE

As given in the INCI Dictionary,[14] formaldehyde functions in cosmetic products as a cosmetic biocide, denaturant, and preservative. According to the 2010 13th Edition of the INCI Dictionary, methylene glycol is reported to function as an artificial nail hardener.[14]

In the FDA's Voluntary Cosmetic Registration Program (VCRP),[15] there are 77 uses of formaldehyde and formaldehyde solution (formalin) reported. Since these all are probably the same ingredient as added to cosmetics, they are combined in Table 1.[2,15,16] Industry surveys of formaldehyde use concentrations and an FDA reports yielded data shown in Table 1.[16-19] No uses of methylene glycol are currently reported to the VCRP, but the use concentration in nail hardeners containing methylene glycol reportedly ranges from 0.8% to 3.5% (corresponding to 0.5% to 2.2% calculated as formaldehyde).[16-19]

The Material Safety Data Sheet (MSDS) provided by Brazilian Blowout for their salon product, however, does include methylene glycol.[20] The list of ingredients provided by the manufacturer is shown in Table 2, with methylene glycol listed at <5.0%.

From a high of 805 reported uses of formaldehyde/formalin in 1984, VCRP data from 2001/2002, 2006/2007, and 2009/2010 show that uses have decreased to less than 100 uses, as shown in Figure 1. The VCRP, however, does not include reporting of ingredients used in cosmetics labeled "for professional use."

In Europe, formaldehyde is also permitted for use in cosmetics at concentrations ≤0.2% (the limit for oral hygiene products is ≤0.1%).[21] Products containing >0.05% formaldehyde must be labeled "contains formaldehyde." The maximum authorized concentration in finished nail hardeners is 5%, provided that the product is labeled "Protect cuticles with grease or oil. Contains formaldehyde" These limits are expressed as "free formaldehyde" or "calculated as formaldehyde." Formaldehyde is prohibited for use in aerosol dispensers. Canada, Australia, China and ASEAN nations have regulatory limits very similar to those of the European Union.[22-27]

Use of Formaldehyde/Methylene Glycol in Nail Hardening Products

The FDA Guide to Inspections of Cosmetic Product Manufacturers[28] states that nail hardeners often contain formaldehyde as the active ingredient and that the Agency has not objected to its use as an ingredient of nail hardeners if the product 1) contained no more than 5% formaldehyde, 2) provided the user with nail shields that restrict application to the nail tip (and not the nail bed or fold), 3) furnished adequate directions for safe use, and 4) warned consumers about the consequences of misuse and potential for causing allergic reactions in sensitized users. Based on comments given at the June 27-28, 2011 CIR Expert Panel meeting, it appears that nail shields are no longer supplied with nail hardeners in the U.S. because consumers did not use the shields.

As noted above, in Europe, formaldehyde is permitted for use in nail hardeners at concentrations ≤5% "calculated as formaldehyde," and the product label must instruct the user to protect cuticles with grease or oil.[29] If the formaldehyde concentration in the product exceeds 0.05%, the label must also state "contains formaldehyde."

In the earlier CIR safety assessment of formaldehyde,[1] the CIR Expert Panel acknowledged reports of use of formaldehyde in nail hardeners at a concentration of 4.5%. It now appears that methylene glycol is considered to be the appropriate ingredient name to use to describe formaldehyde/methylene glycol in nail hardeners. [14] . Recent data provided by the Nail Manufacturers Council (NMC)[30] indicated that, to make a nail hardener nominally "1% formaldehyde"– which should be considered a typical marketplace level – a formulator would add 2.703% formalin (2.703% x 37% = 1%). Because of the well-recognized equilibrium relationship between formaldehyde and methylene glycol, the formaldehyde converts to methylene glycol. Therefore, a product with 2.703% formalin would contain 1.60% methylene glycol (2.703% x 59.2% = 1.60%). A recent survey of U.S. marketers conducted by the NMC indicated that formaldehyde/methylene glycol is not used in all brands of nail hardeners.[18] The survey results indicated that brands using methylene glycol/formaldehyde contain 0.7% to 1.85%, calculated as formaldehyde. Analyses of two finished nail hardener products (brand/origin not identified) indicated that they contained 1.9% and 2% formaldehyde equivalents, expressed as formaldehyde.[19] FDA recently reported finding 2.2% formaldehyde/methylene glycol in a nail hardening product that was cited often in a compilation of customer self-reports from Internet sites indicating adverse effects including skin irritation, burning sensation of nail beds and

exposed skin, and pain[17,31] and two cases of eyelid dermatitis reported by a member of the CIR Expert Panel. The cases reported by the Panel member patched tested negative for 1% formaldehyde equivalents (calculated as formaldehyde) in water; higher concentrations (eg, 2%) were not tested.

Use of Formaldehyde/Methylene Glycol in Hair Smoothing Products

The use of formaldehyde/methylene glycol containing hair smoothing products largely appears to take place in salons, but use in a home is not precluded. Workplace surveys conducted by the Oregon Occupational Safety and Health Administration (OSHA) uncovered a wide variety of ventilation approaches, including simply having a building HVAC system, propping the business's doors open, or operating ceiling fans.[11]

Although the purpose and mechanism of action of formaldehyde/methylene glycol in hair relaxers/straighteners is not well documented, formaldehyde (as part of a formalin solution) is known to induce a fixative action on proteins (eg, keratin).[32] This is at least in accord with formaldehyde's function as a denaturant, in the classic sense of the term (ie, reacting with biological molecules, such as disrupting the tertiary structure of proteins, not just making liquids non-potable). Purportedly, formaldehyde/methylene glycol hair straightening formulations, such as Brazilian-style or keratin-based straightening products, maintain straightened hair by altering protein structures via amino acid crosslinking reactions, which form crosslinks between hair keratins and with added keratin from the formulation.[33]

One proposed reaction scheme involves: 1) hemiacetal formation between a keratin hydroxyl group and formaldehyde, 2) reaction of two such hemiacetals, in a dehydration step, to form a methylene ether crosslink, and 3) formaldehyde elimination to finalize the new methylene crosslink.[34] Stoichiometricaly, this proposed scheme purports that some of the formaldehyde that initially reacts with keratin is eventually released as formaldehyde during the hair straightening process. Formaldehyde can react with multiple protein residue side-chains, although the principal reactions are with the epsilon amino groups of lysine residues.[35] Besides proteins, formaldehyde is known to react with other biological molecules such as nucleic acids and polysaccharides.[36] The action of formaldehyde in intramolecular and intermolecular crosslinking of macromolecules can considerably alter the physical characteristics of the substrates.

The U.S. OSHA has issued a hazard alert concerning hair smoothing products that could release formaldehyde into the air.[37] The alert stated that OSHA investigations uncovered formaldehyde concentrations greater than OSHA's limits of exposure.[38] One investigation reported such levels of formaldehyde even though the product was labeled "formaldehyde-free." The hazard alert stated that formaldehyde gas presents a health hazard if workers are exposed, described the other chemical names to look for on the label that would signal reason for concern, and told businesses what to do to reduce exposure when using formaldehyde-releasing hair smoothing products.

Canada issued health advisories informing consumers of the risks associated with hair smoothing products containing excessive levels of formaldehyde, and has recalled several such products.[39-42] Hair smoothing products with formaldehyde at levels >0.2% are not permitted for sale in Canada.[41]

France's health authority warned consumers and hairdressers against using hair straightening treatments that contain high levels of formaldehyde and has removed a number of such products from the market.[43] Germany's Federal Institute for Risk Assessment (BfR) advised against the use of hair straightening products that contain formaldehyde in high concentrations.[44] The Irish Medicines Board, which is the competent authority in Ireland for cosmetics, took action to remove hair smoothing products from the market if they contain greater than 0.2%, the level established by the European Commission (EC).[45]

TOXICOKINETICS

Formaldehyde is a highly water-soluble, reactive, rapidly metabolized chemical with a relatively short biological half-life. Inhaled formaldehyde is absorbed primarily in the respiratory epithelium lining the upper airways, where it undergoes extensive local metabolism and reactions with macromolecules. Based on the weight of the evidence, the NRC concluded that formaldehyde does not penetrate beyond the superficial layer of the nasopharyngeal epithelium, and is unlikely to appear in the blood as an intact molecule, except possibly at concentrations high enough to overwhelm the metabolic capacity of the epithelium.[4] The NRC concluded that formaldehyde is not

available systemically in any reactive form, and systemic effects are unlikely from the direct delivery of formaldehyde or methylene glycol to distal sites, except possibly in highly exposed people.

TOXICOLOGY

Previous CIR Safety Reports on Formaldehyde- Summary

In low amounts, formaldehyde is generated and present in the body as a normal metabolite, and as such or when taken into the body, it is rapidly metabolized by several pathways to yield carbon dioxide. It is a very reactive chemical. Not surprisingly, formaldehyde is an irritant at low concentrations, especially to the eyes and the respiratory tract. Formaldehyde exposure can result in a sensitization reaction. Under experimental conditions formaldehyde is teratogenic, mutagenic and can induce neoplasms.

Perhaps the single most important attribute common to these toxic effects of formaldehyde is that they are all concentration/time dependent. A higher concentration or duration of exposure than that which produces irritation, for example, induces degenerative changes in the tissues exposed to it. There was no evidence that formaldehyde can induce neoplasia at concentration/time relationships that do not damage normal structure and function of tissues, even under laboratory conditions.

From the Final Report on the Safety Assessment of Formaldehyde[1]

New clinical studies reviewed in 2003 confirmed that formaldehyde can be a skin irritant and sensitizer, but at levels higher than the 0.2% free Formaldehyde upper limit established by the CIR Expert Panel.

The developmental toxicity, genotoxicity, and carcinogenicity of high doses of formaldehyde were also confirmed in the new studies (published between 1984 and 2003). These studies demonstrated that there is a threshold effect; that is, high doses are required before any effect is seen.

From the Published Re-Review of Formaldehyde[2]

New Data on Safety of Formaldehyde

The U.S. EPA National Center for Environmental Assessment (NCEA) released a 4-volume draft toxicological review of formaldehyde for external review on 2 June 2010, including interagency comments on an earlier draft of the document.[3] U.S. EPA is conducting this assessment to support the development of new chronic inhalation toxicity values for formaldehyde. Ultimately, the final versions of these values will be incorporated into the U.S. EPA Integrated Risk Information System (IRIS).

The NRC recently released their review of U.S. EPA's draft assessment [4] and their findings are also summarized below, where appropriate. The NRC noted that the systemic delivery of formaldehyde may not be required for some of the systemic effects attributed to formaldehyde inhalation (eg, lymphohematopoietic cancers and reproductive toxicity). Instead, systemic effects could be secondary, indirect effects of the local effects of exposure, including local irritation and inflammation, and stress.

This document provides a summary of the toxicological literature, including both human and animal studies and all of the major exposure routes of concern (inhalation, ingestion, and skin contact). Much of the significant new toxicology data are related to genotoxicity, carcinogenicity, and reproductive and developmental toxicity. A comprehensive summary of the findings is presented in Tables 3 through 11.

Reproductive and Developmental Toxicity

Several potential modes of action of formaldehyde for reproductive and developmental outcomes have been suggested by animal studies, including endocrine disruption, genotoxic effects on gametes, and oxidative stress or damage.[46,47] However, the evidence for causality is weak. In addition, it is not clear that inhaled formaldehyde or its metabolites can penetrate past the portal of entry or cross the placenta, blood-testis barrier, or blood-brain barrier.

The findings of studies on male reproduction generally used concentrations that result in significant weight loss and overt toxicity. There are no multigenerational tests for reproductive function.[3] These deficiencies, particularly for

male reproductive effects, represent important data gaps in the assessment of risks of reproductive and developmental toxicity associated with inhalation exposures to formaldehyde.[4]

The NRC noted that a small number of epidemiological studies[48-51] suggest an association between occupational exposure to formaldehyde and adverse reproductive outcomes in women.[4]

Genotoxicity

Clear evidence of systemic mutagenicity does not emerge from animal inhalation bioassays, despite the reactivity and mutagenicity demonstrated in isolated mammalian cells.[52-54]

Similarly, the evidence that inhaled formaldehyde may be directly genotoxic to humans systemically is inconsistent and contradictory.[55-60]

Carcinogenicity

Nasopharyngeal Cancers (NPC)

The NRC agreed with EPA that there is sufficient evidence from the combined weight of epidemiologic findings, results of animal studies, and mechanistic data of a causal association between the inhalation of formaldehyde and cancers of the nose, nasal cavity, and nasopharnyx.[4] Formaldehyde is highly reactive, readily forms DNA and protein adducts and crosslinks, and is a direct-acting genotoxicant. Among the potential modes of action that have been considered for the development of NPCs through the inhalation of formaldehyde in animal studies include direct mutagenesis of cells at the site of first contact and cytotoxicity-induced cell proliferation (CICP), which correlates with tumor incidence.[61-68]

The subchronic or chronic inhalation of formaldehyde at high concentrations (eg, ≥6 ppm) clearly can cause NPCs in mice and rats. However, there is still debate in the scientific community about whether this effect should be considered to be a non-threshold effect or a threshold effect in cancer risk assessments.

The NRC concluded that these two primary modes of action contribute to formaldehyde-induced carcinogenicity in nasal tissues, including mutagenicity and CICP.[4] A mutagenic mode of action is generally the reason for adopting the default low-dose linear extrapolation methods in a quantitative cancer risk assessment. However, the NRC noted that formaldehyde is endogenous, that nasal tumors are rare in both rats and humans, and that no increases in tumor frequency are observed in animal studies at formaldehyde concentrations that do not also cause cytotoxicity. Further, the animal studies reveal a substantial nonlinearity in dose-response relationships among formaldehyde uptake, cytotoxicity, cell proliferation, and tumor formation.

Thus, the NRC recommended that the quantitative assessment of the risks of formaldehyde-induced NPCs incorporate the nonlinear phenomenon of CICP, as well as the mutagenicity of formaldehyde.[4]

Lymphohematopoietic (LHP) Cancers

The three proposed modes of action by which formaldehyde exposure may cause leukemia include:[69]

- Transport of formaldehyde/methylene glycol from the portal of entry through the blood to the bone marrow, followed by direct toxic action to hematopoietic stem cells in the marrow
- Direct toxic action of formaldehyde/methylene glycol on circulating blood stem cells and progenitors at the portal of entry, followed by return of the damaged cells to bone marrow
- Direct toxic action of formaldehyde/methylene glycol on primitive pluripotent stem cells at the portal of entry, followed by migration of damaged cells to bone marrow

Similarly, direct toxic action of formaldehyde/methylene glycol on lymphocytes in mucosa-associated lymphoid tissues (MALT) at the portal of entry may cause lymphoid cancers.[3]

Remarkably little evidence from animal studies indicates that formaldehyde exposure can cause LHP cancer. Studies have consistently failed to find elevated levels of free formaldehyde or methylene glycol in the blood of exposed human and animal subjects, or DPCs in the bone marrow of exposed animals.[70] Further, formaldehyde is a highly reactive, rapidly metabolized chemical yielding short-lived DPCs and DNA-adducts that are amenable to rapid reversal and repair.[71,72] These observations are consistent with conventional wisdom, which has been that the expected sites of action of formaldehyde are limited to portals of entry (eg, nasal epithelium), and would not likely include distal sites, such as the bone marrow, where leukemias originate.[70,73-75] Although several possible modes of action have been postulated to explain associations between LHP cancers and formaldehyde exposure in epidemiological studies, little scientific evidence supports these hypotheses, and there is some recent evidence against them. Thus, these proposals remain speculative and continue to represent a highly controversial topic in the scientific community.

The NRC noted that little is known about the potential modes of action by which formaldehyde might cause LHP cancers, other than mutagenicity.[4] A mechanism that would explain the occurrence of LHP cancers has not been established, the epidemiological data are inconsistent, the animal data are weak, and there is a growing body of evidence that formaldehyde is not available systemically in any reactive form. Further, the lack of consistency in exposure-response relationships between several exposure metrics and the LHP cancers in the epidemiological data could reflect the absence of causal mechanisms associating these cancers with formaldehyde exposure.

Irritation and Sensitization

As noted in the original safety assessment of formadehyde,[1] aqueous formaldehyde/formalin solutions can irritate the skin and cause contact urticaria and allergic sensitization in both occupationally and non-occupationally exposed persons. The North American Contact Dermatitis Group (NACDG) reported a 5% incidence of skin sensitization among 2,374 patients exposed to 2% formaldehyde in aqueous solution.[76] Aqueous formaldehyde solutions as low as 0.01% can elicit skin responses in some sensitized persons under occlusive conditions. Most sensitized individuals can tolerate repeated topical axillary application of products containing up to 0.003% aqueous formaldehyde solution on normal skin.[77] Cosmetic products containing 0.0005% to 0.25% formalin (0.000185%-0.0925% calculated as formaldehyde) were essentially nonirritating and non-sensitizing in 1,527 subjects in 18 studies summarized in Table 5 of the original safety assessment.[1]

Recent reviews addressing the human irritation and sensitization potential for aqueous formaldehyde/formalin solutions are consistent with the observations reported in the original assessment.[78,79]

Healthy volunteers (n=30; ≥18 years old) of either sex were exposed to 11 personal care products and 2 controls (ie, deionized water and 0.3% sodium lauryl sulfate) using an occlusive patch-testing protocol.[80] The products included 3 keratin hair straighteners containing methylene glycol (concentration not reported). All of the products were diluted to 8%, presumably with deionized water, before applying 0.2 ml of the diluted product to Webril© disks. Note that, based on the manufacturer's directions, hair straighteners are applied undiluted to the hair. The patches were applied to the skin of the upper arms of each subject and left in place for 23 hours, and removed and examined during the 24th hour, for 4 consecutive days. Each subject was exposed to each of the 11 products and 2 controls on patches applied to the same site of the skin each day. The specific site of application for each product/control varied from subject to subject, depending on the random assignment of each subject to one of 5 groups. None of the diluted products or the negative control elicited any more than minimal erythema throughout the study. In contrast, the positive control elicited substantial erythema.

CLINICAL USE

Adverse Event Reporting

Nail Hardening Products

A compilation of 33 customer self-reports from Internet sites and blogs of nail hardening products indicate adverse effects including skin irritation, burning sensation of nail beds and exposed skin, severe finger pain, scabbing under the nails, and drying, flaking, splitting, crumbling, or peeling of the nails.[31] Two additional reports noted that the product contained formaldehyde and has a strong odor, without noting any other adverse effects. Three reports indicated that the product contained 4%-4.5% formaldehyde.

Hair Smoothing Products

Canada
Some 50-60 individuals have reported adverse reactions to Health Canada resulting from use of hair smoothing products containing formaldehyde. These reports concerned burning eyes, nose, throat and breathing difficulties, with one report of hair loss,[41] but additional reports also were received of headache, arthritis, dizziness, epistaxis, swollen glands and numb tongue (Health Canada, personal communication).

USA
The Center for Research in Occupational and Environmental Toxicology (CROET) at the Oregon Health Sciences University (OHSU) has received numerous phone calls and emails from stylists from around the United States since first posting an alert on a hair product on September 16, 2011.[11] Many of the stylists reported health symptoms associated with the use of this product at work. The health symptoms reported include the following: burning of eyes and throat, watering of eyes, dry mouth, loss of smell, headache and a feeling of "grogginess," malaise, shortness of breath and breathing problems, a diagnosis of epiglottitis attributed by the stylist to their use of the product, fingertip numbness, and dermatitis. Some of these effects were also reported to have been experienced by the stylists' clients. CROET also received emails from persons who report hair loss after having the treatment. Oregon OSHA has received similar, although generally less detailed, reports from individuals who have contacted the agency as a result of recent media coverage.

The U.S. OSHA recently issued a Hazard Alert and identified safeguards that should be in place to keep formaldehyde concentrations below the U.S. OSHA occupational exposure limits.[37]

The FDA has been notified by some state and local organizations of reports from salons about problems associated with the use of Brazilian Blowout, a product used to straighten hair.[81] Complaints include eye irritation, breathing problems, and headaches. State and local organizations with authority over the operation of salons are currently investigating these reports.

The FDA adverse reporting system includes 33 adverse event reports from use of hair smoothing and straightening products from hair stylists, their customers, and individual users from 9/29/08 through 3/1/11.[82] The results clearly link the use of formaldehyde/methylene glycol-containing hair smoothing products to clinical signs and symptoms that would be expected from the vaporization and inhalation of toxic levels of this ingredient. These reported effects include irritation of the eyes, nose and throat, nasal discharge, nose bleeds, congested sinuses, hoarseness, persistent coughing, bronchitis, difficulty breathing, feeling of pressure, tightness, or pain in chest. Two reports note inhalation pneumonitis in a professional hair stylist. Other complaints include headache, dizziness, fainting, and vomiting. Reported effects potentially attributable to direct contact with these products include irritation, inflammation, or blistering of the skin, especially on the scalp, and hair loss. In addition to these 33 reports, there were 7 reports of hair loss that did not indicate whether other possible adverse effects also occurred.

RISK ASSESSMENTS

Carcinogenicity

In 2006, the International Agency for Research on Cancer (IARC)[83] concluded that there was *sufficient* epidemiological evidence that formaldehyde causes NPC in humans and *strong but not sufficient* evidence for a causal association between leukemia and occupational exposure to formaldehyde. They also elevated their evaluation of formaldehyde from probably carcinogenic to humans (Group 2A) to carcinogenic to humans (Group 1).

In 2009, IARC[84] updated their evaluation to conclude that there is *sufficient* evidence for a causal association between leukemia, particularly myeloid leukemia, and occupational exposure to formaldehyde. This conclusion was based primarily on:

- The statistically significant association between embalming and myeloid leukemia, including statistically significant trends for cumulative years embalming and peak formaldehyde exposure.[85]

- The levels of chromosome 7 monosomy and chromosome 8 trisomy in myeloid progenitor cells and hematological changes in formaldehyde exposed workers.[69]

The IARC Working Group was almost evenly split on the prevailing view that the evidence was sufficient for formaldehyde causing leukemia in humans.[84]

The U.S National Toxicology Program (U.S. NTP) concluded that formaldehyde is *known to be a human carcinogen* based on epidemiological reports indicating that exposures are associated with nasopharyngeal, sinonasal, and LHP cancers and data on mechanisms of carcinogenicity from laboratory studies.[86-88]

In 1991, U.S. EPA classified formaldehyde as a B1 carcinogen (ie, a probable human carcinogen), based on limited evidence in humans, and sufficient evidence in animals.[89] They estimated an upper-bound inhalation cancer unit risk of 1.6×10^{-2} per ppm (1.3×10^{-5} per $\mu g/m^3$), using a linearized multistage, additional-risk procedure to extrapolate dose-response data from a chronic bioassay on male F344 rats. An upper-bound 10^{-6} human cancer risk would be associated with continuous inhalation of 0.06 ppb (63 ppt) formaldehyde over a lifetime, based on this unit risk.

Recently, U.S. EPA proposed to identify formaldehyde as carcinogenic to humans.[3] They proposed an upper-bound inhalation cancer unit risk for NPC, Hodgkin's lymphoma, and leukemia, combined, using log-linear modeling and extra risk procedures to extrapolate cumulative exposure estimates from the epidemiological studies.[90] The NRC agreed that the Hauptmann et al (2004) study[91] of the NCI cohort is the most appropriate for deriving cancer unit risk estimates for respiratory cancers and other solid tumors, but noted that this study is being updated.[4] The update will likely address the deaths reported to be missing from this study.[90] However, the NRC explicitly did not recommend that U.S. EPA wait until the release of the update to complete its assessment.

Non-Cancer Effects

In 1990, U.S. EPA published a chronic reference dose (cRfD) of 0.2 mg/kg/day for oral exposure to formaldehyde, based on the results of a 2-year bioassay in rats.[89,92] Formaldehyde (methylene glycol/formaldehyde) was administered to Wistar rats (70/sex/dose) in drinking water, yielding mean doses of 0, 1.2, 15, or 82 mg/kg/day for males and 0, 1.8, 21, or 109 mg/kg/day for females. Severe damage to the gastric mucosa was observed at 82 and 109 mg/kg/day in males and females, respectively, but no tumors were found. The NOAEL was 15 mg/kg/day in this study.

U.S. EPA released a draft risk assessment for formaldehyde for public comment and review by the NRC.[3] They proposed a chronic reference concentration for formaldehyde exposure by inhalation, based on three "cocritical" epidemiological studies. These studies reported associations between formaldehyde exposure and increased physician-diagnosed asthma, atopy[93], and respiratory symptoms,[94] and decreased pulmonary peak expiratory flow rate[95] in residential populations, including children. The NRC agreed with U.S. EPA's assessment of a causal relationship between formaldehyde and respiratory effects, except for incident asthma based on one of the "cocritical" studies.[4,93]

EXPOSURE ASSESSMENTS

Formaldehyde is ubiquitous in both indoor and outdoor air. Substantial sources of airborne formaldehyde include both natural and anthropogenic sources. Formaldehyde concentrations are generally greater in urban air than in agricultural areas, and greater in indoor air than in outdoor air.[3,4,83,96,97] It is estimated that the general population is exposed to an average of 0.016 to 0.032 ppm formaldehyde in indoor air.[98] In addition, formaldehyde is a natural metabolic intermediate in humans and other animals and is, thus, normally present in all tissues, cells, and bodily fluids.[96] The concentration of endogenous formaldehyde in the blood of rats, monkeys, and humans is about 0.1 mM.[99,100] Endogenous tissue formaldehyde concentrations are similar to genotoxic and cytotoxic concentrations observed in vitro.[70] In addition, formaldehyde is likely present normally in exhaled breath at concentrations of a few parts per billion (ppb).[4]

Standards and Guidance for Formaldehyde Inhalation Exposures

U.S. OSHA Enforceable Standards[38]

8-hour Threshold for Hazard Communication Requirements (Threshold-TWA)	0.1 ppm
8 hour Action Level (AL-TWA)	0.5 ppm
8-hour Permissible Exposure Limit (PEL-TWA)	0.75 ppm
15-minute Short Term Exposure Limit (STEL-TWA)	2 ppm

The 8-hour Threshold-TWA is the time-weighted average concentration (0.1 ppm) above which employers are required to meet U.S. OSHA's hazard communication requirements.[38]

NIOSH Recommended Exposure Limits

10-hour Recommended Exposure Limit (REL-TWA)	0.016 ppm
15-minute Recommended Short Term Exposure Limit (REL-STEL-TWA)	0.1 ppm

The U.S. National Institute of Occupational Health (NIOSH) standards and recommendations were developed to protect workers primarily from irritation of the eyes, nose, throat, and respiratory system.[101]

U.S. NAC AEGL Committee

Acute Exposure Guideline Level-1 (AEGL-1)	0.9 ppm

The U.S. National Advisory Committee for Acute Exposure Guideline Levels (U.S. NAC AEGL Committee) for Hazardous Substances interim acute exposure guideline level-1 (AEGL-1) for formaldehyde is defined as a concentration in air above which the general population (including susceptible individuals) could experience notable discomfort, irritation, or other adverse effects.[102]

> The AEGL-1 was based on the NOAEL for eye irritation in a study in which 5 to 28 healthy subjects previously shown to be sensitive to 1.3 or 2.2 ppm formaldehyde were exposed eye-only for 6 minutes to 0, 0.35, 0.56, 0.7, 0.9, or 1.0 ppm.[103] Subjective eye irritation responses ranged from none to slight at 0, 0.35, 0.56, 0.7 and 0.9 ppm. The 0.9 ppm AEGL-1 was applied across all acute exposure durations (10-min to 8 hours) because several studies show that there is adaptation to irritation at such concentrations and because in the absence of exercise, there are no decrements in pulmonary function parameters in healthy or asthmatic subjects inhaling 3 ppm for 3 hours.[104-106]

ACGIH

Threshold Limit Value-Ceiling (TLV®-C)	0.3 ppm.

The American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value-Ceiling (TLV®-C) is defined as the concentration that should not be exceeded during any part of the working exposure.[107]

WHO

30-minute average indoor air guideline	0.08 ppm

The World Health Organization (WHO) 30-minute average indoor air guideline is for the prevention of significant sensory irritation in the general population.[108] WHO notes that this guideline represents a negligible risk of upper respiratory tract cancer in humans, because it is more than an order of magnitude lower than the threshold for cytotoxic damage estimated for the nasal mucosa. Recent reviews of the relevant epidemiological and animal studies concluded that this guideline is protective against acute and chronic sensory irritation, as well as for all types of cancer (including LHP malignancies).[73,108]

Formaldehyde Exposures During use of Nail Products

Time Weighted Average (TWA) formaldehyde exposures of nail technicians and customers were measured simultaneously, during normal operations at 30 nail salons throughout California in winter and summer.[109,110] Nail hardeners containing formaldehyde were used in some of these salons and other products containing formaldehyde resins were used in most, if not all, of the salons during the study.[109] 2,4-dinitrophenylhydrazine (DNPH)-treated silica gel absorption tubes and high-flow pumps were used to collect the samples. One sample inlet tube was placed close to the technician's breathing zone, and another close to the customer's breathing zone during the application of the nail products. A third sampler was placed in the salon about 10 feet from the work station to collect "area samples" to measure concentrations in the salon during the application of the nail products. A fourth sampler was placed inside the salon early in the morning before the salon opened, inside during the first two hours the salon was open, or outside the salon while the salon was open, to provide background data. Preliminary air samples were collected from two office buildings for comparison.

Most of the air samples were collected for approximately 4 hours, and some for about 2 hours or 8 hours.[109] The samples were analyzed using high-performance liquid chromatography (HPLC), in accordance with U.S. EPA method TO-11.[110] The measured concentrations were used to calculate 8-hour TWAs.

The authors reported 8-hour TWA formaldehyde concentrations in the breathing zones ranging from **0.0032 to 0.065 ppm** (median = 0.01 ppm; mean = 0.0187 ppm; SD = 0.0187 ppm) during the application of the nail products.[110] The corresponding area concentrations ranged from 0.0038 to 0.06 ppm (median = 0.01 ppm; mean = 0.0196 ppm; SD = 0.0195 ppm). The background concentrations, pooled, ranged from 0.0023 to 0.12 ppm (0.021 to 0.12 ppm early morning before opening; 0.014 to 0.081 ppm during first two hours after opening; 0.0023 to 0.013 ppm outside; overall: median = 0.014 ppm; mean = 0.033 ppm; SD = 0.038 ppm). The concentrations ranged from 0.015 to 0.021 ppm (mean = 0.018 ppm) in one office building, and was 0.043 ppm in the other office building. The authors did not determine the sources of the formaldehyde measured in the background samples.

Thus, the reported 8-hour TWA formaldehyde concentrations in the breathing zones during the application of the products appear to be indistinguishable from the salon area concentrations, and comparable to the background concentrations. In addition, the reported concentrations measured in the breathing zone, area, and outside background locations were uniformly lower than standards for formaldehyde, including the U.S. OSHA PEL-TWA (0.75 ppm), AL-TWA (0.5 ppm), and Threshold-TWA (0.1 ppm).

One of the 7 remaining inside background concentrations (collected during the first to hours after opening) exceeded the Threshold-TWA, and none exceeded the PEL-TWA, AL-TWA, or AEGL-1.

In another study, aluminum foil over a wooden support was used as the substrate for a nail hardening product in a chamber (1.43 m^3) under two conditions: "Typical:" 70 ºF, 1 air change/hour; "Elevated:" 80 ºF, 0.3 air changes per

hour.[111] Formaldehyde concentrations were measured at 5-minute intervals in the chamber air over a 10.5 hour period. The nail hardener (15 mg/cm^2) was painted on 70 cm^2 of the surface of the substrate (>7 times the total surface of nails on the on a person's 10 fingers, assuming ~1 cm^2/nail). The peak chamber air concentrations (5-minute samples) were 0.15-0.6 ppm under the "Typical" conditions and 0.2 – 0.24 ppm under the "Elevated" conditions. The peak concentrations measured in the chamber in this study are not directly comparable to the OSHA/ACGIH/WHO standards and guidelines, because they are not estimates of the concentrations of formaldehyde in the breathing zones of a customer or manicurist over relevant exposure durations. In any case, the 5-minute peak concentrations in the chamber were all about an order of magnitude less than the 15-min STEL-TWA of 2 ppm.

Formaldehyde Exposure during Use of Hair Smoothing Products

Air samples during use of hair smoothing products were measured in six separate studies. The results are summarized below and in Table 12.

Oregon OSHA and Center for Research in Occupational Toxicology (CROET) collected 15 air samples from seven beauty salons during the use of a "formaldehyde-free" hair-smoothing product.[11] They used DNPH-treated silica gel absorption tubes (SKC 226-119) and high-flow pumps, and analyzed the samples using NIOSH method 2016, which is comparable to U.S. EPA method TO-11. The concentrations of formaldehyde at the stylists' workstations ranged from **0.074 to 1.88 ppm** (median = 0.34 ppm; mean = 0.62 ppm; SD = 0.59 ppm) during sampling/exposure periods ranging from **6 to 48 minutes** (median = 19 minutes; mean = 23 minutes; SD = 12 minutes):

- 4 samples (ranging from 1.26 ppm for 34 minutes to 1.88 ppm for 26 minutes) exceeded the U.S. NAC AEGL-1 (0.9 ppm for ≥10 min).[102]
- 9 samples (0.303 to 1.88 ppm) exceeded the ACGIH TLV®-Ceiling (0.3 ppm).[107]
- All 3 samples collected for ≥30 minutes (1.26 ppm for 34 minutes, 0.34 ppm for 47 minutes, and 1.35 ppm for 48 minutes) exceeded the WHO 30-minute guideline (0.08 ppm).[108]

Further, 2 of 24 area samples collected during the procedures (**0.319 and 0.471 ppm**) exceeded the TLV®-C, and 10 of 12 area samples collected for ~30 minutes or more (eg, 0.226 ppm for 26 minutes and 0.255 ppm for 97 minutes) exceeded the WHO guideline.

Exponent® collected two 30-minute background air samples in a salon before the use of a hair smoothing product, and duplicate samples in the stylist's breathing zone, the customer's breathing zone, and within 3 feet of the customer's location during the application of the product.[112] They used U.S. EPA method TO-11 to collect and analyze the samples. The background formaldehyde concentrations were 0.024 and 0.025 ppm. The concentrations in the samples collected during the procedure ranged from **0.170 ppm for 141 minutes to 0.269 ppm for 95 minutes**. All of these concentrations were from 57% to 90% of the ACGIH TLV®-C (0.3 ppm), and all exceeded the WHO 30-minute guideline (0.08 ppm).

The Tennessee Occupational Safety and Health Administration (Tennessee OSHA) conducted an inspection of a salon, including the collection and analysis of air samples.[113] They used DNPH-treated silica gel absorption tubes (XAD-2) and high-flow pumps (SKC AirChek® 2000) to collect, apparently, one air sample every 15 minutes for 75 minutes during the use of the product. The analytical method was not specified. The 15-minute concentrations ranged from **0.3 to 1.07 ppm**. One of these values is equal to the TLV®-C (0.3 ppm), and the 4 others exceeded the TLV®-C (0.3 ppm) by up to nearly 4-fold. The highest value (1.07 ppm) exceeds the U.S. NAC AEGL-1 (0.9 ppm). In addition, the 75-minute TWA calculated from the reported series of 15-minute concentrations is 0.558 ppm, which is approximately 7-times greater than the WHO 30-minute guideline (0.08 ppm).

The Professional Keratin Smoothing Council (PKSC) submitted the results of the analysis of 15-minute air samples collected during the blow-drying or flat-ironing steps of 4 hair-smoothing treatments.[13,114] They used Sep-Pak® DNPH-Silica Cartridges to collect the samples. No further details were provided about the methodology. Formaldehyde was not detected (reporting limit 0.0082 ppm) in one of the samples collected during blow drying, and was not included in the PKSC summary table, presumably because of technical difficulties encountered with this sample. The 15-minute concentrations in the 7 remaining samples ranged from **0.761 to 1.71 ppm**. None of

these samples exceeded the 15-minute STEL-TWA. However, all of the samples exceeded the ACGIH TLV®-C (0.3 ppm) by 2.5 to 5.7-fold, and all but one of them exceeded the U.S. NAC AEGL-1 (0.9 ppm) by 1.3 to 1.9 fold. TWAs (30-minute) calculated from each complete 15-minute sample pairs (ie, blow drying plus flat ironing) ranged from 0.996 to 1.69 ppm, exceeding the WHO 30-minute guideline (0.08 ppm) by 12 to 21-times.

The PKSC submitted the results of air samples collected to estimate the stylist's and customer's inhalation exposures in a beauty salon during hair-smoothing treatments conducted on two separate occasions.[13,115] They used Sep-Pak® DNPH-Silica Cartridges to collect the samples. No further details were provided. The results ranged from **0.189 ppm for 117 minutes to 0.395 ppm for 86 minutes**. The concentrations in two of the samples (customer exposure to 0.355 ppm for 117 minutes; stylist exposure to 0.395 ppm for 86 minutes) exceeded the ACGIH TLV®-C (0.3 ppm). All of the air samples exceeded the WHO 30-minute guideline (0.08 ppm) by 2.4 to 5 times.

In another study, Exponent® collected 63 air samples at 6 salons where hair-smoothing treatments were performed.[116,117] These included 6 area (background) samples collected before any hair-smoothing procedures were conducted, and 35 samples collected in the stylists' breathing zones during a total of 9 treatments. An additional 22 area samples were collected in the salons within 5 feet of the stylists during and after the procedures. They used DNPH-treated silica gel absorption tubes (SKC 226-119) and followed NIOSH method 2016 to collect and analyze the samples. Following is a summary of the results:

- Concentrations in the 6 background samples ranged from 0.0068 to 0.032 ppm.

- Concentrations in the other 22 area samples ranged from <0.005 ppm for 45 minutes to 0.14 ppm for 73 minutes. The 3 highest area concentrations (ranging from 0.084 ppm for 69 minutes to 0.14 ppm for 73 minutes) were collected during the treatments, and exceeded the WHO 30-minute guideline (0.08 ppm).

- Calculated 8-hour TWAs ranged from 0.02 ppm to 0.08 ppm. The highest of these is equal to the WHO 30-minute guideline.

- Concentrations in 9 samples collected in the breathing zones during the procedures (including application of the product, blow drying and flat ironing) ranged from 0.11 ppm for 63 minutes to 0.33 ppm for 73 minutes. The highest concentration (0.33 ppm) exceeded the ACGIH TLV®-C (0.3 ppm), and all of them exceeded the WHO 30-minute guideline (0.08 ppm) by up to 4 fold.

- Concentrations in the 26 samples collected in the breathing zones during each of the separate steps the procedures ranged from **0.041 ppm for 43 minutes** (during flat ironing) to **0.76 ppm for 17 minutes** (during blow drying). The 4 highest concentrations (ranging from 0.66 for 20 minutes to 0.76 ppm for 17 minutes) were 73% to 84% of the U.S. NAC AEGL-1 (0.9 ppm). Concentrations in 9 of the 26 samples (ranging from 0.31 ppm for 32 minutes to 0.76 for 17 minutes) exceeded the ACGIH TLV®-C (0.3 ppm) by up to 2.5 fold. Concentrations in 6 of the 10 samples collected for 30 minutes or more during each step of the treatments (ranging from 0.084 ppm for 31 minutes to 0.31 ppm for 32 minutes) exceeded the WHO 30-minute guideline (0.08 ppm) by up to 4 times.

ChemRisk collected air samples at a salon during 4 consecutive keratin hair smoothing treatments performed by a licensed cosmetologist (stylist) on 4 separate human hair wigs mounted on mannequin heads over a 6-hour period.[118] Four different hair-smoothing products were used, in random order, during this 1-day study. The mean aqueous formaldehyde concentration was below the limit of detection (LOD $<5 \times 10^{-7}$% w/w) in one product and 3%, 8.3% and 11.5% (w/w) in the others, as measured using a modified NIOSH 3500 method. Background air samples were collected in the stylist's breathing zone immediately before each treatment. Treatment-duration and task-duration samples were collected in the stylist's and mannequin's breathing zones, in areas representing the breathing zones of potential bystanders, and in the salon's reception area. The samples were collected on DNPH-treated silica gel absorption tubes (SKC 226-119) using sample pumps (SKC AirChek® 52) with low-flow adaptors. All of the samples were analyzed using a modified NIOSH 2016 method coupled with high performance liquid chromatography (HPLC) and ultraviolet (UV) detection. Following is a summary of the results:

- The concentrations of formaldehyde in the air samples collected during the treatments were directly related to the concentrations measured in the bulk samples.

- The mean concentrations in the treatment-duration breathing-zone samples for the three products containing measurable concentrations of aqueous formaldehyde ranged from **0.11 ppm for 82-84 minutes** to **1.17 for 56-57 minutes**. The concentrations in 4 of these 16 samples (ranging from 1.13 ppm to 1.21 ppm) exceeded the U.S. NAC AEGL-1 (0.9 ppm), and the concentrations in 8 of them (ranging from 0.58 ppm to 1.21 ppm) exceeded the ACGIH TLV®-C (0.3 ppm) by up to 4 fold. The concentrations in all 16 of these samples (ranging from 0.09 ppm to 1.21 ppm) exceeded the WHO 30-minute guideline (0.08 ppm) by up to 15 times.

- The highest mean concentrations in the treatment-duration samples collected 6-10 meters from the stylist were 0.37 ppm for 51 minutes and 0.52 ppm for 56 minutes. These values exceed both the ACGIH TLV®-C (0.3 ppm) and the WHO 30-minute guideline (0.08 ppm).

- The highest mean concentrations in duplicate samples collected in the breathing-zones during the blow-drying step (task) of the treatments were 2.35 ppm and 3.47 ppm for 10 minutes. The corresponding TWAs of the mean concentrations reported for the blow-drying and flat-ironing steps, combined, approached the OSHA 15-minute STEL-TWA (2 ppm) in the stylist's breathing zone (1.65 ppm for 23 minutes) and exceeded this standard in the mannequin's breathing zone (2.1 ppm for 23 minutes).

- ChemRisk estimated 8-hour TWA concentrations over all 4 treatments conducted sequentially over the 6-hour period. The 8-hour TWAs ranged from 0.25 ppm 6-10 meters from the stylist to 0.46 ppm in the stylist's breathing zone. None of the 8-hour TWAs exceeded the OSHA PEL-TWA (0.75 ppm). However, they approached the OSHA AL-TWA (0.5 ppm) by up to 92%, and they all exceeded the OSHA Threshold-TWA (0.1 ppm).

Simulated Use; Calculated Formaldehyde Levels

Berkeley Analytical placed 0.0946 grams of a hair smoothing product in a glass Petri dish, placed the dish in a small-scale, ventilated environmental chamber (0.067 m^3), and followed ASTM D 5116 procedures for measuring organic emissions from indoor materials and products.[119,120] They collected three consecutive 1-hour air samples from the chamber (1 air change/hour), at room temperature (73.4 ºF), using Sep-Pak XPoSure samplers. They reported emissions factors for formaldehyde ranging from 1,020 µg/gram-hour for the first hour to 1,670 µg/gram-hour for the third hour. Indoor Environmental Engineering calculated formaldehyde concentrations in a hypothetical hair salon (240 ft^2; 8-ft ceiling) from single 90-minute emissions of formaldehyde from the hair smoothing product. They conservatively assumed a 1,020 µg/gram-hour emission rate at room temperature, likely underestimating the emissions during actual use.[34] The emission rates are most probably much higher when the product is heated (eg, during blow-drying and flat-ironing). They modeled TWA exposure concentrations for the customer (110 minutes) and the stylist (8 hours), assuming 3 outdoor air ventilation rates (0.13 to 0.6 $ft^3/min\text{-}ft^2$) and three different amounts of the product applied the customer's hair (12.6 to 37.8 grams). The amounts were selected from recommendations provided in the manufacturer's training video for using the product on short, medium and long hair.

The 110-minute formaldehyde concentrations ranged from 0.033 ppm (12.6 grams product; 0.6 $ft^3/min\text{-}ft^2$) to 0.269 ppm (37.8 grams product; 0.6 $ft^3/min\text{-}ft^2$). Two of the three 110-minute estimates assuming 25.2 grams of product (0.096 to 0.18 ppm at 0.38 and 0.13 $ft^3/min\text{-}ft^2$, respectively) and all of the estimates assuming 37.8 grams (0.098 to 0.269 ppm), exceeded the WHO 30-minute guideline (0.08 ppm). The highest estimate (0.269 ppm) was about 90% of the ACGIH TLV®-C (0.3 ppm). In addition, the highest estimated 8-hour TWA was 0.108 ppm (37.8 grams; 0.13 $ft^3/min\text{-}ft^2$), which exceeds the U.S. OSHA 8-hour Threshold-TWA (0.1 ppm).

DISCUSSION

Based on the available data, the CIR Expert Panel (Panel) considered that formaldehyde and methylene glycol are safe for use in cosmetics when formulated to ensure use at the minimal effective concentration, but in no case should the formalin† concentration exceed 0.2% (w/w), which would be 0.074% (w/w) calculated as formaldehyde or 0.118% (w/w) calculated as methylene glycol. Additionally, formaldehyde and methylene glycol are safe in the present practices of use and concentration in nail hardening products. However, formaldehyde and methylene glycol

are unsafe in the present practices of use and concentration in hair smoothing products. This is a final amended safety assessment.

The Panel emphasized that a large body of data has demonstrated that formaldehyde gas exposure can cause nasopharyngeal cancers (NPCs). While debate is ongoing regarding the dose-response relationship for the induction of NPCs, the Panel continues to believe that formaldehyde gas can produce such cancers at high doses. Epidemiology studies have suggested a weak association between exposure to formaldehyde and lymphohematopoietic (LHP) cancers. The reported association of formaldehyde exposure with LHP cancers is just that, an association, and the Panel is not aware of a plausible mechanism by which formaldehyde exposure could be causally linked to LHP tumors. Based on the testicular effects observed in rats exposed to formaldehyde, the CIR Panel acknowledged that a mechanism of action by which formaldehyde might cause the testicular effects is not known and these effects may be secondary to local effects, such as irritation and inflammation, and stress at high doses.

The Nail Manufacturers Council, the Professional Keratin Smoothing Council (PKSC), the American Chemistry Council, the Personal Care Products Council, and one individual provided new data and comments. After reviewing the comments and additional data, the Panel determined that the data were sufficient to support the safety of these ingredients in nail hardeners.

The additional data confirmed the current use concentration of formaldehyde/methylene glycol in the 1 – 2% range in nail hardeners (one product tested had a value of 2.2%). Given the rapid reaction on the nail surface and the use of nail hardeners at room temperature, the Panel did not consider that formaldehyde/methylene glycol at 1 – 2% in nail hardeners would present a risk of sensory irritation to the eyes, nose, or throat of users. The Panel noted that the present practices of use of nail hardeners include instructions that cautioned users to limit application of the material to the top surface of the nail only, to allow it to dry fully, and to not get the material on the skin.

The Panel noted that the OSHA occupational safety limits include a time-weighted average permissible exposure level of 0.75 ppm for a work day and a short-term exposure limit of 2 ppm. Air monitoring and medical exams are triggered when formaldehyde concentrations in workplace air exceed 0.5 ppm averaged over an 8-hour shift, and ventilation and training when concentrations exceed 0.75 ppm averaged over 8 hours or 2 ppm averaged over 15 minutes. Formaldehyde must be listed in a company's MSDS if formaldehyde is present at 0.1% or more, or if the product releases formaldehyde gas above 0.1 ppm.

While such requirements are mandated by OSHA, the Panel remained concerned about adverse reports of sensory irritation consistent with measured air levels of formaldehyde in salons using hair smoothing products (a.k.a. hair straightening products) containing formaldehyde/methylene glycol. Because the use of these products involves the application of heat, the Panel remained concerned about the amounts of formaldehyde vapor that can be released. The reported levels of formaldehyde gas measured in the air around salon work stations can be below occupational exposure standards and guidelines, but also may be at or only marginally below occupational exposure standards and above indoor air quality guidelines. The Panel noted that the PKSC suggested that these products are manufactured with the expectation that adequate ventilation would be provided during use; ie, safe use requires adequate ventilation. OSHA and other inspections, however, reported a range of ventilation controls, many of which were inadequate.

Additional use studies were done on behalf of the PKSC to demonstrate that exposure to formaldehyde could be minimized with proper procedures and use of personal ventilation devices. The Panel acknowledged that formaldehyde levels in air samples were lower in the most recent data compared to data submitted earlier, but proper safety procedures, including positioning of personal ventilation devices, were not uniformly followed.
In concept, therefore, limits on the concentration of formaldehyde/methylene glycol in hair smoothing products, control of the amount of product applied, use of lower temperatures, and approaches to mandate adequate ventilation, are among the steps that could be taken to ensure that these products would be used safely in the future. However, in the present practices of use and concentration (on the order of 10% formaldehyde/methylene glycol, blow drying and heating up to 450 ^{o}F with a flat iron, inadequate ventilation, resulting in many reports of adverse effects), hair smoothing products containing formaldehyde and methylene glycol are unsafe.

The Panel adopted a suggestion to include limits for formalin concentration because formalin is what formulators actually add to cosmetic products. Formalin is an aqueous solution typically containing 37% (w/w) formaldehyde.

Formalin contains both formaldehyde and methylene glycol because of the equilibrium between formaldehyde and methylene glycol in aqueous solution.

While retaining the concept that formaldehyde and methylene glycol should be used only at the minimal effective concentration, the Panel stated that in no case should the formalin concentration exceed 0.2% (w/w), which would be 0.074% (w/w) calculated as formaldehyde or 0.118% (w/w) calculated as methylene glycol. While these numbers appear to be disparate, they are not. The value of 0.074 % (w/w) of formaldehyde simply reflects that formalin typically contains 37% formaldehyde (0.2% (w/w) formalin multiplied by 0.37 = 0.074% (w/w) formaldehyde). The value of 0.118% (w/w) for methylene glycol simply reflects the difference in molecular weight between formaldehyde and methylene glycol.

The Panel recognized that the most commonly used analytical methods for the detection and measurement of formaldehyde are not specific for non-hydrated formaldehyde, but can accurately indicate the presence and quantity of formaldehyde equivalents. A typical method, for example, can detect formaldehyde equivalents in a formulation, or released into the air, via a two stage process: 1) derivatization of a sample with a hydrazine (which reacts with formaldehyde or methylene glycol, in a formulation sample or in an air sample), and 2) detection and measurement of the resultant hydrazone (ie, the reaction product of the hydrazine and formaldehyde) with a diode array, after separation on a column (eg, high performance liquid chromatography separation followed by ultraviolet/visible light (UV/Vis) detection).

While other formaldehyde/methylene analytical techniques are known, such as nuclear magnetic resonance (NMR) spectrometry, the Panel found that the methodology used by OSHA and FDA produces consistent results that are directly and meaningfully comparable to regulatory standards and guidelines. As the conditions under which formaldehyde is measured in products can affect the results, the method used to measure formaldehyde in products should be appropriate for the conditions, such as temperature and pH, under which the product is used.

The Panel reasoned that the term “formaldehyde equivalents” best captures the idea that methylene glycol is continuously converted to formaldehyde, and vice versa, even at equilibrium, which can be easily shifted by heating, drying, and other conditions to increase the amount of formaldehyde. Any other term would not distinguish the rapid, reversible formaldehyde/methylene glycol equilibrium from the slow, irreversible release of formaldehyde resulting from so-called formaldehyde releaser preservatives (eg, diazolidinyl urea). Formaldehyde releaser preservatives are not addressed in this safety assessment. The formaldehyde releasers may continue to be safely used in cosmetics at the levels established in their individual CIR safety assessments.

CONCLUSION

The CIR Expert Panel concluded that formaldehyde and methylene glycol are safe for use in cosmetics when formulated to ensure use at the minimal effective concentration, but in no case should the formalin† concentration exceed 0.2% (w/w), which would be 0.074% (w/w) calculated as formaldehyde or 0.118% (w/w) calculated as methylene glycol. Additionally, formaldehyde and methylene glycol are safe in the present practices of use and concentration in nail hardening products. However, formaldehyde and methylene glycol are unsafe in the present practices of use and concentration in hair smoothing products (a.k.a. hair straightening products).

†Formalin is an aqueous solution wherein formaldehyde (gas) has been added to water to a saturation point, which is typically 37% formaldehyde (w/w). Because of the equilibrium between formaldehyde and methylene glycol in aqueous solution, formalin is composed of both formaldehyde and methylene glycol.

TABLES AND FIGURE

Table 1. Frequency and Concentration of Use Table Formaldehyde, Formalin and Methylene glycol

	No. of Uses (2010) [15]	*Conc. of Use (2011) (%)* [16-19]	*No. of Uses (2010)* [15]	*Conc. of Use (2011) (%)* [16-19]
	formaldehyde (and formaldehyde solution (formalin))[a]		**methylene glycol**[b]	
Totals[c]	**77**	**0.04 – 2.2**	**NR**[d]	**0.8-3.5**
Duration of Use				
Leave-On	*33*	*0.056 – 2.2*	*NR*	*0.8-3.5*
Rinse Off	*44*	*0.04*	*NR*	*NR*
Product Category				
Bath oils, tablets and salts	1	NR	NR	NR
Bubble baths	1	NR	NR	NR
Hair conditioner	16	NR	NR	NR
Permanent waves	2	NR	NR	NR
Shampoos (non-coloring)	13	0.04	NR	NR
Hair grooming aids	6	0.056	NR	NR
Other hair preparation	7	NR	NR	NR
Other hair coloring preparation	2	NR	NR	NR
Manicure basecoats and undercoats	2	NR	NR	NR
Nail Hardeners	6	<0.5-2.2	NR	<0.8-3.5
Bath soaps and detergents	7	NR	NR	NR
Other personal care products	2	NR	NR	NR
Shaving cream	1	NR	NR	NR
Depilatories	2	NR	NR	NR
Body and hand (excl. shave prep.)	2	NR	NR	NR
Skin moisturizing preparations	1	NR	NR	NR
Paste masks (mud packs)	1	NR	NR	NR
Other skin care preparations	5	NR	NR	NR

[a]Reported as formaldehyde
[b]Calculated as methylene glycol
[c]Totals = Rinse-off + Leave-on Product Uses
[d]NR = Not Reported

Table 2. List of ingredients in Brazilian Blowout from the Brazilian Blowout MSDS dated 10/26/10

Ingredient	Percentage
Water	≤85%
Methylene glycol	<5%
Behenyl methylammonium methosulfate/N-hexadecanol/butylene glycol	≤5%
Isoparaffin	≤3%
Cetrimonium chloride	≤2%
Petrolatum	≤1%
Hypnea musciformis extract/Gellidiela acerosa extract/Sargassum filipendula extract/sorbitol	≤1%
Theobroma grandiflorum seed butter (cupuacu butter)	≤0.5%
Panthenol	≤0.25%
Hydrolyzed keratin	≤1%
Fragrance (parfum)	≤1%
Methylchloroisothiazolinone	≤0.1%
Methylisothiazolinone	≤0.1%

Table 3. Skin irritancy/sensitization studies of formaldehyde/methylene glycol in test animals

Species (n)	Concentrations; volume; duration	Results	Reference
	Multiple dose studies		
Hartley guinea pigs (n = 5/group)	1%, 3%, 10% formalin; 100 µl/d,10 days	Dose-dependent increase in skin-fold thickness was observed, with shorter latencies at higher concentrations; e.g., erythema on treatment day 6 for 1%, day 5 for 3%, and day 2 for 10% formalin.	[121]
English smooth-haired guinea pigs (n = 4 or 8 males/group)	**Induction, Dermal:** (a) 100% formalin; 100 µl/d, 2 days (b) 50% formalin w/50% adjuvant; 200 µl/d, 1 day (c) 0.13, 1.3, 13, 54, 100% formalin; 25 µl/d, 1day **Induction, Inhalation:** (a) 6, 10 ppm; 6 h/d, 5 days (b) 10 ppm; 8h/d, 5 days **Challenge, Dermal:** 5.4% formalin; 20 µl/d, 1 day	Dose-dependent contact sensitivity was observed in all of the animals exposed dermally during the induction phase and challenged on day 7 of the experiment. Two of the 4 guinea pigs challenged on day 31exhibited signs of contact sensitivity (mild) after inhalation of 10 ppm, 8 h/d for 5 days. No contact sensitivity was observed in the other inhalation groups or in any of the control groups.	[122]
Wistar and BN rats (n = 4 females/group)	2.5, 5, 10% formalin in 4:1 acetone/raffinated olive oil; 75 µl/d, 3 days	Increase in the weights of the lymph nodes and dose-related increase in the proliferation of paracortical cells were observed in both strains in response to 5% and 10% formalin (1.9% and 3.7% formaldehyde equivalents) in a local lymph node assay (LLNA). No statistically significant increase in serum IgE concentrations were observed in BN rats (high IgE responders) in a parallel experiment.	[123]

Table 4. Genotoxicity inhalation studies of formaldehyde/methylene glycol in test animals

Species (n)	Concentrations; duration	Results	Reference
		Multiple dose studies	
Sprague-Dawley rats (n = 10 males/group)	0, 5, 10 ppm; 6 h/d, 5 d/wk, 2 weeks	Statistically significant, dose-dependent increases in Comet Olive tail moments were observed in blood lymphocytes, liver cells, and lung tissue. **Comment:** A critical review noted that formaldehyde-induced formation of DNA-protein crosslinks (DPCs) and DNA-DNA crosslinks (DDCs) in the cells should have decreased, rather than increased, DNA migration in these assays.	52,53,124
F344/DuCrl rats (n = 6 males/group)	0, 0.5, 1, 2, 6, 10, 15 ppm; 6 h/d, 5 d/wk, 4 weeks	No statistically significant differences were found between the exposed and negative control groups in Comet tail moment or intensity, or sister chromatid exchange (SCE) and micronuclei (MN) frequencies in peripheral blood samples. The results of the Comet assay were negative even after irradiating the blood samples to increase sensitivity for detecting DNA-protein crosslinks (DPCs). Statistically significant effects were observed in the positive controls (ie, orally administered methyl methanesulfonate or cyclophosphamide), demonstrating the sensitivity of the tests.	54

Table 5. Genotoxicity inhalation studies of formaldehyde/methylene glycol in human subjects

Subjects (n)	Concentrations; duration	Results	Reference
(a) Workers at a formaldehyde manufacturing plant (n = 10) (b) Waiters (n = 16) (c) Students (n = 23)	(a) 0.80 ± 0.23 ppm 8-h TWA, 1.38 ppm Ceiling; average 8.6 years, range 1 to 15 years (b) 0.09 ± 0.05 ppm 5-h TWA; 12 weeks (c) 0.009 ppm 8-h TWA; not reported	Statistically significant increases in mononucleus (MN) and sister chromatid exchange (SCE) frequencies were found in nasal mucosa cells of the workers compared to student controls. The MN and SCE frequencies in nasal mucosa cells from the waiters were not different from the controls.	58
(a) Workers at two plywood factories (n = 151) (b) Workers at a machine manufacturing facility (n = 112)	(a) 0.08-6.42 ppm TWA (b) <0.008 ppm TWA	Exposure-related, statistically significant increases were found in Comet Olive tail moments and lengths and MN frequencies in lymphocytes from the plywood-manufacturing workers compared to controls (ie, machine-manufacturing workers).	59
(a) Pathology and anatomy laboratory workers (n = 59) (b) Individuals matched for gender, age, smoking (n = 37)	(a) 2 ppm 15-min TWA (range <0.1-20.4 ppm), 0.1 ppm 8-h TWA (range <0.1-0.7 ppm) (b) Not determined	No increase in DNA damage was observed in the lymphocytes of the pathologists/anatomists after one day of exposure, using a chemiluminescence microplate assay. Statistically significant increases in mono- and bi-nucleated lymphocyte frequencies were found in pathologists/anatomists compared to the controls using cytokinesis-blocked micronucleus (CBMN) & fluorescence in-situ hybridization (FISH) assay. No statistically significant differences were observed in the frequencies of centromeric or acentromeric MN. The authors suggested that the results are attributable to an aneugenic rather than clastogenic mode of action.	56
Volunteers (n = 10 women, 11 men)	0.15 to 0.5 ppm (concentration randomly assigned to each subject each day) w/ four 15-min 1-ppm peaks & three 15-min bicycling exercises during each exposure; 4 h/d, 10 days (Cumulative: 13.5 ppm-hour, 10 days)	A statistically significant decrease in MN frequency was observed in buccal mucosal cells collected 21days after the end of the exposure period compared with the control samples collected from the subjects 1 week before exposure. MN frequencies in samples collected immediately, 7 days, or 14 days after exposure did not differ from the control samples.	57
(a) Hospital pathological anatomy laboratory workers (n = 30) (b) Matched administrative personnel in the hospitals (n = 30)	(a) 0.44 ± 0.08 ppm mean 8-h TWA (range 0.04−1.58 ppm) (b) Not determined	Statistically significant increase in MN and SCE frequencies and Comet tail lengths were observed in lymphocytes collected from laboratory workers (employment duration averaging 11±7 years, ranging from 0.5 to 27 years) compared with controls. A statistically significant, positive correlation between exposure and both MN frequency and Comet tail length was found in the lymphocytes of the laboratory workers.	55

Table 5. Genotoxicity inhalation studies of formaldehyde/methylene glycol in human subjects

Subjects (n)	Concentrations; duration	Results	Reference
Healthy, non-smoking male volunteers (n = 41); 12 groups (n = 2 to 4/group)	Each subject exposed once to 0, 0.3 w/ four 15-min 0.6-ppm peaks, 0.4 w/ four 0.8 ppm peaks, and 0.5 ppm; 4 h/d, 5 days (subjects performed four 15-min bicycling exercises during each exposure period, including 2 during peaks)	A small but statistically significant increase in Comet tail intensity was observed in lymphocytes after the 5-day exposure period compared to the values determined before exposure. The authors concluded that this finding was not biologically significant, because formaldehyde-induced DPCs would be expected to decrease, not increase, Comet tail intensity. No statistically significant differences were found in Comet tail moments or SCE and MN frequencies in lymphocytes, MN frequencies in nasal epithelial cells, or biologically significant changes in gene expression in nasal biopsies collected after exposure compared with those collected before exposure.	[60]

Table 6. Nasal tissue studies of formaldehyde/methylene glycol in test animals

Species (n)	Concentrations; duration(s)	Results	Reference
		Multiple dose studies	
F344 CDF(F344)/CrlBr rats (n = 6 males/group)	0, 0.7, 2, 6, 10, 15 ppm; 6 h/d, 5d/wk, 1, 4, 9, 42 days (short-term) or 3, 6, 12, 18, 24 months (long-term)	Statistically significant increases in nasal cell proliferation were found only at ≥6.0 ppm (short-term) and ≥10.0 ppm (long-term). **Comment:** The authors and their co-workers interpreted these data to indicate that the dose-response curve is non-monotonic (ie, highly-nonlinear), because cell proliferation was diminished at lower doses and elevated at the higher, cytotoxic doses. This view is consistent with the hypothesis that formaldehyde exposure must be sufficient to stimulate regenerative cell proliferation, thereby increasing the likelihood that mutations that would otherwise be repaired will become permanent, and could then lead to tumor formation. Others have disputed this interpretation, because of the considerable uncertainty and variability in the data.	[64-66,125,126]
F344/CrlBR (n = 8 males/group)	0, 0.7, 2, 6, 10, 15 ppm; 6 h/d, 1,4,13 weeks	Transcriptional and histological changes at ≥6 ppm corresponded to doses for which pharmacokinetic modeling predicted substantial decrease in free glutathione (GSH) and increase in methylene glycol in nasal tissue. **Comment:** The authors concluded that formaldehyde exposure below 1 to 2 ppm in air would not perturb formaldehyde homeostasis in epithelial cells or elevate the risk of cancer in any tissue, consistent with a threshold for tissue responses and carcinogenicity.	[127]
F-344/NCrl rats (n = 5 males/group)	0, 0.7, 2, 6, 10, 15 ppm; 6 h/d, 13 weeks	Mutation levels were not elevated above the low spontaneous background levels, even in the rats exposed to 15 ppm formaldehyde, and showed no dose-related increases. Bromodeoxyuridine (BrdU) incorporation increased with dose and was statistically significantly elevated in the rats exposed to either 10 ppm or 15 ppm formaldehyde. **Comment:** The results support the view that cytotoxicity-induced cell proliferation (CICP) plays a pivotal role in the formation of NPCs in rats and, thus, formaldehyde-induced carcinogenicity is largely a threshold effect.	[62]
F344 (n = 10 to 30 males/group)	0.7, 2, 5.8, 9.1, 5.2 ppm; 6 hours	Formation of endogenous DNA adducts did not change in a dose-related manner in nasal epithelium. In contrast, the formation of exogenous adducts was highly non-linear, increasing 286-fold with a 21.7-fold increase in the exposure concentration. About 1% and 3% of the total number of adducts (endogenous plus exogenous) were exogenous adducts at 0.7 ppm and 2 ppm, respectively.	[61]
Cynomolgus macaques (n = 8 males)	1.9, 6.1 ppm; 6 h/d, 2 days	Endogenous and exogenous DNA adducts were detected in the nasal tissues at both exposure concentrations. **Comment:** The monkeys exposed to 6.1 ppm exhibited greater numbers of endogenous adducts and lower numbers of exogenous adducts in nasal tissues, compared with rats exposed to 5.8 ppm. Based on these results, the authors' suggested that the percentage of exogenous adducts would be lower in primates than in rats at equivalent exposure concentrations.	[63,68]

Table 7. Epidemiological studies of formaldehyde/methylene glycol and nasopharyngeal cancers

Study design; subjects (n)	Exposure metrics	Results	Reference
Retrospective Cohort mortality; Men employed after 1937 at six British factories where formaldehyde was produced or used, followed through 2000 (n = 14,014), compared with the general population	(a) Background: <0.1 ppm (b) Low: 0.1 to 0.5 ppm (c) Moderate: 0.6 to 2.0 ppm (d) High: >2.0 ppm	One nasopharyngeal cancer (NPC) mortality was identified among the factory workers, which included 3,991 workers exposed to >2 ppm. The single NPC case worked in a job with low exposure; two NPC cases were expected. Two sinonasal cancer deaths were identified, both having high exposures; 2.3 cases were expected. Fifteen pharyngeal tumor deaths were observed; 9.7 cases were expected.	128,129
Retrospective cohort mortality; Textile workers (82% female) employed after 1955 at 3 U.S. garment facilities, followed through 1998 (n = 11,039), compared with U.S. and local populations	(a) 8-h TWA (across all departments and plants) mean 0.15 ppm, range 0.09 to 0.2 ppm (b) Age at first exposure: median 26.2, range 15.2–79.8 years (c) Duration: <3, 3 to 9, ≥10 years (d) Time since first exposure: <10, 10 to 19, ≥20 years (e) Year first exposed: <1963, 1963 to 1970, ≥ 1971	No cases of NPC or nasal cancers were found; 1 case was expected.	129,130
Retrospective cohort mortality; Workers first employed before 1966 at 10 formaldehyde manufacturing plants (NCI cohort; Plants #1-#10) and followed through 1994 (n = 25,619)	(a) Average intensity: 0, ≤0.5, 0.5 to <1.0, ≥1.0 ppm (b) Cumulative: 0, >0 to <1.5, 1.5 to <5.5, ≥5.5 ppm-years (c) Duration: 0, >0 to <5, 5 to <15, ≥15 years (d) Ever vs. never exposed (e) Peak: 0, >0 to <2.0, 2.0 to <4.0, or ≥ 4.0 ppm	Nine deaths from NPC were identified in this cohort, including 7 classified as "ever exposed" and 2 as "never exposed." The highest relative risk (RR) estimates were 4.14 for ≥5.5 ppm-years cumulative exposure and 4.18 for ≥15 years exposure duration. Although confidence limits were not specified, the authors' footnotes indicate that they included 1 for these RR estimates. However, statistically significant dose-response trends were apparent for both peak exposure and cumulative exposure. **Comment:** Other researchers have demonstrated critical weaknesses in the model used in this study, including instability problems related to the data from Plant #1.	91,131-133
Retrospective cohort mortality; Workers employed in a plastics-manufacturing plant in Wallingford CT (NCI cohort; Plant #1) from 1941 to 1984 followed through 1998 (n = 7,328) compared with general population of 2 CT counties	(a) Average intensity: 0 to <0.03, 0.03 to 0.159, ≥0.16 (b) Cumulative: 0 to <0.004, 0.004 to 0.219, ≥0.22 ppm-years (c) Duration: 0 to <1, 1 to 9, ≥ 10 years (d) Duration exposed to >0.2 ppm: 0, 0 to <1, 1 to 9, ≥10 years (e) Short-term (<1 year) vs. long-term (>1 year) worker	Seven NPC cases were identified in this cohort, including 6 cases specifically identified as NPC and 1 case of pharyngeal cancer that was not identified specifically as NPC in the records. Several formaldehyde exposure metrics were associated with NPC for Plant #1, including "ever exposed," exposure duration ≥10 years, and cumulative exposure ≥0.22 ppm-years. The standardized mortality ratios (SMRs) estimated for these metrics were 6.03, 12.46, and 7.51, respectively, all with confidence limits >1. **Comment:** The authors suggested that their findings do not support a causal relationship between formaldehyde exposure and NPC mortality because elevated risks were seen in both short-term (<1 year; 4 cases) and long-term workers (3 cases), 5 NPC cases worked <5 years at the plant, the NPC cases among the long-term workers (>1 year) had relatively low average-intensity exposures (0.03-0.60 ppm), and the NPC deaths were concentrated among workers hired during 1947-1956.	134
Retrospective cohort mortality; Workers first employed before 1966 at 10 formaldehyde manufacturing plants (NCI cohort; Plants #1-#10) and followed through 1994 (n = 25,619	(a) Average intensity: <1.046, 1.046 to 1.177, ≥1.178 ppm (b) Cumulative: <0.734, 0.734 to 10.150, ≥10.151 ppm-years (c) Duration: <0.617, 0.617 to 2.258, ≥2.259 years (d) Highest peak: >0 to 1.9, 2.0 to 3.9, ≥4.0	Six of 10 NPC deaths (ie, identified specifically as NPC) in this cohort were associated specifically with employment at Plant #1, the remaining 4 cases distributed among 4 of the other 9 plants studied. A regional rate-based SMR of 10.32 (95% CI: 3.79-22.47) was estimated for exposed workers at Plant #1, compared to 0.65 (95% CI: 0.08 to 2.33) for exposed workers at Plants #2 through #10 combined. The statistically significant peak exposure-response relationship in the cohort was driven by excess NPC risk associated with the highest peak exposure category (≥4 ppm) at Plant #1. None of the exposure-response relationships for any of the four exposure metrics were statistically significant for Plants #2 through #10, combined. The authors concluded that the suggestion of a causal relationship between	135

Table 7. Epidemiological studies of formaldehyde/methylene glycol and nasopharyngeal cancers

Study design; subjects (n)	Exposure metrics	Results	Reference
	ppm	formaldehyde exposure and NPC mortality in previous studies was based entirely on anomalous findings at Plant #1.	
Retrospective cohort mortality; Workers employed in a plastics-manufacturing plant in Wallingford CT (NCI cohort; Plant #1) from 1941 to 1984 (n = 7,345) followed through 2003, nested case-control and comparison with general populations of U.S. and local counties	(a) Average intensity: 0 to <0.03, 0.03 to 0.159, ≥0.16 (b) Cumulative: 0 to <0.004, 0.004 to 0.219, ≥0.22 ppm-years (c) Duration: 0 to <1, 1 to 9, ≥10 ppm (d) Exposed vs. unexposed	SMRs of 4.43 (95% CI: 1.78-9.13) and 4.34 (95% CI: 1.74-8.94) were calculated for the 7 NPC mortalities among the exposed Plant #1 workers compared with local and U.S. rates, respectively. Four of the 7 NPC cases also held silver-smithing jobs, and 5 of the 7 NPC cases held silver-smithing or other metal-working jobs, and this type of work was relatively rare in the remaining study population. The authors noted possible exposures to several suspected risk factors for upper respiratory system cancer (eg, sulfuric acid mists, mineral acid, metal dusts and heat) associated with this type of work.	[136]
Nested case-control; Deceased embalmers and funeral directors (n = 6,808)	(a) Average intensity while embalming: 0, >0 to 1.4, >1.4 to 1.9, >1.9 ppm (b) Cumulative: 0, >0 to 4058, >4058 to 9253, >9253 ppm-hours (c) Duration in jobs involving embalming: 0, >0 to 20, >20 to 34, >34 years (d) Ever vs. never embalming (e) Lifetime 8-h TWA: 0, >0 to 0.1, >0.1 to 0.18, >0.18 ppm (f) Number of embalmings conducted: 0, >0 to 1422, >1422 to 9253, >9253 (g) Peak: 0, >0 to 7, >7 to 9.3, >9.3 ppm	Four cases of NPC were identified, only two of which had "ever embalmed" (Odds ratio = 0.1; 95% CI: 0.01-1.2). Exposure estimates for these 2 cases were indistinguishable from controls.	[85]

Table 8. Comparative tissue studies of formaldehyde/methylene glycol in test animals

Species (n)	Concentration(s); duration(s)	Results	Reference
		Multiple dose studies	
F344 (n = 30 males)	10 ppm; 6 h/d, 1 or 5 days	Exogenous formaldehyde-induced DNA monoadducts and DNA-DNA crosslinks (DDCs) were found exclusively in the nasal tissues after exposure. No exogenous products were detected in any other tissue even though, for example, the analytical method can detect ~3 monoadducts/10^9 deoxyguanosine (dG). This detection limit is ~30 times less than the endogenous monoadducts/10^9 dG measured in white blood cells (on-column detection limits ~240 and 60 amol for monoadducts and crosslinks, respectively). Endogenous products were found in all of the tissues examined, including blood and bone marrow. The levels of endogenous products were comparable across all tissues examined. The authors concluded: (1) Neither formaldehyde nor methylene glycol from formaldehyde reaches sites distant from the portal of entry, even when inhaled at high concentrations known to stimulate nasal epithelial cell proliferation and cause nasal tumors in rats. (2) Genotoxic effects of formaldehyde/methylene glycol are not plausible at sites distant from the portal of entry. (3) The idea that formaldehyde/methylene glycol transforms cells in the peripheral circulation or the nasal epithelium at the portal of entry, which can then migrate and incorporate into the bone marrow or other distant tissues to cause cancer, is not plausible.	[137]
F344 (n = 10 to 30 males/group)	0.7, 2, 5.8, 9.1, 15.2 ppm; 6 hours	Measurable numbers of endogenous adducts were found in both the nasal mucosa and bone marrow, and exogenous adducts in the nasal mucosa. No exogenous adducts were detected in the bone marrow (on-column detection limit ~20 amol).	[61]
Cynomolgus macaques (n = 8 males)	1.9, 6.1 ppm; 6 h/d, 2 days	Measurable numbers of endogenous and exogenous adducts were detected in the nasal tissues of both exposure groups, but only endogenous adducts in the bone marrow (on-column detection limit ~20 amol).	[63]

Table 9. Epidemiological studies of formaldehyde/methylene glycol and lymphohematopoietic cancers

Study design; subjects or studies (n)	Exposure concentration or metrics	Results	Reference
		Cohort, case-control and molecular studies	
Retrospective cohort mortality; Men employed after 1937 at six British factories where formaldehyde was produced or used, followed through 2000 (n = 14,014), compared with the general population	(a) Background: <0.1 ppm (b) Low: 0.1 to 0.5 ppm (c) Moderate: 0.6 to 2.0 ppm (d) High: >2.0 ppm	There were 31 leukemia deaths in this cohort, which included 3,991 workers exposed to >2 ppm; 34 cases were expected.	[128,129]
Retrospective cohort mortality; Textile workers (82% female) employed after 1955 at 3 U.S. garment facilities, followed through 1998 (n = 11,039), compared with U.S. and local populations	(a) 8-h TWA (across all departments and plants) mean 0.15 ppm, range 0.09 to 0.2 ppm (b) Age at first exposure: median 26.2, range 15.2–79.8 years (c) Duration: <3, 3 to 9, ≥10 years (d) Time since first exposure: <10, 10 to 19, ≥20 years (e) Year first exposed: <1963, 1963 to 1970, ≥ 1971	There were 59 leukemia cases in this cohort; 61 cases were expected.	[129,130]
Retrospective cohort	(a) Average intensity (8-h	This study reported and included 1,006 death certificates that a previous	[90,138]

Table 9. Epidemiological studies of formaldehyde/methylene glycol and lymphohematopoietic cancers

Study design; subjects or studies (n)	Exposure concentration or metrics	Results	Reference
mortality; Workers first employed before 1966 at 10 formaldehyde manufacturing plants (NCI cohort; Plants #1-#10) and followed through 2004 (n = 25,619), compared with U.S. population	TWA): 0, 0.1 to 0.4, 0.5 to <1, ≥1.0 ppm (b) Cumulative: 0, 0.1 to 1.4, 1.5 to 5.4, ≥5.5 ppm-years (c) Ever vs. never exposed (d) Peak: 0, 0.1 to 1.9, 2 to 4, ≥ 4.0 ppm (e) Peak frequency: hourly, daily, weekly, monthly	paper missed for this cohort. There were proportionally greater numbers of missing deaths among the un-exposed and low-exposed groups used as internal referents in the previous paper. There were 319 deaths from all LHP cancers (from a total of 13,951 deaths) in this cohort, including 286 "exposed" and 33 "non-exposed" cases. Based on U.S. mortality rates, neither of these groups showed statistically significant elevations in SMRs estimated for all LHP cancer, all leukemia, lymphatic leukemia, myeloid leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, or multiple myeloma. Statistically significant dose-response trends were reported for peak exposure and all LHP, all leukemia and Hodgkin's lymphoma deaths, as well as for average intensity of exposure and Hodgkin's lymphoma deaths. However, the relative risk (RR) for Hodgkin's lymphoma in workers with the highest average intensity was lower than for workers with lower average exposure. No statistically significant trends were found among the LHP cancers and peak frequency or cumulative exposures.	
Nested case-control mortality; Deceased embalmers and funeral directors (n = 6,808)	(a) Average intensity while embalming: 0, >0 to 1.4, >1.4 to 1.9, >1.9 ppm (b) Cumulative: 0, >0 to 4058, >4058 to 9253, >9253 ppm-hours (c) Duration in jobs involving embalming: 0, >0 to 20, >20 to 34, >34 years (d) Ever vs. never embalming (e) Lifetime 8-hour TWA: 0, >0 to 0.1, >0.1 to 0.18, >0.18 ppm (f) Number of embalmings: 0, >0 to 1422, >1422 to 9253, >9253 (g) Peak: 0, >0 to 7, >7 to 9.3, >9.3 ppm	There were 168 deaths attributable to LHP cancers in this cohort, including 99 lymphoid and 48 non-lymphoid cancers. Non-lymphoid cancers included 34 cases of myeloid leukemia. Statistically significant increases in risks of LHP cancers of non-lymphoid origin were found for several exposure metrics, including the highest levels of exposure for cumulative, TWA, and peak exposures, as well as for subjects who embalmed for >20 years. For myeloid leukemia, strong, statistically significant associations with exposure duration, number of embalmings performed, and cumulative exposure were found. Statistically-significant dose-response relationships were reported between myeloid leukemia deaths and both exposure duration and peak exposure. **Comment:** Several methodological issues have been identified for this study study. For example: (1) Myeloid leukemia cases among the study subjects were 50% more likely than controls to have begun employment in the funeral industry before 1942; This suggests that they belonged primarily to an older and earlier population than the controls and likely explains why they performed more embalmings (2) The single myeloid leukemia case in the control group yielded large, unstable confidence intervals; The odds ratios (ORs) were substantially reduced when the referent group included both the controls and the subjects performing <500 embalmings (3) The myeloid leukemia cases and controls had nearly identical mean estimated average, 8-h TWA, and peak exposures; The cases had higher estimated number of embalmings and cumulative exposure than the controls, which can be explained by their earlier first employment, younger age at hire, and longer average employment in the industry, compared with controls.	85,139-141
Molecular epidemiology of formaldehyde workers and frequency-matched controls in China (n = 43; 51 controls)	Median (10^{th}-90^{th} percentile): (a) Formaldehyde workers: 1.28 (0.63-2.51) ppm (b) Controls: 0.026 (0.0085-0.026) ppm	Statistically significant decreases were observed in mean red blood cell (RBC), white blood cell (WBC), granulocyte, and platelet counts in the subjects compared with the controls. Statistically significant increases were found in mean corpuscular volume (MCV) and in frequencies of chromosome 7 monosomy and chromosome 8 trisomy. No occupational co-exposures to benzene or other hemotoxic or genotoxic solvents were detected in this study. In a parallel experiment, statistically significant, dose-related decreases were observed in the number of colonies formed per plated cells from the subjects compared with controls. **Comment:** Numerous problems in this preliminary study have been identified. For example: (1) All of the blood counts in the exposed workers were within the reference range. (2) The frequencies of the aneuploidies reported were seen only after 14 days of in vitro incubation, were high for cells from both the workers	142-146

Table 9. Epidemiological studies of formaldehyde/methylene glycol and lymphohematopoietic cancers

Study design; subjects or studies (n)	Exposure concentration or metrics	Results	Reference
		and controls, and were not reported in either the factory workers or the controls in vivo. (3) The most frequent chromosome aberrations associated with myeloid leukemia are translocations, but this study investigated neither translocations nor aneuploidies other than monosomy 7 and trisomy 8. (4) Formaldehyde appears to be mutagenic predominantly by a clastogenic, not an aneugenic mode of action. (5) Formaldehyde has been shown to damage several cell types directly exposed in vitro, an effect therefore not unique to myeloid progenitor cells.	
		Meta-analyses	
Meta-analysis of cohort and case-control studies that reported leukemia rates in professional or industrial workers; (n = 18)	Not detailed	No statistically-significant associations were found between leukemia and exposure across all of the studies, across all cohort studies, or across all case-control studies. Slightly elevated risk of leukemia was reported among embalmers and pathologists/anatomists, but none for industrial workers, even those with the highest reported exposures.	147
Meta-analysis of cohort studies of professional or industrial workers through February 2007 (n = 25)	Not detailed	A "modestly elevated" pooled RR for LHP cancers was calculated for professionals (ie, embalmers, anatomists and pathologists; 8 studies), but not for industrial workers (4 studies). Similar results were reported for leukemia.	129
Meta-analysis of cohort and case-control studies that reported LHP cancer rates in professional or industrial workers (n = 26)	Not detailed	Summary RRs for professional and industrial workers combined were increased for all LHP cancers combined (19 studies). Statistically significant increases in RRs were reported for all leukemias (15 studies) and myeloid leukemia (6 studies). **Comment:** These authors attempted to increase the statistical power of their analysis by focusing only on the highest exposure groups in each study, selecting exposure duration from some studies, and peak, average, or cumulative exposure from others. They preferentially selected results for myeloid leukemia, rather than results for all types of leukemia combined, when available. They did not stratify the data to distinguish low-exposure professionals from high-exposure industry workers.	148
Meta-analysis of case-control and cohort studies that reported myeloid leukemia rates in professional or industrial workers (n = 14)	Not detailed	Statistically significant increases in summary RRs for professional and industrial workers combined were observed for leukemia and myeloid leukemia. Statistically significant increases in summary RRs were calculated for industrial workers (6 studies) and professionals (8 studies) considered separately. **Comment:** These authors attempted to increase the statistical power of their analysis by focusing only on the highest exposure groups in each study, selecting exposure duration from some studies, and peak, average, or cumulative exposure from others. They preferentially selected results for myeloid leukemia, rather than results for all types of leukemia combined, when available.	149
Meta-analysis of cohort and case-control studies of professional and industrial workers through May 2009 (n = 17)	Not detailed	For leukemia, no statistically significant increases in summary RRs were found in the cohort or the case-control studies for professionals (ie, embalmers and technical workers) and industrial workers combined. No statistically significant increases was observed in the summary RRs calculated specifically for professional workers (15 studies), for industrial workers (2 studies), or for myeloid leukemia from the cohort studies. Although the authors found that their summary proportionate mortality ratio (PMR) for leukemia was elevated (PMR = 1.44; 95% CI: 1.25- 1.67; 3 studies), they explained that PMRs are unreliable and sugested that the inclusion of PMR studies may have caused inaccurately elevated summary risk estimates in previous meta-analyses.	150

Table 10. Reproductive and developmental toxicity studies of formaldehyde/methylene glycol in test animals

Species (n)	Concentration(s); volume; duration	Results	Reference
		Multiple dose studies	
Wistar rats (n = 6 males/group)	0, 5, 10 ppm; 8 h/d, 5 d/wk, 91 days	Exposure to 5 or 10 ppm caused unsteady breathing, excessive licking, frequent sneezing, and hemorrhage of nasal mucosa. Statistically significant decreases in serum testosterone concentrations and seminiferous tubule diameters were found in both groups of exposed rats compared with controls. Hsp70 levels were increased in the spermatogonia, spermatocytes, and spermatids of the treated rats compared with controls.	[46]
Sprague-Dawley rats (n = 10 males/group)	8 ppm; 12 h/d, 2 weeks	Significant decrease in testicular weight was found in the exposed rats compared with the controls. Histopathological examination revealed seminiferous tubule atrophy, interstitial vascular dilatation and hyperemia, disintegration and shedding of seminiferous epithelial cells into azoospermic lumina, and interstitial edema in the testes of the exposed rats. Statistically significant decreases were reported in epididymal sperm count, percentage of motile sperm, activities of testicular superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and in glutathione (GSH) levels, and increase in malondialdehyde (MDA) levels in the exposed rats compared with controls. All of these effects were markedly decreased in exposed rats that were also treated with Vitamin E. These authors did not report the overt toxic effects of the exposures.	[151]
Wistar rats (n = 7 males/group)	1.5 ppm; 4 h/d, 4 d/wk; 2 h/d, 4 d/wk; or 4 h/d, 2 d/wk; 18 weeks	Statistically significant decreases in diameter and height of seminiferous tubules/testis were observed in the exposed rats compared with controls. Severe decreases were found in the number of germ cells in the seminiferous tubules and evidence of arrested spermatogenesis after exposure 4 h/d, 4 d/wk, decrease in the number of germ cells and increased thickness of the tubule basement membrane after exposure 2 h/d, 4 d/wk, and disruption in the arrangement of Sertoli and germinal cells, with increased spacing between germ cells, after exposure 4 h/d, 2 d/wk. The authors did not report the overt toxic effects of the formaldehyde exposures.	[152]
Mice, strain not specified (n = 12 males/group)	0, 16.9, 33.8, 67.6 ppm; 2 h/d, 6 d/wk, 13 weeks	A statistically significant increase in the sperm aberration rate and decrease in mean live fetuses/litter in a dominant-lethal test were observed after exposure to 67.6 ppm. Resorption rates were statistically significantly increased for all groups of exposed rats. The English abstract of this Chinese paper does not detail the exposure method or report the overt toxic effects of the exposures.	[153]
Wistar rats (n = 10 males/group)	0, 6, 12 ppm; 6 h/d, 5 d/wk, 30 days	Lower numbers of both granular cells in the hippocampal dentate gyrus and pyramidal cells in the cornu ammonis of the hippocampus were observed at post-natal day 90 (PND90), compared to PND30, in rats exposed to 12 ppm. The authors did not report the overt toxic effects of the formaldehyde exposures.	[47,154]
Sprague-Dawley rats (n = 6 dams/group)	0, 6 ppm; 8 h/d, 6 weeks, starting on gestation day 1 (GD1), post-natal day 1 (PND1), or at 4 weeks of age or adulthood	Statistically significant decreased mean body and liver weights were observed in the offspring when exposure began on GD1. Liver weights were statistically significantly increased when exposure began at 4 weeks of age compared with controls. In the liver, statistically significant increases in catalase (CAT) activity and malondialdehyde (MDA) concentration, and decreases in glutathione (GSH) concentration and superoxide dismutase (SOD) activity were observed in the offspring when exposure began on GD1, PND1, or at 4 weeks of age. The authors did not report the overt toxic effects of the formaldehyde exposures.	[155]

Table 11. Epidemiological studies of formaldehyde/methylene glycol and reproductive effects

Study design; subjects or studies (n)	Exposure concentration or metrics	Results	Reference
Case control; Women who worked full-time in cosmetology and had a spontaneous abortion or a live baby during 1983−1988 (n = 376; 61 with spontaneous abortions, 315 with live births)	Exposed vs. unexposed	An association was reported between spontaneous abortion and use of "formaldehyde-based" disinfectants (crude odds ratio = 2.0; 95% CI: 1.1-3.8). The association was still apparent (adjusted odds ratio = 2.1; 95% CI: 1.0−4.3) after adjusting for maternal characteristics (eg, age, smoking, glove use, other jobs) and other workplace exposures (eg, chemicals used on hair, use of manicure products).	49
Case-control; Women occupationally exposed to formalin in hospital laboratories and having a spontaneous abortion, compared to controls who delivered a baby without malformations, during 1973−1986 (n = 208; 329 controls)	Mean: 0.45 ppm (range: 0.01-7 ppm) reported in similar laboratories	A statistically significant association was found between exposure to formalin/formaldehyde 3 to 5 d/wk and incidence of spontaneous abortions, after adjusting for employment, smoking, alcohol consumption, parity, previous miscarriage, birth control failure, febrile disease during pregnancy, and exposure to other organic solvents in the workplace. Exposures to toluene and xylene were also statistically significantly associated with the incidence of spontaneous abortions. No association was found between formalin exposure and congenital malformations in laboratory workers (n = 36) compared with controls (n = 5).	50
Case-control; Women occupationally exposed in woodworking industries, compared with employed, unexposed women (n = 602; 367 controls)	TWAs: (a) Low: 0.1 to 3.9 ppm (b) Medium: 4.0 to 12.9 ppm (c) High: 13.0 to 63 ppm	Statistically significant decrease was observed in fecundability density ratios (FDRs; ie, the average pregnancy incidence density of the exposed women divided by that of the unexposed women) for the high exposure group, and in the women in the high exposed group who did not wear gloves (n = 17). The reduced FDR among women in the high exposed group who wore gloves was not statistically significant (n=22). Associations were found between exposure and spontaneous abortions in 52 women who had worked in their workplace during the year of the spontaneous abortion and at the beginning of the time-to-pregnancy period. The odds ratios (ORs) were 3.2 (95% CI: 1.2−8.3), 1.8 (95% CI: 0.8−4.0), and 2.4 (95% CI: 1.2−4.8) for the low, medium, and high exposure categories, respectively. Endometriosis also appeared to be associated with exposure in women in the high exposure category (OR = 4.5; 95% CI: 1.0−20.0).	51
		Meta-analysis	
Meta-analysis of cohort, case-control and cross-sectional studies of professional or industrial workers through September 1999 (n = 8)	Up to 3.5 ppm	An overall meta-relative risk (meta-RR) estimate of 1.4 (95% CI: 0.9-2.1) was calculated, suggesting an association between occupational exposure and spontaneous abortion. However, no increased risk was observed after adjusting this estimate for reporting and publication biases (meta-RR = 0.7; 95% CI: 0.5-1.0).	156

Table 12. Measured formaldehyde levels during use of hair smoothing products

Test	Form Levels (ppm)	Exposure Time (min)	Samples ≥ Guidelines		
			US NAC AEGL-1[a] 0.9ppm ≥ 10 min	ACGIH TLV®-Ceiling[b] 0.3 ppm	WHO 30 min Guideline[c] 0.08 ppm
Oregon OSHA	0.074-1.88	6-48	Yes (4)	Yes (9)	Yes (All ≥30 min)
Exponent 1	0.170-0.269	95-141	No	No	Yes (All)
Exponent 2	0.041-0.76	17-43	No	Yes (9)	Yes (6 ≥30 min)
Tennessee OSHA	0.3-1.07	15	Yes (1)	Yes (5)	Yes[d]
PKSC 1	0.761-1.71	15	Yes	Yes (All)	Yes[e]
PKSC 2	0.189-0.395	86-117	No	Yes	Yes[f]
ChemRisk	0.11-1.17	56-82	Yes (4)	Yes (8)	Yes[g]

[a]National Advisory Committee Interim Acute Exposure Guideline Level-1 (concentration above which the general population could experience notable discomfort, irritation, or other effects)
[b]American Conference of Government Industrial Hygienists Threshold Limit Value Ceiling (concentration that should not be exceeded during any part of the working day)
[c]World Health Organization Guideline for Indoor Air Quality
[d]Calculated levels exceed by up to 4 fold
[e]Calculated levels exceed by 12-21 fold
[f]Calculated levels exceed by up to 5 fold
[g]Calculated levels exceed by up to 15 fold

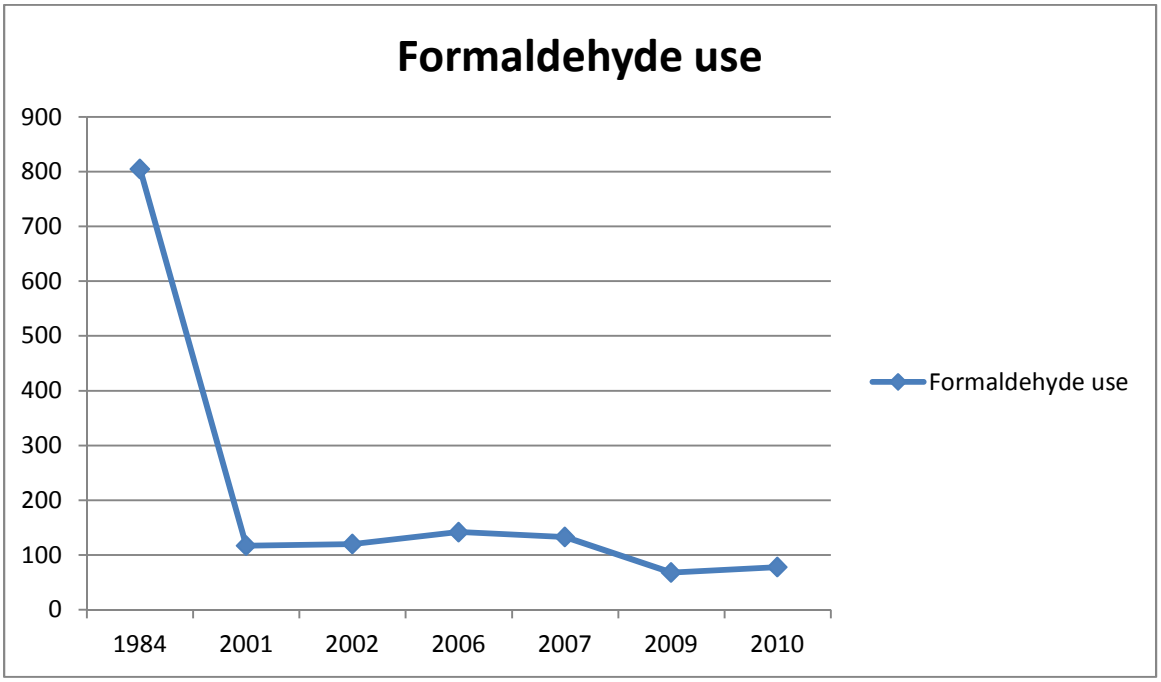


Figure 1. Declining use of formaldehyde in cosmetic products as reported to the FDA VCRP (The x-axis is not linear).

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Cosmetic Ingredient Review

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: May 31, 2013
Subject: Wave 2 Data on Methyl Glucose Polyethers and Esters

Toxicity study summaries on isostearic acid, esters with methyl α-D-glucoside, available at the European Chemical Agency's (ECHA) website (http://echa.europa.eu/), are included in this wave 2 data submission for the Panel's review, including: acute oral toxicity, repeated dose toxicity/reproductive and developmental toxicity, skin irritation, skin sensitization, and genotoxicity.

To avoid confusion, please note that a table with results from these studies was included in one of the data submissions (megluc062013data1 pdf file) that the Panel received on May 17. Study details were not provided in this data submission, but are now available. The Panel also received current use concentration data (megluc062013data2 pdf file) on this date. An updated use/concentration table is also included in the wave 2 summary.

Just to be clear, these new safety data were generated for isostearic acid, esters with methyl α-D-glucoside, defined as follows: 80% methyl glucoside isostearate esters (mainly Di-), 16% isostearic acid, and 4% methyl glucoside. This is the test material that was evaluated in the combined repeated dose toxicity study with the reproductive/developmental toxicity screening test (and the other studies mentioned above). That is good because data of this type were requested by the Panel, based on the use of methyl glucose polyethers and esters in lipsticks. There is uncertainty as to whether the material tested will allow the Panel to extrapolate to methyl glucose sesquistearate, PEG-20 methyl glucose sesquistearate, and PEG-20 methyl glucose distearate. The Panel would need to determine whether these data are sufficient for evaluating the safety of these ingredients in lipstick products.

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WAVE 2 DATA SUMMARIES ON ISOSTEARIC ACID, ESTERS WITH METHYL α-D - GLUCOSIDE

Acute Oral Toxicity

In an acute oral toxicity study performed according to the OECD TG 423 protocol**,** a single oral dose (gavage**)** of isostearic acid, esters with methyl α-D-glucoside (in 1% carboxymethyl cellulose and water) was administered to groups of fasted, young adult female Wistar rats. Initially, the test material was administered at a dose of 300 mg/kg body weight. According to a stepwise procedure, additional groups received doses of 300 and 2000 mg/kg body weight. The animals were observed daily and macroscopic examination was performed after terminal sacrifice on day 15. Body weight gain was classified as normal, and none of the animals died. Hunched posture and/or piloerection were observed in all animals on day 1, and, in the first group of animals, on day 2. There was no evidence of abnormalities at macroscopic, postmortem examination. The test material was classified as practically non-toxic (LD_{50} > 2000 mg/kg body weight).

Repeated Dose Toxicity/Reproductive and Developmental Toxicity

A combined repeated dose toxicity study with a reproduction/developmental toxicity screening test was performed according to the OECD 422 test protocol. Isostearic acid, esters with methyl α-D-glucoside (in1% aqueous carboxymethyl cellulose) was administered orally (gavage) to the following dose groups (10 male and 10 female Han rats/dose group) daily: 50, 150, and 1000 mg/kg body weight per day. The fourth group served as the negative control. The males were dosed for 2 weeks prior to mating, during mating, and up to termination (30 days total). Females were dosed for 2 weeks prior to mating, during mating, during post-coitum, and for at least 4 days of lactation (42 to 44 days total). Ten litters per dose group were delivered. Findings for the 1000 mg/kg dose group were as follows: statistically significant reduction in hemoglobin, cholesterol, and protein levels (males), and elevated white blood cell counts (determined for only 2 females) plus alkaline phosphatase levels (males), and increased liver weights (absolute and relative) in males and females.

In adult rats, there were no treatment-related changes in the following remaining parameters investigated: mortality, clinical appearance, functional observations, body weight, food consumption, and macroscopic and microscopic examination. There were also no treatment-related changes in reproduction, breeding, or pup development. Based on the findings observed at 1000 mg/kg/day, the parental NOEL was defined as 150 mg/kg/day. The parental NOAEL was defined as ≥ 1000 mg/kg/day, based on the findings observed at 1000 mg/kg/day. It was noted that the findings at this dose level were not considered adverse and were without any corroborative findings, such as histopathological changes. The reproduction, breeding, and developmental NOAEL was defined as ≥ 1000 mg/kg/day.

Skin Irritation

A primary dermal irritation study was performed according to OECD Guideline 404 using 3 young adult, male New Zealand White rabbits. Isostearic acid, esters with methyl α-D-glucoside (0.5 g) was applied, under a semi-occlusive dressing, to the skin for 4 h. The dermal application period was followed by a 14-day observation period. Very slight erythema was observed at the application sites of all 3 animals at 60 min post-application. In 2 rabbits, the reaction had resolved within 24 h. The reaction had resolved within 7 days in the remaining animal. Scaliness at the application site was observed in one animal at 72 h and 7 days post-application, but had resolved within 14 days. It was concluded that the test material was not a dermal irritant.

Skin Sensitization

Isostearic acid, esters with methyl α-D-glucoside (100% UVCB substance, defined as substances of unknown or variable composition, complex reaction products or biological materials) was evaluated in the maximization test using Dunkin-Hartley guinea pigs. The test material was evaluated at concentrations of 0.5% and 75% in sesame oil during induction, and the challenge concentration was 15% in sesame oil.

2-Mercaptobenzothiazole served as the positive control. Slight skin reactions were observed after induction. However, neither erythema nor edema was observed in test or control animals during the challenge phase. It was concluded that the test material was not a dermal sensitizer.

Genotoxicity

In Vivo

The genotoxicty of isostearic acid, esters with methyl α-D-glucoside (100% UVCB-substance [80% methyl glucoside isostearate esters (mainly Di-), 16% isostearic acid, and 4% methyl glucoside]) in DMSO was evaluated in the mouse lymphoma assay using L5178Y mouse lymphoma cells. In the first experiment, the test material was evaluated at concentrations up to 500 µg/ml (with metabolic activation) and 300 µg/ml (without metabolic activation). In the second experiment, the test material was evaluated at concentrations up to 375 µg/ml (with metabolic activation) and 240 µg/ml (without metabolic activation). In both experiments, the test material did not induce a significant increase in the mutation frequency with or without metabolic activation. The positive controls induced the appropriate response. The spontaneous mutation frequencies in the solvent-treated control cultures were between the minimum and maximum values of the historical control data range. Under the conditions of this test, it was concluded that the test material was not genotoxic.

Isostearic acid, esters with methyl α-D-glucoside (80% methyl glucoside isostearate esters (mainly Di-), 16% isostearic acid, and 4% methyl glucoside) in DMSO was evaluated in a cytogenetics assay using peripheral human lymphocyte cultures. In the first assay, the test material was evaluated at concentrations up to 333 µg/ml with and without metabolic activation. In the second assay, the test material was evaluated at concentrations up to 300 µg/ml (with metabolic activation) and 800 µg/ml (without metabolic activation). In both assays, the test material did not induce a statistically significant or biologically relevant increase in the number of cells with chromosome aberrations either with or without metabolic activation. The positive controls induced the appropriate response. The number of cells with chromosome aberrations in solvent control cultures was within the laboratory historical control data range.

In Vitro

Isostearic acid, esters with methyl α-D-glucoside was evaluated in a reverse gene mutation assay using the following bacterial strains: *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *E. coli* strain WP2 uvr A. The test material was evaluated at doses up to 3330 µg/plate both with and without metabolic activation. For each strain tested, dosing did not result in a significant dose-related increase in the number of revertant colonies, with or without metabolic activation. There was no evidence of cytotoxicity. Precipitation was observed at doses of 1000 and 3330 µg/plate. The positive controls induced the appropriate responses in the corresponding strains. It was concluded that the test material was not mutagenic in any of the tester strains used in this study.

Table 6. Current Frequency and Concentration of Use According to Duration and Type of Exposure Provided in 2012.[16,17]

	MG Dioleate		**MG Sesquioleate**		**MG Sesquiisostearate**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	4	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	0.6	NR	NR	NR	NR
Dermal Contact	10	0.2 to 0.6	1	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	1	4	NR	NR	NR	0.1
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use					NR	NR
Leave-On	11	0.2 to 0.6	1	NR	NR	NR
Rinse off	NR	4	NR	NR	NR	0.1
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	11	0.2 to 4	1	NR	NR	0.1
	MG Sesquistearate		**PPG-10 MG Ether**		**PPG-20 MG Ether**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	29	0.3 to 2	1	NR	NR	0.5
Incidental Ingestion	13	1	NR	NR	NR	NR
Incidental Inhalation- Sprays	7	0.5 to 0.8	2	NR	8	0.1 to 1
Incidental Inhalation- Powders	NR	NR	NR	0.8	1	0.4
Dermal Contact	169	0.3 to 5.19	9	0.8	42	0.1 to 3
Deodorant (underarm)	NR	NR	NR	NR	5	0.1
Hair - Non-Coloring	2	0.5 to 2	11	2	14	NR
Hair-Coloring	NR	0.5	1	0.5	NR	NR
Nail	NR	0.8	1	NR	1	NR
Mucous Membrane	17	0.4 to 1	4	NR	2	NR
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	161	0.3 to 5.19	11	0.8 to 2	36	0.1 to 3
Rinse off	25	0.4 to 4	11	0.5	21	0.1 to 0.5
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	186	0.3 to 5.19	22	0.5 to 2	57	0.1 to 3
	PPG-20 MG Ether Distearate		**Methyl Gluceth-10**		**Methyl Gluceth-20**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	NR	NR	2	1 to 5	15	2 to 6
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	NR	1	1	12	0.5 to 2
Incidental Inhalation- Powders	NR	NR	NR	0.02 to 15	1	1 to 10
Dermal Contact	2	4	63	0.02 to 15	386	0.04 to 15
Deodorant (underarm)	NR	NR	NR	NR	3	NR
Hair - Non-Coloring	NR	NR	10	0.0003 to 11	39	0.2 to 5
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	5	3	2 to 5
Mucous Membrane	NR	NR	6	0.02	207	0.04 to 6
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	2	4	60	0.02 to 15	154	0.2 to 10
Rinse off	NR	NR	13	0.0003 to 15	252	0.04 to 15
Diluted for (bath) Use	NR	NR	NR	NR	22	0.08 to 1
Totals/Conc. Range	2	4	73	0.0003 to 15	428	0.04 to 15

Table 6. Current Frequency and Concentration of Use According to Duration and Type of Exposure Provided in 2012[17]

	PEG-120 MG Dioleate		PEG-20 MG Distearate		PEG-20 MG Sesquistearate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	3	6	NR	NR	19	0.1 to 1
Incidental Ingestion	NR	NR	NR	0.05	1	NR
Incidental Inhalation- Sprays	2	NR	NR	NR	1	0.9 to 1
Incidental Inhalation- Powders	NR	0.4 to 4	NR	NR	NR	1 to 10
Dermal Contact	370	0.2 to 6	2	NR	121	0.1 to 10
Deodorant (underarm)	1	NR	NR	NR	NR	NR
Hair - Non-Coloring	74	0.1 to 2	1	NR	2	0.9 to 3
Hair-Coloring	NR	NR	NR	NR	1	0.5
Nail	NR	NR	NR	NR	1	1 to 3
Mucous Membrane	292	0.2 to 4	NR	0.05	20	2 to 4
Baby Products	4	1	NR	NR	NR	NR
Duration of Use						
Leave-On	11	0.4 to 4	3	0.05	88	0.1 to 10
Rinse off	415	0.1 to 6	NR	NR	40	0.5 to 6
Diluted for (bath) Use	20	0.8 to 3	NR	NR	NR	2
Totals/Conc. Range	446	0.1 to 6	3	0.05	128	0.1 to 10

	PEG-120 MG Trioleate	
	# of Uses	Conc. (%)
Exposure Type		
Eye Area	NR	NR
Incidental Ingestion	NR	NR
Incidental Inhalation- Sprays	NR	0.1
Incidental Inhalation- Powders	NR	0.1 to 0.5
Dermal Contact	3	0.1 to 0.5
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	4	NR
Hair-Coloring	NR	NR
Nail	NR	NR
Mucous Membrane	1	0.1 to 0.5
Baby Products	NR	NR
Duration of Use		
Leave-On	NR	0.1 to 0.5
Rinse off	7	0.1 to 0.5
Diluted for (bath) Use	NR	NR
Totals/Conc. Range	7	0.1 to 0.5

MG = Methyl Glucose; 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.

Cosmetic Ingredient Review

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett
Scientific Writer/Analyst
Date: May 31, 2013
Subject: Wave 2 for Polyvinylpyrrolidone (PVP)

The Council has provided an updated concentration of use survey for PVP. The 94% that was reported in a non-spray body and hand product should have been 0.94%.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

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

DATE: May 22, 2013

SUBJECT: Concentration of Use by FDA Product Category: Updated information on PVP

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Concentration of Use by FDA Product Category
PVP

FDA Code*	Product Category	Maximum Concentration of Use
03A	Eyebrow pencil	2-2.5%
03B	Eye liner	0.1-12%
03C	Eye shadow	1-8%
03D	Eye lotion	0.05-0.71%
03E	Eye makeup remover	8%
03F	Mascara	1-12%
03G	Other eye makeup preparations	1-9%
05A	Hair conditioners	0.0005-2%
05B	Hair sprays aerosol pump spray	 0.1-3.5% 0.02-5%
05D	Permanent waves	0.1%
05E	Rinses (noncoloring)	0.1%
05F	Shampoos (noncoloring)	0.0005-0.29%
05G	Tonics, dressings and other hair grooming aids aerosol pump spray	0.1-10.5% 3% 0.5-3%
05H	Wave sets	2%
05I	Other hair preparations (noncoloring)	0.8%
06F	Hair lighteners with color	3.3%
06G	Hair bleaches	1.6%
07C	Foundations	0.06-3%
07D	Leg and body paints	0.1%
07E	Lipstick	0.1-3%
07G	Rouges	0.1%

08E	Nail polish and enamel	0.3%
08G	Other manicuring preparations	2-5%
09A	Dentifices	10.5%
09B	Mouthwashes and breath fresheners	0.38%
10B	Deodorants not spray	 0.5%
11A	Aftershave lotions	0.005%
11D	Preshave lotions (all types)	0.0005%
12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.015-0.2%
12C	Face and neck products not spray	 0.4-2%
12D	Body and hand products not spray spray	 0.1-0.94% 0.42%
12J	Other skin care preparations	0.2-1.4%

*Product category codes used by FDA

Information collected in 2012
Table prepared: January 23, 2013
Updated May 22, 2013: Corrected 94% body and hand product to 0.94%

Cosmetic Ingredient Review

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: F. Alan Andersen, PhD
Director, CIR
Date: May 31, 2013
Subject: Wave 2 for Retinol

The Council has forwarded information provided by the Industrieverband Korperpflege- und Waschmittel e.V. (IKW) regarding safety test data for retinol and retinyl palmitate. IKW pulled these guinea pig and human data together based on unpublished studies from the late 1980's through the mid-1990's and extracted relevant information for our use. You may note that the original unpublished studies listed at the end of Dr. Schilling's extracted summary include the phrase "confidential data." That applies to those original unpublished studies, not to the extracted information prepared specifically by Dr. Schilling for the IKW so that we could use the data.

Included in these data are:

1. Guinea pig phototoxicity study. 5 female and 5 male animals. Retinyl palmitate, undiluted, applied at 0.25 ml/cm^2 to shaved flank. UVA exposure from FS40 black light lamps at 20 J/cm^2. Sites examined at 24, 48, and 72 hours. Unirradiated site was used as control. No phototoxicity.

2. Guinea pig maximization study. 5 female and 5 male control animals. 10 female and 10 male test animals. Induction: Retinyl palmitate at 10% in olive oil. Freund's adjuvant injected followed by retinyl palmitate surface application followed by UVA (FS40 black light lamps) at 10 J/cm^2 and UVB (sunlamp) at 1.8 J/cm^2. Repeated 4x (without the adjuvant). Control group received Freund's only. Challenge: Retinyl palmitate at 3, 10, 30, or 100% followed by 10 J/m^2 UVA. Study found adjuvant related effects only. No photosensitization.

3. Human phototoxicity study. 12 (both sexes) volunteers with predetermined MED. Moisturizer cream with 0.15% retinol for 24 hours. UVA at 10 MED and UVB at 0.5 MED (sequentially, using filters) from a solar simulator. Retinol alone, retinol with UV, and UV alone were evaluated in each subject. Sites examined at 24 and 48 hours post-irradiation. No phototoxicity.

4. Human phototoxicity study. 11 female volunteers. Formulation with 0.04% retinol 2x per day for 4 wks. Before first application of test material and at end of wks 1, 2, and 4 each subject received 1 MED of UVA+IVB (solar simulator). No phototoxicity.

5. Human phototoxicity study summary. 10 volunteers. Anti-wrinkle cream with 0.04% retinol. No other details. No phototoxicity.

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6. Human photosensitization study. 29 (both sexes) volunteers with predetermined MED. An antiperspirant without retinol was mentioned but not further discussed. Induction: moisturizer cream with 0.15% retinol was applied and 24 hours later the sites were exposed/unexposed to UV (1 MED (?) from a solar simulator) – repeated for a total of 6x. Rest for 2 wks. Challenge: test material applied for 24 hours then UVA only at 10 MED. Sites scored at 0, 24, and 48 hours post-irradiation. No photosensitization.

7. Human photosensitization study summary. 28 volunteers. Anti-wrinkle cream with 0.04% retinol. No other details. No photosensitization.

8. Use study. No information on UV exposure. Moisturizer with 0.15% retinol for 6 wks in 12 female volunteers. Follicular biopsy for micro-comedones performed. No micro-comedones reported.

9. Clinical effectiveness test. No information on UV exposure. 34 patients with sun-damaged skin used a moisturizer cream with 0.04% retinol for 16 wks. Three patients reported frequent redness.

10. Clinical effectiveness test. No information on UV exposure. Number of patients not given. Patients with sun-damaged skin used a moisturizer with 0.3% retinol, or an anti-wrinkle cream with 0.04, 0.075, or 0.1% retinol. Slight scaling/edema were reported in the first week for the 0.1% retinol-containing anti-wrinkle cream users only.

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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

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

DATE: May 31, 2013

SUBJECT: Retinol and Retinyl Esters: Summary of Phototoxicity Data

Schilling K. 2013. Extract on phototoxicity of the dossier on retinol and retinyl ester to hand over to CIR.

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Extract on Phototoxicity of the dossier on Retinol and Retinyl ester to hand over to CIR

[redacted]

Sponsor:

Industrieverband Körperpflege- und Waschmittel e.V.(IKW)
The German Cosmetic, Toiletry, Perfumery and Detergent Association
Mainzer Landstr. 55
60329 Frankfurt am Main, Germany

on behalf of a consortium of interested parties

Author:

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Am Grenzgraben 11
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Dr. Karsten Schilling
Senior Manager Regulatory Affairs

SCC-Project: CH108-00001

Date: 24 May 2013

1 Phototoxicity/photoirritation in experimental animals

Study design

Guideline/method:	CTFA Safety Testing Guidelines for evaluating photodermatitis and according to Harber LC and Shalita AR (1975)
Species/strain:	Guinea pig/Himalayan
Group size:	5 male and 5 females
Test substance:	Retinyl palmitate
Batch:	710758 (purity: about 95%, 1.7 mio IU/g (935 mg RP/g))
Route:	Open epicutaneous induction
Carrier:	Not applicable
Dose level:	Undiluted
Light source:	UV Lamp Westinghouse FS 40 "Black Lamp (1x10 exp. 4 Ergs/cm²/sec, 320 – 400 nm)
Irradiation:	20 J/cm² UV-A
GLP:	Yes
Published:	No

The phototoxic property of retinyl palmitate (1.7 mio IU/g) was evaluated according CTFA safety testing guidelines and methodology published by Harber and Shalita (1975) using male and female albino Himalayan guinea pigs.

One day prior to application, the animals were fasted. Both flanks were shaved and the animals were anaesthetized. Thereafter, each 5 male and 5 female animals received 0.025 mL/cm² by an open application using a syringe on the right and left flanks. About 30 min after application of the test item to the left flank, the animals were exposed to non-erythrematogenic UV-A irradiation (20 J/cm²). After irradiation the right flanks were treated with the test item but remained unexposed to light serving as control sites. The animals were examined 24, 48 and 72 after application for signs of erythema and edema and the skin reactions were graded according a scale from 0 – 4. In addition, the animals were observed at least once daily for mortality and clinical signs and were weighed five days prior to treatment and at day one and at termination.

Results

No mortality or clinical sign of toxicity occurred and the body weight gain was not affected. A transient discoloration of the treated skin was observed during day 2 – 4 of the study but no skin finding indicative for a phototoxic reaction could be noted in any of the treated male and female animals either with or without UV-A irradiation.

Conclusion

Under the conditions of the study undiluted retinyl palmitate (1.7 mio IU/g (935 mg RP/g)) was shown to have no phototoxic or irritant potential in male and female guinea pigs.

(Reference: 125)

2 Photosensitization in experimental animals

Study design

Guideline/method:	CTFA Safety Testing Guidelines for evaluating photodermatitis and according to Harber LC and Shalita AR (1975)
Species/strain:	Guinea pig/Himalayan
Group size:	Control group: 5 males and 5 females Test group: 10 males and 10 females
Test substance:	Retinyl palmitate
Batch:	710758 (purity: about 95%, 1.7 mio IU/g (935 mg RP/g))
Route:	Epicutaneous induction and challenge
Carrier:	Olive oil
Dose level:	Induction: 10% in olive oil Challenge: 3, 10, 30% in olive oil and 100%
Light source:	UV-A: Westinghouse FS 40 "Black Lamp (1x10 exp. 4 Ergs/cm²/sec, 320 – 400 nm) UV-B: Philips UV-B Sunlamp TL/12
Irradiation:	UV-A: 10 J/cm² UV-B: 1.8 J/cm²
GLP:	Yes
Published:	No

The contact photosensitizing potential of retinyl palmitate (1.7 mio IU/g) was evaluated according CTFA safety testing guidelines and methodology published by Harber and Shalita (1975) using male and female albino Himalayan guinea pigs.

Within the induction procedure, the animals of the test group were shaved shortly prior to the test item application in the nuchal skin area on an area of 8 cm², marked by 4 intradermal injections of Freund's complete adjuvant and physiological saline 50:50 (0.1 mL) into the corners. This was followed by application of 0.1 mL of 10% test item. About 30 min thereafter, the test site was exposed to 1.8 J/cm² UV-B and 10 J/cm² UV-A irradiation. The topical application and irradiation procedure was repeated 4 times in 2 weeks without adjuvant injection.

The animals of the control group received only 4 intradermal adjuvant injections in the corner at the 8 cm² nuchal skin area but without any further treatment.

For challenge, three weeks after the start of the induction period, the animals were fasted one day prior to application, both flanks were shaved and the animals were anaesthetized. Thereafter, the animals received 0.025 mL/2 cm² of 3, 10, 30 and 100% test item preparations by an open application using a syringe on left flanks. About 30 min thereafter, the animals were exposed to 10 J/cm² UV-A irradiation. The right flanks were treated accordingly but without irradiation. The animals were examined 24, 48 and 72 after application for signs of erythema and edema and the skin reactions were graded according a scale from 0 – 4. In addition, the animals were observed at least once daily for mortality and clinical signs and were weighed at pre-treatment, start of treatment and at termination.

Results

With the exception of one spontaneous death of a control female, no mortality or clinical sign of toxicity occurred and the body weight gain was not affected.

The animals of the vehicle control and of the test group showed a transient edema and erythema in the neck area of grade 1–4 lasting from day 2–7; skin necroses were observed from day 8–12 and exfoliation from day 13–26. These finding originated from the intradermal adjuvant injections.

Beside these adjuvant related skin findings neither erythema nor edema formation could be observed at any challenge readings in any of the irradiated or non-irradiated animals.

Conclusion

Under the conditions of this study retinyl palmitate (1.7 mio IU/g (935 mg RP/g)) was shown to possess no photoallergic potential in male and female guinea pigs when tested either as oily emulsion of up to a concentration of 30% or undiluted as supplied.

(Reference: 128)

3 Phototoxicity In Human

Study Design:

Guideline/Method:	Human phototoxicity test according an approved study protocol and standard operating
Species:	Human
Group size:	12 volunteers (males and females)
Test substance:	Moisturizer cream [redacted] 0.15% retinol)
Batch:	No information available
Route:	Occlusive epicutaneous application
Light source:	UV-A and B from an Oriel 1000 W solar simulator
GLP:	Yes
Published:	No

The phototoxicity of the moisturizer cream containing 0.15% retinol was assessed in 12 human volunteers according to an approved study protocol and under GLP conditions.

Prior to application, a slurry was prepared and an unchanged amount of 0.2 – 0.3 g of the test substance was applied to each patch. There were 3 sets of treatment sites. One set received the material and UV light, the 2nd set received the material but no UV light and the 3rd set was a single site receiving only UV light. A maximum of 2 duplicate patches were applied to the backs or other non- tanned areas of the body. A maximum of 10 days prior to the start the individual minimum erythema dose (MED) was determined according to specific guidelines by exposing the skin to gradually increasing UV light doses using a Oriel 1000 W solar simulator.

Each test substance was applied under occlusive conditions and remained for about 24 h. One set of treated sites was a control for inherent Irritation potential of the formulations and was covered to avoid irradiation. The second set of treated sites was always irradiated first with ultraviolet A-wavelength range (UV-A), then with ultraviolet B-wavelength range (UV-B). A site adjacent to the irradiated sites was not treated with formulations and was irradiated at the same time as the treated-irradiated sites. This site was a control for inherent irritation potential.

For irradiation two sets of stripped sites were treated as described. After the sites had dried, one set of treated sites was covered and the other set of treated sites and the untreated site were exposed to UV-A light. The time exposure was equal to 10 times the MED equivalent (MED previously determined separately for each subject with UV-A and UV-B light). The light was

filtered with clear window glass. Following irradiation with UV-A, the filter was removed from the light source and the irradiated sites further exposed to 0.5 MED of UV-A/UV-B light.

An evaluation of the amount of skin reactions present at all sites was made about 5minutes after the second irradiation. Sites were then lightly covered with non-woven cotton cloth. All sites were re-examined for irritation at 24 and 48hours after irradiation (48 and 72hours alter test material application). Forty-eight and 72 hours alter test material application, each site was evaluated and skin reactions recorded.

Results

On the irradiated sites all volunteers had faint, minimal erythema at the immediately grading, but no skin reactions were observed at the 48 and 72 h readings and the respective score was0.

Concluslon

In this phototoxicity test the investigated moisturizing cream containing 0.15% retinol did not induce a dermal phototoxic response under the conditions of the study in male and female Human volunteers.

(Reference: 81)

A further phototoxicity study with a 0.04% containing retinol formulation [redacted] was carried out in 11 healthy female volunteers when applied to the volar forearm for a period of 4 weeks. The test substance was applied unchanged twice daily (in the morning and evening) under occlusive condition at an amount of about 2 mg/cm². Prior to the start and at the end of 1, 2 and 4 weeks all volunteers received their MED of solar simulated light determined on both forearms. The simulated light was generated using a model I5S Solar UV simulator model XPS 200 Xenon lamp power supply and a model DCS-1 dose controller system. The skin reactions were evaluated prior to the start and at the end of 1, 2 and 4 weeks and scored using a 4 grade scoring system. The 0.04% retinol containing formulation caused no phototoxic response under the respective study conditions.

(Reference: 88)

A safety test summary report on a anti-wrinkle cream [redacted] containing 0.04% retinol did not report a phototoxic potential in 10 human volunteers.

(Reference: 113)

4 Photosensitization In Human

Study Design:

Guideline/Method:	Human photoallergy test according to an approved study protocol and standard operating procedures
Species:	Human
Group size:	29 volunteers (males and females)
Test substance:	Moisturizer cream [redacted] 0.15% retinol)
Batch:	No information available
Route:	Occlusive epicutaneous application
Light source:	UV-A and B from an Oriel 1000 W solar simulator
Light dose:	UV-A = 2.85 mJ/cm²/sec UV-B = 3.78mJ/cm²/sec
GLP:	Yes
Published:	No

A comparative photoallergy study using an antiperspirant roll-on without retinol (product A) and a moisturizer cream containing 0.15% retinol (product B) was performed in a panel of 29 male and female volunteers according to an approved study protocol and under GLP conditions.

An occlusive clinical patch was used. For product A, a slurry was prepared and 0.2 to 0.3 g applied to each patch. Solid test material product B was left dry and 0.2 to 0.3 g applied to each patch (1 cm²). Each test material was applied under an occlusive patch to the designated test site two times a week for a total of six applications. Both sites on the back were patched with the test material in the same sequence. The patches remained in place and the area was kept dry for 24 hours at which time the study coordinator removed them at the test laboratory. The test sites were observed by the study coordinator and graded.

After the test sites were graded, one of the test sites on the back was irradiated with UV-B and UV-A. The other sites were not irradiated (control sites) and were protected from the light source by a flexible lead shield. The test sites (irradiated back sites) were irradiated with UV A and UV-B from the Orial Solar Simulator. This simulator delivered both UV-A and UV-B (UV-A = 2.85 mJ/cm²/sec, UV-B = 3.78mJ/cm²/sec).The irradiation site also had a control site that received light only and had no product applied. The irradiation period was based on the subject's MED. A maximum of 10 days before the start of the study, each subject's Minimal Erythema Dose (MED) was determined.

After each rest period, the test sites were graded by the study coordinator and fresh test material and patches were applied to the identical test sites until six induction patching and irradiations were completed.

Subjects were cautioned to protect the test sites from exposure to sunlight during the study.

A rest period of approximately two weeks followed the application of the last induction patches; no test materials were applied during this rest period. The minimum test interval was 10 days.

Following the test period, a challenge patch of each test material was applied adjacent to the induction site. Twenty-four hours later, the patches were removed by the study coordinator and the challenge sites were scored. The designated test sites were irradiated with UV -A only at an irradiation time of ten times the MED. Immediately after the irradiation, the challenge sites were scored again. The sites were scored again at 24 and 48 hours post-irradiation.

Results

There was no adverse reaction reported. The non irradiated sites showed no skin reaction during the induction and at the challenge. The irradiated sites had numerous faint, minimal irritation during the induction and at the challenge but no skin reaction could be seen anymore at the second and third readings.

Conclusion

There was no indication for a phototoxic or photoallergic skin reaction and thus, the moisturizing cream containing 0.15% retinol was shown to be not a photosensitizer when tested in male and female human volunteers.

(Reference: 83)

The same results was observed on an anti-wrinkle cream [redacted] containing 0.04% retinol as no indication for a photo-allergic potential was reported in a safety test summary report after investigation in 28 human volunteers.

(Reference: 113)

A study comprising a six-week assessment of two moisturizer creams containing 0.15% retinol (application under an semi-occlusive patch) in each 12 female human volunteers showed no comedogenic effects for either test item, when each test site was sampled by cyano-acrylate follicular biopsy technique and the slides were examined for the presence of micro-comedones. The controlled, randomized and blind study was performed according to a previously approved protocol under GLP conditions.

(Reference: 85)

For completeness sake, it should also be mentioned that various products were also tested in clinical trials using patients suffering from sun-/or photo-damages facial skin under a double blind study design with controlled, randomized conditions. In a 16-week clinical evaluation of two night time moisturizer creams (0.04% retinol and placebo creams), very few adverse experiences were reported during the treatment period. Three of 34 patients recorded frequent redness. In another three-month clinical investigation with a night time moisturizer cream (0.3% retinol) and anti-wrinkle creams (0.04, 0.075 or 0.1% retinol) resulted in slight scaling or edema after the first week only in participants using the 0.1% formulation.

(References: 79, 87)

5 References

Ref 79 [redacted] (1991) Nighttime facial moisturizer with 0.3% Retinol cosmetic use of formula [redacted] in Italy, Protocol 16375, Pharmacy Code 90-119, [redacted] unpublished data, 08 May 1991, confidential data

Ref 81 [redacted] (1993) Phototoxicity test, Study No. JJ PT-3, Education & Research Foundataion, Lynchburg, Virgina, USA, unpublished data,17 December 1993, confidential data

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Ref 85 [redacted] (1995) An assessment of the reaction profile of two moisturizer creams in human subjects, Ref. No. 95-4689-74, Hill Top Res. Inc., East Brunswick, NJ, USA, unpublished data, 31 March 1995, confidential data

Ref 87 [redacted] (1996) A clinical evaluation of two moisturizer creams (Retinol night cream versus placebo night cream), study report, 19321.27A, [redacted] Skin Study Center, Broomall, PA, USA, unpublished data, 19 February 1996, confidential data

Ref 88 [redacted] (1996) Evaluation of the photoreactivity potential of a retinol product during 4 weeks application to human skin, S.K.I.N. Incorporated Project No. 95-46, S.K.I.N. Inc., Conshokocken PA, USA, unpublished data, 15 May 1996, confidential data

Ref 113 [redacted] (1997), Product Safety Report, Healthy Skin Anti-Wrinkle Cream for Europe, Formula# [redacted], Safety Test Summary Report, [redacted] unpublished data, 16 September 1997

Ref 125 [redacted] (1988) Investigation of phototoxic potential of [redacted] Vitamin A palmitate 1.7 mio IU/g L, [redacted] – in albino Guinea pigs, [redacted] unpublished data, 29 December 1988, confidential data

Ref 128 [redacted] (1989) Determination of photoallergenicity of [redacted] Vitamin A palmitate 1.7 mio IU/g L, [redacted] in albino Guinea pigs [redacted] unpublished data, 08 March 1989, confidential data

Cosmetic Ingredient Review

Commitment & Credibility since 1976

May 24, 2013

MEMORANDOM

To: CIR Expert Panel and Liaisons

From: Lillian C. Becker, M.S.
Scientific Analyst and Writer

Subject: Tentative Safety Assessment For Tromethamine, Aminomethyl Propanediol, And Aminoethyl Propanediol As Used In Cosmetics; Wave 2

Industry has submitted data on impurities in tromethamine. Below is a summary of the attached information.

Impurities

A manufacturer reported that cosmetic grade tromethamine was 99% pure. Secondary amines, anhydrous were present at a maximum of 0.5% wt; nitrosamines at 50 ppb, and water at 0.5% wt. Nickel, used in the manufacture of tromethamine, was present at < 10 ppm; other metals are not expected to be present due to non-use. Methanol, used as a solvent in the manufacture process, is limited to 3000 ppm; the typical value is < 100 ppm.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

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

DATE: May 21, 2013

SUBJECT: Tromethamine Purity/Impurities

Troester LE. 2013. Letter to Carol Eisenmann concerning the composition purity and impurity information for TRIS AMINO Ultra PC Tromethamine.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

The Dow Chemical Company
1500 E. Lake Cook Road
Buffalo Grove, IL 60089
U.S.A.

21 May 2013

VIA E-MAIL

Carol Eisenmann
Personal Care Products Council
1101 17th Street, NW, Suite 300
Washington, D.C. 20036-4702
Tel: (202) 331-1770
Fax: (202) 331-1969
E-Mail: eisenmannc@personalcarecouncil.org

Dear Carol,

This letter is in response to your request from the Personal Care Products Council and Cosmetic Ingredient Review Board (CIR) for composition, purity, and impurity information for **TRIS AMINO** Ultra PC Tromethamine**, a product of ANGUS Chemical Company, a subsidiary of The Dow Chemical Company.

Composition:

Component	CAS #	EC No:	Weight
Tris(hydroxymethyl)aminomethane	77-86-1	201-064-4	99%

INCI (CTFA) Name: Tromethamine

Product Grade:
This product is a cosmetics grade product. It does not have USP or FDA grade status. EU Cosmetics Directive 76/768/EEC and Amendments

Purity:
This product meets the following requirements as per the sales specifications for this product:

Purity, Anhydrous	99.0 Min	% wt.
Secondary Amines, Anhydrous	0.5 Max	% wt.
Nitrosamines	50 Max	ppb
Water	0.2 Max	% wt.

Metals:
Although we do not test for the materials routinely, this product is not produced with or manufactured using cadmium, chromium, lead, arsenic, or mercury, and therefore, would not be expected to contain any of these substances. This product is made from an ingredient which uses a very small amount of nickel in the manufacture of that ingredient. Although we do not analyze for nickel routinely, occasional samples of similar Tris(hydroxymethyl)aminomethane products have been tested for nickel and the results of those tests have shown less than 10 ppm of nickel.

Residual Solvents According to ICH (International Conference Harmonization):
Due to the manufacturing process, this product does not contain any residual solvent except traces of methanol. This solvent is classified as Class 2 according to ICH and our product contains less than the allowed limit of 3000 ppm of methanol. In fact, a recent assessment indicated a typical value of less than 100 ppm of methanol.

I hope that this information meets your needs. Please do not hesitate to contact me if you have any further questions regarding ANGUS products.

Sincerely,

Linda E. Troester

Linda E. Troester
ANGUS EH&S Product Stewardship Manager
Tel: 847-808-3432 / Fax: 847-808-3701
E-mail: LTroester@dow.com
LET/nf

C: Brian Hughes, The Dow Chemical Company

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

Date: June 10, 2013

From: Bart Heldreth, Ph.D., Chemist CIR

To: CIR Expert Panel Members, Liaisons and the general public

Re: Memo on CIR SSC 2014 Priority List comments

The CIR Science and Support Committee (CIR SSC) has submitted a second set of comments on the 2014 Priorities List, which is to be finalized at this CIR Expert Panel meeting. The first suggestion made by the CIR SSC is to add rationale and/or description of the parameters for each ingredient group as proposed. We have independently come to the same conclusion and plan to implement this addition starting with the 2015 Priorities List.

The CIR SSC has questioned the rationale for grouping all of the algae ingredients of different species in one report, as proposed. We believe this grouping is appropriate in light of the definition of Algae Extract (805 reported cosmetic uses), which recites "Algae Extract is the extract of various species of *Algae*." It is difficult to see how we would NOT include different algae species.

That said, we agree with the CIR SCC's opinion that only eukaryotic algae should be reviewed in the proposed Algae report. However, as the INCI Dictionary does not provide an indication of each ingredient's life domain, and all of the ingredients proposed for this group have the INCI chemical class "Botanical Products and Botanical Derivatives" (i.e. are purportedly plant products and not prokaryotes), the removal of any algae ingredients, which fall under the bacteria or archeae domains, will be finalized when each ingredient has been analyzed in further detail at the SLR preparation stage (or earlier if such information is provided to us prior to our own investigations).

Regarding comments to the limits of the proposed *Ginko biloba* ingredient group, we have endeavored to provide the CIR Expert Panel with the greatest opportunity for efficient productivity, and chose to be all inclusive of *Ginko* ingredients, rather than limit to the plant leaf ingredients. While the Expert Panel has elected to limit some groupings to a specific plant part in a past report, the Panel has also retained groupings that encompass ingredients prepared from different parts of a plant, in another report. We believe that it will be evident to the Panel as to setting limitations on the grouping of *Ginko* ingredients once all of the information on the ingredients is before them in a Draft Report.

We again concur with the CIR SSC on their opinion that a description of ingredient grouping rationales would be helpful at the priorities setting stage, including for example the Phospholipids grouping. This type of further information will be provided in future Priorities setting drafts, starting with those for 2015. The rationale behind this particular grouping was to combine phospholipid ingredients which share the commonality of having one of the primary hydroxyl groups of glycerin esterified with phosphoric acid (where the phosphoric acid may carry an additional ester grouping), and the two

remaining hydroxyl groups of glycerin are esterified with simple long chain, saturated or unsaturated fatty acids (i.e. are phosphoglycerides).

The CIR SSC has pointed out that there are additional vinyl-type styrene copolymers that could be added to the proposed Styrene and Vinyl-Type Styrene Copolymers report, which are non-obvious from their INCI names of Polyacrylate-x. We concur and propose adding these eight ingredients to the report: Polyacrylate-2, -5, -12, -15, -16, -18, -21, and -30.

We fully agree with the CIR SSC that the search strategy of the cell culture ingredients in the proposed *Centella* report should specifically include information on culture-derived ingredients. If no information is found, the Expert Panel will then have the options, *inter alia*, to limit this report to non-cultured ingredients or make a culture-derived ingredients-specific insufficient data announcement.

The point provided by the CIR SSC to expand the grouping in the proposed Alky Phosphates report to other salts and alkyl ester chain lengths is conceded and we propose to add the following 15 additional ingredients to the report: 1) C8-10 Alkyl Ethyl Phosphate, 2) C9-15 Alkyl Phosphate, 3) C20-22 Alkyl Phosphate, 4) Castor Oil Phosphate, 5) Cetearyl Phosphate, 6) Cetyl Phosphate, 7) Disodium Lauryl Phosphate, 8) Disodium Oleyl Phosphate, 9) Lauryl Phosphate, 10) Myristyl Phosphate, 11) Octydecyl Phosphate, 12) Oleyl Ethyl Phosphate, 13) Oleyl Phosphate, 14) Sodium Lauryl Phosphate, and 15) Stearyl Phosphate.

While there is no requirement for CIR to publish an annual list of scheduled re-reviews or groupings (or to seek comment), we greatly appreciate the comments received on our potential expansions to include additional ingredients. The Expert Panel's consideration of these comments is requested, but such consideration need not impede the issuance of a Final 2013 Priorities List, as finalization of re-reviews and re-review groupings is not required.

Additionally, the CIR policy for re-reviewing ingredients has adhered to a somewhat arbitrary fifteen year cycle. Perhaps, for greater planning flexibility and efficiency, it would be beneficial to have a more flexible fifteen *to twenty year* cycle for re-reviewing ingredients. The Panel has authority to make changes to this policy if they so desire. In our planning for future years beyond 2014, we have noticed that the number of re-reviews that would be thrust, by a rigid fifteen year clock, on the Panel between 2015 and 2019 would be quite high. We therefore request that an adjustment be made to this policy, so that we may better arrange the schedule in those and future years.

We have proposed that Glyceryl Stearate and Glyceryl Stearate SE could be re-opened in a re-review to add a multitude of other glycerol esters. The CIR SSC has suggested that we might wait to re-review Glyceryl Stearate and Glyceryl Stearate SE (last reviewed in 1984) until we re-review Glyceryl Monoesters (reviewed in 2004 and due for re-review in 2019) and include them therein. However, a third option may exist, according to the Expert Panel's prerogative. In this third option, the Panel may elect to simply add Glyceryl Stearate and Glyceryl Stearate SE, administratively, to the Glyceryl Monoesters report now, as these two ingredients fit well within the parameters of the Glyceryl Monoesters report grouping, have already been concluded as safe, and could (and maybe should) have been added to the Glyceryl Monoesters report originally. The Expert Panel has set some precedence for adding ingredients to reports administratively, in the Alkyl PEG Ethers report, the PEG's report, and the PPG's report (therein, if additional ingredients fitting within the parameters of the grouping were to be added to the dictionary after the final report was issued, those additional ingredients would be covered by Panel's conclusion). These options may warrant some discussion, if not a full conclusion on the subject matter should time permit.

The CIR SSC suggested that the proposed Ascorbic Acid, Esters, and Phosphates report grouping should not include any phosphate ingredients, as the original report did not contain any phosphates. We respectfully disagree. The proposed grouping of Ascorbic Acid, Esters, and Phosphates is actually an amalgamation of two prior reports. The first is the final safety assessment of L-Ascorbic Acid, Calcium Ascorbate, Magnesium Ascorbate, ***Magnesium Ascorbyl Phosphate***, Sodium Ascorbate, and ***Sodium Ascorbyl Phosphate*** (2005). The second report is the final safety assessment of Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid (D-enantiomer of Ascorbic Acid), and Sodium Erythorbate (1999). Since these two reports are the basis for the proposed Ascorbic Acid, Esters, and Phosphates report, we believe that the addition of phosphate containing ingredients therein is reasonable.

In 1984, the Expert Panel reviewed Nonoxynol-2, -4, and -8 through -50, concluding those ingredients were safe as used. In 1998, the Panel reviewed Nonoxynol-1 through -8, concluding those ingredients were safe at concentrations less than 5%. We have proposed that the Panel may now combine all of the Nonoxynol ingredients into one re-review, possibly with a split conclusion. The CIR SSC has suggested that the Panel may wish to keep them separate. It is the Panel's prerogative as to whether all of the Nonoxynol ingredients should now be reviewed together or not. However, both reports are due for re-review.

Hydroxystearic Acid was originally reviewed as a one ingredient report and is now due for re-review. The CIR SSC has suggested that this report may be re-opened to add the two simple salts, Sodium Hydroxystearate and Potassium Hydroxystearate. The Expert Panel, however, may consider an alternative plan to not re-open this report, but to administratively add these two ingredients to the original Hydroxystearic Acid report. The CIR SSC has also recommended that the Panel should consider whether it is appropriate to re-open this report to add other hydroxy fatty acids to this report. Seven such additional ingredients were found in the INCI Dictionary for the Panel's consideration: C10-40 Hydroxyalkyl Acid, Hydroxycapric Acid, Hydroxycaprylic Acid, 10-Hydroxydecanoic Acid, 10-Hydroxydecenoic Acid, Hydroxylauric Acid, and Hydroxyundecanoic Acid.

The CIR SSC suggested that PEG-9 and PEG-25 Diethylmonium Chloride be deleted from the re-review priority list, and we concur.