Memo
Agenda
Minutes
Formaldehyde
Human Umbilical Extract

CIR EXPERT PANEL MEETING
DECEMBER 13-14, 2010
Memorandum

To: CIR Expert Panel Members and Liaisons
From: Director, CIR
Subject: 117th Meeting of the CIR Expert Panel
Date 18 November 2010


We will reserve rooms for the nights of Sunday, December 12th and Monday December 13th. If you encounter any problems, please contact me on my cell phone at 301-512-7846.

The agenda includes consideration of 14 reports. The .pdf's of each book will be accessible on the CIR website.

I’m very pleased to let you know that Dr. Ivan Boyer has joined CIR as senior toxicologist. Ivan received his MS and PhD degrees in toxicology from the University of Rochester School of Medicine and Dentistry. He did a post-doc at the Robert Wood Johnson Medical School in N.J. He has over 20 years of work experience including the FDA’s CFSAN early in his career, a stint at the Waste Policy Institute in San Antonio, and most recently at Noblis, Inc. (formerly The MITRE Corporation and Mitretek Systems) in Virginia.

Team Meetings - remember, juice and coffee on day 1 will be available at 8:00 am and meeting starts at 8:30 am.

Two new re-review packages (buff book) are provided for your consideration.

1. Formaldehyde – this ingredient was re-reviewed in 2003, and the limit of 0.2% free formaldehyde was confirmed. As a reminder from the original safety assessment back in 1984, formaldehyde in water (aka formalin) rapidly converts to methylene glycol (itself a cosmetic ingredient) and an equilibrium is maintained that strongly favors methylene glycol. FDA has asked CIR to look at these two ingredients and consider how to address the safety of not-yet-reviewed methylene glycol. Doing a re-review of formaldehyde appeared to be a good way to kill two birds with one stone, so to speak. Recent reports from OSHA in Oregon and from Health Canada have found levels of formaldehyde on the order of 10% in Brazilian hair treatments. New epidemiology studies are available addressing the possible link between formaldehyde and leukemia. If the Panel determined to reopen this safety assessment, then an opportunity exists to add methylene glycol.

2. MEA/DEA/TEA – the scientific issues surrounding ethanolamine fatty acid carcinogenesis have been discussed several times at CIR Expert Panel meetings. In doing the housekeeping for the re-review process, however, we found that we have never re-reviewed the original 1983 safety assessment of monoethanolamine (now, just ethanolamine), diethanolamine, and triethanolamine. That is an error of omission. Since there are lots of other re-reviews coming up over the next year or two that involve ethanolamine containing ingredients, we figured we’d better do this re-review as a baseline for all the others and fix the error of omission. For your convenience, we have separated each of the three ingredients into its own little report within the package. If you decide to reopen any or all, that will make it easier.
New data have been provided on one ingredient (buff book) for which the Panel had reached an insufficient data conclusion.

3. **Human Umbilical Extract** – back in 2002, CIR published its final safety assessment of ingredients derived from human and animal placentas and umbilical cords. The available data were considered insufficient to support safety for Human Umbilical Extract, for which there were no reported uses.

   Procedurally, that means that Human Umbilical Extract is on the “Insufficient Data – No current uses” list with the expectation that if there were to be usage in the future, sufficient data to support safety would be provided. We have received correspondence indicating that a company intends to use Human Umbilical Extract in cosmetic products. They have provided a number of pieces of information to support the safety of this ingredient. The question for the teams: Do these additional data provide a basis for reopening this safety assessment to change the conclusion for Human Umbilical Extract?

There are four reports advancing in the process.

4. **Alkyl Benzoate group** – back in August, the Panel issued an insufficient data announcement, asking for irritation and sensitization data, genotoxicity data, dermal penetration data (and if significantly absorbed, reproductive and developmental toxicity data), and clarification of mode of action (if significantly absorbed) for increased tumor growth in oral studies. Some data have been received and are incorporated into the draft report. We expect that more data will be included in the wave 2 package you will receive.

   So, we are asking you to sort through what you now have, make your own check marks on the available data profile when you see that a new piece has become available, and we can try to move this forward at this meeting. We will provide an up-to-the-minute available data profile at the meeting.

5. **Caprylyl Glycol group** – back in August, the Panel issued an insufficient data announcement. We received technical comments from the Council, along with new unpublished data. The technical comments have been addressed and the new data added. We have also received a request from the Council to shorten the list of ingredients included in this safety assessment.

   So, there are two questions for the teams to resolve: Are the data now sufficient for any/all the ingredients? Is their a basis for deleting certain ingredients from the safety assessment? This report should be issued as a tentative safety assessment at this meeting.

6. **Disperse Blue 1** – back in April, the Panel decided to re-open this safety assessment and re-evaluate the carcinogenicity and risk assessment data. After a presentation by Dr. Sam Cohen at the August meeting, the Panel tabled discussion to include an updated MOS determination. Approaches to evaluating the mode of action in the formation of smooth muscle tumors also were sought. We have an updated MOS and a risk assessment for smooth muscle tumors based on input from the Council and additional evaluation by Dr. Boyer. Based on this information, the Panel may proceed to issue a tentative amended safety assessment or determine that the original conclusion is valid and not reopen.

7. **Edible oils group** – back in August, the Panel combined vegetable and nut oils into one report. While the Panel agreed that the edible oils for which fatty acid profiles were available (i.e., most of the ingredients) could be determined to be safe in the present practice of use and concentration, there was concern over ingredients for which fatty acid profile data were not available (46 ingredients).
Just in the time since we finalized the draft report and the cover memo for this package, more data have become available and we now appear to have winnowed the list at list down to just 23 that don’t yet have fatty acid profiles — and that number is decreasing daily! The problem of course is that means there will be more data that will come in wave 2, including an updated table that shows how many ingredients have composition data. Again we hope you will be able to sort through those data, add to the list of oils for which fatty acid profiles are available and move this forward.

There is no reason why the report conclusion could not list those edible oils that are considered safe and those for which the data are insufficient (maybe as few as 3!). The report should be issued as a tentative safety assessment and an opportunity again provided to submit data.

There are seven reports to be issued as final safety assessments.

8. Alkyl PEG Ethers – Back in June, the Panel issued a tentative report that encompassed the entire family of alkyl PEG ethers used in cosmetics. This report was originally brought forward as a possible re-review of laureth-4 and laureth-23, but Dr. Heldreth noted that there are many ingredients, all of which are alkyl PEG ethers. On that basis, a grouping of ingredients based on structural and functional similarities was created, and the Panel agreed to re-review laureth-4 in order to include these ingredients. Many of the ingredients included in this family have been reviewed previously, as have many of the components of these ingredients, and the information included in those original reports is used to support the safety of the entire Alkyl PEG Ether family. While not a new approach, this was the first time an ingredient family of this size was created. Technical comments have been addressed and this report is ready to be issued as a final safety assessment.

9. CAPB group – back in August, the Panel considered the presentation on the quantitative risk assessment approach given by the Personal Care Products Council’s Task Force on Sensitization Risk from CAPB Impurities. From the information presented at the meeting and the data in the report, the Panel issued a Tentative Report with the conclusion that CAPB and its related amidopropyl betaines are safe in cosmetics as long as they are formulated to be non-sensitizing based on a QRA. Technical comments have been received and should be discussed. This report should be issued as a Final Amended Safety Assessment.

10. Dicarboxylic Acids group – back in August, the Panel, issued a Tentative Report with the conclusion that the 12 dicarboxylic acids and salts and 44 esters of dicarboxylic acid that are included in this report are safe for use in cosmetic products in the present practices of use and concentration. Were ingredients in these groups not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to other in these groups. Technical comments have been addressed and this report is ready to be issued as a final safety assessment.

11. Dimethiconol group – back in August, the Panel issued a tentative report with a safe as used conclusion on these ingredients. All of the unpublished data received prior to that meeting have now been incorporated into the report. Technical comments were received from the Council and addressed. After careful review of the discussion, this report is ready to be issued as a final safety assessment.

12. Isoparaffins group – back in August, the Panel issued a tentative report with a conclusion stating that the isoparaffins are safe for use in cosmetics when formulated to be non-irritating. Technical comments from the Council have been addressed. After considering recent additional comments from industry, this report is ready to be issued as a final safety assessment.
13. Triclosan – back in August, the CIR Expert Panel issued a tentative report, concluding that triclosan is safe for use in cosmetics in the present practices of use and concentration. The Panel focused on the exposure to triclosan that could be expected from all cosmetic products, but also noted that a risk assessment using an appropriate no-observable-adverse-effect-level and considering exposures to triclosan from all product sources, including OTC drugs, also supported safety. Technical comments from the Council have been received and addressed. This report is ready to be issued as a final safety assessment.

14. Trimonium group - at the June meeting, the Panel issued a tentative amended report with a safe as used when formulated to be non-irritating conclusion. Technical comments were provided by the Council and have been addressed. After carefully reviewing the “read-across” rationale in this report, the Panel should issue a final amended report.

Full Panel Meeting - remember, juice and coffee at 7:30 am and meeting starts at 8:00 am on day 2.

The Panel will consider the 7 reports to be issued as final safety assessment, followed by the reports advancing in the process, the re-reviews and the new data.

While the number of reports is high and there are several very significant reports being considered, I think it likely that the full Panel session will conclude by lunch time on day 2, so plan your travel accordingly.

Post-Panel Meeting Event 🎉

Although we were not able to make suitable arrangements this year for a special event tour of the holiday decorated White House for CIR Expert Panel members, liaisons, and guests, we promise that we will start earlier next year and try to bring it all together for next December’s meeting.

Have a safe journey.
117th Cosmetic Ingredient Review Expert Panel Meeting  
December 13-14, 2010

Embassy Suites Washington D.C. - Convention Center  
900 10th Street NW  
Washington, DC 20001  
Tel: 202-739-2001  
Fax: 202-739-2099

Monday, December 13, 2010

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<th>Time</th>
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<td>8:00 am</td>
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<td>8:30 am</td>
<td>WELCOME TO THE 117th EXPERT PANEL TEAM MEETINGS</td>
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<td>9:00 am</td>
<td>TEAM MEETINGS</td>
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<td>Pink (CB/MF) Edible oils group</td>
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Noon Lunch

1:00 pm TEAM MEETINGS (continued as needed)

5:00 pm ADJOURN DAY 1 SESSION

NOTE: The order of presentation and discussion of each topic will be maintained. However, the scheduled times may be accelerated or delayed depending upon the time required for the Expert Panel to complete its review of each subject.
Tuesday, December 14, 2010

7:30 am  Juice and coffee

8:00 am  WELCOME TO THE 117th FULL CIR EXPERT PANEL MEETING

8:15 am  MINUTES OF THE August 30-31, 2010 EXPERT PANEL MEETING (Buff)  Dr. Bergfeld

8:25 am  DIRECTOR’S REPORT  Dr. Andersen

8:45 am  FINAL REPORTS, REPORTS ADVANCING TO THE NEXT LEVEL, and RE-REVIEWS

Final Reports

Blue (WJ)  Dimethiconol group - Dr. Belsito reports
Blue (WJ)  Isoparaffins group - Dr. Marks reports
Blue (MF/BAH)  Dicarboxylic Acids group – Dr. Belsito reports
Blue (MF/BAH)  Alkyl PEG Ethers group - Dr. Marks reports
Blue (CB/BAH)  CAPB - - Dr. Belsito reports
Blue (LB)  Trimonium group - Dr. Marks reports
Blue (FAA)  Triclosan – Dr. Belsito reports

Reports Advancing

Pink 2 (WJ)  Caprylyl Glycol group-Dr. Marks reports
Pink (LB)  Alkyl Benzoate group – Dr. Belsito reports
Pink (CB/MF)  Edible oils group - Dr. Marks reports
Green (MF)  Disperse Blue 1 - Dr. Belsito reports

Re-Reviews

Buff (FAA))  Formaldehyde – Dr. Marks reports
Buff (MF)  MEA/DEA/TEA – Dr. Belsito reports

New Data

Buff (FAA)  Human umbilical Extract - Dr. Marks reports

ADJOURN  - Next meeting Thursday and Friday, March 3-4, 2011
ONE HUNDRED SIXTEENTHMEETING

OF THE

EXPERT PANEL

August 30-31, 2010

Embassy Suites Washington D.C. – Convention Center

Washington, D.C.

Expert Panel Members

Wilma F. Bergfeld, M.D., Chairman
Donald V. Belsito, M.D.
Ronald A. Hill, Ph.D.
Curtis D. Klaassen, Ph.D.
Daniel C. Liebler, Ph.D.
James G. Marks, Jr., M.D.
Ronald C. Shank, Ph.D.
Thomas J. Slaga, Ph.D.
Paul W. Snyder, D.V.M., Ph.D.

Liaison Representatives

Consumer
Rachel Weintraub, Esq.

Industry
John Bailey, Ph.D.

Government
Linda Katz, MD., M.P.H.

Adopted (Date)

Wilma F. Bergfeld, M.D.
Others Present at Meeting

F. Alan Andersen  CIR
Michelle Andriot  SEHSC
Jay Ansell  The Council
Louise Aust  Dial/Henkel
Dan Bagley  Colgate
Lillian Becker  CIR
Don Bjerke  Procter & Gamble
Ivan Boyer  Noblis, Inc.
Halyna Breslawec  CIR
Robert Bronaugh  FDA
Christina Burnett  CIR
Steve Cantrell  Unilever
Sam Cohen  University of Nebraska Medical Center
Kapal Dewan  FDA
Robert Finking  BASF
Monice Fiume  CIR
Kevin Fries  CIR
Christina Griffin  Delta Analytical
Tracy Guerrero  SEHSC
Steve Gutsell  Unilever
Don Havery  FDA
Bart Heldreth  CIR
Wilbur Johnson, Jr.  CIR
Akihiv Kinoshita  Shiseido
Pam Kloepfer Sans  Procter & Gamble
Linda Loretz  The Council
David Mallon  Unilever
Stanley R. Milstein  FDA
Lauren Nardella  The Rose Sheet
Damani Parra  Akzu Nobel
Thomas Re  L’Oreal USA
Josephine Robinson  CIR
Diego Rua  FDA
Noriko Shibuya  Shiseido
Julie Skare  Procter & Gamble
David Steinberg  Steinberg & Associates
CHAIRMAN'S OPENING REMARKS

The 116th meeting of the CIR Expert Panel was called to order by Dr. Bergfeld at 8:00 a.m on Tuesday, August 31, 2010. More than 300 ingredients were reviewed in Teams on the preceding day, and the Panel was also presented with a new format for use concentration tables. Dr. Bergfeld noted that the practice of sending lengthy documents received after the mail date to the Panel via CIR’s website is problematic in terms of the need for printed material and needs to be refined. She also remarked that the 3 presentations to the Panel on cocamidopropyl betaine, disperse blue 1, and triclosan, respectively, on the preceding day were very helpful in deliberations on the safety of these ingredients.

APPROVAL OF MINUTES

The minutes of the June 28-29, 2010 CIR Expert Panel meeting were unanimously approved, with corrections.

DIRECTOR’S REPORT

♦ Dr. Andersen stated that the publication of CIR’s findings in the International Journal of Toxicology continues, and that CIR maintains a good relationship with the new editor of this journal, Dr. Mary Beth Genter. Mr. Kevin Fries and Dr. Halyna Breslawec were thanked for their expertise in the process of CIR report submission to the journal.

♦ Recruitment for a new toxicologist at CIR is nearing completion.

♦ CIR needs to improve its process of handling late data submissions prior to a Panel meeting. The indexing of materials for easier access is being considered. The Council and CIR Science and Support Committee were thanked for their efforts to provide large amounts of data.

♦ The 2011 Expert Panel meeting schedule is as follows: March 3-4, June 27-28, September 26-27, and December 12-13. The Panel will be polled for 2012 meeting dates shortly after this Expert Panel meeting.

♦ Recognizing the large number of ingredients that may be included in a safety assessment, a chart identifying the available data on each ingredient would be useful and will be developed for each ingredient report. Such information would allow the Panel to identify data gaps and determine the extent to which read across would be used to evaluate the safety of ingredients with very limited or no available data.

APPROVAL OF FINAL REPORT

Methyl Acetate, Simple Alkyl Acetate Esters, Acetic Acid and its Salts

Methyl acetate, propyl acetate, isopropyl acetate, t-butyl acetate, isobutyl acetate, butoxyethyl acetate, nonyl acetate, myristyl acetate, cetyl acetate, stearyl acetate, isostearyl acetate, acetic acid, sodium acetate, potassium acetate, magnesium acetate, calcium acetate, zinc acetate, propyl alcohol, and isopropyl alcohol are safe in the present practices of use and concentration. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group.

While most of these ingredients function as fragrances in cosmetics, additional functions such as solvent, buffering agent, or even skin conditioning agent are reported for these ingredients. No new data were received during the 60-day comment period of the Tentative Report. Accordingly, the CIR Expert Panel finalized the conclusion unchanged and issued the final safety assessment for these 19 ingredients.
APPROVAL OF AMENDED FINAL REPORT

Stearyl Heptanoate and Related Stearyl Alkanoates

Stearyl heptanoate, stearyl caprylate, stearyl palmitate, stearyl stearate, stearyl behenate, and stearyl olivate are safe in the present practices of use and concentration.

In 1995, the CIR Expert Panel determined that Stearyl heptanoate was safe for use in cosmetics in the (then) present practices of use. The Panel has affirmed that stearyl heptanoate is safe in the (now) present practices of use and concentration and has determined that the available safety information also support the safety of related stearyl alkanoates including stearyl caprylate, stearyl palmitate, stearyl stearate, stearyl behenate, and stearyl olivate. In particular, stearyl olivate, a recent addition to the International Cosmetic Ingredient Dictionary and Handbook, while a mixture of fatty acids esterified to stearyl alcohol, is not significantly different in properties from the other stearyl alkanoates. These 6 ingredients function primarily as skin conditioning agents in cosmetics.

TENTATIVE SAFETY ASSESSMENTS

Dicarboxylic Acids and Their Salts and Esters

The CIR Expert Panel concluded that these ingredients are safe for use in cosmetic products in the present practices of use and concentration. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group.

The following 12 dicarboxylic acids and salts are included: malonic acid, succinic acid, sodium succinate, disodium succinate, glutaric acid, adipic acid, azelaic acid, dipotassium azelate, disodium azelate, sebacic acid, disodium sebacate, and dodecanedioic acid. The dicarboxylic acids function in cosmetics primarily as pH adjusters and fragrance ingredients.

The 44 following esters of these dicarboxylic acids are included: diethyl malonate, decyl succinate, dimethyl succinate, diethyl succinate, dicapryl succinate, dicetearyl succinate, disobutyl succinate, diethyhexyl succinate, dimethyl glutarate, dibutyl glutarate, diisostearyl glutarate, dimethyl adipate, diethyl adipate, dipropyl adipate, dibutyl adipate, dihexyl adipate, dicapryl adipate, di-c12-15 alkyl adipate, ditridecyl adipate, dicetyl adipate, diisopropyl adipate, disobutyl adipate, diethylhexyl adipate, diisooctyl adipate, diisodecyl adipate, dihexyldodecyl adipate, dioctyldodecyl adipate, disoctyl adipate, disostearyl adipate, isostearyl sebacate, diethyl sebacate, dibutyl sebacate, dicaprylyl/capryl sebacate, diisopropyl sebacate, diethyhexyl sebacate, dibutyl/octyl sebacate, diisooctyl sebacate, dioctyldodecyl sebacate, dioctylisostearate sebacate, diisostearate sebacate, dioctylisododecyl dodecanedioate, and diisocetyl dodecanedioate. The esters have a wide range of functions in cosmetics, including skin conditioning agent, fragrance, plasticizer, solvent, and emollient.

A concern was expressed regarding the extent of dermal absorption for certain long-chain, branched diesters (which have molecular weights greater than 500 g/mol and log Kow values of 10 or greater). In this view, were it demonstrated that dermal penetration was low for the entire group, no further data would be needed. If not, then a concern was expressed about the absence of toxicity data for several branched-chain alcohols that would be produced if dermal penetration and subsequent metabolism could occur.

Dimethiconol and its Esters and Reaction Products

The CIR Expert Panel concluded that dimethiconol and its esters and reaction products are safe for use in cosmetics in the present practices of use and concentration. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group. Most of these ingredients function in cosmetics either as a skin conditioning agent or a hair conditioning agent.

In all, 28 ingredients are included. In addition to dimethiconol, included are:
1) end-capped homopolymers: dimethiconol arginine, dimethiconol beeswax, dimethiconol behenate, dimethiconol borageate, dimethiconol candelillate, dimethiconol carnaubate, dimethiconol cysteine, dimethiconol dhupa butterate, dimethiconol hydroxystearate, dimethiconol illipe butterate, dimethiconol isostearate, dimethiconol kokum butterate, dimethiconol lactate, dimethiconol meadowfoamate, dimethiconol methionine, dimethiconol mohwa butterate, dimethiconol panthenol, dimethiconol sal butterate, and dimethiconol stearate; and

2) copolymers: hydrolyzed collagen PG-propyl dimethiconol, dimethiconol/methylsilanol/silicate crosspolymer, dimethiconol/silica crosspolymer, dimethiconol/silsesquioxane copolymer, dimethiconol/stearyl methicone/phenyl trimethicone copolymer, isopolyglyceryl-3 dimethiconol, trimethylsiloxydimethiconol crosspolymer, and acrylates/dimethiconol acrylate copolymer.

The Expert Panel focused on the available data on methods of manufacture and the composition data provided, all deemed sufficient, and the potential for dermal absorption. The composition data relate to materials registered under the dimethiconol INCI name as well as tested materials containing dimethyl siloxane, hydroxyl-terminated. The available data on octanol-water partition coefficients and molecular weights suggested that the ingredients reviewed do not penetrate the skin, obviating any concerns that would relate to systemic toxic effects. Additionally, the potential for inhalation exposure was noted, in that some of the ingredients are being used in hair sprays. However, the particle size associated with these aerosolized products would preclude any concern over toxic effects related to inhalation exposure.

**Isoparaffins**

The CIR Expert Panel concluded that the isoparaffins are safe for use in cosmetics when formulated to be non-irritating. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group.

The 24 ingredients include C7-8 isoparaffin, C8-9 isoparaffin, C9-11 isoparaffin, C9-12 isoparaffin, C9-13 isoparaffin, C9-14 isoparaffin, C9-16 isoparaffin, C10-11 isoparaffin, C10-12 isoparaffin, C10-13 isoparaffin, C11-12 isoparaffin, C11-13 isoparaffin, C11-14 isoparaffin, C12-14 isoparaffin, C12-20 isoparaffin, C13-14 isoparaffin, C13-16 isoparaffin, C18-70 isoparaffin, C20-40 isoparaffin, C15-35 isoparaffin/isoalkylcycloalkanes, isododecane, Isoeicosane, isohexadecane, and isooctane. Many of these ingredients function as solvents in cosmetics, but other functions include skin conditioning and fragrance.

The Panel appreciated the extensive submission of unpublished skin irritation/sensitization data by industry. The Panel noted the potential for inhalation exposure, in that some of these ingredients are being used in hair sprays, but the particle size associated with these aerosolized products would preclude any concern over exposure-related toxic effects.

**REVISED TENTATIVE AMENDED SAFETY ASSESSMENT**

**Cocamidopropyl Betaine and Related Amidopropyl Betaines**

The total of 31 ingredients includes the following related amidopropyl betaines: almondamidopropyl betaine, apricotamidopropyl betaine, avocadamidopropyl betaine, babassuamidopropyl betaine, behenamidopropyl betaine, canolamidopropyl betaine, capryl/capramidopropyl betaine, coco/oleamidopropyl betaine, coco/sunfloweramidopropyl betaine, cupuassuamidopropyl betaine, isostearmidopropyl betaine, lauramidopropyl betaine, meadowfoamamidopropyl betaine, milkanamidopropyl betaine, minkamidopropyl betaine, myristamidopropyl betaine, oatamidopropyl betaine, oleamidopropyl betaine, olivamidopropyl betaine, palmamidopropyl betaine, palmmitamidopropyl betaine, palm kernelamidopropyl betaine, ricinoleamidopropyl betaine, sesamidopropyl betaine, shea butteramidopropyl betaine, soymamidopropyl betaine, stearamidopropyl betaine, tallowamidopropyl betaine, undecylenamidopropyl betaine, and wheat germamidopropyl betaine.

These ingredients were found safe as cosmetic ingredients when formulated to be non-sensitizing based on a quantitative risk assessment (QRA).
A QRA is needed because of the demonstrated sensitization potential of two impurities of cocamidopropyl betaine and related amidopropyl betaines, DMAPA and aminoamine, that may be present in these ingredients as supplied to cosmetics manufacturers. By using a QRA approach, a manufacturer can evaluate all of the relevant factors from use concentration to product usage and combine that information with the weight of evidence no expected sensitization induction level (NESIL). Manufacturers must ensure finished products have an acceptable exposure level (AEL) to consumer exposure level (CEL) ratio greater than 1 for each product.

**INSUFFICIENT DATA ANNOUNCEMENTS**

**Alkyl Benzoates**

The available data are insufficient to complete the safety assessment of these 17 alkyl benzoates, which included: methyl benzoate, ethyl benzoate, propyl benzoate, butyl benzoate, amyl benzoate, lauryl/myristyl benzoate, C12-15 alkyl benzoate, C16-17 alkyl benzoate, stearyl benzoate, behenyl benzoate, isopropyl benzoate, isobutyl benzoate, isostearyl benzoate, ethylhexyl benzoate, butyloctyl benzoate, hexyldecyl benzoate, and octyldodecyl benzoate.

The CIR Expert Panel is aware that a dossier is being prepared for submission in Europe to meet requirements imposed by the REACH program there. These data likely will resolve many of the data needs below, but the Panel determined that it would expedite the process of evaluation to identify the data needs.

Additional data needs include: (1) irritation and sensitization of the low molecular weight ingredients (≤ 12 C), especially methyl benzoate; (2) genotoxicity data; (3) dermal penetration data; and (4) if there is significant dermal penetration, then reproductive/developmental toxicity data may be needed; and (5) if there is significant dermal penetration, clarification (e.g., mode of action) of existing carcinogenicity data that appear to show increased tumor growth in rats given oral doses of methyl benzoate, or additional carcinogenicity data.

**Caprylyl Glycol and Other 1,2-Glycols**

The 1,2-glycols are used mostly as skin and hair conditioning agents and viscosity increasing agents in cosmetics. This safety assessment includes: caprylyl glycol, arachidyl glycol, cetyl glycol, hexacosyl glycol, lauryl glycol, myristyl glycol, octacosanyl glycol, stearyl glycol, decylene glycol, pentylene glycol, 1,2-butanediol, 1,2-hexanediol, C14-18 glycol, C15-18 glycol, C18-30 glycol, C20-30 glycol. Of these 16 ingredients, the following 4 are being used in personal care products: caprylyl glycol, pentylene glycol, 1,2-hexanediol, and C15-18 glycol.

The Panel determined that additional data are needed to complete this safety assessment, including: (1) dermal absorption data on caprylyl glycol or similar lipid-soluble 1,2-glycol; (2) if significant dermal absorption occurs, then reproductive/developmental toxicity study may be needed; (3) if significant dermal absorption occurs, a 28-day dermal toxicity study to evaluate other systemic toxicity endpoints, using caprylyl glycol or another appropriate lipid-soluble 1,2-glycol; and (4) genotoxicity data.

**Plant-Derived Edible Oils**

The Panel determined that oils derived from nuts posed no unique toxicological issues and that the review of these ingredients could be combined with the review of the oil ingredients derived from vegetables and fruits under the umbrella “plant-derived edible oils.” This combined group contains 244 individual cosmetic ingredients. The list of ingredients under current review can be found at [http://cir-safety.org/current.shtml](http://cir-safety.org/current.shtml).

The Panel agreed that the composition data that were available for these ingredients, combined with the available data on method of manufacture, impurities, safety test data on fatty acids, a long history of safe use in foods, and an absence of adverse reactions in clinical experience would be a sufficient basis for determining safety. The Panel also would expect to limit pesticide residues and heavy metal impurities. Composition data, however, were not available for all of the oils. Additional data are needed to complete the safety assessment of edible oils derived from plants. Chemical composition, specifically fatty acid profiles, of the oils for which those data were not given in the report are needed. These 50 oils include:
REPORT TABLED

The CIR Expert Panel tabled the report on Disperse Blue 1 following a robust discussion of the results of the existing carcinogenicity studies. The Expert Panel has requested a calculation of a margin of safety using the renal carcinogenicity data and will consider issues relating to the mode of action of Disperse Blue 1 in the formation of the rarely seen smooth muscle tumors. The existing conclusion will then be re-evaluated. Disperse Blue is no longer reported to be used, nor is it being manufactured.

2011 PRIORITY LIST AND RE-REVIEWS

The list is available at http://cir-safety.org/priorities.shtml.

Fifteen years or more have elapsed since the original safety assessment of MEA/DEA/TEA, Cocamide DEA, Glutaral (Listed As Glutaraldehyde), Methyldibromo Glutaronitrile, Polyvinyl Acetate, 4-Chlororesorcinol and HC Red No. 1. These ingredients will be re-reviewed in 2011. In addition, based on new questions regarding safety, retinyl palmitate and benzophenone-3 (aka oxybenzone) will be re-reviewed.

PRESENTATIONS

Donald L. Bjerke, PhD, DABT representing the Personal Care Products Council Task Force on Sensitization Risk from cocamidopropyl betaine (CAPB) Impurities reviewed the available sensitization data for DMAPA and amidoamine impurities in the context of active CAPB levels. Using a weight of evidence no expected sensitization induction level, CAPB use concentrations, and product usage, he demonstrated how a quantitative risk assessment could be used to ensure that dermal sensitization would not be a concern for use CAPB and related ingredients.
Samuel M. Cohen, M.D., Ph.D., University of Nebraska Medical Center, reviewed available genotoxicity and carcinogenicity data for the anthraquinone hair dye, Disperse Blue 1. While the levels at which a carcinogenicity concern appears to exist are significantly higher than the exposures that could result from the use of a hair dye product containing Disperse Blue 1, he did note that the late-developing smooth muscle tumors were rare and that no mode of action explained their appearance, suggesting that there may be insufficient data to support the safety of this hair dye.

Dr. Robert Finking, Regulatory Toxicologist and Head of Product Safety at BASF presented his perspective on the available data supporting the safety of triclosan in cosmetics. He indicated that it would be important to limit the dioxin levels that may be present, but that industry appears to be doing that now. Dioxin produced by ultraviolet radiation (UVR) of triclosan occurs at 254 nm, which is not relevant to cosmetics because that wavelength is not found in solar UVR. He argued that antimicrobial resistance would not occur in vivo. And finally, he reported that the Scientific Committee on Cosmetic Products in Europe would be considering further input in its determination of a no-observable-adverse-effect level (NOAEL), but that a NOAEL based on hematological parameters in the absence of any demonstrable anemia was not reasonable.

CIR PROCEDURES FOR INSUFFICIENT DATA INGREDIENTS

Industry liaison to the CIR Expert Panel, Dr. John Bailey, reviewed a proposed change in CIR Procedures that would alert all interested parties that an insufficient data conclusion had been reached and that a 2-year period would begin, within which data could be provided to CIR to allow completion of the safety assessment. Were data not submitted to CIR, after two years the ingredient would be re-classified as “Use not supported by the Data Submitted to CIR”. Other aspects of procedural changes to address insufficient data ingredients already existing and those with no uses were also addressed.

The CIR Expert Panel supported this effort to redefine this category of conclusion and to clearly state the impact of an insufficient data finding on the use of an ingredient in cosmetics.
FORMALDEHYDE
Memorandum

To: CIR Expert Panel

From: Director, CIR

Subject: Formaldehyde – early second re-review

Date November 18, 2010

In 1984, CIR published its original safety assessment of formaldehyde, concluding that this preservative is safe for use in cosmetics if free formaldehyde was minimized, but in no case >0.2%. The Panel also said that it can’t be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized. The Panel re-reviewed formaldehyde in 2003 confirming the original conclusion. That finding was published in 2006.

FDA has asked CIR to consider the safety of formaldehyde given its detection in hair straightening products and to address the safety of methylene glycol in cosmetics. The Personal Care Products Council has supported such an effort.

Included in this package are:

- 2010 re-review report on formaldehyde
- Original 1984 formaldehyde safety assessment
- 2003 re-review package
- 2003 re-review summary
- Health Canada advisory
- FDA website public health focus item
- Council statement
- Brazilian Blowout MSDS

If the CIR Expert Panel determines to reopen the safety assessment of formaldehyde, such a step should add methylene glycol, since the two chemicals are inexorably linked. Methylene glycol first appeared as a cosmetic ingredient in the 2010, 13th edition of the International Cosmetic Ingredient Dictionary and Handbook.

Interested parties would have the opportunity to submit published or unpublished information relevant to a determination of the safety of formaldehyde and methylene glycol in any product type. CIR would include such information, along with new published data as described above, in a scientific literature review for public review and consider a draft report next year.
**SAFETY ASSESSMENT FLOW CHART**

**Public Comment**
- 60 day public comment period
- ANNOUNCE

**CIR**
- Draft Priority List

**Expert Panel**
- Priority List
- INGREDIENT

**Re-Reviews**
- 15 years; or
  - Original 1984
  - New Data; or
- Re-review to Panel 2003
- November 2010
- reaffirmed 2006

**Report Color**
- Buff Cover
- Green Cover
- Pink Cover
- Blue Cover

**Flow Chart Details**
- **SLR**
  - Decision not to reopen the report*
  - Draft Report
    - TABLE
    - Draft TR ISD
      - TABLE
      - Draft FR
        - TABLE
        - Final Report

*The CIR Staff notifies the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

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

- Expert Panel Decision
  - Document for Panel Review
  - Option for Re-review

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*This diagram outlines the process for safety assessment and decision-making, including public comment periods, expert panel reviews, and report decisions.*
Introduction

In 1984, CIR published its original safety assessment of formaldehyde\(^1\), concluding that this preservative is safe for use in cosmetics if free formaldehyde was minimized, but in no case > 0.2%. The Panel also said that it can’t be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized. This safety assessment acknowledged the equilibrium relationship between formaldehyde and methylene glycol, but did not address the safety of methylene glycol (which was NOT listed as a cosmetic ingredient at the time).

The Panel re-reviewed formaldehyde in 2003 confirming the original conclusion.\(^2\) That finding was published in 2006.\(^3\) As best we can determine, methylene glycol was not yet listed as a cosmetic ingredient.

Recently, an Oregon OSHA laboratory, following up on a salon worker complaint, measured the formaldehyde levels in Brazilian Blowout, one of the current popular salon Brazilian hair treatments. They found a range of values from 8 – 10%. Health Canada is working to stop distribution and use of Brazilian Blowout in Canada, predicated on their validated testing that found 8.4% formaldehyde in Brazilian Blowout. Recognizing that methylene glycol now is listed as a cosmetic ingredient, FDA has asked CIR to consider the safety of formaldehyde given its detection in hair straightening products and to address the safety of methylene glycol.

Chemistry

Formaldehyde, a gas, is not commercially available, but is instead produced as a related solution called formalin.\(^4\) Formalin is industrially produced from methanol. In a first step, 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. In a second step, the formaldehyde is reacted with water in an absorption column, because formaldehyde in its pure, gaseous form is highly unstable.

Formaldehyde quickly reacts with water to synthesize methylene glycol and, without a polymerization inhibitor (e.g., methanol), polymethylene glycols via a series of reversible reactions (Scheme 1).

Scheme 1

The apparent good solubility of formaldehyde in water is actually the good solubility of methylene glycol in water and the capacity of the solution to accommodate small polymethylene glycols (i.e. two to ten methylene glycol units long).\(^5\) Formaldehyde itself is only sparingly soluble in water. The rate of the hydration reaction is relatively fast (i.e. the half-life of formaldehyde in water is 70 ms) and the equilibrium between methylene glycol and formaldehyde strongly favors methylene glycol, at room temperature.\(^6\)
The formation rate of the higher polymethylene glycols is much slower and can be inhibited by the addition of a small amount of methanol. Accordingly, an average solution of formalin consists of water (~40-60%), methylene glycol (~40%), methanol (~1-10%), small methylene glycols (e.g., dimers and trimers; ~1%), and a very small amount of formaldehyde (~0.02-0.1%). All of the components of formalin are in a series of equilibriums that favor methylene glycol at room temperature. However, removal of water, the addition of heat, reduction of pH, and/or the reaction of the small amount of free formaldehyde in the solution will drive the equilibrium back towards formaldehyde. Accordingly, a product application process, wherein a formalin containing formulation is dried, heated, acidified, and/or applied to a formaldehyde reactive substrate, could potentially lead to the shift of these equilibriums towards free formaldehyde.

Cosmetics Use

As given in the International Cosmetic Ingredient Dictionary and Handbook (INCI Dictionary), the cosmetic functions of formaldehyde are: cosmetic biocide, denaturant, and preservative.

In the FDA’s Voluntary Cosmetic Registration Program (VCRP), there are 58 uses of formaldehyde and 20 uses of formaldehyde solution (formalin) reported. Since these all are probably the same ingredient as added to cosmetics, they are combined in Table 1a. Formaldehyde and formalin are listed separately in Table 1b, prepared using the new use table format.

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 leveled-off to less than 100 uses as shown in Figure 1.

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 (e.g., keratin). Formaldehyde is extremely reactive and, among a multitude of potential reactions, can react with protein residue sidechains of arginine, lysine, tyrosine, tryptophan, histidine, and cysteine/cystine. Additionally, the primary amides, glutamine and asparagine, are known to be capable of reaction with formaldehyde. Some of these reactions can be bi-functional as well as mono-functional. In addition to simple methylene crosslinkages (i.e. -CH2-), formaldehyde has a known propensity for self-condensation so that polymethylene glycol crosslinkages (i.e. -(OCH2)n-) are feasible. Besides proteins, formaldehyde is known to react with other biological molecules such as glycoproteins, nucleic acids, and polysaccharides. The action of formaldehyde in intramolecular and intermolecular crosslinking of macromolecules can considerably alter the physical characteristics of the reacted substrates.

According to the 2010 13th Edition of the INCI Dictionary, methylene glycol is reported to function as an artificial nail builder. Methylene glycol is not reported to FDA to be used in cosmetics according to the VCRP database.

Current usage reports of formaldehyde (336 uses), formalin (15 uses), and methylene glycol (2 uses) in Canada are given in Table 2 as a function of duration of use (leave-on vs. rinse off) and exposure type (eye area, nail, etc.).

The MSDS provided by Brazilian Blowout for their salon product, however, does include methylene glycol. The list of ingredients provided by the manufacturer is shown in Table 3, with methylene glycol listed at <5.0%.

Summary of Data from 2003 Re-Review

Most of the significant new data related to skin irritancy/sensitization or developmental toxicity, genotoxicity, and carcinogenicity.

Other items of note from the 2003 re-review were the two Danish product surveys in which the formaldehyde levels were measured. In the first survey of 84 shampoos and skin creams, 8 products contained formaldehyde at levels above 0.05%. In the second survey of 67 skin creams, 22 products were found to contain formaldehyde, 18 of which had levels <0.003%. The author suggested that these 18 with low levels may represent products in which formaldehyde was not intentionally used in the formulation. The author did not report whether methylene glycol was listed as an ingredient.
Skin irritancy/sensitization

The dermal studies described in the 2003 re-review included the work in Denmark in 1985 and 1986 done using aqueous formaldehyde. Based on the chemistry, that suggests that most of the exposure was to methylene glycol.

Six intradermal (0.01%-3%), and 6 topical (0.5%-20%) concentrations were used for induction, and formaldehyde 1% and 0.1% was used for challenge. The incidence of contact sensitivity depended on the intradermal, but not on the topical induction dose.

Statistical analyses showed a non-monotonous (non-linear) dose response relationship. The estimated maximal sensitization rate in Copenhagen was 80% after intradermal induction with 0.65% formaldehyde; in Stockholm it was 84% after induction with 0.34%.

The data from the two laboratories could be described by parallel displaced dose response curves suggesting that the guinea pig strain used in Stockholm was significantly more susceptible to formaldehyde than the strain used in Copenhagen.

The EC50 (formaldehyde concentration at which 50% of the guinea pigs were sensitized) at the 72 h scoring and a 1% challenge concentration, was 0.061% in Copenhagen and 0.024% in Stockholm.

It would be interesting to look further at data such as these to determine if a Quantitative Risk Assessment approach could be used to come up with a better “number” for formaldehyde in cosmetic products to ensure no sensitization.

Genotoxicity

There were a mix of studies that used formaldehyde gas (DNA replication in vivo, fruit fly mosaic and sex-linked recessive lethal tests, chromosome damage to bone marrow and pulmonary lavage cells in vivo) and studies of formaldehyde in aqueous media (S. typhimurium resistance, E. coli DNA-repair, E. coli SOS repair, newt micronucleus test) which probably means that these assays measured the effect of methylene glycol.

In none of the genotoxicity testing was formaldehyde gas a significant genotoxicant and where positive results were found, the effect appeared only at high levels.

In aqueous media, formaldehyde/methylene glycol was not a significant genotoxicant.

Carcinogenicity

Rat carcinogenicity data similar to that in the original safety assessment were included in the re-review summary. These data are primarily inhalation of formaldehyde gas and show a threshold response.

A 2-year drinking water study using rats was performed, so it would be expected that the animals were exposed mostly to methylene glycol with a little formaldehyde. Leukemia incidence was increased at 500, 1000, and 1500 mg/l, but not at 10, 50, or 100 mg/l based on just eye-balling the numbers. No statistical analysis was provided. The 2003 re-review package went on to cite the criticisms of this study and give results of 2 other 2-year drinking water studies in rats that found no increases in leukemia incidences. In one of those studies, a NOEL of 0.02% formaldehyde in drinking water (10 mg/kg/day) was reported.

Reproductive and Developmental Toxicity

A dermal study using 37% formaldehyde (presumably, formalin) in pregnant (exposures on days 8, 9, 10, or 11 of gestation) hamsters resulted in no skeletal or other malformations.

Male rats given formaldehyde injections (10 mg/kg total over 30 days, presumably as formalin) was linked with reduced tissue DNA content in the testis and decreased sperm count, sperm viability, and sperm motility.

Presumably, these studies also relate to methylene glycol.
Formaldehyde gas was not teratogenic, but slightly fetotoxic at levels that were not maternally toxic in one study, but was non-toxic to the fetus in another. There were a series of Russian studies that reported maternal and embryo/fetal effects of inhaled formaldehyde.

New Data

We have not performed an exhaustive literature search to identify additional studies that may be relevant to the safety of formaldehyde/methylene glycol. We have found epidemiology and laboratory studies that address the link between formaldehyde exposure and leukemia. This is a significant body of work that was not included in the original safety assessment or in the 2003 re-review.

New Epidemiology

Three epidemiology studies were published in the 2003-2004 timeframe, but not captured in the 2003 re-review and one of those studies has a 10-year follow-up published in 2009. Presumably all of these occupational studies related to formaldehyde gas exposures.

One study, conducted by NCI, looked at 25,619 workers in industries with the potential for occupational formaldehyde exposure and estimated each worker's exposure to the chemical while at work and found an increased risk of death due to leukemia, particularly myeloid leukemia, among workers exposed to formaldehyde. The finding persisted in the 10-year follow-up study published in 2009. The analysis continued to show a possible link between formaldehyde exposure and cancers of the hematopoietic and lymphatic systems, particularly myeloid leukemia.

A cohort study of 11,039 textile workers performed by the National Institute for Occupational Safety and Health (NIOSH) found that an association between the duration of exposure to formaldehyde and leukemia deaths, but a cohort study of 14,014 British industry workers found no association between formaldehyde exposure and leukemia deaths.

Apropos of these epidemiology studies that examined the possible association between occupations that involve exposure to formaldehyde and the incidence of leukemia, follow-up meta-analyses have been reported.

One of these efforts combined study results for leukemia and nasopharyngeal cancer and confounder information from selected cohort and case-control studies in a meta-analysis. The authors reported leukemia relative risks of 1.05 (95% CI from 0.93 to 1.20) for cohort studies and odds ratios of 0.99 (95% CI from 0.71 to 1.28) for case-control studies. Discounting one manufacturing plant at which a cluster of nasopharyngeal cancers had occurred, they reported a cohort study relative risk of 0.72 (95% CI from 0.40 to 1.28). Nasopharyngeal cancers were increased in case-control studies, but the authors determined that the odds ratio for smoking-adjusted studies was 1.10 (95% CI from 0.80 to 1.50).

A second meta-analysis was performed focusing on high-exposure groups and myeloid leukemia. The authors reported that formaldehyde was associated with increased risks of leukemia (relative risk = 1.53; 95% confidence interval from 1.11 to 2.21) and specifically myeloid leukemia (relative risk = 2.47; 95% confidence interval from 1.42 to 4.27).

New Laboratory studies

Male Fischer 344 rats were exposed to formaldehyde gas and DNA from respiratory nasal mucosa was analyzed by highly sensitive liquid chromatography tandem mass spectrometry. [13CD2]-formaldehyde exposures were used, allowing differentiation of DNA adducts and DNA-DNA crosslinks originating from endogenous and inhalation-derived formaldehyde exposure. The authors concluded that these data provide a plausible explanation for the local site nasopharyngeal tumors commonly reported with formaldehyde exposure. Because exogenous formaldehyde induced DNA monoadducts and dG-dG cross-links only occur in nasal mucosa, the authors suggested that genotoxic
effects at sites remote to the portal of entry of inhaled formaldehyde are implausible and do not support that inhaled formaldehyde could also cause leukemia.

Basis for reopening

Formaldehyde use in hair straightening products was not considered in the original safety assessment or the re-review. At the time, methylene glycol was not listed as a cosmetic ingredient. While there remain (2010 VCRP data) no uses reported to FDA in the hair straightener category of either formaldehyde or methylene glycol. The MSDS for Brazilian Blowout does include methylene glycol.

Next steps

If the CIR Expert Panel determines to reopen the safety assessment of formaldehyde such a step should add methylene glycol, since the two chemicals are inexorably linked.

Interested parties would have the opportunity to submit published or unpublished information relevant to a determination of the safety of formaldehyde and methylene glycol in any product type and CIR would prepare a new scientific literature review.

CIR would include unpublished information, along with new published data as described above, in a draft report for consideration by the Panel next year.

REFERENCES


Figure 1. Declining use of formaldehyde in cosmetic products as reported to the FDA VCRP (note x-axis is not linear...).
Table 1a. Current and historical uses and concentrations of formaldehyde and formalin (combined) in cosmetics.

<table>
<thead>
<tr>
<th>Product Category</th>
<th>2002 uses&lt;sup&gt;1&lt;/sup&gt;</th>
<th>2010 uses&lt;sup&gt;10&lt;/sup&gt;</th>
<th>2002 use concentrations&lt;sup&gt;1&lt;/sup&gt; (%)</th>
<th>2010 use concentrations&lt;sup&gt;4&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td><strong>Bath Preparations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oils, tablets and salts</td>
<td>6</td>
<td>1</td>
<td>0.08</td>
<td></td>
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<tr>
<td>Bubble baths</td>
<td>4</td>
<td>1</td>
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<td>Soaps and detergents</td>
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<td>&lt;0.002 – 0.08</td>
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<tr>
<td>Other bath preparations</td>
<td>1</td>
<td>-</td>
<td>0.08</td>
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<tr>
<td><strong>Eye Makeup Preparations</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Mascara</td>
<td>-</td>
<td>-</td>
<td>0.0002</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Other fragrance preparations</td>
<td>-</td>
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<td>0.02</td>
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<td><strong>Non-coloring Hair Preparations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair conditioners</td>
<td>11</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td>Permanent waves</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td></td>
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<tr>
<td>Rinses</td>
<td>2</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Shampoos</td>
<td>59</td>
<td>13</td>
<td>&lt;0.005 – 0.08</td>
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<tr>
<td>Hair tonics, dressings, etc.</td>
<td>9</td>
<td>6</td>
<td>&lt;0.005</td>
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<tr>
<td>Wave sets</td>
<td>8</td>
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<td>-</td>
<td></td>
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<td>Other non-coloring hair preparations</td>
<td>3</td>
<td>7</td>
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<td><strong>Hair Coloring Preparations</strong></td>
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<tr>
<td>Shampoos</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Other hair coloring preparations</td>
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<td><strong>Makeup Preparations</strong></td>
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<tr>
<td>Leg and body paints</td>
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<td>0.02</td>
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<td>Other makeup preparations</td>
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<tr>
<td><strong>Nail Care Products</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Creams and lotions</td>
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<td>Other nail care products</td>
<td>1</td>
<td>6</td>
<td>2&lt;sup&gt;2&lt;/sup&gt;</td>
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<td><strong>Oral Hygiene Products</strong></td>
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<tr>
<td>Dentifrices</td>
<td>-</td>
<td>-</td>
<td>0.04</td>
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<tr>
<td><strong>Personal Hygiene Products</strong></td>
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<td></td>
</tr>
<tr>
<td>Other personal hygiene products</td>
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<td>2</td>
<td>0.07 – 0.08</td>
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<td><strong>Shaving Preparations</strong></td>
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</tr>
<tr>
<td>Shaving cream</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Skin Care Preparations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin cleansing creams, lotions, liquids, and pads</td>
<td>1</td>
<td>1</td>
<td>0.0001 – 0.002</td>
<td></td>
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<tr>
<td>Depilatories</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td></td>
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<tr>
<td>Body and hand skin care preparations</td>
<td>2</td>
<td>2</td>
<td>0.0001</td>
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<tr>
<td>Moisturizers</td>
<td>1</td>
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<td>-</td>
<td></td>
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<tr>
<td>Paste masks (mud packs)</td>
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<tr>
<td>Other skin care preparations</td>
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<td>5</td>
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<td><strong>Total uses/ranges for formaldehyde</strong></td>
<td>120</td>
<td>78</td>
<td>&lt;0.0001 – 0.08%</td>
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</tbody>
</table>

<sup>1</sup> 2010 survey underway

<sup>2</sup> product sold only in Europe and no longer marketed
Table 1b. Current uses of formaldehyde and formalin separated in the new use table format.

<table>
<thead>
<tr>
<th></th>
<th>Formaldehyde</th>
<th>Formaldehyde Solution (Formalin)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Totals</strong></td>
<td>58</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Duration of Use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leave-On</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>Rinse Off</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Exposure Type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye Area</td>
<td>NR</td>
<td>NS</td>
</tr>
<tr>
<td>Possible Ingestion</td>
<td>NR</td>
<td>NS</td>
</tr>
<tr>
<td>Inhalation</td>
<td>NR</td>
<td>NS</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>Deodorant (Underarm)</td>
<td>NR</td>
<td>NS</td>
</tr>
<tr>
<td>Hair - Non-Coloring</td>
<td>35</td>
<td>NS</td>
</tr>
<tr>
<td>Hair - Coloring</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Nail</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Mucous Membrane</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Bath Products</td>
<td>NR</td>
<td>NS</td>
</tr>
<tr>
<td>Baby Products</td>
<td>NR</td>
<td>NS</td>
</tr>
</tbody>
</table>

NR = Not Reported; NS = Not Surveyed; 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.
Table 2. Uses of formaldehyde, formalin, and methylene glycol reported to Canada.14

Table. Use and concentration data from Health Canada.

<table>
<thead>
<tr>
<th></th>
<th>Formaldehyde</th>
<th>Formaldehyde Solution (Formalin)</th>
<th>Methylene Glycol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of Uses</td>
<td>Conc. of Use (%)</td>
<td># of Uses</td>
</tr>
<tr>
<td>Totals</td>
<td>336</td>
<td>&lt;0.1-30</td>
<td>15</td>
</tr>
<tr>
<td>Duration of Use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leave-On</td>
<td>146</td>
<td>&lt;0.1-10</td>
<td>4</td>
</tr>
<tr>
<td>Rinse Off</td>
<td>190</td>
<td>&lt;0.1-30</td>
<td>11</td>
</tr>
<tr>
<td>Exposure Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye Area</td>
<td>3</td>
<td>&lt;0.1-0.3</td>
<td>NR</td>
</tr>
<tr>
<td>Possible Ingestion</td>
<td>1</td>
<td>&lt;0.1</td>
<td>1</td>
</tr>
<tr>
<td>Inhalation</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Dermal Contact</td>
<td>43</td>
<td>&lt;0.1-30</td>
<td>11</td>
</tr>
<tr>
<td>Deodorant (underarm)</td>
<td>1</td>
<td>3-10</td>
<td>NR</td>
</tr>
<tr>
<td>Hair - Non-Coloring</td>
<td>149</td>
<td>&lt;0.1-10</td>
<td>NR</td>
</tr>
<tr>
<td>Hair-Coloring</td>
<td>1</td>
<td>0.1-0.3</td>
<td>NR</td>
</tr>
<tr>
<td>Nail</td>
<td>72</td>
<td>&lt;0.1-10</td>
<td>3</td>
</tr>
<tr>
<td>Mucous Membrane</td>
<td>1</td>
<td>&lt;0.1</td>
<td>1</td>
</tr>
<tr>
<td>Bath Products</td>
<td>33</td>
<td>&lt;0.1-0.3</td>
<td>2</td>
</tr>
<tr>
<td>Baby Products</td>
<td>6</td>
<td>&lt;0.1-0.3</td>
<td>1</td>
</tr>
<tr>
<td>Other*</td>
<td>11</td>
<td>&lt;0.1-1</td>
<td>NR</td>
</tr>
</tbody>
</table>

*Types of products not defined by Health Canada.
NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.

Table 3. List of ingredients in Brazilian Blowout from the Brazilian Blowout MSDS dated 10/26/10.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>≤85%</td>
</tr>
<tr>
<td>Methylene glycol</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Behenyl methylammonium methosulfate/N-hexadecanol/butylene glycol</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Isoparaffin</td>
<td>≤3%</td>
</tr>
<tr>
<td>Cetrimonium chloride</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>≤1%</td>
</tr>
<tr>
<td>Hypnea musciformis extract/Gellidiela acerosa extract/Sargassum filipendula extract/sorbitol</td>
<td>≤1%</td>
</tr>
<tr>
<td>Theobroma grandiflorum seed butter (cupuacu butter)</td>
<td>≤0.5%</td>
</tr>
<tr>
<td>Panthenol</td>
<td>≤0.25%</td>
</tr>
<tr>
<td>Hydrolyzed keratin</td>
<td>≤1%</td>
</tr>
<tr>
<td>Fragrance (parfum)</td>
<td>≤1%</td>
</tr>
<tr>
<td>Methylchlorosothiazolinone</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Methylisothiazolinone</td>
<td>≤0.1%</td>
</tr>
</tbody>
</table>
Final Report on the Safety Assessment of Formaldehyde

ABSTRACT
The report selectively reviews the extensive literature available on the toxicity of Formaldehyde. It is concluded that Formaldehyde in cosmetic products is safe to the great majority of consumers. Because of the skin sensitivity of some individuals to this agent, the formulation and manufacture of a cosmetic product should be such as to ensure use at the minimal effective concentration of Formaldehyde, not to exceed 0.2% measured as free Formaldehyde. It cannot be concluded that Formaldehyde is safe in cosmetic products intended to be aerosolized.

INTRODUCTION
The following report reflects the position of the CIR Expert Panel on the safety of formaldehyde in cosmetics. The report is a synopsis of the chemistry, use, biology and toxicology of formaldehyde. In developing this document, members of the Expert Panel reviewed selected references pertaining to formaldehyde safety, as well as a number of unpublished research reports\(^{(1,2)}\) and published literature surveys.\(^{(3-12)}\)

CHEMISTRY
Formaldehyde is a colorless, flammable, readily polymerizable gas having a pungent, suffocating odor. It has the following structural formula.\(^{(9,10,13,14)}\)

\[
\begin{align*}
\text{H} & \quad \text{C} \quad \text{H} \\
O & \\
\text{H} & \quad \text{C} & \quad \text{H}
\end{align*}
\]

Formaldehyde is generally supplied commercially as a 30%-56% (by weight) aqueous solution known as formalin. In aqueous solution, the dominant form of the formaldehyde is methylene glycol; in concentrated solution, it is one of many polymer molecules such as polyoxymethylene glycol. Formaldehyde is also available as its solid cyclic trimer, trioxane; and as its solid, linear, low-molecular-weight homopolymer, paraformaldehyde. Anhydrous gaseous formaldehyde is not available commercially.\(^{(8,10)}\)
Formaldehyde is produced by the oxidation of methanol with air in the presence of a metal catalyst (silver or copper), or an iron-oxide molybdenum oxide catalyst. When used in cosmetics, formaldehyde solution (formalin) typically has the following specifications: assay as HCHO: 37%-52%; assay as methanol: 1.5%-12%; acidity as formic acid: 0.04% maximum; iron: 2 ppm maximum; copper: 10 ppm maximum; ash: 0.01% maximum. Methanol is present in formalin to inhibit polymerization.

Formaldehyde is soluble in water, acetone, benzene, diethyl ether, chloroform, and ethanol. In the absence of water, formaldehyde exists as a monomer and is stable. In the presence of small amounts of water, however, the gas may slowly trimerize to metaformaldehyde. When in aqueous solution, formaldehyde slowly polymerizes to form paraformaldehyde and other products including higher polymers of polyoxymethylene. The uncatalyzed decomposition of formaldehyde is very slow below 300°C. The gas is relatively stable to polymerization at 80°-100°C, but slowly polymerizes at lower temperatures. Decomposition products resulting from the photooxidation of formaldehyde include carbon monoxide, hydrogen, hydrogen peroxide, formic acid, and some other metastable products. A proprietary stabilization process permits prolonged storage of formaldehyde.

Formalin is a powerful reducing agent, especially in the presence of alkali. It is soluble in water, acetone and alcohol. In air, formalin slowly oxidizes to formic acid. Formation of various polymers may occur in formalin as evidenced by development of a cloudy solution; the rate of polymer formation is dependent on methanol content and storage temperature. On exposure of formalin to “very low” temperatures, a precipitate of trioxymethylene is formed.

Paraformaldehyde is a colorless or white granular solid soluble in hot water or strong alkali solution, but insoluble in alcohol or ether. Its formula is HO(CH₂O)ₙH, where n equals 8-100. The higher polymers of paraformaldehyde are insoluble in water. Paraformaldehyde is prepared by the evaporation of formalin. Commercial grades of paraformaldehyde usually contain not less than 95% formaldehyde by weight. At room temperature, paraformaldehyde gradually vaporizes to yield monomeric formaldehyde.

Formaldehyde contains a highly reactive carbonyl group, and it undergoes chemical reactions typical of aldehydes. Among some of the reactions of formaldehyde are: hydration in the presence of water to yield CH₂(OH)₂; reaction with the active hydrogen of ammonia, amines or amides; reaction with other compounds having active hydrogens, such as thiols, nitroalkanes, hydrogen cyanide, and phenol; and condensation with HCL (and possibly other inorganic chlorides) in the presence of water to form the human carcinogen, bis(chloromethyl)ether. Formaldehyde is reported to undergo self-condensation, particularly under alkaline conditions. It may also condense with numerous compounds to produce methylol (CH₂OH) or methylene (=CH₂) derivatives.

Reaction of formaldehyde with the active hydrogen of ammonia, amines or amides is of particular concern because of the ubiquity of nitrogen compounds.
FORMALDEHYDE

(DNA, RNA, proteins, amino acids, etc.) in all biological systems. The reaction with purines and other amines yields an intermediate methylol product which is labile; the reaction product with a second amine moiety is stable.\(^6\) Reaction of formaldehyde with the free amino group in protein probably accounts for the characteristic irritant effects of formaldehyde on mucous membranes.\(^9\)

A diverse group of organic compounds, including alcohols, amines, amides, proteins, phenols, and hydrocarbons, form resins with formaldehyde. For example, urea and phenol can react with formaldehyde to form thermoplastic or thermosetting resins. These latter materials are widely used in the production of plywood, particleboard, foam insulation, and a variety of molded or extruded plastic items.\(^8\) Formaldehyde may also react with acetaldehyde in the presence of a strong base to form pentaerythritol, a compound used in the production of various pharmaceuticals, plasticizers, insecticides, varnishes, resins, and esters.\(^15\)

Various cosmetic ingredients such as albumin, casein, gelatin, agar, and starch may combine directly with formalin to form insoluble compounds. Formalin may also react with such cosmetic materials as perfume, coloring agents, ammonia, alkalis, iron preparations, and hydrogen peroxide.\(^13,21,22\) Because of the highly reactive nature of formaldehyde, the possibility of its interaction with numerous other cosmetic ingredients should not be discounted.

It has been reported that aqueous solutions of formaldehyde generally contain less than 0.1% of the formaldehyde monomer. Polymeric forms of the monomer are the principle molecular species.\(^1,4\) In the cosmetic product, it is not certain whether these same species predominate or if other condensation products or adducts are the predominant forms. It is reasonable to assume, however, that the free formaldehyde monomer concentration is a very small percentage of the amount of formaldehyde added to a cosmetic formulation, and that it exists in some equilibrium with the reacted monomer of polymeric forms.\(^1,1\)

COSMETIC USE

Formaldehyde is typically used in cosmetics as a 37%-52% by weight aqueous solution.\(^15\) The principal function of this cosmetic ingredient is that of an antimicrobial agent.\(^16,23\) Both the concentration and antimicrobial effectiveness of formaldehyde in cosmetic products may decline over time.\(^21,22\) Minimum inhibitory concentrations of formaldehyde against common cosmetic contaminants are presented in Table 1.

<table>
<thead>
<tr>
<th>Agar pH (^b)</th>
<th>Pseudomonas and other gram-negatives</th>
<th>Yeasts</th>
<th>Molds</th>
<th>Cocci</th>
<th>Bacillus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>20-200</td>
<td>90-600</td>
<td>90</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5.5</td>
<td>100-550</td>
<td>350-400</td>
<td>100-450</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>7</td>
<td>70-400</td>
<td>200-750</td>
<td>200-400</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

\(^a\) Data from Ref. 24.

\(^b\) Gradient plate method.
Data submitted to the Food and Drug Administration (FDA) in 1981 by cosmetic firms participating in the voluntary registration program indicated that this preservative (formaldehyde, paraformaldehyde and/or formalin) was used in a total of 805 formulations at concentrations of >5%-10% (2 products), >1%-5% (8 products), >0.1%-1.0% (429 products), and ≤0.1 percent (366 products) (Table 2). Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators conforms to the prescribed format of preset concentration ranges and product categories as described in Title 21 Part 720.4 of the Code of Federal Regulations. Since formaldehyde is primarily supplied for cosmetic use in a 37%-52% aqueous solution, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration might be one-third to one-half of that reported to the FDA (frequently, a reported concentration of “formaldehyde” is actually the concentration of formalin). The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to 10-fold error in the assumed ingredient concentration.

<table>
<thead>
<tr>
<th>Product category</th>
<th>Total no. of products in product category</th>
<th>Total no. of products containing formaldehyde</th>
<th>No. of products containing formaldehyde at each percent concentration rangeb</th>
<th>&gt;5-10</th>
<th>&gt;1-5</th>
<th>&gt;0.1-1</th>
<th>≤0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby shampoos</td>
<td>35</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby lotions, oils, powders, and creams</td>
<td>56</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bath oils, tablets, and salts</td>
<td>237</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bubble baths</td>
<td>475</td>
<td>109</td>
<td>25</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other bath preparations</td>
<td>132</td>
<td>24</td>
<td>5</td>
<td>18</td>
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<td></td>
</tr>
<tr>
<td>Mascara</td>
<td>397</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other eye makeup preparations</td>
<td>230</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sachets</td>
<td>119</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair conditioners</td>
<td>478</td>
<td>95</td>
<td>1</td>
<td>66</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent waves</td>
<td>474</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair rinses (noncoloring)</td>
<td>158</td>
<td>32</td>
<td>18</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair shampoos (noncoloring)</td>
<td>909</td>
<td>316</td>
<td>2</td>
<td>181</td>
<td>133</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonics, dressings, and other hair grooming aids</td>
<td>290</td>
<td>21</td>
<td>1</td>
<td>13</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wave sets</td>
<td>180</td>
<td>37</td>
<td>2</td>
<td>20</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other hair preparations (noncoloring)</td>
<td>177</td>
<td>13</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair dyes and colors (all types requiring caution statement and patch test)</td>
<td>811</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair shampoos (coloring)</td>
<td>16</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face powders</td>
<td>555</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Makeup foundations</td>
<td>740</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Makeup bases</td>
<td>831</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle softeners</td>
<td>32</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nail creams and lotions</td>
<td>25</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 2. (Continued.)

<table>
<thead>
<tr>
<th>Product category</th>
<th>Total no. of products in product category</th>
<th>Total no. of products containing formaldehyde</th>
<th>No. of products containing formaldehyde at each percent concentration range&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;5-10</td>
</tr>
<tr>
<td>Mouthwashes and breath fresheners (liquids and sprays)</td>
<td>53</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Bath soaps and detergents</td>
<td>148</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Deodorants (underarm)</td>
<td>239</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Feminine hygiene deodorants</td>
<td>21</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other personal cleanliness products</td>
<td>227</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aftershave lotions</td>
<td>282</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Shaving cream (aerosol, brushless, and lather)</td>
<td>114</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Other shaving preparation products</td>
<td>29</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Skin cleansing preparations (cold creams, lotions, liquids, and pads)</td>
<td>680</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Face, body, and hand skin care preparations (excluding shaving preparations)</td>
<td>832</td>
<td>47</td>
<td>37</td>
</tr>
<tr>
<td>Foot powders and sprays</td>
<td>17</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Moisturizing skin care preparations</td>
<td>747</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Night skin care preparations</td>
<td>219</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Paste masks (mud packs)</td>
<td>171</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Skin fresheners</td>
<td>260</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other skin care preparations</td>
<td>349</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Suntan gels, creams, and liquids</td>
<td>164</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>1981 TOTALS</strong></td>
<td><strong>805</strong></td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from Ref. 25.

<sup>b</sup> Preset concentration ranges are used by firms in reporting data to FDA in order to conform to federal filing regulations outlined in 21 CFR 720.4.<sup>28</sup>

Cosmetic products containing formaldehyde, formalin and/or paraformaldehyde are applied to or have the potential to come in contact with hair (shampoos and hair preparations, etc.); skin (deodorants, bath products, skin preparations and lotions, etc.); eyes (mascara and eye makeup preparations, etc.); mouth mucosa (mouthwashes and breath fresheners); vaginal mucosa (feminine hygiene deodorants); and nails (cuticle softeners and nail creams and lotions). Aerosol products (shaving creams, for example) also present the potential that formaldehyde may be inhaled.

The FDA permits use of formaldehyde as an ingredient in nail hardeners provided that the product: (1) contains no more than 5% formaldehyde, (2) provides the user with nail shields which restrict application to the nail tip, (3) furnishes adequate directions for safe use, and (4) warns consumers about the consequences of misuse and potential for causing allergic reactions in sensitized users. The FDA has taken action against nail hardeners not meeting these safety requirements.<sup>26</sup>

The European Economic Community<sup>27</sup> has adopted a Directive which im-
poses concentration limits for formaldehyde and paraformaldehyde in cosmetics. These substances are permitted at maximum concentrations of 0.2% (expressed as free formaldehyde) in all cosmetic formulations except nail hardeners, oral hygiene products, and aerosol dispensers. Nail hardeners and oral hygiene products may contain maximum formaldehyde concentrations of 5% and 0.1%, respectively, whereas formaldehyde and paraformaldehyde are prohibited for use in aerosol dispensers (except for foams). Cosmetic product labels are required to list formaldehyde and paraformaldehyde as ingredients when the concentration of either exceeds 0.05%.

U.S. Federal regulations require that formaldehyde and other cosmetic ingredients be listed on the package of each cosmetic product in descending order of predominance. The labeling of ingredients is to “appear with such prominence and conspicuousness as to render it likely to be read and understood by ordinary individuals under normal conditions of purchase.”

NONCOSMETIC USE

Formalin is used as a preservative in many human and veterinary drugs and biologicals. Viral vaccines contain formalin at a level of 0.05% as an inactivating agent. The numerous applications of formalin also include use in tissue preservation, embalming and vaccine production, pesticides, brake lining and pharmaceutical manufacturing, printing, insulation, plastic molding, and as a lubricant and ingredient in paint pigment. The OTC Panel on Dentifrices and Dental Care Products has concluded that there are insufficient data to assess the effectiveness of formalin as a tooth desensitizer.

Nearly three-fourths of the nine billion pounds of formaldehyde now produced annually in the United States is used in various resinous products. Formaldehyde is an essential component in urea-formaldehyde foam insulation, and wrinkle-resistant and shrink-proof textiles. It is also widely used as a binder and adhesive in the manufacture of paper, plywood and particleboard. Federal regulations permit the use of formaldehyde as an indirect food additive in a number of materials having contact with food including adhesive coatings and components, acrylate ester copolymer coatings, resinous polymeric coatings, xylene-formaldehyde resins condensed with 4,4'-isopropylidene-diphenol-eponichlorohydrin epoxy resins; zinc–silicon dioxide matrix coatings, paper and paperboard, defoaming agents used in coatings, defoaming agents used in the manufacture of paper and paperboard, cellophane (as urea formaldehyde), closures with sealing gaskets for food containers (as paraformaldehyde), phenolic resins in molded articles, textiles and textile fibers, and animal glue. Formaldehyde is also allowed as a direct food additive in defoaming agents and as an additive to animal feed.

New installations of urea–formaldehyde foam insulation in residences and schools have been banned by the Consumer Product Safety Commission. The Commission's ban, effective August 10, 1982, is based on findings that suggest urea–formaldehyde foam insulation presents an unreasonable risk of injury from irritation, sensitization, and cancer because of the release of formaldehyde gas from the product after it is installed.
Several recent documents provide detailed literature reviews of the published biological data on formaldehyde. Unpublished information on the toxicity of cosmetic products containing formaldehyde noted in this document is available from the Cosmetic Ingredient Review.

Normal Metabolism of One-Carbon Units

A dietary source of one-carbon units is essential (for example, from the methyl groups of methionine or choline). The vitamin folic acid functions as a metabolic carrier of the one-carbon units obtained from dietary and metabolic sources. The one-carbon derivatives of tetrahydrofolate provide methyl groups for such vital processes as the synthesis of DNA and for the control of protein synthesis. Tetrahydrofolate serves as a carrier of one-carbon units at three levels of oxidation corresponding to methanol, formaldehyde and formic acid. Under normal conditions, the content of free formaldehyde is very low in animal tissues. Sources include formaldehyde in equilibrium with N-5,N-10-methylene tetrahydrofolate and that which may be formed by the direct degradation of methionine, as well as by various demethylation reactions. There is a formaldehyde-forming enzyme present in mammalian tissues (pig brain and rat kidney) that functions in the formation of certain alkaloids from, for example, dihydroxyphenylalanine. Formaldehyde may also be formed in tissue by action of mixed function oxidases on the N-methyl groups of various xenobiotics. Free formaldehyde in concentrations that exceed the dissociation constant of methylene tetrahydrofolate is quickly incorporated into the one-carbon pool. Certainly, minor quantities of formaldehyde are encountered normally and are rapidly metabolized.

Absorption, Metabolism, and Excretion of Exogenous Formaldehyde

Formaldehyde can enter the body through skin and ocular contact, inhalation, and ingestion. Once absorbed into the blood stream, formaldehyde disappears rapidly because of condensation reactions with DNA, protein, amino acids and other amines, as well as by oxidation to CO₂. The half-life of formaldehyde in monkey blood has been estimated to be 1.5 min; similar half-lives have been observed in the blood of rats, guinea pigs, rabbits, and cats. The liver and erythrocytes appear to be primary sites of rapid oxidation of formaldehyde to formic acid and CO₂. Rapid oxidation of formaldehyde to formate has also been shown to occur in many other tissues, including human brain; sheep liver; rat brain, kidney and muscle; rabbit brain; and bovine brain and adrenals. The conversion of formaldehyde to formate has been observed following intravenous infusion, subcutaneous injection, gastric intubation and inhalation. The plasma half-life of formate in dogs following i.v. infusion or oral administration of 0.2 M formaldehyde has been estimated to be 80-90 min.

Oxidation of formaldehyde may be initiated by formation of S-formyl glutathione, which is then oxidized by nicotinamide-adenine dinucleotide, and finally cleaved by a thiol esterase releasing formic acid and glutathione. Studies with rats, monkeys, and rat liver perfusates have demonstrated that the
primary pathway to CO$_2$ from formaldehyde and formate occurs via the tetrahydrofolate pathway.\cite{61-64} Whereas the conversion of formaldehyde to CO$_2$ occurs in a similar manner in the different species studied, the relative importance of each reaction varies among species and tissues.\cite{6} Thus, the rat is able to convert formate to CO$_2$ at more than twice the rate of monkeys (or humans) and, as a result, has lower blood formate levels and does not excrete formate in the urine.\cite{65} With regard to tissue differences, mouse (C3Hf/A) and hamster (Syrian Golden) lung tissue does not convert formate to CO$_2$ as efficiently as liver tissue.\cite{66}

Absorption of formaldehyde through the upper respiratory tract of dogs was shown in one study to exceed 95% of the inhaled dose.\cite{67} Humans exposed to formaldehyde gas (0.78 mg/m$^3$) for 3 h had a rapid rise in blood and urine formate concentrations.\cite{66} Following subcutaneous injection of $^{14}$C-formaldehyde into rats, approximately 81% of the radioactivity appeared as CO$_2$; a small amount of the radioactivity was found in choline.\cite{69} In rats given formaldehyde by intraperitoneal injection, 82% of the radiolabel was recovered as CO$_2$, whereas 13%-14% was recovered as urinary methionine, serine and a cysteine adduct; it was postulated that CO$_2$ was derived from serine by deamination to pyruvate and oxidation in the Krebs cycle.\cite{65} Incorporation of $^{14}$C-formaldehyde into the nucleic acid protein fraction of WI 38 human diploid fibroblasts was also demonstrated; most of the radiolabel was found in RNA with lesser amounts in DNA and protein.\cite{70}

**Effect on Macromolecules of Biological Importance**

The adverse effects of formaldehyde seen in many in vivo and in vitro systems may be related to its high reactivity with amines, and its formation of methylol adducts with nucleic acids, histones, proteins, and amino acids.\cite{8} The irritant effects of formaldehyde on mucous membranes is likely the result of its reaction with the free amino group in proteins.\cite{9} The interaction of formaldehyde with proteins and nucleic acids, particularly RNA, results in tissue fixation and denaturation; the denaturation observed with DNA is irreversible. If permanent cross-links are formed between DNA reactives sites and formaldehyde, these links could interfere with the replication of DNA and may result in mutations.\cite{8}

Grafstrom et al.\cite{71} recently suggested that formaldehyde could exert its mutagenic and carcinogenic effects by both damaging DNA and inhibiting DNA repair. In their studies with cultured bronchial epithelial and fibroblastic cells from humans, formaldehyde induced DNA protein cross-links and DNA single-strand breaks at HCHO concentrations of 100 $\mu$M and 500 $\mu$M, respectively. Formaldehyde also inhibited the unscheduled DNA synthesis that occurs after exposure of cells to ultraviolet irradiation or to benzo[a]pyrene dioleloxipoxide, as well as inhibited the resealing of DNA single-strand breaks produced by ionizing radiation. It was suggested that the high reactivity of formaldehyde probably causes methylolation of chromatin or other proteins, including enzymes critical to DNA repair processes.

**Animal Toxicology**

LD$_{50}$ values for formaldehyde in various species are given in Table 3.

Cosmetic products containing formaldehyde concentrations of 0.074% (2
TABLE 3. Acute LD₅₀ Values for Formaldehyde in Various Species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>LD₅₀ (mg/kg)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat</td>
<td>oral</td>
<td>800</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>420</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>87</td>
<td>74</td>
</tr>
<tr>
<td>mouse</td>
<td>s.c.</td>
<td>300</td>
<td>73</td>
</tr>
<tr>
<td>rabbit</td>
<td>dermal</td>
<td>270</td>
<td>75</td>
</tr>
<tr>
<td>guinea pig</td>
<td>oral</td>
<td>260</td>
<td>72</td>
</tr>
</tbody>
</table>

Formalin produced severe skin irritation following application to rabbit skin using an occlusive dressing technique; however, no significant irritant effects were noted following exposure to a 1% aqueous solution of formaldehyde. Cosmetic formulations containing 0.074% and 0.0925% formaldehyde were minimal to slight irritants to the skin of rabbits. In studies with guinea pigs, 5%, 10%, and 20% aqueous solutions of formaldehyde, and 0.01% and 0.02% saline solutions of formaldehyde, were mildly to moderately irritating to the skin.

Formalin produced severe skin irritation following application to rabbit skin using an occlusive dressing technique; however, no significant irritant effects were noted following exposure to a 1% aqueous solution of formaldehyde. Cosmetic products containing 0.074% (2 formulations) and 0.0925% (1 formulation) formaldehyde were at most minimally irritating to the rabbit eye. Severe ocular irritation was observed in rabbits given 15% formaldehyde in aqueous solution.

Results of guinea pig sensitization studies varied according to formaldehyde concentration and test methodology. Formalin (37% formaldehyde in aqueous solution) elicited skin sensitization when tested by the Draize, Buehler, and Magnusson–Kligman maximization procedures. In two separate studies in which the Buehler technique was employed, 2% formaldehyde in aqueous solution elicited sensitization, whereas 5% formaldehyde in aqueous solution elicited no allergic reaction. Skin sensitization was observed in guinea pigs following repeated intradermal dosing (optimization test); in this study, a 0.04% aqueous formaldehyde solution was used for induction. In four separate guinea pig sensitization studies, the Magnusson–Kligman maximization test was used to evaluate formaldehyde in aqueous solution at various concentrations. Formaldehyde was sensitizing in two of these studies following induction and challenge applications of 2% and 0.8%, respectively. Formaldehyde was nonsensitizing to guinea pigs in a third study where the induction and challenge concentrations were 0.703% and 0.222%, respectively; as well as in a fourth study where the induction, booster, and challenge concentrations were 1.85%, 3.7%, and 0.925% (or 0.463%), respectively.
Formaldehyde was administered for 90 days either in the drinking water of rats on a w/v basis at 50, 100, or 150 mg/kg/day; or, in the diet of dogs at doses of 50, 75, or 100 mg/kg/day. There were no significant changes observed in the organs examined microscopically, or in hematological and biochemical tests (hematocrit, hemoglobin, total and differential leukocyte counts, blood sugar, blood urea nitrogen, alkaline phosphatase, serum glutamic oxaloacetic transaminase). A decrease in weight gain was noted in the high dose group of each species.\(^{83,84}\)

A 13-week study was conducted with rats to determine the dermal toxicity of a moisturizer containing 0.074% formaldehyde. The daily dose of formaldehyde was 2.3 mg/kg. There were no cumulative, systemic toxic effects. Urinalysis, clinical chemistry, hematologic values, appearance, behavior, survival, body and organ weights were normal, and no gross or microscopic lesions were found. Mild hyperkeratosis at the site of application was the only change noted which could be related to treatment.\(^{1,2}\) Similar parameters were examined in another 13-week dermal toxicity study involving two cosmetic formulations. With each product, formaldehyde was applied daily to the rat skin at a dose of 1.78 mg/kg. At week seven, rats treated with one of the two products had a decrease in brain-to-body weight ratio, an altered neutrophile/lymphocyte ratio, increased uterine weight, and hyperkeratosis; other parameters were normal. No cumulative systemic toxicity was observed with either product.\(^{1,2}\)

Acute, subacute, and chronic studies have been conducted in rats, mice, rabbits, guinea pigs, hamsters, cats, dogs, and monkeys to determine the effects of inhalation exposure to formaldehyde. These studies are reviewed extensively by Fielder.\(^9\) Acute and subacute exposure to low (< 1 ppm) or moderate (10–50 ppm) concentrations of formaldehyde vapor is known to cause increased airway resistance, decreased sensitivity of the nasopalatine nerve, irritation of eyes and of the respiratory system, and changes in the hypothalamus. Exposure to high doses (> 100 ppm) of formaldehyde vapor can cause salivation, acute dyspnea, vomiting, cramps and death of the test animals.\(^{10}\) Rats, guinea pigs, rabbits, monkeys and dogs exposed continuously to 3.7 ppm formaldehyde for 90 days exhibited interstitial inflammation of the lungs.\(^{85}\)

Several recent inhalation studies have been reviewed by IARC.\(^{10}\) Exposure of rats to 2.0, 5.6, or 14.3 ppm formaldehyde vapor 6 h/day, five days/week for up to 24 months resulted in a variety of nasal cavity lesions including dysplasia and squamous metaplasia of respiratory epithelium, and purulent or seropurulent rhinitis. Similar lesions were observed in mice exposed for the same length of time to 5.6 or 14.3 ppm formaldehyde; whereas, no effects were observed after exposure to 2 ppm.\(^{86}\) Acute cellular degeneration, necrosis and inflammation were present in the nasal mucosa of rats exposed to 15 ppm formaldehyde vapor 6 h/day for 1–9 days.\(^{87}\) Hyperplasia of respiratory epithelial cells has been observed in rats and mice exposed to 15 ppm formaldehyde 6 h/day for three days.\(^{87}\) In yet another study, monkeys, rats, and hamsters were exposed to 0.0, 0.02, 1.0, or 3.0 ppm formaldehyde vapor for 22 h/day, seven days/week for 26 weeks. Squamous metaplasia of the nasal turbinate epithelium was evident in 6/6 monkeys at 3 ppm and in 1/6 at 1 ppm; squamous metaplasia and basal cell hyperplasia of the respiratory epithelium were significantly increased in rats exposed to 3 ppm. No exposure-related effects were observed in the hamsters.\(^{88}\)

Rats and mice are obligatory nose breathers; therefore, nasal defense
mechanisms may be more important in these animals. Thus, with respect to target organs for formaldehyde, it may be inappropriate to extrapolate results of rat and mouse formaldehyde-inhalation experiments directly to humans.\(^8\)

**Special Studies**

**Embryotoxicity/Teratogenicity**

The International Agency for Research on Cancer has concluded there are insufficient data to evaluate adequately the embryotoxicity/teratogenicity of formaldehyde.\(^9\)

Formaldehyde was given to pregnant CD-1 mice as an aqueous solution (containing about 0.2% formaldehyde) at oral doses of 74, 148, and 185 mg/kg on Days 6–15 of gestation. The 185 mg/kg dose was lethal by Day 18 to most of the females (23/34), but no deaths were noted at the 74 mg/kg dose; one rat of the mid-dose group died. The number of resorption sites was increased and mean litter size was decreased slightly in the high dose group; these parameters were normal in the other two dose groups. No effects on fetus size, and no skeletal or visceral abnormalities (gross or microscopic) were observed.\(^9\)

No teratogenic effects were observed in the offspring of pregnant beagles given formaldehyde at dietary concentrations of 125 or 375 ppm on Days 4–56 of gestation. No compound-related effects were noted in the female parents. Litter size, number of stillborn pups, and pup survival were not different from controls. A few of the pups were observed for up to nine months, during which time no abnormalities were noted in appearance or behavior.\(^9\)

In an inhalation study, female rats were exposed continuously at reported concentrations of 0.012 mg/m\(^3\) (0.01 ppm) or 1 mg/m\(^3\) (0.8 ppm) from 10 days prior to mating and throughout the gestation period. No gross malformations occurred in fetuses at either dose level, and the only evidence of toxicity in females was a slight increase in the length of the gestation period. Average weight of offspring from both treatment groups was increased at birth. At necropsy of the neonates, the weights of thymus, kidney, and adrenal were increased and liver and lung weights were decreased.\(^9\)

No alteration of reproductive function was seen in male rats given formaldehyde for six months at 0.1 ppm in drinking water, or 0.4 ppm in air.\(^9\)

**Mutagenicity**

The mutagenicity of formaldehyde has been reviewed.\(^8-10,93,94\) There is evidence that formaldehyde does not react with native double stranded DNA, but that it does react with single stranded DNA, or “open” DNA in which the hydrogen bond is disrupted.\(^9\) Grafstrom et al.\(^7\) recently suggested that formaldehyde could exert its mutagenic and carcinogenic effects both by damaging DNA and inhibiting DNA repair.

The mutagenic activity of formaldehyde has been demonstrated in studies with bacteria (*Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus*); RNA containing virus; yeast (*Saccharomyces cerevisiae*); fungi (*Neurospora crassa, Aspergillus nidulans*); grasshopper (*Tristria pulvinata*) and fruitflies (larval and adult *Drosophila*). The mutagenic effect of formaldehyde in *Drosophila melanogaster* is dependent on the route of administration. Both positive and negative mutagenic results have been obtained with *Salmonella*.
typhimurium. Formaldehyde did not induce mutations in the silkworm (Bombyx mori). (8-10)

The mutagenic activity in various mammalian systems has also been studied. An increase in the mutation frequency was observed when formaldehyde was tested in the L5178Y mouse lymphoma assay, but not in the Chinese hamster ovary cell assay. (97) No mutagenic effect was noted in a dominant-lethal study in which Swiss mice were given intraperitoneal injections of formaldehyde; however, it should be noted that several known mutagens were inactive in this test as well. (98) Early fetal deaths and preimplantation losses in Q strain mice were reported after males were given intraperitoneal injections of formaldehyde, but no chromosomal aberrations were observed in preparations of meiotic spermatocytes from the treated animals. (99) Treatment of C3H/10T1/2 Cl eight mouse embryo fibroblasts with formaldehyde did not result in significant rates of transformation; however, when formaldehyde exposure was followed by continuous treatment with the tumor promoter 12-O-tetradecanoyl phorbol-13-acetate, transformed foci were produced. (100) Formaldehyde has also been shown to induce sister chromatid exchanges in cultured Chinese hamster ovary cells and human lymphocytes; unscheduled DNA synthesis in Hela cells; preferential killing of xeroderma pigmentosum cells; DNA-protein crosslinks in both mouse L1210 cells and Chinese hamster V79 cells; and transformation in mouse BALB/c 3T3 cells. (101)

The relevance of these studies is difficult to assess for any systemic, mutagenic, or carcinogenic effects in mammals since it is known that formaldehyde is rapidly metabolized in the bloodstream, and hence, may be detoxified before it can produce critical damage to cellular DNA. (9)

Carcinogenicity

In a recent study at the Chemical Industry Institution of Toxicology (CIIT), groups of B6C3F mice and Fischer 344 rats were exposed to 0, 2.0, 5.6, or 14.3 ppm (0, 2.0, 6.9, 17.6 mg/m³) formaldehyde (>97.5% pure) vapor by whole body exposure for 6 h/day, 5 days/week for up to 24 months. Animals were killed at 6, 12, 18, 24, 27, and 30 months. Histopathological examinations were made of the tissues lining the nasal cavity as well as tissues from each major organ system. In mice of the high-dose group, the incidence of nasal mucosal squamous cell carcinoma was not statistically significant. However, the incidences of a variety of nonneoplastic lesions of the nasal mucosa were significantly increased at formaldehyde concentrations of 5.6 and 14.3 ppm. (86) In rats exposed to 14.3 ppm, a significant increase in the incidence of nasal mucosal squamous cell carcinoma was noted; no other neoplasm was significantly increased. The incidences of a variety of nonneoplastic lesions of the nasal mucosa were also significantly increased in rats at formaldehyde concentrations as low as 2 ppm; these increased in extent and severity with increasing concentrations. (86,106)

With regard to the CIIT study, signs of chronic irritation to the nasal passages were noted prior to the development of the tumors. It has been suggested that the marked increase in cell turnover associated with chronic irritation is necessary for the expression of any mutagenic effects of formaldehyde on the cells of the nasal turbinates. (9)

In a second inhalation study, 100 Sprague-Dawley rats were exposed from
nine weeks of age to 14.2 ppm (17.23 mg/m³) formaldehyde vapor for 6 h/day. After a total of 382 exposures over a period of 588 days, 10 histologically confirmed, grossly visible nasal squamous-cell carcinomas were observed. No nasal tumors were seen in 1,920 control rats over a period of 14 years.\(^{107}\)

Ninety-nine Sprague–Dawley rats were exposed from eight weeks of age to a mixture of 14.7 ppm (17.9 mg/m³) formaldehyde vapor and 10.6 ppm (17.3 mg/m³) hydrogen chloride gas 6 h/day, 5 days/week for life. The average concentration of bis(chloromethyl)ether (BCME) formed was 1 ppb (5.13 ng/m³). Of the exposed animals, 28 developed nasal tumors (25 had squamous cell carcinomas and three had papillomas). No nasal tumors were seen in controls.\(^{109}\)

Various reports indicate that BCME, a recognized human carcinogen, should not be formed in substantial amounts if concentrations of both hydrogen chloride and formaldehyde gas are less than 100 ppm at ambient temperature and humidity.\(^{8}\) However, Frankel et al.\(^{108}\) found that BCME was formed in glass vessels at less than 0.5 ppb when formaldehyde and hydrogen chloride are each present at 20 ppm.

C3H mice were exposed to formaldehyde vapor at concentrations of 0, 0.05, 0.1, or 0.20 mg/l (0, 50, 100, or 200 mg/m³) for 1 h/day, three times per week for 35 weeks. There were no pulmonary tumors in any dose group. The nasal epithelium was not examined either grossly or microscopically. Basal cell hyperplasia and squamous and atypical metaplasia were seen in the trachea and bronchi of treated mice. In the same experiment, an additional group of mice were exposed to 100 mg/m³ formaldehyde vapor for 35 weeks and then to a coal-tar aerosol for 35 weeks; the formaldehyde did not modify the pulmonary carcinogenesis of coal tar.\(^{109}\)

Ten rats were injected subcutaneously once weekly for 15 months with 1 ml of 0.4%-0.5% formaldehyde in aqueous solution. Sarcomas were observed in four rats: two in the skin at the injection site, one in the liver, and one in the peritoneal cavity. No control animals were used.\(^{110}\)

Hamsters were given 10 weekly subcutaneous injections of 0.5 mg N-nitrosodiethylamine concurrently with weekly 5-hour exposures to 30 ppm (36.7 mg/m³) formaldehyde for life. The number of tumors per tumor-bearing animal of the “concurrently exposed group” was increased over the group receiving N-nitrosodiethylamine alone.\(^{111}\)

Hamsters were exposed in an inhalation study to 10 ppm formaldehyde five times/week (5 h/day) for life. No tumors were observed in sections of respiratory tract tissues from either unexposed or treated animals. In a separate experiment, hamsters were exposed once per week to 30 ppm formaldehyde (5 h/day) for life. No tumors were observed in the respiratory tract of the formaldehyde-only control group; however, hamsters exposed to formaldehyde at two days prior to each of 10 weekly diethylnitrosamine injections had a higher incidence of tracheal tumors/tumor-bearing animal at necropsy than those receiving diethylnitrosamine alone.\(^{112}\)

Six rabbits were fitted by a muzzle-like holder to tanks containing a 3% formaldehyde solution for 90 min, five times per week for 10 months. Animals were sacrificed after 11 months of exposure. Leucoplakia was grossly visible in 2/6 rabbits; in these lesions, dyskeratosis and intraepithelial carcinoma of the exposed mucosa were confirmed microscopically.\(^{113}\)

The International Agency for Research on Cancer concluded “there is sufficient evidence that formaldehyde gas is carcinogenic to rats.” They noted that
concentrations of formaldehyde that cause nasal tumors also cause acute degeneration, necrosis, inflammatory changes and increased cell replication (hyperplasia) of the nasal mucosa of rats and mice following inhalation exposure.\(^{(10)}\)

The evidence of formaldehyde-induced neoplasia in rats had led the National Institute of Occupational Safety and Health to recommend that this material be handled as a potential human carcinogen.\(^{(11,14)}\)

**Clinical Assessment of Safety**

Table 4 summarizes data on human responses to formaldehyde at various airborne concentrations. The severity of specific health effects appears to be dose-related.\(^{(8)}\) Among some of the reported effects are neurophysiologic changes (as demonstrated by alterations in optical chronaxy, EEG, etc.); eye, skin, nose, throat, and bronchial irritation; and pulmonary lesions (pneumonia, bronchial inflammation, pulmonary edema). Death may result from exposure to formaldehyde vapor at concentrations of 100 ppm and greater.\(^{(8,9)}\) The effects of formaldehyde arising from occupational exposure have been reviewed in some detail by Fielder.\(^{(9)}\) The American Conference of Governmental Industrial Hygienists recommends a limit of 2 ppm (approximately 2.5 mg/m\(^3\)) for occupational exposure.\(^{(14)}\)

Formaldehyde is intensely irritating to the eyes. Ocular irritation to atmospheric formaldehyde generally occurs at concentrations of 0.05–0.5 ppm; lacrimation occurs at concentrations of 4–20 ppm. Aqueous solutions of formaldehyde accidently splashed into the eye have caused such injuries as eyelid and conjunctival edema, corneal opacity, and loss of vision.\(^{(8,9)}\) Numerous studies demonstrating the eye irritating ability of formaldehyde have been reviewed by the National Research Council.\(^{(8)}\)

Upper airway (nose and throat) irritation to formaldehyde vapor frequently occurs at 1–11 ppm (irritation has been recorded at concentrations as low as 0.1 ppm). Formaldehyde can cause alterations in the nasal defense mechanisms, which may include a decrease in mucociliary clearance and loss of olfactory sensitivity. Lower airway irritation frequently is reported at 5–30 ppm. Chest radiographs of persons exposed to these concentrations are usually normal, ex-

<table>
<thead>
<tr>
<th>Health effects reported</th>
<th>Approx. formaldehyde conc. (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0–0.05</td>
</tr>
<tr>
<td>Neurophysiologic effects</td>
<td>0.05–1.50</td>
</tr>
<tr>
<td>Odor threshold</td>
<td>0.05–1.0</td>
</tr>
<tr>
<td>Eye irritation</td>
<td>0.01–2.0(^b)</td>
</tr>
<tr>
<td>Upper airway irritation</td>
<td>0.10–25</td>
</tr>
<tr>
<td>Lower airway and pulmonary effects</td>
<td>5–30</td>
</tr>
<tr>
<td>Pulmonary edema, inflammation, pneumonia</td>
<td>50–100</td>
</tr>
<tr>
<td>Death</td>
<td>100+</td>
</tr>
</tbody>
</table>

\(^{a}\) Data from Ref. 8.

\(^{b}\) The low concentration (0.01 ppm) was observed in the presence of other pollutants that may have been acting synergistically.
cept for occasional reports of accentuated bronchovascular marks; however, pulmonary function tests may be abnormal. Pulmonary edema, and pneumonitis and death can result from very high airborne formaldehyde concentrations (50–100 ppm). (8)

Formaldehyde inhalation has been shown to cause bronchial asthma and asthma-like symptoms in humans. Although asthmatic attacks are in some cases specifically attributable to formaldehyde sensitization or allergy, the gas seems to act more commonly as a direct airway irritant in persons who have bronchial asthmatic attacks from other causes. The exact mechanism for asthma induction by formaldehyde is not known. (8)

Formaldehyde has been reported to cause contact urticaria, and it is a known skin irritant and sensitizer. Allergic contact dermatitis in persons both occupationally and nonoccupationally exposed to formaldehyde is well recognized. (9) The North American Contact Dermatitis Group reported a 5% incidence of skin sensitization (124 reactors) among 2,374 patients exposed to 2% formaldehyde in aqueous solution. (115) Most sensitized persons can tolerate topical axillary products containing formaldehyde at up to 30 ppm; (116) with increasing concentrations, a higher frequency of responders is seen. (117) The National Research Council reported that aqueous formaldehyde solutions elicit skin responses under occlusive conditions in some sensitized individuals at concentrations as low as 0.01%. It was also noted that underarm products containing up to 0.003% formaldehyde are tolerated by most sensitized individuals. (8) In unpublished data reported by the Cosmetic, Toiletry and Fragrance Association, cosmetic products containing 0.000185%–0.0925% formaldehyde were practically nonirritating and nonsensitizing in a total of 1,527 subjects in 18 separate tests (Table 5). Bruynzeel et al. (118) have noted that allergens which are marginal skin irritants such as formaldehyde, often give weak positive reactions which may be lost at retesting.

Jordan et al. (116) have evaluated threshold skin sensitization responses to formaldehyde in formaldehyde-sensitive individuals. In one double-blind experiment, closed patches containing 0, 30, or 100 ppm formaldehyde in a vehicle of water and 12 percent methanol were applied to the upper backs of nine formaldehyde-sensitive subjects. Patches were applied on Friday and reapplied the following Monday (72 h) and Wednesday (120 h). The last reading was conducted on Friday (168 h). Four nonallergic control subjects underwent identical testing. Results for the formaldehyde-sensitive subjects are presented in Table 6. By the 168 hr. reading, a total of six of nine subjects reacted to 100 ppm, five of nine reacted to 60 ppm, and four of nine to 30 ppm. None of the four nonallergic control subjects reacted to 0, 30, 60, or 100 ppm formaldehyde in the methanol-in-water vehicle. In a second experiment, 13 formaldehyde-sensitive subjects pump-sprayed 28.86 ppm formaldehyde in a methanol-in-water vehicle into one axilla twice per day for two weeks. The vehicle served as a control in the opposite axilla. Two of 13 subjects exhibited minimal dermatitis, whereas another three individuals had subjective complaints of itching or burning skin. No responses to the vehicle were seen. Findings from these two studies indicate that formaldehyde concentrations below 30 ppm can be tolerated by most sensitive subjects when repeatedly applied to normal skin. According to Jordan et al. (116) any response on normal, semioccluded skin should be a “very mild, self-limited problem, provided no additional therapeutic insults are added.”

The dose needed to elicit a skin sensitization response depends on such fac-
TABLE 5. Human Skin Irritation and Sensitization to Cosmetic Products Containing Formaldehyde.

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Material tested</th>
<th>Actual Formaldehyde conc. tested (%)</th>
<th>Method</th>
<th>No. of subjects</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin irritation</td>
<td>Skin moisturizer containing 0.25% formalin.</td>
<td>0.0925</td>
<td>24 h occlusive patch</td>
<td>20</td>
<td>Nineteen subjects showed no skin reactions and one had a “barely perceptible” erythemic response. The PIL(^b) was 0.03.</td>
<td>119</td>
</tr>
<tr>
<td>Skin irritation</td>
<td>Noncoloring hair rinse containing 0.2% formalin.</td>
<td>0.074</td>
<td>24 h occlusive patch</td>
<td>20</td>
<td>Nineteen subjects showed no skin reaction and one had a “barely perceptible” erythemic response. The PIL(^b) was 0.03.</td>
<td>120</td>
</tr>
<tr>
<td>Skin irritation</td>
<td>Facial cleanser containing 0.2% formalin.</td>
<td>0.074</td>
<td>21 daily applications under an occlusive patch</td>
<td>8</td>
<td>Three of eight subjects showed cumulative skin irritation. The three reactors had a total cumulative score of 19/504 to the facial cleanser, whereas the test group as a whole had a total cumulative score of 299.5/504 to the positive control. The average cumulative scores to the facial cleanser and positive control were 2.38 and 37.44, respectively.</td>
<td>121</td>
</tr>
<tr>
<td>Skin irritation</td>
<td>Skin cleanser containing 0.2% formalin.</td>
<td>0.074</td>
<td>24 h occlusive patch</td>
<td>20</td>
<td>No skin irritation observed. The PIL(^b) was 0.</td>
<td>122</td>
</tr>
<tr>
<td>Skin irritation</td>
<td>Skin Cleanser containing 0.2% formalin.</td>
<td>0.074</td>
<td>24 h occlusive patch</td>
<td>19</td>
<td>No skin irritation observed. The PIL(^b) was 0.</td>
<td>122</td>
</tr>
<tr>
<td>Skin irritation</td>
<td>Skin Cleanser containing 0.2% formalin.</td>
<td>0.074</td>
<td>24 h occlusive patch</td>
<td>19</td>
<td>Eighteen subjects showed no skin reaction and one had a mild erythemic response. The PIL(^b) was 0.05.</td>
<td>122</td>
</tr>
</tbody>
</table>
Skin irritation/sensitization

Facial Cleanser containing 0.2% formalin.

Two subjects showed one or more skin reactions (1+ and 2+) to the induction patches. Two others showed skin reactions (1+) on challenge as well as to the induction patches (1+ and 2+). One of the latter two subjects agreed to a follow-up rechallenge with the product “as is” under occlusion, diluted 1:4 under occlusion, and “as is” under semi-occlusive conditions. The material was applied three times daily for five consecutive days. At 48 h, a 1+ reaction was noted in the subject under “as is” occluded conditions. No reaction was observed at 96 h. No reactions were observed at 48 or 96 h under the “diluted occlusive” or the “as is semi-occlusive” regimens. The investigators determined that this was evidence of an irritant reaction, and was not sensitization. The other subject who had reacted at challenge declined to participate in the rechallenge. It was noted that this subject had reacted to several other materials being evaluated, and she was categorized as an “angry back” type subject. The facial cleanser was considered to possess a minimal potential for inducing irritant or allergic contact dermatitis under foreseeable conditions of product use.
<table>
<thead>
<tr>
<th>Type of test</th>
<th>Material tested</th>
<th>Actual Formaldehyde conc. tested (%)</th>
<th>Method</th>
<th>No. of subjects</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin irritation/</td>
<td>Facial Moisturizer containing 0.1% formalin.</td>
<td>0.037</td>
<td>RIPT</td>
<td>200</td>
<td>None of the subjects had any skin changes as a result of the first induction application. During subsequent induction applications, six subjects showed skin reactions determined to be “artifacts” on the procedure in that the changes were minimal, of less than 24 h duration, noncumulative in producing scores of 2+ or greater, and not consistently repetitive. One subject showed irritant responses considered to be evidence of skin fatigue. On challenge none of the subjects showed any response.</td>
<td>124</td>
</tr>
<tr>
<td>sensitization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin irritation/</td>
<td>Hair care product containing 0.2% formalin.</td>
<td>0.0185</td>
<td>RIPT</td>
<td>101</td>
<td>Four subjects exhibited one or more skin reactions (minimal to moderate erythema) during the induction phase. No subjects reacted on challenge.</td>
<td>125</td>
</tr>
<tr>
<td>sensitization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Medicated cleanser containing 0.2% formalin.</td>
<td>0.074</td>
<td>NR</td>
<td>83</td>
<td>No sensitization observed.</td>
<td>126</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Liquid cleanser containing 0.1% formalin.</td>
<td>0.000185</td>
<td>NR</td>
<td>83</td>
<td>No sensitization observed.</td>
<td>126</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Moisturizer containing 0.2% formalin.</td>
<td>0.074</td>
<td>NR</td>
<td>118</td>
<td>No sensitization observed.</td>
<td>126</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Cleansing cream containing 0.2% formalin,a</td>
<td>0.074</td>
<td>NR*</td>
<td>118</td>
<td>No sensitization observed.</td>
<td>126</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Hand cream containing 0.1% formalin,a</td>
<td>0.037</td>
<td>NR*</td>
<td>113</td>
<td>No sensitization observed.</td>
<td>126</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Moisturizer containing 0.2% formalin,a</td>
<td>0.074</td>
<td>NR*</td>
<td>92</td>
<td>No sensitization observed.</td>
<td>126</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Hair treatment product containing 0.2% formalin,a</td>
<td>0.0185</td>
<td>NR*</td>
<td>98</td>
<td>No sensitization observed.</td>
<td>126</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Hair conditioning rinse containing 0.2% formalin,a</td>
<td>0.0185</td>
<td>NR*</td>
<td>98</td>
<td>No sensitization observed.</td>
<td>126</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Daytime moisturizer containing 0.1% formalin,a</td>
<td>0.037</td>
<td>NR*</td>
<td>113</td>
<td>No sensitization observed.</td>
<td>126</td>
</tr>
</tbody>
</table>

Total: 1527

---

a Formalin: 37% (w/w) aqueous formaldehyde solution.
b PII = Primary Irritation Index: a value depicting the average skin response of the test panel as a whole. It is calculated by adding the irritation scores and dividing by the total no. of test subjects. The PII is based on a scale of 0 (no skin reaction) to 4 (severe skin erythema and/or edema).
c For each subject, scores were graded daily on a scale of 0 to 4; however, testing ceased when a score of 3 was reached. A score of 3 was then recorded thereafter for each of the remaining days that the subject was not tested. Thus, for 8 subjects over 21 days of testing, the maximum possible score was 504 (8 × 21 × 3 = 504).
d RIPT = Repeat Insult Patch Test. Each induction application was made every other day for 3 weeks for a total of 9–10 induction exposures. The challenge application followed 10–14 days after the induction phase. Scores were based on a scale of 0 (no skin reaction) to 4+ (marked erythema and edema).
e NR = Not reported.
TABLE 6. Number of Formaldehyde-Sensitive Subjects Having Allergic Responses.a,b

<table>
<thead>
<tr>
<th>Formaldehyde (ppm)</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/9</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4/9</td>
</tr>
<tr>
<td>60</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>5/9</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>6/9</td>
</tr>
</tbody>
</table>

aData from Ref. 116.
bPatch tests applied on Friday with reading and reapplication on Monday (72 h) and Wednesday (120 h). Final reading Friday (168 h).

Skin sensitization to cosmetic products may result from a number of formaldehyde-releasing agents used in formulations. It has been reported that formaldehyde-releasing preservatives, such as Quaternium-15, show a greater reaction frequency than formaldehyde itself. Quaternium-15 at the usual preservative concentration of 0.1% releases about 100 ppm of free formaldehyde. Repeated topical application of creams and lotions utilizing Quaternium-15 can pose a problem for formaldehyde-sensitive individuals.

Ingestion of formaldehyde has been reported to cause allergic reactions, cor-rosive effects on the gastrointestinal and respiratory tracts, and systemic damage. Following ingestion, there may be loss of consciousness, vascular collapse, pneumonia, hemorrhagic nephritis, fatty degeneration of the liver, "involvement of the brain," and spontaneous abortion. Death may occur after the swallowing of as little as 30 ml of formalin. Paresthesia, soft-tissue necrosis and sequestration of bone have occurred when formaldehyde preparations were used for devitalization of dental pulps.

Central nervous system responses to formaldehyde have been evaluated in a
variety of ways including determination of optical chronaxy, electroencephalography, and by the sensitivity of dark-adapted eyes to light. Responses are observed in some individuals at 0.05 ppm formaldehyde and are maximized at approximately 1.5 ppm.\(^{(18)}\)

Hemolytic anemia occurred in patients undergoing chronic hemodialysis following contamination of the dialysis water with formaldehyde. Subsequent in vitro experiments to determine the mechanism of formaldehyde action revealed that the substance converts NAD to NADH in the erythrocyte. The alteration of the redox state leads to inhibition of glycolysis at the level of glyceraldehyde 3-phosphate dehydrogenase and rapid decline in cellular ATP content. A formaldehyde concentration as low as 0.1 mM caused decreased ATP content in erythrocytes, whereas the maximal inhibiting effect on red blood cell metabolism occurred after exposure to 1.0 mM formaldehyde.\(^{(127)}\)

Numerous studies have been conducted to determine the incidence of cancer and mortality in industries where formaldehyde is used. However, these studies are of limited value since workers in such industries were exposed to many other chemicals in addition to formaldehyde.\(^{(10)}\)

Medical personnel, particularly pathologists and certain laboratory technicians, have an increased likelihood of exposure to formaldehyde.\(^{(109)}\) According to data recorded in the Danish Cancer Registry during 1943–1976, only three cases of cancer of the nasal cavities, sinuses, or nasopharynx were observed in Danish doctors. None of these three doctors had ever worked in a pathology department or as anatomists.\(^{(128)}\) In a mortality study of pathologists and medical laboratory technicians in the U.K., male pathologists had a significant increase in lymphoid and hematopoietic neoplasms, but similar findings were not observed in the laboratory technicians.\(^{(129)}\) In a study of 34,400 British doctors, no significant increase in respiratory cancers was found among nonsmokers.\(^{(13)}\) The mortality rates within 11 occupation groups (including scientific research, pathology and biochemistry) among 20,540 male doctors indicated reduced numbers of oral, esophageal and pulmonary cancer.\(^{(131)}\)

The result of three recent mortality studies of workers using formaldehyde or manufacturing formaldehyde and other chemicals were inconclusive. In a study of embalmers who used embalming fluid containing formaldehyde and a variety of other chemicals, a proportional excess of deaths from skin cancer was observed.\(^{(132)}\) Mortality as a result of skin cancer increased with both duration of employment in embalming and intensity of exposure (as judged by whether a person was involved in both embalming and funeral directing or just in embalming). The group involved only in embalming had increased proportional mortality from cancers of the kidney and brain; there was no proportionate excess of deaths from respiratory cancer and no deaths from cancer of the nose or nasal sinuses. In a second study of white male employees of a chemical factory where formaldehyde and a variety of other chemicals were manufactured, overall mortality was significantly less than expected.\(^{(133)}\) In addition, the number of deaths observed from all cancers equalled that expected. However, excess numbers of deaths over those expected occurred from Hodgkin's disease as well as prostatic and brain cancers. The number of deaths from respiratory cancer equalled that expected, and there were fewer digestive system cancers than expected. There were no deaths from cancers of the nose or nasal sinuses. Analysis of causes of deaths that occurred more than 20 years after first employment in the factory indicated a significant excess of deaths from prostatic cancer. In a third mortality
study of white and nonwhite male employees from a chemical plant where formaldehyde was both produced and used as a raw material in the production of other chemicals, a proportional excess of deaths from digestive-tract cancer was reported for white workers. These latter individuals were each exposed to formaldehyde for a total of less than five years. Cancers of the nose or nasal cavities were not observed among the workers. It should be noted that the number of deaths observed after a suitable latent period in all three of the aforementioned studies was small and would be insufficient to show increased risk of an uncommon cancer.\textsuperscript{(134)}

The International Agency for Research on Cancer has concluded that epidemiological studies to date provide inadequate evidence to assess the carcinogenicity of formaldehyde in man. Several epidemiological studies to determine the relationship between formaldehyde and cancer are currently in progress.\textsuperscript{(110)}

\section*{DISCUSSION}

Formaldehyde is a useful compound manufactured on a huge scale and employed as such or in various forms in numerous industries and in a wide variety of products. It seems unlikely that one can escape exposure to this compound in one form or another whether it be at work, at home, in the clothes we wear, or in air contamination from combustion engines or tobacco smoke. In low amounts, it 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.

Because of this reactivity, it is both useful and hazardous. It is useful as an ingredient in cosmetic formulations principally for the prevention of microbial contamination, but for other reasons as well. It is an irritant at low concentration, especially to the eyes and the respiratory tract in all people. It induces hypersensitivity, but not as often as might be expected, considering the frequency and extent of exposure. Under experimental conditions it is teratogenic, mutagenic and it can induce neoplasms.

Perhaps the single most important attribute common to these toxic effects of formaldehyde is that they are all concentration/time dependent. Formaldehyde can be employed usefully at concentrations that do not induce lacrimation, or irritation to the nose or throat. Still higher concentration/duration exposure than that which produces irritation induces degenerative changes in the tissues exposed to it. For that matter, there is 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. It may or may not be relevant that no creditable epidemiological studies in humans support a carcinogenic potential for formaldehyde.

It is expected that there will be future studies on the carcinogenic potential of formaldehyde. It is possible that some of these studies will suggest or demonstrate that formaldehyde is an activator of known carcinogens, that it combines with other normal body constituents to cause cancer, or that perhaps it acts as a cocarcinogen. Such work will have to be judged on its own merits and as a part of total knowledge of the safety or hazard of formaldehyde.
ANALYSIS OF SUBMITTED COMMENTS

The CIR Expert Panel publicly reviewed submitted comments relating to the use of formaldehyde at a concentration of 4.5% in nail hardeners.* In its deliberations, the Panel concurred that the submitted evidence was inadequate to assure that formaldehyde could be safely used above 0.2% in cosmetic products. Further information on the Panel’s discussion regarding nail hardeners may be found in the Minutes of the CIR Expert Panel meeting held on July 25–26, 1983.

CONCLUSION

Formaldehyde in cosmetic products is safe to the great majority of consumers. The Panel believes that because of skin sensitivity of some individuals to this agent, the formulation and manufacture of a cosmetic product should be such as to ensure use at the minimal effective concentration of formaldehyde, not to exceed 0.2% measured as free formaldehyde. It cannot be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized.

ACKNOWLEDGMENT

Mr. Jonathon T. Busch, Scientific Analyst and writer, prepared the literature summary used by the Expert Panel in developing this report.

REFERENCES


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29. CFR. (Revised as of April 1, 1982). Title 21, Part 701.3. Designation of ingredients.

30. CFR. (Revised as of April 1, 1982). Title 21, Part 630.22. Manufacture of Adenovirus Vaccine.


34. CFR. (Revised as of April 1, 1982). Title 21, Part 175.105. Adhesives.

35. CFR. (Revised as of April 1, 1982). Title 21, Part 175.210. Acrylate ester copolymer coating.

36. CFR. (Revised as of April 1, 1982). Title 21, Part 175.300. Resinous and polymeric coatings.

37. CFR. (Revised as of April 1, 1982). Title 21, Part 175.380. Xylene-formaldehyde resins condensed with 4,4'-isopropylidene-di-phenolphlorohydrin epoxy resins.

38. CFR. (Revised as of April 1, 1982). Title 21, Part 175.390. Zinc-silicon dioxide matrix coatings.

39. CFR. (Revised as of April 1, 1982). Title 21, Part 176.170. Components of paper and paperboard in contact with aqueous and fatty foods.

40. CFR. (Revised as of April 1, 1982). Title 21, Part 176.180. Components of paper and paperboard in contact with dry food.

41. CFR. (Revised as of April 1, 1982). Title 21, Part 176.200. Defoaming agents used in coatings.
42. CFR. (Revised as of April 1, 1982). Title 21, Part 176.210. Defoaming agents used in the manufacture of paper and paperboard.
43. CFR. (Revised as of April 1, 1982). Title 21, Part 177.1200. Cellophane.
44. CFR. (Revised as of April 1, 1982). Title 21, Part 177.1210. Closures with sealing gaskets for food containers.
45. CFR. (Revised as of April 1, 1982). Title 21, Part 177.2410. Phenolic resins in molded articles.
46. CFR. (Revised as of April 1, 1982). Title 21, Part 177.2800. Textiles and textile fibers.
47. CFR. (Revised as of April 1, 1982). Title 21, Part 178.3120. Animal glue.
48. CFR. (Revised as of April 1, 1982). Title 21 Part 573.460. Formaldehyde.


About Health Canada

Brazilian Blowout Solution Contains Formaldehyde: Update

Advisory
2010-182
October 26, 2010
For immediate release

The issue:

This is an update to Health Canada's advisory posted on October 7, 2010 regarding Brazilian Blowout Solution.

Health Canada is informing Canadians that Brazilian Blowout Solution manufactured by Brazilian Blowout of California has been confirmed to contain unacceptable levels of formaldehyde. Note that at this time, this advisory pertains only to the Brazilian Blowout Solution.

Formaldehyde is permitted at low levels when used as a preservative in cosmetics. In liquid form, formaldehyde is referred to as methylene glycol or formalin. The terms "formaldehyde" and "methylene glycol" are often used interchangeably in toxicity studies pertaining to formaldehyde.

Testing previously conducted by Health Canada found that the Brazilian Blowout Solution contained 12% formaldehyde. Health Canada has continued to analyze this product. Validated test results show formaldehyde levels of 8.4%, which is consistent with results found in other jurisdictions. This amount is 42 times the acceptable limit when used as a preservative in certain cosmetic products, and remains well above the amount known to cause injury.

Health Canada continues to receive complaints of burning eyes, nose, and throat, breathing difficulties, and hair loss associated with use of this product. Health Canada believes that the reactions are being caused primarily by formaldehyde being released and inhaled during the blow drying and flat ironing stages of the treatment.

Health Canada remains concerned that elevated levels of formaldehyde in any form places people at increased risk. Possible long-term effects are also of concern to Health Canada, since formaldehyde is a known irritant, sensitizer, and is linked to cancer in humans when inhaled chronically over a long period of time. As a result, the Department has worked to stop distribution of this product in Canada.

Who is affected:

Consumers receiving hair smoothing treatments using Brazilian Blowout Solution and stylists performing the treatment may experience reactions of burning eyes, nose, throat, and breathing troubles. Effects may also be associated with exposure to the skin.

What consumers should do

- Consumers who have had adverse reactions to Brazilian Blowout treatments are advised to


11/16/2010
seek medical attention. There is no cause for concern for consumers who have used this product and not experienced any reactions.

- Stylists who use Brazilian Blowout treatments should immediately stop using the affected product.
- Adverse reactions to cosmetics can be reported to Health Canada using a form available on Health Canada's website: [www.healthcanada.gc.ca/reportaproduct](http://www.healthcanada.gc.ca/reportaproduct)

## What Health Canada is doing:

Health Canada has worked with the exclusive Canadian distributor to address concerns regarding Brazilian Blowout Solution, and to stop distribution of this product to salons in Canada. Health Canada is also informing consumers of the risks associated with this product.

Health Canada is continuing its investigation by following up on complaints and continuing to monitor the marketplace. In addition, Health Canada is testing similar products for which complaints have been received. If Health Canada identifies any other products of concerns, the appropriate information will be communicated to the public.

## Background:

Testing previously conducted by Health Canada found that the Brazilian Blowout Solution contained 12% formaldehyde. Health Canada has continued to analyze this product. Validated test results confirmed that the formaldehyde releasing potential is 8.4%, as measured by High Performance Liquid Chromatography (HPLC).

Due to the nature and number of complaints received by the Department and the elevated levels of formaldehyde found, an advisory was issued based on the initial results, in order to communicate health risks in a timely manner and prevent additional injuries from occurring.

This product remains a concern to Health Canada and the Department will continue to follow-up on incident reports associated with these types of products.

### Products recalled/affected:

This advisory is only for the Brazilian Blowout Solution at this time, since it is known to contain formaldehyde. At this time, other hair smoothing treatment products are not known to be affected. The affected product was only available in salons.

### For more information:

Consumers and health professionals can contact Consumer Product Safety toll free at 1-866-662-0666.

Media enquiries related to this Advisory should be directed to Health Canada Media Relations at 613-957-2983.

### How to report problems with consumer products:

Health Canada is interested in receiving reports of incidents or injury reports related to consumer products and cosmetics that have been previously recalled (health and safety related complaints).
Incidents or injuries may be about the same hazard or may be about a different hazard related to the same product. An incident report form can be accessed on the Health Canada website.

**Send us your feedback:** Help improve Health Canada's risk communications by sharing your thoughts on the new format for our Advisories.

Stay connected with Health Canada and receive the latest advisories and product recalls using these social media tools:

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Date Modified: 2010-10-26
FDA Receives Complaints Associated With the Use of Brazilian Blowout

October 8, 2010; updated October 18, 2010

FDA has recently received a number of inquiries from consumers and salon professionals concerning the safety of "Brazilian Blowout" and similar "professional use only" hair care products. The following information is intended to answer questions people may have on this subject.

Has FDA received reports of problems related to these products?

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. Complaints include eye irritation, breathing problems, and headaches. State and local organizations with authority over the operation of salons are currently investigating these reports. FDA has recently received some adverse event reports on Brazilian Blowout from salon personnel or consumers. These reports included symptoms similar to those in the reports received by state and local organizations, as well as symptoms such as rashes and fainting.

What is FDA's role in regulating these products?

Although FDA does not have authority over the operation of salons, we do have certain authority over hair straighteners and similar cosmetic products. To learn more, please see "FDA Authority Over Cosmetics."1

Who regulates salon safety?

Workplace safety in general, including air quality issues, is regulated by the Occupational Safety and Health Administration (OSHA). Salons are generally subject to state and local authorities as well, which may specify safety practices such as assuring proper ventilation.

What is FDA doing?

FDA is working with state and local organizations, as well as OSHA, to determine whether the products or ingredients would be likely to cause health problems under the intended conditions of use. The composition of the products and the labeling, including use instructions and any warning statements, will be factors in this determination. One safety issue we'll be evaluating is whether formaldehyde may be released into the air after the product is applied to the hair and heated.

Where can I learn more?

Some information regarding workplace exposure to formaldehyde is available on OSHA's Web page, "Formaldehyde."2 The National Institute for Occupational Safety and Health (NIOSH) at the Centers for Disease Control and Prevention also has published resources on formaldehyde on its Web page, "Formaldehyde: NIOSH Resources."3

How can I report an adverse experience related to a cosmetic?

Consumers and salon professionals are urged to report adverse experiences to FDA in either of the following ways:

1) Reporting to the nearest FDA district office. Phone numbers for their complaint coordinators are posted on FDA's Web page, "Consumer Complaint Coordinators"4 and in the Blue Pages of the phone book, generally under United States Government/Health and Human Services.

2) Reporting online to FDA's MedWatch adverse event reporting system5. You also may call Medwatch at 1-800-332-1088 to request a reporting form by mail.

Will FDA provide updates as more information becomes available?

FDA will continue to monitor this problem and will report on any new developments.

Links on this page:

Statement by John Bailey, Chief Scientist, Personal Care Products Council: Industry Concerned About Safety of Ingredient in Professional Hair Smoothing Products

November 4, 2010

Contact: Kathleen Dezio, 202/454-0302 or Lisa Powers, 202/466-0489

Cosmetic Ingredient Review (CIR) Expert Panel to Review its Safety

Background: The U.S. Food and Drug Administration (FDA) has reported receiving adverse event reports from consumers and salons on “professional use only” hair smoothing products. Adverse reactions to products used to smooth and straighten hair that have been reported include eye irritation, breathing problems, headaches, and rashes.

FDA has authority over hair straighteners and similar cosmetic products, including those intended for professional use only, but does not have authority over the operation of salons.

The Personal Care Products Council (PCPC) and its members are concerned about recent consumer reports of adverse reactions to ‘professional use only’ hair smoothing products. These products have been reported to contain high levels of formaldehyde, which under some conditions of use can be sensitizing and irritating to users. One of the specific issues to be evaluated by FDA is whether unsafe levels of formaldehyde are being released into the air once this product is applied to the hair and then heated. When hair smoothing products that contain formaldehyde are heated, they can release low levels of formaldehyde gas. Formaldehyde and methylene glycol are sensitizing agents, and consumers may experience allergic reactions if they become sensitized.

“The Cosmetic Ingredient Review (CIR) Expert Panel, an independent, non-profit body of scientific and medical experts that assesses the safety of ingredients used in cosmetics in the U.S., last reviewed the use of formaldehyde in beauty products in 2005 and concluded that, ‘...because of skin sensitivity of some individuals to this agent, the formulation and manufacture of a cosmetic product should be such as to ensure use at the minimal effective concentration of formaldehyde, not to exceed 0.2 percent measured as free formaldehyde. It cannot be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized.’”

“The primary application considered by the CIR during their review was the use of formaldehyde as a preservative to prevent the growth of potentially harmful microorganisms in cosmetic products. The CIR did not examine the use of formaldehyde in hair straightening and smoothing treatments. Therefore, PCPC has joined with FDA in asking CIR to review the safety of formaldehyde and methylene glycol in professional use hair smoothing products.

“In addition, we urge FDA to work with state and local organizations, as well as with the federal Occupational Safety and Health Administration (OSHA), which is responsible for regulating workplace safety, to objectively determine if salon hair smoothing products emit levels of formaldehyde gas that are unsafe for consumers and salon workers under their intended conditions of use. We recommend FDA take prompt and appropriate action to make sure that these products have been fully tested and substantiated for safety under the conditions of use.

Safe and proper use depends largely on the ventilation in the salon and the application procedure, which is why we advise consumers not to use professional hair straightening products at home, but to visit a salon for proper application by trained salon workers. Consumers who do visit a salon to receive hair smoothing treatments should be certain that the salon is properly ventilated and that the products and application process meet the OSHA safety guidelines. The federal OSHA has established limits for safe levels of inhalation exposure to formaldehyde gas.

“We strongly encourage consumers to report any adverse reaction to FDA and to visit the FDA website for more information on this important consumer health issue

http://www.fda.gov/Cosmetics/ProductandIngredientSafety/ProductInformation/ucm228898.htm"

Formaldehyde present in water or water-containing formulations exists mostly as methylene glycol with virtually no gaseous formaldehyde remaining. However, when heated and dried during use, it is possible that formaldehyde may be released from the product into the air and may be inhaled by the customer or salon worker.

For more information on cosmetic and personal care products, please visit www.CosmeticsInfo.org.
Based in Washington, D.C., the Personal Care Products Council is the leading national trade association representing the global cosmetic and personal care products industry. Founded in 1894, the Council’s more than 600 member companies manufacture, distribute, and supply the vast majority of finished personal care products marketed in the U.S. As the makers of a diverse range of products, millions of consumers rely on everyday items, from sunscreens, toothpaste and shampoo to moisturizer, lipstick and fragrance, personal care products companies are global leaders committed to product safety, quality and innovation.

"30"
MATERIAL SAFETY DATA SHEET

1. PRODUCT IDENTIFICATION

1.1 Product Name:
BRAZILIAN BLOWOUT PROFESSIONAL SMOOTHING SOLUTION

1.2 Chemical Name:
NA

1.3 Synonyms:
NA

1.4 Trade Name:
Brazillian Blowout Professional Smoothing Solution

1.5 Product Use:
PROFESSIONAL USE ONLY

1.6 Distributor’s Name:
Brazillian Blowout

1.7 Distributor’s Address:
6855 Tujunga Ave., North Hollywood, CA 91605

1.8 Emergency Phone:
CHEMTREC: +1 (703) 527-3887 / +1 (800) 424-3887

1.9 Business Phone:
+1-818-232-8775 / +1-877-779-7706

2. HAZARD IDENTIFICATION

2.1 Hazard Identification:
This product IS NOT classified as a HAZARDOUS SUBSTANCE or as DANGEROUS GOODS according to the classification criteria of [NOHSC: 1088 (2004)] and ADG Code (Australia). In case of eye contact, rinse immediately with water. For external use only. Keep out of reach of children. Use only as directed.

2.2 Routes of Entry:
INHALATION: YES
Absorption: YES
Ingestion: YES

2.3 Effects of Exposure:
 INGESTION: If product is swallowed, may cause nausea, vomiting and/or diarrhea.
EYES: Direct contact may cause mild irritation. Symptoms of overexposure may include redness, itching, irritation and watering.
SKIN: May be irritating to skin. The product can cause allergic skin reactions (e.g., rashes, welts, dermatitis) in some sensitive individuals.
INHALATION: May cause irritation and sensitization of the respiratory tract, especially if proper ventilation is not used or otherwise used improperly, e.g., excessive amounts of product are applied. When exposed to high heat (e.g., flat iron), may cause sore throat, coughing, and shortness of breath.

2.4 Symptoms of Overexposure:
Overexposure in eyes may cause redness, itching and watering. Symptoms of skin overexposure may include redness, itching, and irritation of affected areas. The product can cause allergic skin reactions (e.g., rashes, welts, dermatitis) in some sensitive individuals.

2.5 Acute Health Effects:
Moderate irritation to eyes. Moderate irritation to skin near affected areas.

2.6 Chronic Health Effects:
No harmful or chronic health effects are expected to occur from a single accidental ingestion. Frequent or prolonged exposure to product may cause hypersensitivity leading to contact dermatitis. Repeated or prolonged skin contact with product may cause an allergic reaction in some people. No harmful or chronic health effects related to the respiratory system are expected. Proper ventilation must be used to ensure the OSHA TLV’s are not exceeded. Avoid prolonged contact to concentrated vapors.

2.7 Target Organs:
Respiratory system, Skin, Eyes.

NA = Not Available; ND = Not Determined; NE = Not Established; NF = Not Found; C = Ceiling Limit; See Section 16 for Additional Definitions of Terms Used
NOTE: All WHMIS required information is included. It is located in appropriate sections based on the ANSI Z400.1-2004 format.
### 3. COMPOSITION & INGREDIENT INFORMATION - continued

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<th>CHEMICAL NAME(S)</th>
<th>CAS No.</th>
<th>RTECS No.</th>
<th>EINECS No.</th>
<th>%</th>
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<th>TLV</th>
<th>STEL</th>
<th>ES-TWA</th>
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### 4. FIRST AID MEASURES

#### 4.1 First Aid:

**INGESTION:** DO NOT INDUCE VOMITING. Contact ChemTrec at 703-527-3887 or the nearest Poison Control Center or local emergency telephone number for assistance and instructions. Seek immediate medical attention. If vomiting occurs spontaneously, keep victim's head lowered (forward) to reduce the risk of aspiration.

**EYES:** If product gets in the eyes, flush eyes thoroughly with copious amounts of water for at least 15 minutes, holding eyelid(s) open to ensure complete flushing. If irritation persists, seek medical attention.

**SKIN:** Remove contaminated clothing and wash affected areas with soap and water. If irritation persists, seek prompt medical attention. Do not use contaminated clothing until after it has been properly cleaned.

**INHALATION:** Should overexposure occur or victim shows signs of immediate distress, remove victim to fresh air at once. Under extreme conditions, if breathing stops, perform artificial respiration. Seek immediate medical attention.

#### 4.2 Medical Conditions Aggravated by Exposure:

Pre-existing dermatitis, other skin conditions, and pre-existing skin or eye disorders.

Overexposure to product may cause lung damage and aggravate pulmonary conditions.

Do no use on broken skin or irritated areas. In some individuals contact with skin may aggravate skin diseases such as eczema and contact dermatitis.

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<tr>
<th>EYES</th>
<th>SKIN</th>
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</thead>
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5. FIREFIGHTING MEASURES

5.1-flashpoint & method:
This product is not flammable.

5.2-autoignition temperature:
ND

5.3-flammability limits:  Lower Explosive Limit (LEL): NE  Upper Explosive Limit (UEL): NE

5.4-fire & explosion hazards:
This product is not a flammable liquid. However, if involved in a fire, this product may ignite and decompose to form toxic gases (e.g., CO, CO₂, and NOₓ, formaldehyde).

5.5-extinguishing methods:
Water Fog, Foam, Dry Chemical, CO₂

5.6-firefighting procedures:
As in any fire, wear MSHA/NIOSH approved self-contained breathing apparatus (pressure-demand) and full protective gear. Keep containers cool until well after the fire is out. Use water spray to cool fire-exposed surfaces and to protect personnel. Prevent runoff from fire control or dilution from entering sewers, drains, drinking water supply, or any natural waterway. Firefighters must use full bunker gear including NIOSH-approved positive pressure self-contained breathing apparatus to protect against potential hazardous decomposition products.

6. ACCIDENTAL RELEASE MEASURES

6.1-spills:
Before cleaning any spill or leak, individuals involved in spill cleanup must wear appropriate Personal Protective Equipment. For small spills (e.g., <1 gallon (3.785 liters)) wear appropriate personal protective equipment (e.g., goggles, gloves). Maximize ventilation (open doors and windows). Remove spilled material with absorbent material and place in appropriate closed container(s) for disposal. Dispose of properly in accordance with local, state and federal regulations. Wash all affected areas and outside of container with plenty of warm water and soap. Remove any contaminated clothing and wash thoroughly before reuse. For spills ≥ 1 gallon (3.785 liters), deny entry to all unprotected individuals. Dike and contain spill with inert material (e.g., sand or earth). Transfer product to containers for recovery or disposal and solid taking material to separate containers for proper disposal. Remove contaminated clothing promptly and wash affected skin areas with soap and water. Keep spills and cleaning runoffs out of municipal sewers and open bodies of water.

7. HANDLING & STORAGE INFORMATION

7.1-work & hygiene practices:
Avoid eye contact. Avoid breathing vapors. Wear protective gloves and avoid direct skin contact whenever possible. Wash hands thoroughly after using this product and before eating, drinking, or smoking. Keep away from foodstuffs and beverages.

7.2-storage & handling:
Use and store in a cool, dry, well-ventilated location (e.g., local exhaust ventilation is preferred, fans, open doors and windows). Do not store in damaged or unmarked containers or storage devices. Keep away from heat and direct sunlight. Keep containers securely closed when not in use.

7.3-special precautions:
Spilled material may present a slipping hazard if left unattended. Clean all spills promptly.
8. EXPOSURE CONTROLS & PERSONAL PROTECTION

8.1 Ventilation: Engineering Controls:
Use with adequate ventilation (e.g., local exhaust ventilation is preferred, fans, open doors and windows). Ensure appropriate decontamination equipment is available (e.g., sink, safety shower, eye-wash station). Use in a chemical fume hood when working with large quantities of product and provide adequate ventilation (e.g., local exhaust ventilation, fans).

8.2 Respiratory Protection:
No special respiratory protection is required under typical circumstances of use or handling, however local source capture exhaust is recommended. In instances where vapors or sprays of this product are generated, and respiratory protection is needed, use only protection authorized by 29 CFR §1910.134, applicable U.S. State regulations, or the Canadian CAS Standard 294.4-93 and applicable standards of Canadian Provinces, EC member States, or Australia.

8.3 Eye Protection:
Avoid eye contact. Safety glasses with side shields should be used when handling large quantities (e.g., ≥1 gallon (3.785 liters)) of this product.

8.4 Hand Protection:
None required under normal conditions of use. However, may cause skin irritation and/or allergic sensitivity in some sensitive individuals, so when product is being mixed, applied or handled, wear gloves made of rubber (nitrile), vinyl or other impervious material.

8.5 Body Protection:
No apron required when handling small quantities. When handling large quantities (e.g., ≥1 gallon (3.785 liters)), eye wash stations and deluge showers should be available.

9. PHYSICAL & CHEMICAL PROPERTIES

9.1 Density: NA
9.2 Boiling Point: NA
9.3 Melting Point: NA
9.4 Evaporation Rate: NA
9.5 Vapor Pressure: NA
9.6 Molecular Weight: NA
9.7 Appearance & Color: Lotion with a slight pungent odor.
9.8 Odor Threshold: NA
9.9 Solubility: Partially soluble in water.
9.10 pH: 4.0-5.0
9.11 Viscosity: NA
9.12 Other Information: NA

10. STABILITY & REACTIVITY

10.1 Stability:
This product is stable when stored in a cool dry location out of direct sunlight and away from other sources of heat.

10.2 Hazardous Decomposition Products:
Oxides of carbon (CO, CO₂) and sulfur (SO₂), and formaldehyde gas.

10.3 Hazardous Polymerization:
Will not occur.

10.4 Conditions to Avoid:
Open flames, sparks, high heat, direct sunlight and close proximity to incompatible substances.

10.5 Incompatible Substances:
High temperatures, sources of heat and direct sunlight.
11. TOXICOLOGICAL INFORMATION

11.1 Toxicity Data:
This product has not been tested on animals to obtain toxicological data. There are toxicology data for the components of this product, which are found in the scientific literature. These data have not been presented in this document.

11.2 Acute Toxicity:
See section 2.5

11.3 Chronic Toxicity:
See section 2.6

11.4 Suspected Carcinogen:
No

11.5 Reproductive Toxicity:
This product is not reported to produce reproductive toxicity in humans.

Mutagenicity:
This product is not reported to produce mutagenic effects in humans.

Embryotoxicity:
This product is not reported to produce embryotoxic effects in humans.

Teratogenicity:
This product is not reported to produce teratogenic effects in humans.

Reproductive Toxicity:
This product is not reported to produce reproductive effects in humans.

11.6 Irritancy of Product:
See Section 2.3

11.7 Biological Exposure Indices:
NE

11.8 Physician Recommendations:
Treat symptomatically.

12. ECOLOGICAL INFORMATION

12.1 Environmental Stability:
The following statements refer to the environmental fate of methylene glycol. When released into the soil, this material is expected to leach into groundwater. When released into water, this material is expected to readily biodegrade. When released into water, this material is not expected to evaporate significantly. This material is not expected to significantly bioaccumulate. When released into the air, this material is expected to be readily degraded by reaction with photochemically produced hydroxyl radicals.

12.2 Effects on Plants & Animals:
There is no specific data available for this product.

12.3 Effects on Aquatic Life:
There is no specific data available for this product.

13. DISPOSAL CONSIDERATIONS

13.1 Waste Disposal:
Dispose of in accordance with federal, state and local regulations.

13.2 Special Considerations:
Dispose of in accordance with federal, state and local regulations.
14. TRANSPORTATION INFORMATION

The basic description (ID Number, proper shipping name, hazard class & division, packing group) is shown for each mode of transportation. Additional descriptive information may be required by 49 CFR, IATA/CAO, IMDG, CTDGR, SCT and ADGR.

14.1 49 CFR (GND):
    - NOT REGULATED

14.2 IATA (AIR):
    - NOT REGULATED

14.3 IMDG (GDN):
    - NOT REGULATED

14.4 TDGR (Canadian GND):
    - NOT REGULATED

14.5 ADR/RID (EU):
    - NOT REGULATED

14.6 SCT (MEXICO):
    - NOT REGULATED

14.7 ADGR (Australia):
    - NOT REGULATED

15. REGULATORY INFORMATION

15.1 U.S. EPA SARA Reporting Requirements:
    - This product contains methylene glycol, a substance subject to SARA 313 reporting requirements.

15.2 U.S. EPA SARA Threshold Planning Quantity:
    - NA

15.3 TSCA Inventory Status:
    - All chemical substances of this product are listed on the TSCA inventory or are otherwise exempted from inventory status.

15.4 U.S. EPA CERCLA Reportable Quantity (RQ):
    - NA

15.5 Other U.S. Federal Requirements:
    - This product complies with the appropriate sections of the Food and Drug Administration's 21 CFR subchapter G (Cosmetics).
    - Clean Air Act: This material does not contain any Class 1 Ozone depletors. This material does not contain any Class 2 Ozone depletors.
    - Clean Water Act: None of the chemicals in this product are listed as Priority Pollutants under the CWA. None of the chemicals in this product are listed as Toxic Pollutants under the CWA.

15.6 Other Canadian Regulations:
    - This product has been classified according to the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all of the information required by the CPR. The components of this product are not listed on the DSL/NDSL.

15.7 U.S. State Regulatory Information:
    - Methylene Glycol is on the following state criteria lists: California, New Jersey, Pennsylvania, Minnesota, and Massachusetts. This product contains methylene glycol. When used as directed, this product releases trace amounts of formaldehyde.
    - The amount of formaldehyde released during use, and any exposure to an employee, will depend and vary widely based on the operating conditions. The precise amount of the exposure is difficult to determine and subject to scientific debate and varying protocols for measurement. Based on current scientific methodology, there is no reason to believe that any occupational exposure to formaldehyde from this product during normal use will exceed 40 micrograms per day.

15.8 67/548/EEC (European Union) and Australia NOHSC.2011 (2003) Requirements:
    - The primary components of this product are listed in Annex I of EU Directive 67/548/EEC.
    - HazChem Code: None allocated.
    - Poison Schedule: None allocated.
## 16. OTHER INFORMATION

16.1 Other Information:
Do not ingest. If swallowed, do not induce vomiting; seek medical attention. Avoid eye contact. Keep out of reach of children. If redness or other signs of adverse reaction occur, discontinue use immediately. If irritation persists, seek medical attention. FOR PROFESSIONAL USE ONLY.

16.2 Terms & Definitions:
Please see last page of this MSDS.

16.3 Disclaimer:
This Material Safety Data Sheet is offered pursuant to OSHA’s Hazard Communication Standard, 29 CFR §1910.1200. Other government regulations must be reviewed for applicability to this product. To the best of ShipMate’s & BRAZILIAN BLOWOUT’s knowledge, the information contained herein is reliable and accurate as of this date; however, accuracy, suitability or completeness are not guaranteed and no warranties of any type, either expressed or implied, are provided. The information contained herein relates only to the specific product(s). If this product(s) is combined with other materials, all component properties must be considered. Data may be changed from time to time. Be sure to consult the latest edition.

16.4 Prepared for:
Brazilian Blowout
6856 Tujunga Ave.
North Hollywood, CA 91605
+1-877-779-7706
+1-818-232-8775
http://www.brazilianblowout.com/

16.5 Prepared by:
ShipMate, Inc.
P.O. Box 787
780 Buckaroo Trail, Suite D
Sisters, OR 97759-0787
Tel: +1 (503) 370-3600
Fax: +1 (503) 370-5700
http://www.shipmate.com
A large number of abbreviations and acronyms appear on a MSDS. Some of these that are commonly used include the following:

**GENERAL INFORMATION:**
- CAS No.: Chemicals Abstract Service Number

**EXPOSURE LIMITS IN AIR:**
- ACGIH: American Conference on Governmental Industrial Hygienists
- TLV: Threshold Limit Value
- OSHA: U.S. Occupational Safety and Health Administration
- PEL: Permissible Exposure Limit
- IDLH: Immediately Dangerous to Life and Health

**FIRST AID MEASURES:**
- CPR: Cardiopulmonary resuscitation - method in which a person whose heart has stopped receives manual chest compressions and breathing to circulate blood and provide oxygen to the body.

**HAZARDOUS MATERIALS IDENTIFICATION SYSTEM: HMIS**
- Health, Flammability & Reactivity Ratings:
  - 0: Minimal Hazard
  - 1: Slight Hazard
  - 2: Moderate Hazard
  - 3: Severe Hazard
  - 4: Extreme Hazard

**PERSONAL PROTECTION RATINGS:**
- A to F

**FLAMMABILITY LIMITS IN AIR:**
- Autoignition Temperature: Minimum temperature required to initiate combustion in air with no other source of ignition.
- LEL: Lower Explosive Limit - lowest percent of vapor in air, by volume, that will explode or ignite in the presence of an ignition source.
- UEL: Upper Explosive Limit - highest percent of vapor in air, by volume, that will explode or ignite in the presence of an ignition source.

**TOXICOLOGICAL INFORMATION:**
- LD₅₀: Lethal Dose (pups & liquid) which kills 50% of the exposed animals.
- LC₅₀: Lethal concentration (gases) which kills 50% of the exposed animal.
- ppm: Concentration expressed in parts per million parts.
- TD₅₀: Lowest dose to cause a symptom.
- TCₕ₀: Lowest concentration to cause a symptom.
- LNTₙₐ or log LNTₙₐ: Coefficient of Oil/Water Distribution

**REGULATORY INFORMATION:**
- WHMIS: Canadian Workplace Hazardous Material Information System
- DOT: U.S. Department of Transportation
- TC: Transport Canada
- EPA: U.S. Environmental Protection Agency
- DSSL: Canadian Domestic Substance List
- NDSL: Canadian Non-Domestic Substance List
- FSL: Canadian Priority Substances List
- TSCA: U.S. Toxic Substance Control Act
- CPR: Canada's Controlled Product Regulations

**EC INFORMATION:**
- Classes: C, E, F, N, O, T, XI, Xn

**WHMIS INFORMATION:**
- Classes: A, B, C, D1, D2, D3, E, F
- Compressed, Flammable, Oxidizing, Toxic, Infectious, Infectious, Corrosive, Reactive
August 8, 2003

Memorandum

To: CIR Expert Panel
From: F. Alan Andersen, PhD
       Director, CIR

Subject: Re-review of Formaldehyde

In 1984, the Panel published its conclusion that: “Formaldehyde in cosmetic products is safe to the great majority of consumers. The Panel believes that because of skin sensitivity of some individuals to this agent, the formulation and manufacture of a cosmetic product should be such as to ensure use at the minimal effective concentration of formaldehyde, not to exceed 0.2 percent measured as free formaldehyde. It cannot be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized.”

The safety assessment (see attached) was prepared in an unusual style, with little detail regarding the available scientific studies and mostly summary statements regarding findings.

Since that time, a large number of Formaldehyde studies have appeared in the scientific literature. Sonal Iyer uncovered 149 publications and I included 126 of them in the write-up!

The task of the Panel at this meeting is to determine if the original conclusion regarding Formaldehyde is still valid. If the conclusion is still valid and the Panel decides to not reopen, the summary of new information would be a long one, but we have experience now in preparing the long ones in addition to the short ones.
# Formaldehyde Re-review

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INTRODUCTION

A safety assessment of Formaldehyde was published in 1984 (Elder, 1984) with a complex conclusion that stated: “Formaldehyde in cosmetic products is safe to the great majority of consumers. The Panel believes that because of skin sensitivity of some individuals to this agent, the formulation and manufacture of a cosmetic product should be such as to ensure use at the minimal effective concentration of formaldehyde, not to exceed 0.2 percent measured as free formaldehyde. It cannot be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized.”

The discussion section acknowledged that Formaldehyde can be a developmental toxin, a genotoxin, and a neoplastic agent in experimental studies. The expectation that future studies on the carcinogenic potential of Formaldehyde would be done was clearly stated. The fact that “several” epidemiology studies on cancer incidence in populations exposed to Formaldehyde were underway was acknowledged.

USE

Cosmetic

Data reported to the Food and Drug Administration (FDA) by industry in 1981 indicated that Formaldehyde was used in a total of 805 cosmetic products. Table 1 presents the frequency of use and the concentration of use as a function of product category for 1981 and for the most current frequency of use data in 2002. Usage of Formaldehyde in cosmetic products reported to FDA appears to have decreased substantially from 805 in 1981 to 120 uses in 2002. For example, almost 35% (316/909) of all non-coloring shampoos reportedly contained Formaldehyde in 1981, yet in 2002 that figure was down to 7% (59/884). Most current uses are reported as Formaldehyde Solution (Formalin). [Note: in the original safety assessment, it was stated that a 30-56 percent aqueous solution of Formaldehyde known as formalin was generally supplied.]

Current concentration of use data submitted directly by industry (CTFA, 2003) are also included in Table 1. Generally current concentrations of use are lower than reported in 1981.
Table 1. Frequency of use and concentration of use of Formaldehyde in cosmetic products.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number of products with Formaldehyde (total products in category)</td>
<td>Concentration of use</td>
</tr>
<tr>
<td><strong>BABY PRODUCTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby shampoos</td>
<td>7 (35)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Baby lotions, oils, powders and creams</td>
<td>1 (56)</td>
<td>&gt;0.1 - 1%</td>
</tr>
<tr>
<td><strong>BATH PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bath oils, tablets and salts</td>
<td>10 (237)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Bubble baths</td>
<td>109 (475)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Other bath preparations</td>
<td>24 (132)</td>
<td>≤0.1 - 5%</td>
</tr>
<tr>
<td><strong>EYE MAKEUP PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mascara</td>
<td>1 (397)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Other eye makeup preparations</td>
<td>3 (230)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td><strong>FRAGRANCE PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sachets</td>
<td>2 (119)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Other fragrance preparations</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NON-COLORING HAIR PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair conditioners</td>
<td>95 (478)</td>
<td>≤0.1 - 5%</td>
</tr>
<tr>
<td>Permanent waves</td>
<td>11 (474)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Rinses (non-coloring)</td>
<td>32 (158)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Shampoos (non-coloring)</td>
<td>316 (909)</td>
<td>≤0.1 - 5%</td>
</tr>
<tr>
<td>Tonics, dressings, and other hair grooming aids</td>
<td>21 (290)</td>
<td>≤0.1 - 10%</td>
</tr>
<tr>
<td>Wave sets</td>
<td>37 (180)</td>
<td>≤0.1 - 10%</td>
</tr>
<tr>
<td>Other hair preparations</td>
<td>13 (177)</td>
<td>≤0.1 - 5%</td>
</tr>
<tr>
<td><strong>HAIR COLORING PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair dyes and colors (with caution statement)</td>
<td>5 (811)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Shampoos (coloring)</td>
<td>3 (16)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td><strong>MAKEUP PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face powders</td>
<td>1 (555)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Foundations</td>
<td>2 (740)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Leg and body paints</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Makeup bases</td>
<td>3 (831)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Other makeup preparations</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NAIL CARE PRODUCTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle softeners</td>
<td>1 (32)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Nail creams and lotions</td>
<td>1 (25)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Other manicuring preparations</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ORAL HYGIENE PRODUCTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dentifices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouthwashes and breath fresheners</td>
<td>2 (53)</td>
<td>≤0.1 - 1%</td>
</tr>
</tbody>
</table>
Table 1 (continued). Frequency of use and concentration of use of Formaldehyde in cosmetic products.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of products with Formaldehyde (total products in category)</td>
<td>Concentration of use</td>
</tr>
<tr>
<td>Personal cleanliness products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bath soaps and detergents</td>
<td>5 (148)</td>
<td>$\leq 0.1 - 1%$</td>
</tr>
<tr>
<td>Deodorants</td>
<td>7 (239)</td>
<td>$&gt;0.1 - 1%$</td>
</tr>
<tr>
<td>Feminine hygiene deodorants</td>
<td>1 (21)</td>
<td>$&gt;1 - 5%$</td>
</tr>
<tr>
<td>Other personal cleanliness products</td>
<td>1 (227)</td>
<td>$\leq 0.1%$</td>
</tr>
<tr>
<td>Shaving preparations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aftershave lotions</td>
<td>1 (282)</td>
<td>$&gt;0.1 - 1%$</td>
</tr>
<tr>
<td>Shaving creams</td>
<td>2 (114)</td>
<td>$\leq 0.1%$</td>
</tr>
<tr>
<td>Other shaving preparations</td>
<td>1 (29)</td>
<td>$&gt;1 - 5%$</td>
</tr>
<tr>
<td>Skin care preparations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin cleansing creams, lotions, liquids, and pads</td>
<td>13 (680)</td>
<td>$\leq 0.1 - 1%$</td>
</tr>
<tr>
<td>Face and neck skin care preparations</td>
<td>47 (832)*</td>
<td>$\leq 0.1 - 1%$</td>
</tr>
<tr>
<td>Body and hand skin care preparations</td>
<td>11 (747)</td>
<td>$\leq 0.1 - 1%$</td>
</tr>
<tr>
<td>Foot powders and sprays</td>
<td>5 (219)</td>
<td>$\leq 0.1 - 1%$</td>
</tr>
<tr>
<td>Skin fresheners</td>
<td>3 (171)</td>
<td>$\leq 0.1 - 1%$</td>
</tr>
<tr>
<td>Night skin care preparations</td>
<td>1 (260)</td>
<td>$&gt;0.1 - 1%$</td>
</tr>
<tr>
<td>Paste masks (mud packs)</td>
<td>4 (349)</td>
<td>$\leq 0.1 - 1%$</td>
</tr>
<tr>
<td>Other skin care preparations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunscreen preparations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunscreen gels, creams and liquids</td>
<td>2 (164)</td>
<td>$\leq 0.1 - 1%$</td>
</tr>
</tbody>
</table>

*product sold only in Europe

The Japan Ministry of Health, Labor, and Welfare (MHLW) has issued new regulations for cosmetics (MHLW, 2001). These regulations include a negative list, and a list of preservatives with concentration limitations. Formaldehyde is not mentioned on either list. Formalin appears on the negative list, but does not appear on the list of preservatives, suggesting that it may not be used in cosmetics in Japan.

According to Annex VI of the European Commission (EC) Cosmetic Directive 76/768/EC, the maximum authorized concentration of free Formaldehyde is 0.2%, except 0.1% for oral hygiene products and a prohibition on use in aerosol dispensers; any product with free Formaldehyde $>0.05\%$ must include be labeled as containing Formaldehyde (EC, 1976). In 2002, the EC’s Scientific Committee on Cosmetic
Products and Non-Food Products Intended for Consumers (SCCNFP) addressed the question of Formaldehyde releasers (e.g., diazolidinyl urea) and concluded that the safety of these preservatives could be assured by assessing the total content of Formaldehyde in the finished cosmetic product, which must not exceed 0.2% (EC, 2002).

According to the Ministry of Health, Labor and Welfare (MHLW) in Japan, formalin may not be used in cosmetic preparations (MHLW, 2001).

Rastogi (1992) performed a survey of Formaldehyde in 84 shampoos and skin creams marketed in Denmark. The content of Formaldehyde ranged from 0.001 to 0.147%. The Formaldehyde content in leave-on products did not exceed 0.095%. It was noted that 8 products were found to contain >0.05% Formaldehyde, but were not labeled as “contains Formaldehyde” and, therefore, do not comply with European regulations.

Rastogi (2000) analyzed the preservatives in 67 skin creams to determine if these products complied with the Cosmetic Directive with respect to ingredient labeling and maximum allowed concentration. While Formaldehyde was not included in the label of 22 products, 18 contained less than 30 ppm (0.003%) and the author suggested these may represent products in which Formaldehyde was not intentionally used in their formulation.

Non-Cosmetic

The US Environmental Protection Agency (EPA) offers the following characterization of the health effects of Formaldehyde on their web site: Formaldehyde, a colorless, pungent-smelling gas, can cause watery eyes, burning sensations in the eyes and throat, nausea, and difficulty in breathing in some humans exposed at elevated levels (above 0.1 parts per million). High concentrations may trigger attacks in people with asthma. There is evidence that some people can develop a sensitivity to formaldehyde. It has also been shown to cause cancer in animals and may cause cancer in humans. Health effects include eye, nose, and throat irritation; wheezing and coughing; fatigue; skin rash; severe allergic reactions. May cause cancer (EPA, 2003a).

EPA’s IRIS assessment describes the carcinogenic potential as Classification -- B1; probable human carcinogen, based on limited evidence in humans, and sufficient evidence in animals (EPA, 2003b).
In 1996, FDA announced that the Agency is amending the food additive regulations to provide for the safe use of formaldehyde (37 percent aqueous solution), at the rate of 5.4 pounds per ton (2.5 kilograms per ton) as an antimicrobial food additive for maintaining complete poultry feeds salmonella negative for up to 14 days (the actual amendment was effective October 6, 1998).

In a poster presented at the 2003 FDA Science Forum, Wang et al. (2003) reported on the development of an HPLC method for the determination of formaldehyde in human vaccines. They noted that Formaldehyde is often used in bacterial vaccines either as a stabilizer or inactivating agent. Vaccines against anthrax, diphtheria, hepatitis A, influenza, Japanese encephalitis, and tetanus contain residual amounts of free formaldehyde. Less than 0.02% formaldehyde is permitted in vaccine products by FDA based on four decades of safety research.

**ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION**

**Percutaneous Absorption**

Bartnik et al. (1985) studied the absorption of Formaldehyde from an o/w cream using rats. $^{14}$C-labeled Formaldehyde (specific activity of 47 mCi/mmole) at 0.1% in an o/w cream was applied to the clipped dorsal skin of 8 male and 4 female Bor:WISW (SPF Cpb) rats with a non-occlusive dressing (perforated glass capsule) and to 2 male rats with an occlusive dressing (closed glass capsule). Over an area of 8 cm$^2$, 200 mg of material was applied. Urine and feces were collected over a 48-h period and $^{14}$CO$_2$ in expired air was trapped. At the end of the study, the animals were killed and the skin from the treated area was collected. The remainder of the carcasses were homogenised. An additional 4 male and 4 female rats were treated in a similar way, but housed in open metabolic cages.

The authors reported the data in Table 2, showing the small amount of $^{14}$C-Formaldehyde detected in the urine, feces, and $^{14}$CO$_2$. Most of the radioactive label was found adhered to the walls of the glass capsule (10-15%) or in the treated skin (~70%). The authors stated that it was unlikely that the material in the treated skin would be absorbed over a longer period of time because the excretion pattern decreased with time and that autoradiographs of the skin showed the radiolabel to be in the uppermost layers. They speculated that this localization resulted from reaction of Formaldehyde with a component of the skin (Bartnik et al., 1985).
Table 2. Percutaneous absorption of Formaldehyde

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time after exposure</th>
<th>Percent applied radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-occlusive - males</td>
</tr>
<tr>
<td>Urine</td>
<td>0 - 8 h</td>
<td>0.3 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>8 - 24 h</td>
<td>0.9 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>24 - 48 h</td>
<td>1.1 ± 0.43</td>
</tr>
<tr>
<td>Total urine</td>
<td></td>
<td>2.3 ± 0.60</td>
</tr>
<tr>
<td>Feces</td>
<td>0 - 24 h</td>
<td>0.3 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>24 - 48 h</td>
<td>0.4 ± 0.33</td>
</tr>
<tr>
<td>Total feces</td>
<td></td>
<td>0.7 ± 0.64</td>
</tr>
<tr>
<td>expired ( ^{14} \text{CO})</td>
<td>0 - 8 h</td>
<td>0.8 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>8 - 24 h</td>
<td>0.3 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>24 - 48 h</td>
<td>0.2 ± 0.16</td>
</tr>
<tr>
<td>Total expired ( ^{14} \text{CO})</td>
<td></td>
<td>1.3 ± 0.55</td>
</tr>
<tr>
<td>Cage rinsing water</td>
<td></td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>Carcass</td>
<td></td>
<td>1.8 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>Total percutaneous absorption</td>
<td>6.1 ± 1.28</td>
</tr>
<tr>
<td>Glass capsule</td>
<td></td>
<td>5.7 ± 1.74</td>
</tr>
<tr>
<td>Volatile ( ^{14} \text{C})-Formaldehyde</td>
<td>0 - 48 h</td>
<td>3.8 ± 2.61</td>
</tr>
<tr>
<td>Treated skin</td>
<td></td>
<td>70.2 ± 4.61</td>
</tr>
<tr>
<td>Total recovery</td>
<td></td>
<td>85.8 ± 4.55</td>
</tr>
</tbody>
</table>

* Open metabolic cage - no gas trapped or measured.

Lodén (1986) reported on the in vitro permeability of human skin to Formaldehyde. \(^{14}\text{C}\)-labeled Formaldehyde (specific activity 10 mCi/mmol), diluted with either concentrated formalin (37% Formaldehyde in water containing 10-15% methanol) or with a 10% solution of formalin in 0.1 M phosphate buffer at pH 7.4. Full thickness human skin was mounted in flow-through diffusion cells. Phosphate buffered saline, pH 7.4 was used as a receptor medium. A flow rate of \(-5\) ml/h was maintained.
The time course of absorption was determined over 21 h. After an initial lag time, the total penetration increased in a linear fashion from 10 h to 21 h. The total absorption (skin + receptor fluid) of $^{14}$C-labeled Formaldehyde from the concentrated formalin solution was 6.02 $\pm$ 1.70 mg/cm$^2$ and from the 10% formalin diluant the value was 0.48 $\pm$ 0.10 mg/cm$^2$. $^{14}$C-labeled Formaldehyde found in the skin was 1.75 $\pm$ 0.30 and 0.23 $\pm$ 0.05, respectively (Lodén, 1986).

**Inhalation**

Casanova et al. (1988) measured the concentration of Formaldehyde in the blood of Rhesus monkeys after inhalation exposure. Young adult Rhesus monkeys (6 animals, 3-5 years old, 6-7 kg) were individually housed with food and water provided ad libitum. Exposures were done in inhalation chambers. Three monkeys were exposed and three served as controls. Formaldehyde was generated by thermal depolymerization of para-formaldehyde in a canister in an isothermal oven and captured by a 6 l/min air stream through the canister. The inhalation chamber Formaldehyde concentration was 6.00 $\pm$ 0.22 ppm. Animals were exposed 6 h/day, 5 days/week for 4 weeks. Blood samples were drawn immediately after exposure and at 45 h after exposure. A $[^{13}$C,$^2$H$_3$] Formaldehyde standard was added to each sample and gas chromatography - mass spectrometry performed.

The average blood concentrations of Formaldehyde in exposed monkeys did not differ significantly from that in the three unexposed monkeys. The authors state that these findings are consistent with those from previous experiments in their laboratory involving rats and humans which indicated that a single exposure to Formaldehyde had no effect on blood levels of Formaldehyde. They expressed the view that the rapid oxidation of Formaldehyde to formate and CO$_2$ or its incorporation into macromolecules by one-carbon metabolic pathways accounts for the absence of Formaldehyde in the blood (Casanova et al., 1988).

Kimbell et al. (2001) modeled the distribution of inhaled Formaldehyde in the rat, monkey, and human nasal passages in an attempt to explain the regional appearance of nasal squamous cell carcinomas in rats and squamous metaplasia in rats and monkeys seen with Formaldehyde inhalation. A computational fluid dynamics (CFD) model of rat, monkey, and human airflow and gas uptake was developed.
The average and maximum flux estimates in the rat nasal passages were 336 and 3210 pmol/[mm$^2$ h ppm], respectively. The average and maximum flux estimates for nonsquamous epithelium regions were less, at 284 and 2650 pmol/[mm$^2$ h ppm], respectively. The authors stated that in the rat, the incidence of Formaldehyde induced squamous cell carcinoma in chronically exposed animals was high in the anterior lateral meatus. The flux estimated for that region was 825 pmol/[mm$^2$ h ppm]. This flux was comparable to the estimate of flux near the anterior or proximal aspect of the inferior turbinate and adjacent lateral walls and septum of 988 pmol/[mm$^2$ h ppm]. The authors suggested that these results can be used in risk modeling of Formaldehyde carcinogenesis (Kimbell et al., 2001).

**ANIMAL TOXICOLOGY**

**Inhalation Toxicity**

Rusch et al. (1983) reported the results of a 26-week inhalation toxicity study with Formaldehyde using rats, hamsters, and monkeys. Simultaneous exposure of five groups of 20 male and 20 female Fischer 344 rats, 10 male and 10 female Syrian golden hamsters, and six male Cynomologus monkeys was done 22 h/day, 7 days/week, for 26 weeks. The Formaldehyde dose levels corresponding to the five groups were 0, 0, 0.19, 0.98, and 2.95 ppm for the two control groups and the three treatment groups.

In rats, squamous metaplasia in the nasal turbinates, decreased body weights (starting during the 2nd week), and decreased liver weights at 2.95 ppm were reported. There were no signs of toxicity in the two lower dose treatment groups.

No signs of toxicity in hamsters were seen at any dose.

In monkeys, squamous cell metaplasia in the nasal turbinates and hoarseness and congestion were noted in the 2.95 ppm treatment group. There were no signs of toxicity in the two lower dose treatment groups.

The authors concluded that doses below 1 ppm, even with almost continuous exposure over six months, produced no adverse effects. They also noted that the rat and monkey appear to be more sensitive to inhaled Formaldehyde, compared to hamsters (Rusch et al., 1983)

Dallas et al. (1986) determined the response in the lower respiratory tract to inhaled Formaldehyde using rats. Male Sprague-Dawley rats were exposed to 0, 0.5, or 15 ppm Formaldehyde for 6 h/day, 5
days/week, for 8 or 16 weeks. Animals were removed immediately after either 8 or 16 weeks and allowed an 18-22 hour equilibration period. The minute volume, respiratory rate, and tidal volume were then recorded. Both control and treated rats were then administered 30 ppm Formaldehyde challenges by tracheal exposure and respiratory responses were measured for 10 minutes.

Control animals receiving the tracheal challenge exposure exhibited a characteristic minute volume depression and correlated tidal volume decreases, but no significant change in respiration rate.

The tracheal challenge response of rats who had been exposed to Formaldehyde vapor for 8 weeks to 0.5 ppm was similar to control animals. The tracheal challenge response of rats who had been exposed to Formaldehyde vapor for 8 weeks to 15 ppm was similar to control animals, except for an increased respiration rate.

The response of rats who had been exposed to Formaldehyde vapor for 16 weeks at 0.5 ppm was similar to controls. Rats exposed to 15 ppm for 16 weeks, however, the minute volume depression on challenge was less than either the controls or the animals pre-exposed for 8 weeks. Respiration rate was increased in rats pre-exposed to 15 ppm for 16 weeks.

The authors compared and contrasted their results with those found in mice and suggested that a species difference in respiratory response could explain the significantly greater nasal carcinogenic response in rats over mice (Dallas et al., 1986).

Morgan et al. (1986) measured the distribution, progression, and recovery of acute Formaldehyde exposure induced inhibition of nasal mucociliary function using rats. Male F-344 rats were exposed (head-only) to 15 ppm Formaldehyde generated by thermal depolymerization of para-formaldehyde for 6 h. Rats were decapitated and mucus flow rates were determined after rapid dissection to expose the nasal mucosa. Treatment groups of 3 rats were exposed to 15 ppm for 10, 20, 45, or 90 minutes. One treatment group of 6 rats was exposed to 15 ppm for 6 h. Treatment groups of 3 rats were also exposed to 2 ppm Formaldehyde for 90 minutes or 6 h. Control animals breathed only room air.

Nasal ciliary activity and mucus flow patterns in control animals were consistent with historical controls. Exposure to 15 ppm Formaldehyde resulted in ciliastasis and mucostasis. The extent of areas of ciliastasis increased progressively with length of exposure and was site specific, appearing on the anterior
and ventral septum, the antero-medial and dorsal maxilloturbinate, and the lateral wall and lateral
nasoturbinate. Areas of mucus flow inhibition followed the same site-specific pattern, but were generally
more extensive. Inhibition of mucus flow Animals exposed to 15 ppm, but allowed a 1 h recovery period,
had extensive recovery of both ciliary activity and mucous flow.

There was no impaired ciliary activity or mucus flow in animals exposed to 2 ppm Formaldehyde
for 90 minutes or 6 h.

The authors noted that these findings of ciliary activity and mucus flow patterns in rats exposed to
Formaldehyde correlate well with both histologic evidence of Formaldehyde-induced acute cytotoxicity and
squamous cell carcinomas in the nasal passages of rats, namely the lateral aspect of the nasoturbinates
and adjacent lateral wall, and the mid-ventral septum (Morgan et al., 1986).

Adams et al. (1987) examined the effect of Formaldehyde inhalation on the mononuclear
phagocyte system of mice. According to the authors, increased incidence of upper respiratory infections in
humans and animals exposed to Formaldehyde vapor raised the question of systemic immune effects. In
previous work in this laboratory, most immune functions were not impaired by Formaldehyde, but
enhanced function in the mononuclear phagocyte system was observed. This study examines that effect
in more detail.

Female B6C3F₁ mice (6-8 weeks old, 18-22 g) were exposed to -15 ppm Formaldehyde 6 h/day, 5
days/week for 3 weeks. Age matched control mice breathed room air. A pyran copolymer (MVE-2) was
used to elicit peritoneal macrophages in one group of Formaldehyde-treated mice, but not in another.
Peritoneal macrophages from both treatment groups (elicited and non-elicited) were tested in vitro against
P815 tumor cells and for their competence to release reactive oxygen intermediates (ROI).

Formaldehyde did not increase the number of macrophages in either elicited or non-elicited mice.
Formaldehyde did not affect the binding of macrophages to tumor cells. Formaldehyde exposure,
however, did enhance the ROI release in response to a phorbol myristate acetate (PMA) stimulus. No
detectable H₂O₂ was seen in macrophages from elicited or non-elicited mice with no PMA stimulus,
whether treated with Formaldehyde. No detectable H₂O₂ was seen in macrophages from elicited mice with
no PMA stimulus. In elicited mice, the PMA stimulus released 42.5 ± 3.3 nmoles of H₂O₂ without
Formaldehyde, but released 78.2 ± 1.7 nmoles of H₂O₂ (p < 0.001) with Formaldehyde treatment. The authors concluded that Formaldehyde had no impact on macrophage tumoricidal function, but did enhance macrophage development for ROI release. The speculated that a reason for the effect may be the local inflammation produced in the upper airway by Formaldehyde (Adams et al., 1987).

The TNO-CIVO Toxicology and Nutrition Institute in the Netherlands conducted a series of studies examining the impact of Formaldehyde exposure regimens on inhalation toxicity. In a subacute study at this laboratory, Wilmer et al. (1987) exposed groups of 10 male Wistar rats to 0, 5, or 10 ppm Formaldehyde for 8 h/day, 5 days/week for 4 weeks; or to 10 or 20 ppm Formaldehyde for 30 min followed by 30 minutes non-exposure, 8x/day, 5 days/week for 4 weeks. Three rats from each group were given a single dose (2 μCi/g) of ³H-Thymidine (specific activity 43 Ci/mmol) by intraperitoneal injection 18 h after the 3rd day and after the last day of exposure. Two hours after the final ³H-Thymidine injection, the animals were killed and 6 standard cross-sections of the nose were prepared.

Focal thinning and disarrangement of the respiratory epithelium were seen in a few rats in the 10 ppm groups and in all animals in the 20 ppm group. Squamous metaplasia of the respiratory epithelium, often accompanied by basal cell hyperplasia, was observed in most rats of the 10 ppm groups and the 20 ppm group, as well as a few in the 5 ppm group.

Overall, the degree of effect appeared to be dose concentration dependent rather than related to cumulative dose. For example, the lesions in the 20 ppm intermittent exposure group (80 ppm/day) were more severe than those in the 10 ppm continuous exposure group (80 ppm/day). Also, the percent of cells with ³H-Thymidine label was the same for both 10 ppm groups (Wilmer et al., 1987).

In a subchronic study at this laboratory, Woutersen et al. (1987) exposed male and female albino Wistar rats to 0, 1, 10 or 20 ppm Formaldehyde 6h/day, 5 days/week for 13 weeks. Blood and individual urine samples were taken in week 13 and analyzed. Animals were killed in week 14. In addition to examining the nasal respiratory epithelium, the authors weighed the adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus and thyroid. Microscopic examination was made of lung, trachea, larynx (3 longitudinal levels), and the standard 6 nasal cross-sections. Tissue from other organs were examined in the control and 20 ppm groups. Protein and glutathione levels in liver post-mitochondrial
fractions were determined.

A separate cell-proliferation assay was performed using 2 extra male rats in each inhalation chamber on three successive days. These animals were killed 18 h after the 3rd exposure, and the nasal turbinates dissected free and placed in tissue culture with 2.5 μCi/ml $^3$H-Thymidine (specific activity 40 Ci/mmol) for 2 h. Radiolabel uptake was determined by autoradiography.

Yellowish fur was noted in 10 ppm and 20 ppm animals, but no other gross changes were noted. Body weights demonstrated growth retardation in males and females in the 20 ppm group. The effect was not statistically significant in the 10 ppm group. No differences in liver or urine parameters were seen between any treated animals and controls. Aspartate amino transferase, alanine amino transferase, and alkaline phosphatase levels were statistically significantly higher in 20 ppm males, but not females. Total plasma protein was lower than controls. In 20 ppm males, increased relative weights for 6 of the 11 organs weighed was noted, but in 20 ppm females, only the brain weight was elevated.

Minimal histopathological changes in nose of a few males and one female of the 1 ppm group, but the authors were unclear that these findings related to treatment. Half the males in the 20 ppm group exhibited squamous metaplasia of the epithelium lining the vocal cord region of the larynx. None of the females in any dose group had this result, nor did any of the males in the 1 or 10 ppm groups.

Cell proliferation as measured by $^3$H-Thymidine uptake was localized in the areas of the turbinates showing focal squamous metaplasia in the 10 and 20 ppm groups (Woutersen et al., 1987).

In a 1 year study at this laboratory, Appelman et al. (1988) examined the inhalation toxicity of Formaldehyde in male Wistar rats as a function of bilateral nasal mucosal damage. Endpoints included nasal mucosal damage and organ toxicity, as done in the above study. Blood samples and individual urine were collected in week 52. Dose levels were 0, 0.1, 1.0, and 9.4 ppm. Each group had 40 animals. Bilateral nasal damage was accomplished by electrocoagulation in half the animals in each exposure group. After 13 weeks of exposure, 10 animals with nasal damage and 10 animals without nasal damage were killed. The remaining animals were killed in week 53.

No differences in blood or urine parameters was seen between groups, except for frequent oliguria seen in the 9.4 ppm group without nasal damage. In rats with nasal damage, liver protein was elevated.
compared to rats without nasal damage. Hepatic glutathione levels appeared to be decreased in rats with damaged noses killed after 13 weeks. The authors speculated that these findings may indicate an hepatotoxic effect of Formaldehyde. The authors concluded that nasal damage made the nose more susceptible to the cytotoxic effects of Formaldehyde, that there were no systemic effects in animals with undamaged noses, and that 1 ppm Formaldehyde had no adverse effect (Appleman et al., 1988).

Finally, this laboratory (Wilmer et al., 1989) reported on the effect of continuous versus intermittent exposure to lower doses of Formaldehyde. Male Wistar rats were exposed for 8 h/day, 5 days/week for 13 weeks to 0, 1 or 2 ppm Formaldehyde, or 30 min followed by 30 minutes non-exposure, 8x/day, 5 days/week for 4 weeks to 2 or 4 ppm Formaldehyde (25 animals in each group). In addition to the nasal histopathological examination performed at the end of the study, 5 animals received $^3$H-Thymidine injections after exposure day 3, and were killed 2 hours later, and the nasal tissue analyzed to determine nasal cell proliferation. The only nasal histopathological changes noted were in the 4 ppm group. The 4 ppm group also demonstrated an increase in radiolabel uptake compared to controls. While an unusually high incidence of rhinitis in the group intermittantly exposed to 2 ppm Formaldehyde complicated their analysis, the authors concluded that this incidence was not treatment related and that 2 ppm was a correct no effect level.

Biagini et al. (1989) exposed monkeys for 10 minutes to 2.55 ppm Formaldehyde to determine the extent of airway narrowing. Nine adult male Cynomolgus monkeys were exposed to this dose level of Formaldehyde that had been generated from formalin using a new system. Before Formaldehyde exposure, animals were challenged with methacholine to determine if any exhibited bronchial hyperreactivity. After Formaldehyde exposure, average pulmonary flow resistance, dynamic compliance, peak expiratory flow rate, forced vital capacity, forced expiratory volume, and forced expiratory flow were measured. The ratio of forced expiratory flow to forced vital capacity was calculated and reported.

Exposure to 2.55 ppm Formaldehyde caused significant increases in the average pulmonary flow resistance that persisted 5 and 10 minutes post-exposure. This effect was not correlated with methacholine hyperreactivity (Biagini et al., 1989).

Dallas et al. (1989) examined the effect of Formaldehyde on cytochrome P450 in the rat. Previous
work had identified that Formaldehyde could inactivate P450 in vitro in rabbit lung microsomes and this study was undertaken to determine if P450 inactivation would occur with Formaldehyde inhalation.

Male Sprague-Dawley rats were treated by whole-body exposure to 0, 0.5, 3.0 or 15 ppm Formaldehyde for 6 h/day, 5 days/week for 1 day, 1 week, 12 weeks, or 24 weeks. Cytochrome P450 was measured in a lung microsomal fraction. The experiment was replicated. P450 levels were below the level of detection after a single 6 h exposure (1 day) at all Formaldehyde exposure levels. Control P450 levels were normal. Exposure for 4 days resulted in significant P450 increases at 3 and 15 ppm in one replication and at 0.5, 3 and 15 ppm in the other. There appeared to be a dose effect. Exposure for 12 weeks resulted in significant P450 increases at 0.5, 3 and 15 ppm in an apparent dose-response manner in one replication. The other replication resulted in no significant increases in P450 at any exposure. The 24 week exposure resulted in a significant P450 increase with the 0.5 ppm exposure in one replication and with the 15 ppm exposure in the other.

The authors stated that their finding of induced lung cytochrome P450 following repeated exposures of 4 days or more adds Formaldehyde to the long list of P450 inducers. They speculated that the initial reduction in P450 followed by an increase could be important when Formaldehyde exposure occurs simultaneously with other toxic agents (Dallas et al., 1989).

Bhalla et al. (1991) reported the effects of acute exposure to formaldehyde on the surface morphology of the nasal epithelia in rats. Male Sprague-Dawley rats were exposed to clean air (4 animals) or 10 ppm Formaldehyde for 4 h (6 animals). Two clean air and 3 treated animals were killed 1 h after the end of the exposure. The remaining animals were killed 1 day after the end of exposure. The study was repeated with the same numbers of animals.

Both scanning electron and light microscopy were performed on nasal tissue. The authors found that damage to the nasal passages by Formaldehyde could be seen in scanning electron microscopy (e.g. disorganized cilia and secretory globules) and that this damage likely correlates with earlier reports of ciliary stasis. They also noted similarities in the pattern of damage done by other inhalation toxins and suggested that the location of cells in the path of the air flow may be the relevant factor in their damage (Bhalla et al., 1991).
Behavioral Toxicity and Neurotoxicity

Guy and Abbott (1991) reported on their extension of work in which formalin injection in a rear paw of rats is used as a model pain stimulus. The classic response in the adult rat is an initial reaction lasting as long as 10 minutes, followed by a 5-15 minute period in which the pain is not apparent, followed by re-emergent pain that can last up to an hour, a so-called biphasic response. This study examined the response in rat pups, 1 - 20 days of age, to subcutaneous injection of 10 μl of 1% formalin in a rear paw. The authors concluded that rats make behavioral responses to formalin injection from day 1 after birth. The response pattern is not biphasic initially, but converts to a biphasic response during the third week of life. The intensity of the response was greatest in day 1 animals and gradually decreased to the point where the concentration of formalin had to be increased on day 20 to produce a consistent response.

Omote et al. (1998) reported the formalin-induced release of excitatory amino acids in the skin of the rat hindpaw. The excitatory amino acids glutamate and aspartate were measured peripherally after subcutaneous injection of formalin in male Sprague-Dawley rats. A 50 μl volume of either formalin or saline was injected into the plantar surface of the right hindpaw with a 26 gauge needle. A probe inserted into the bilateral glabrous skin of the hind paw was used to collect perfusate for analysis. Glutamate and aspartate were analyzed by HPLC with electrochemical detection. Intradermal concentration of these excitatory amino acids increased on the same side as the formalin injection. The authors concluded that the release of glutamate and aspartate would contribute to pain reactions and inflammatory pain.

Pitten et al. (2000) evaluated the neurotoxicity of inhaled Formaldehyde in rats. Wistar rats (40) were trained to find food in a maze within a set time. When all rats were trained to an equal level, 13 animals inhaled 2.6 ppm and 13 animals inhaled 4.6 ppm Formaldehyde 10 min.day, 7 days/week for 90 days. The remaining animals were placed in a control group and inhaled water steam according to the same regimen. During the exposure period and up to 30 days after exposure ended the time required to find food and the number of mistakes on the way were recorded.

The animals exposed to Formaldehyde needed more time and made more mistakes than the animals of the control group (p < 0.05). There were no significant differences between the animals exposed to 2.6 or 4.6 ppm Formaldehyde. The authors noted that treated animals appeared to retain their
appetite, since food consumption and body weights did not differ between the three groups. The authors classified Formaldehyde as probably neurotoxic and cautioned that precautions in the case of occupational or dwelling related exposure was warranted (Pitten et al., 2000).

Liu et al. (2001) studied the formalin-evoked peripheral release of adenosine in the rat hind paw pain model. Based on data suggesting that low doses (0.5 - 2.5%) of formalin induce a spike of adenosine release, but that high doses (5%) result in a more sustained adenosine release, this study examined the effect of 1.5 and 5% formalin under conditions where the neurogenic and non-neurogenic components of the response could be isolated. Male Sprague-Dawley rats were used. Capsaicin pretreatment was used to reduce the function of unmyelinated C-fibre afferents; 6-hydroxydopamine pretreatment was used to reduce the function of sympathetic postganglionic nerves, and compound 48/80 was used to degranulate mast cells. Formalin was injected at 1.5 or 5% in a volume of 50 µl into the dorsal aspect of the hind paw.

Formalin at 1.5% caused a rapid increase and decrease (spike) in adenosine levels. Pretreatment with capsaicin decreased that response. Formalin at 5% also caused a rapid increase, but the decrease was slow. Capsaicin decreased the initial response so that overall the adenosine release simply increased with time. Pretreatment with 6-hydroxydopamine had no effect on the 1.5% formalin-induced spike, but did reduce the overall magnitude of the response to 5%, but not the shape. Compound 48/80 did not produce a significant effect on the 1.5 or 5% formalin response, although the peaks were somewhat depressed.

The authors concluded that small diameter afferent nerves are involved in the adenosine release response to low doses of formalin, while those small diameter afferents and sympathetic postganglionic nerve terminals are involved at high doses (Liu et al., 2001).

**Hepatotoxicity**

Beall and Ulsamer (1984) reviewed the hepatotoxicity of Formaldehyde. A check of the reference list of some 80 studies found that only 7 of these references had been cited in the CIR safety assessment published the same year (Elder, 1984).

While the authors failed to find sufficient data on which to base a dose-response, they did conclude there was ample evidence that inhalation, ingestion, or injection of Formaldehyde is associated with liver changes in mice, rats, hamsters, guinea pigs, rabbits, dogs, and humans. These changes were
qualitative, such as size, weight, and color, and included microscopic and biochemical alterations. Quantitative relationships were not possible to establish. Striking in that regard was the lack of more pronounced liver changes following inhalation exposure to what was termed “obviously toxic” Formaldehyde at 38 ppm.

Increases in liver weight were linked to passive congestion, swelling of hepatocytes, and possible hepatic inflammation, hyperemia, and edema. Liver weight increases were accompanied by or followed closely by biochemical alterations such as nucleic acid clumping and an increased concentration of alkaline phosphatase. Later changes included a decrease in formation and excretion of hippuric acid and in ascorbic acid. Longer term changes included centrilobular vacuolar degeneration, formation of cytoplasmic vacuoles, and hepatocellular degeneration.

The authors speculated that the catabolism of Formaldehyde by reactions involving glutathione may deplete glutathione levels, which in turn could increase the toxicity of hepatotoxins that require glutathione for detoxification (Beall and Ulsamer, 1984).

Dermal Irritation/Sensitization

Andersen et al. (1985) evaluated the Formaldehyde dose-response in two laboratories, each using a different guinea pig strain in a maximization test. Dunkin-Hartley strain animals were used in one laboratory and Ssc:AL strain animals were used in the other. The usual maximization test procedure was followed with the induction concentrations shown in Table 3. Challenge was done with 0.1 or 1% Formaldehyde in water and reactions read at 48 and 72 h.

The number of animals with a positive response to the 0.1% challenge was too small to allow a reliable dose response analysis. The authors reported a clear dose-response exhibiting an S-shaped curve up to a maximal response, followed by a reduction in response when the intradermal concentration of Formaldehyde was increased. While almost identical in shape, the curves were displaced from each other, with the Dunkin-Hartley strain being the more sensitive. For example, at 72 h after challenge, the intradermal concentration of Formaldehyde at maximal response was 0.34 for the Dunkin-Hartley strain and 0.65 for the Ssc:AL strain. Likewise, the half-maximum response was 0.019 for the Dunkin-Hartley strain and 0.046 for the Ssc:AL strain. The actual maximum response was not significantly different.
between the two strains. The authors reported that the 2% concentration used in the topical exposure and the 1% concentration in the intradermal injection were likely in error, but that the overall results were unchanged when these data were excluded.

The authors noted that the shape of the response curve is similar to that seen with DNCB, sultones, nitrobenzyl compounds, and partially for chlorocresol (Andersen et al., 1985).

Table 3. Guinea pig maximization test dosing regimen (Andersen et al., 1985).

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<thead>
<tr>
<th>Induction concentration (% v/v)</th>
<th>Intradermal Day 0</th>
<th>Topical Day 7</th>
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In a follow-up report, Andersen (1985) presented a review of guinea pig and clinical sensitization data. The guinea pig findings are summarized here. The clinical sensitization data will be discussed in the section on Clinical Assessment of Safety. Without providing details of Formaldehyde concentrations used, the author notes that the sensitization rate can vary as a function of the type of sensitization test performed. While the modified Draize test had a 10% incidence in one report, another had 33%. In maximization tests, two reports give 100% incidence, but one reported 18%. One report of the Buehler test found a 0% incidence of sensitization.

Dossou et al. (1985) reported a method for assessment of experimental allergy in guinea pigs adapted to cosmetic ingredients and used 5% Formaldehyde as one of the test materials. Two protocols were presented: (1) an open epidermal induction and challenge, maximized by foot pad injection of Freund’s complete adjuvant, and (2) injection delivery of the test substance and adjuvant into the foot pad. In both cases, challenge was made with a single topical application. By the topical route, the intensity of the sensitization reaction was 0.8, with a sensitization rate of 20%. By the injection route, the intensity was 3.8 and the sensitization rate was 80%.

Riedel et al. (1996) reported that Formaldehyde exposure enhances inhalative allergic sensitization in the guinea pig. Female Perlbright-white Dunkin-Hartley guinea pigs were exposed (12 in each group) to Formaldehyde at 0, 0.13, or 0.25 ppm for 8 h/day for 5 days, followed by sensitization to 0.5% ovalbumin. After two weeks (day 19), the 0.5% ovalbumin sensitizing inhalation was repeated. Pulmonary function, ovalbumin antibody levels, lung and trachea tissue histology, and measurement of the wall thickness of the respiratory bronchioles and alveolar septa were the endpoints used. No anaphylactic reactions were noted to the second ovalbumin sensitization. The low-dose and control groups were not different in airway obstruction, although there was one animal with a high degree of airway obstruction in the low-dose group. Airway obstruction in the high-dose group was significantly increased compared to controls. In the low-dose group, 3/12 animals had increased ovalbumin specific antibody titers. For the high-dose group, that ratio was 6/12 and in control animals, 0/12. Histologic examination found edema of the bronchial mucosa with Formaldehyde exposure without mucosal inflammation. Bronchial wall thickness was significantly higher in Formaldehyde-exposed animals.
The authors concluded that relatively low concentrations of Formaldehyde can enhance allergic sensitization in the guinea pig by other antigenic agents (Riedel et al., 1996).

Bergh and Karlberg (1999) determined the sensitizing potential of formaldehyde using a modified cumulative contact enhancement test. They also evaluated acetaldehyde and cross-reactivity between it and Formaldehyde. Female Dunkin-Hartley guinea pigs (15) were induced with 5% Formaldehyde in saline (4 closed topical applications on days 0, 2, 7, and 9) on a 2 x 4 cm² area of the upper back (200 mg of material/application). Freund’s complete adjuvant (0.1 ml) was injected intradermally at the same site. Closed challenge testing was done on day 21 on the shaved flank with ~15 mg of test material applied to each patch. Three concentrations of Formaldehyde were used at challenge, 0.03, 0.10, and 0.30%. After 24 hours, the patches were removed and assessment made at 48 and 72 hours.

Both Formaldehyde and acetaldehyde showed significant sensitization in this assay (p < 0.01) with a clear dose-response at challenge. No cross-reactivity was seen between the two. A rechallenge was done 78 days after the start of the study. Significant reactions were seen to 1.0 and 0.3% Formaldehyde (Bergh and Karlberg, 1999).

Ushio et al. (1999) examined the effects of environmental pollutants, including Formaldehyde, on the production of pro-inflammatory cytokines by normal human dermal keratinocytes in vitro. Keratinocytes were incubated with Formaldehyde at 0.25, 0.5, 1, or 5 μg/ml and cytokine production determined by enzyme-linked immunosorbent assay. In other cultures, keratinocytes were stimulated with PMA before Formaldehyde treatment.

Formaldehyde alone did not increase the production of IL-1β or IL-8, but Formaldehyde did significantly increase the IL-1β and IL-8 production in cells stimulated with PMA. IL-1α and TNF-α were unaffected by Formaldehyde with or without PMA stimulation. The authors speculated that Formaldehyde might be involved in the initiation or pathogenesis of allergic or non-allergic cutaneous inflammation (Ushio et al., 1999).

Xu et al. (2002) studied the expression of cytokine mRNAs in mice cutaneously exposed to Formaldehyde. Female Balb/C mice were skin-painted with three topical applications of 100 μl of 17.5% Formaldehyde or distilled water. Applications were made to shaved abdominal skin with a 1-day interval.
between applications. Mice were killed on day 3, 5, 7, 9, and 12 after the last skin painting and the spleen and draining lymph nodes removed (3-5 animals per group). Other mice were challenged with 2% Formaldehyde on both ears on day 3 after the last skin painting.

In the draining lymph nodes, IL-4 and IFN-γ mRNAs were significantly increased in treated mice on days 3-12. IL-13 mRNA was normal on day 3, significantly increased on days 5 and 7, but returned to normal on days 9 and 12. IL-2, IL-5, IL-18, and IL-12p40 mRNAs were all detected, but showed no treatment related changes.

In the spleen, IL-2 mRNA was significantly increased on days 7-12; IL-15 mRNA on days 5-12; IL-4 mRNA on days 3-12; IL12p40 mRNA on days 5-12; IFN-γ mRNA on days 5-12; and IL-13 mRNA on days 3-7, returning to normal on days 9 and 12.

A weak response to the 2% Formaldehyde challenge (ear swelling) was found. IL-2 was detected in the ears, but not elevated. IL-4 and IFN-γ mRNAs were significantly elevated in the ears of rechallenged animals. The authors state that these findings extend the pattern of induction of TH1 and TH2 cytokines in contact hypersensitivity and speculate that IL-4 and IFN-γ mRNAs may be used as in vivo markers for assessing chemical sensitization potential Xu et al. 2002).

**Respiratory Sensitization**

Dearman et al. (1999) compared the cytokine secretion profiles provoked in mice by glutaraldehyde and Formaldehyde. These authors had previously characterized the cytokine secretion profiles of topically applied contact allergans and respiratory allergans. Respiratory allergans selectively activated TH2-type cells and contact allergans selectively activated TH1-type cells.

In this study, BALB/c mice were exposed topically to 50% Formaldehyde or to various concentrations of glutaraldehyde in acetone. A reference contact allergan (DNCB) and a reference respiratory allergan (trimellitic anhydride) were also used as positive controls. Formaldehyde produced TH1-type cytokine interferon γ, but little TH2-type products such as interleukin 4 or 10. Glutaraldehyde did the opposite. The authors concluded that Formaldehyde did not have a significant potential to cause allergic sensitization of the respiratory tract (Dearman, 1999).
Ocular Irritation

Hayasaka et al. (2001) evaluated the effects of intravitreal injection of Formaldehyde on rabbit eyes. Male albino rabbits were injected in the right eye through the sclera into the central vitreous cavity with 100 µl of 0.1 or 1% Formaldehyde (5 animals each) in a sodium phosphate buffer. The right eye of 5 control animals received the vehicle. After injection, 0.3% ofloxacin was topically instilled. At day 1, 2, 7, 14, and 30 the overall condition of the animals was evaluated and the eyes examined by biomicroscopy and ophthalmoscopy. At 30 days the animals were killed and histologic examination performed on ocular tissues, including the retina, choroid, sclera temporal to the optic disc, and the optic nerve behind the lamina cribrosa.

No overall signs of animal distress were noted. Animals receiving 0.1% Formaldehyde showed retinal vessel dilation and those receiving 1% showed mild posterior subcapsular cataract, retinal vessel dilation, and retinal hemorrhages. Histologic examination of the eyes that received 0.1% Formaldehyde had a disorganized ganglion cell layer and outer nuclear layer. That progressed to a markedly disorganized retina in animals receiving 1% Formaldehyde. Both Formaldehyde concentrations resulted in vacuolizations in the optic nerve (Hayasaka et al., 2001).

Maurer et al. (2001) reported the pathology of ocular irritation with Formaldehyde and other materials in the rabbit low-volume eye test. This study was undertaken to better understand the mechanisms by which ocular irritation occurs in order to better assess possible in vitro approaches. The authors proposed that differences in ocular irritation are related to differences in the extent of initial injury and devised this protocol to examine this hypothesis.

For the low-volume eye test, male New Zealand rabbits were treated with 37% Formaldehyde. The right eyelid was held open and 10 µl of the material placed directly above the center of the cornea. The eyelid was released immediately. For light microscopy, 12 rabbits were used and the left eye of each animal served as the control. For in vivo confocal microscopy, 16 rabbits were used and the untreated eyes of a separate group of 16 were used as the controls because of the potential for corneal damage due to desiccation during the examination.

While the main focus of this study was to extend the authors previous work by demonstrating that
the extent of initial injury is the best indicator of ocular irritation with a broader range of irritants.

Formaldehyde was described as an example of a severe ocular irritant in this study. The results of all parameters confirmed that both incidence of damage and severity were high for Formaldehyde and no eye, regardless of the assay, showed recovery by 35 days (Maurer et al., 2001).

**GENOTOXICITY**

**DNA Assays**

Heck and Casanova (1999) applied a model that describes the arrest of DNA replication as a function of DNA-protein cross-links to measurements of DNA replication in the nasal mucosa of rats and monkeys exposed to Formaldehyde gas. Male Fischer 344 rats were exposed to 6.1 ± 0.2 ppm of radiolabeled (\(^{3}\)H and \(^{14}\)C) Formaldehyde in a nose-only chamber for 6 h. Rats were killed immediately after the exposure. Nasal mucosal samples were homogenized and DNA extracted and sheared by passes through a 20-gauge needle. The number of DNA-protein cross-links per nucleotide in the interfacial fraction (none were found in the aqueous fraction) and the thymidine-methyl-\(^{14}\)C per nucleotide in the interfacial (IF) and aqueous (AQ) DNA fractions were measured. The IF/AQ ratio of the thymidine-methyl-\(^{14}\)C was considered the replication efficiency, a parameter predicted in the mathematical model. By assuming values for the mean number of DNA-protein cross-links in a given length of DNA, they worked the model to yield replication efficiency that matched the experimental data.

Using previous measured data on DNA-protein cross-links in nasal tissues of rats and monkeys, the replication efficiency was calculated. The mathematical model predicts that the replication efficiency will be unity if there is no significant increase in DNA-protein cross-links to interfere with DNA synthesis. In both species, the replication efficiency was 1 at airborne Formaldehyde concentrations below 2 ppm. At higher exposures, the replication efficiency decreased. The authors concluded that this model predicts that the effect of Formaldehyde on DNA replication is mainly a high-dose phenomenon (Heck and Casanova, 1999).

**Bacterial Assays**

Temcharoen and Thilly (1983) measured the lethal and genotoxic effects of various concentrations of Formaldehyde in *S. typhirurium* TM677 with and without metabolic activation. Formaldehyde, analytical
grade 37% in aqueous solution with 10-15% ethyl alcohol stabilizer was diluted to yield 0.33, 0.67, 1.0, 1.33, 1.67, and 2.0 mM solutions with 1.0 ml bacterial suspensions in 15-ml centrifuge tubes for times ranging from 15 to 120 min. Cell survival was determined by plating on non-selective media and forward mutations to 8-azaguanine resistance were determined by plating on media with 50µg/ml 8-azaguanine.

Both reduction in cell survival and increased mutations were concentration and time dependent, but the effect was reduced with metabolic activation. The minimum concentration required to produce a detectable increase in mutations was 0.167 mM in the absence of metabolic activation and 0.33 mM with metabolic activation (Temcharoen and Thilly, 1983).

De Flora et al. (1984) retested 30 agents (including Formaldehyde) that were positive in an E. coli DNA-repair test but had failed to produce measurable mutations in S. Typhirurium strains TA1535, TA1537, TA1538, TA98, and TA100 were retested in strains TA97 and TA102. A dose-dependent increase in mutations was seen in TA102 with Formaldehyde.

Schmidt et al. (1986) reported the number of Formaldehyde-induced revertants of S. typhimurium TA100. Formaldehyde 37% with 10% methanol at a dose range of 0 - 1.5 mM was used with and without metabolic activation in a pre-incubation and a plate incorporation assay. In the plate assay, only a weak mutagenic response was seen. In the pre-incubation assay, a 1.6 fold increase in the number of revertants without metabolic activation (geodesic dome shaped response with peak around 0.15 mM), and a 2.7 fold increase with metabolic activation (capital dome shaped response with definite peak at 0.2 mM).

Le Curieux et al. (1993) compared the results of three short-term genotoxicity assays on seven chemical, including Formaldehyde. In the SOS chromotest, E. coli strain PQ37 was incubated in the presence of Formaldehyde then divided to assay separately for β-galactocidase activity and alkaline phosphatase activity - a ratio of the former to the latter is the genotoxic activity. If the genotoxic activity is >1.5, the β-galactocidase activity is increased above controls, there is a dose-effect, and the result is reproduced, then the assay is positive. In the fluctuation test, S. typhimurium strains TA100, TA98, and TA102 were used. Tests were first done on TA100 and, if negative, on the other two. In this assay Formaldehyde is exposed to the bacteria in liquid medium in a 96-well microplate for 3 days. Prototrophic mutants turn yellow on the addition of bromothymol blue and no-effect cells remain green. In the newt
micronucleus test, larvae at stage 53 (max. mitotic index) are maintained in a glass container with water containing the test concentration, which is renewed daily. After 12 days, blood samples are taken and slides made. The number of micronucleated erythrocytes is counted. Because the distribution in controls is not a normal distribution, the mean is not calculated; median and quartiles are calculated. An agent is clastogenic in this assay if the number of micronucleated erythrocytes is increased relative to controls (p<0.05) and the median for the treated group is 2x the control.

In the SOS chromotest, Formaldehyde was studied at concentrations from 1 to 30,000 μg/ml in the assay. The toxicity threshold was 50 μg/ml and the range of genotoxic concentrations was between 20 and 50 μg/ml. In the fluctuation test, Formaldehyde was studied at concentrations from 0.1 to 30 μg/ml. The threshold toxic response was seen at 30 μg/ml and the range of mutagenic concentrations was reported as 10 μg/ml. In the newt micronucleus test, the threshold toxic dose was 10 μg/ml and at 5 μg/ml there was no clastogenic response (Le Curieux et al., 1993).

Muller et al. (1993) reported the results of genotoxicity testing of several agents, including Formaldehyde, using S. typhimurium strain TA102 in three different laboratories. Formaldehyde was weakly mutagenic in one laboratory, but negative in the other two.

Dillion et al. (1998) tested several aldehydes and peroxides, including Formaldehyde, using S. typhimurium strains TA97a, TA100, TA102, and TA104 with and without metabolic activation in either pre-incubation or vapor phase protocols. Formaldehyde was tested in only strains TA100, TA102, and TA104 and was positive in each, with or without metabolic activation.

Overall there does not seem to be a consistent pattern of effect, especially in S. typhimurium testing between strains and as a function of metabolic activation.

Saccharomyces cerevisiae Assays

Zimmermann and Mohr (1992) reported the effect of several agents, including Formaldehyde, on chromosome loss and mitotic recombination in Saccharomyces cerevisiae. Formaldehyde at 37% in aqueous solution was added to the incubation mixture at 0, 50, 75, 87, 100, 112, 124, and 137 nl/ml for 16 h at 28°C. All concentrations except the highest were also used in a cold shock treatment regimen in which incubation for 4 h at 28°C was followed by 16 h in an ice bath, followed by a final 4 h at 28°C.
Formaldehyde exposures in combination with 14.39 mg/ml propionitrile (a strong inducer of chromosome malsegregation) were at 0, 9.8, 14.7, 19.6, 24.5, 29.4, 34.3, 39.3, 44.2, 49.1 and 58.9 nl/ml.

In combination with propionitrile, Formaldehyde produced a dose-dependent increase in chromosome loss, in an apparent synergistic fashion. Alone, there was no such effect. Formaldehyde alone, however, did induce mitotic recombination and respiratory deficiency (Zimmerman and Mohr, 1992).

**Mammalian Cell Assays**

Grafstrom et al. (1985) investigated the repair of O\(^{6}\)-methylguanine and 7-methylguanine lesions induced by N-methyl-N-nitrosourea (NMU) in cultured human bronchial fibroblasts as a function of Formaldehyde treatment. Because the former is repaired relatively rapidly and the latter slowly, the ratio of the two is actually a measure of the repair of O\(^{6}\)-methylguanine. After 1 h treatment of these cells with 200 μM NMU, the ratio of O\(^{6}\)-methylguanine to 7-methylguanine had a mean value of 0.041. After 5 h of post-treatment incubation in NMU-free medium, repair of these promutagenic lesions occurred and the ratio fell to a mean value of 0.09, indicating repair of O\(^{6}\)-methylguanine lesions consistent with previous results in human skin fibroblasts. When cells were incubated post-treatment in NMU-free medium plus 100 μM Formaldehyde, the ratio had a mean value of 0.14 and with 300 μM Formaldehyde, the ratio was 0.021. These values were significantly different from the ratio seen with post-incubation with medium alone. The authors interpreted this finding to suggest that Formaldehyde inhibits repair of the O\(^{6}\)-methylguanine lesion.

The authors then went on to investigate the toxic and mutagenic effects of NMU and Formaldehyde separately and together in these human bronchial fibroblast cells. Colony-forming efficiency was the measure of toxicity in cells treated with 100 - 800 μM NMU for 1 h, or to 50 - 175 μM Formaldehyde for 5 h. The frequency of mutation to 6-thioguanine-resistance was the measure of mutagenic effect. On a molar basis, Formaldehyde was 3x more mutagenic than NMU, but each was a weak mutagen. Addition of 50 or 75 μM Formaldehyde to 200 μM NMU resulted in a mutation frequency greater than adding the two results when tested separately. The authors speculated that the enzyme systems responsible for the metabolism of aldehydes are important in the carcinogenic susceptibility of organs to N-nitrosamines (Grafstrom et al., 1985).

Schmid et al. (1986) measured the toxic and genotoxic effect of Formaldehyde in human
lymphocytes, with and without metabolic activation. Formaldehyde doses were from 0 - 1.0 mM. Cell proliferation was determined by measuring the yield of M3 metaphases. At doses above 0.063 mM Formaldehyde, the number of M3 metaphases declined. The effect was more pronounced without metabolic activation, but the patterns were similar with and without. Without metabolic activation, no mitoses could be observed in M3 cells at 0.5 mM Formaldehyde.

Structural chromosome abnormalities associated with Formaldehyde exposure were of the chromatid type, with breaks and exchanges accompanied by gaps. Sister chromatid exchange (SCE) frequencies were increased at 0.125 mM Formaldehyde and higher doses. At concentrations up to 0.25 mM Formaldehyde, the SCE frequency was not influenced by metabolic activation, but at 5 mM Formaldehyde, the SCE frequency was significantly reduced with metabolic activation (Schmid et al., 1986).

Snyder and Van Houten (1986) failed to find Formaldehyde-induced DNA strand breaks in a traditional strand-break assay, so they used a nick translation assay which did detect DNA damage in human fibroblasts treated with Formaldehyde. In this system, damage induced by Formaldehyde was not repaired by typical excision repair. The authors also examined the effect of Formaldehyde on the repair of X ray or bleomycin induced DNA strand breaks, on the excision of pyrimidine dimers, and on unscheduled DNA synthesis. In each case, Formaldehyde had no effect. The authors attributed previous findings of Formaldehyde inhibition of unscheduled DNA synthesis was most likely an inhibition of radiolabeled precursor uptake.

Grafstrom et al. (1993) used cultured Chinese hamster V79 cells to measure the toxicity and mutagenicity of Formaldehyde (0.3 - 1.0 mM) separately and in combination with ionizing radiation (2 and 4 Gy) and NMU (0.2 and 0.5 mM). Each agent alone caused a dose-dependent decrease in colony forming efficiency and an increase in 6-thioguanine resistant colonies. Exposure of cells to ionizing radiation or NMU followed by a submutagenic concentration of Formaldehyde increased the toxicity and mutagenicity seen with each agent alone.

Frenzilli et al. (2000) validated the alkaline single cell gel (SCG) assay - aka comet assay - using 18 reference compounds, including Formaldehyde. Human leukocytes at the highest tested Formaldehyde...
dose of 0.8 mM had a 96% viability. Formaldehyde induced an increase in DNA migration at low doses and a decrease at high doses. The authors speculated that Formaldehyde-induced DNA strand breaks at low concentration predominate, while Formaldehyde-induced DNA cross-linking reverses that effect and then some.

Hamaguchi and Tsutsui (2000) assessed the genotoxicity of a series of dental antiseptics, including Formaldehyde, on unscheduled DNA synthesis in cultured Syrian hamster embryo (SHE) cells. SHE cells (2.5 x 10^5 cells) were plated into 75-cm² flasks, incubated overnight, and then treated for 48 h with Formaldehyde concentrations of 0.3, 1.0, and 3.0 μg/ml. Relative cell survival percentages were 86.6 ± 7.3, 81.5 ± 8.4, and 27.7 ± 4.1, for the three Formaldehyde concentrations, respectively. In the absence of metabolic activation, the radiolabel incorporation (in counts per minute) was 249.1 ± 12.4, 358.6 ± 18.6, and 539.4 ± 41.7 for the three Formaldehyde concentrations, respectively. No radiolabel incorporation was detected in the presence of metabolic activation.

**Drosophila Assays**

Szabad et al. (1983) determined the mutagenicity of malondialdehyde and Formaldehyde in the Drosophila mosaic and sex-linked recessive lethal tests. Eye mosaicism was used to detect genetic changes involving the X chromosome. Eyes of w^co/w females were screened for both single and twin mosaic spots and eyes of w^co/Y males were screened for single spots. Wing mosaicism was used to detect changes involving the 3rd chromosome. One type of mosaic spot was characterized by higher trichome content in wing-blade cells and the other by abnormal wing hairs. Germ-line mosaicism was also used to detect changes in the X chromosome. Abnormally shaped eggs can be linked to mitotic recombination. Sex-linked recessive lethal mutations were present if the F_2 and F_3 generations of males exposed to treatment agents produced no w^co/Y males. Treatment was done in feed at the larval stage for 3-4 days (until pupation). Range-finding tests determined the concentration of Formaldehyde that allowed 50% of the larvae to develop to the adult stage, and that concentration was used throughout the rest of the work.

Formaldehyde did produce a small but significant increase in eye mosaic spots compared to controls, a large and significant increase in wing mosaicism, no effect on germ line mosaicism, and a
significant increase in sex-linked recessive mutations (Szabad et al., 1983).

Woodruff et al. (1985) presented the results of 53 coded compounds tested for the National Toxicology Program, including Formaldehyde, in Drosophila. Exposures were conducted by adult feeding (12,000 ppm Formaldehyde in feed). The results of a sex-linked recessive assay on those flies showed no significant increase in lethal mutations compared to controls. As designed, exposure was then done by injection of 2,000 ppm Formaldehyde in meiotic and postmeiotic germ cell stages of adult males. This produced 0.38% lethal mutations, which was significantly higher than controls (0.09%). According to the protocol, the brood in which there were a significantly higher number of lethal mutations was then used in a reciprocal translocation test. Formaldehyde did not produce reciprocal translocations.

In Vivo Animal Assays

Dallas et al. (1992) conducted cytogenetic analyses on bone marrow and pulmonary lavage cells from rats that inhaled Formaldehyde repeatedly. Male Sprague-Dawley rats were exposed to 0, 0.5, 3, or 15 ppm Formaldehyde for 6 h/day, 5 days/week for 1 and 8 weeks. Each exposure group consisted of 4 or 5 animals.

No detectable increases were seen in chromosomal abnormalities in the bone marrow of rats exposed up to 8 weeks. The authors stated that this was an expected finding given the lack of penetration of Formaldehyde vapor beyond the respiratory passages. There was a significant increase in chromosomal aberrations in pulmonary lavage cells in animals exposed to the highest concentration for either 1 or 8 weeks. The authors compared the increase to that previously reported for benzene and found the increase associated with Formaldehyde to be small in comparison (Dallas et al., 1992).

Odeigah (1997) used sperm head abnormality and dominant lethal mutation assays to examine the in vivo mutagenicity of Formaldehyde in rats. Isogenic strains of albino rats received 5 daily injections of Formaldehyde (37% aqueous with 10% methanol) at 0.125, 0.250, and 0.50 mg/kg body weight. A control received distilled water injections. Each group consisted of 6 animals. Rats were killed 3 weeks after the last injection. Spermatozoa (1,000) from each of 6 rats in each treatment group were scored for sperm head abnormalities.

Formaldehyde treatment resulted in a significant increase in sperm head abnormalities as a
function of dose. The authors also noted a significant general decrease in the sperm count as a function of
dose. For each dose there was a significant increase in the dominant mutation index, and there is a
suggestion of a dose-response (Odeigah, 1997).

CARCINOGENICITY

Inhalation

Kerns et al. (1983) reported on the carcinogenicity of Formaldehyde in rats and mice after long-
term inhalation exposure. All animals were exposed for 6h/day, 5 days/week for up to 24 months.
Exposure was followed by a 6-month period of nonexposure. The mean exposure concentrations for
Formaldehyde were 14.3 ± 0.04 ppm (target 15 ppm), 5.6 ± 0.02 (target 6 ppm), and 2.0 ± 0.01 ppm (target
2 ppm). A control group breathed room air. Rats were 7-week old Fischer 344 animals and mice were 6-
week old C57BL/6 x C3H F1 animals. Each exposure and control group had between 119 and 121 animals
of each sex. Gross pathological examinations were performed on all animals that died or were killed at 6,
12, 18, 24, 27, and 30 month intervals. Tissues from each organ system in the control and high exposure
groups were evaluated histologically. Multiple sections of the nasal turbinates were evaluated in all rats
and mice.

Mildly decreased body weights in rats of both sexes were observed from week 3 to week 103.
Animals in the 2 ppm group had sporadic reduced body weights throughout the exposure period. At 27
months, however, the body weights of the male rats in the high exposure group and males and females in
the 5.6 and 2 ppm groups were not statistically different from controls.

In male mice, no pattern of body weight decrease was discernable. In female mice in the high
exposure group, generally lower body weights were seen beginning after 72 weeks, but the weights
returned to controls levels after exposure terminated.

Cumulative survival of male rats remained around 100% up until nine months where the 14.3 ppm
group began to decline to 0% at 27 months. The 5.6 ppm group began its dip at 12 months and declined
to 30% at 29 months. The 2 ppm group began its dip at 15 months and declined to 55% at 29 months.
Decreases in control group survival began at 20 months and decreased to 73% at month 29. Cumulative
survival of female rats was similar to males at the high exposure level, but was very similar in the 5.6 ppm,
2 ppm, and control groups.

Cumulative survival of male mice began a steady, slow decrease at 2 months in all groups, with the exposed groups generally lower than controls. There were no differences in cumulative survival between the groups of female mice.

No effect on clinical pathology or ophthalmological or neurofunctional data was seen related to Formaldehyde exposure in rats or mice.

As shown in Table 4, exposure to 14.3 ppm Formaldehyde for 24 months produced a high incidence of squamous cell carcinoma in male (51/117) and female (52/115) rats. At 18, 24, and 27 months virtually all rats exhibited squamous metaplasia.

Two male mice in the 14.3 ppm group at 24 months had squamous cell carcinomas similar to those observed in rats. Squamous cell metaplasia incidence in all mice in the 14.3 ppm group was around 90% at 18 and 24 months, but decreased to around 25% at 27 months. No significant compound related lesions were found in the 2.0 and 5.6 ppm mouse groups.

Table 4. Neoplastic lesions in the nasal cavity of rats exposed to Formaldehyde by inhalation (Kerns et al., 1983).

<table>
<thead>
<tr>
<th>Exposure level</th>
<th>Sex</th>
<th>No. Nasal cavities examined</th>
<th>Squamous cell carcinoma</th>
<th>Nasal carcinoma</th>
<th>Undifferentiated carcinoma or sarcoma</th>
<th>Carcinosarcoma</th>
<th>Polypoid adenoma</th>
<th>Osteochondroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>0  M</td>
<td>118</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
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<tr>
<td>F</td>
<td>114</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.0  M</td>
<td>118</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>118</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5.6  M</td>
<td>119</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>116</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14.3  M</td>
<td>117</td>
<td>51</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>115</td>
<td>52</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Woutersen et al (1989), from the TNO-CIVO Toxicology and Research Institute in the Netherlands,
reported nasal tumors in rats after severe injury to the nasal mucosa associated with prolonged exposure of Formaldehyde. Male Wistar rats (720) were assigned to different treatment groups. Half the animals were exposed to 0, 0.1, 1.0, or 10 ppm Formaldehyde (6 h/day, 5 days/week) for 24 months and the other half to those same levels for 3 months. Electrocoagulation of the nasal cavity was performed on 2/3 of the animals before treatment. All animals were killed at month 28.

Body weights were determined regularly and the general condition of the animals was observed daily. Pathology and histopathology was performed on dead or killed animals, with special emphasis on the nasal cavity.

The authors reported that the incidence of squamous metaplasia and rhinitis was greater in control rats who received electrocoagulation compared to control rats who did not.

In the groups exposed for three months, followed by a 25 month observation period, increased squamous metaplasia of the respiratory epithelium and rhinitis were noted in the high exposure group with no electrocoagulation. With electrocoagulation, again the only changes were in the high exposure group with squamous metaplasia and basal cell/pseudoepithelial hyperplasia of the respiratory epithelium, and a thinning of the olfactory epithelium. Increased rhinitis in all treatment groups was noted.

In the groups exposed for 28 months, increased squamous metaplasia and basal cell/pseudoepithelial hyperplasia of the respiratory epithelium, and a thinning of the olfactory epithelium were seen in the high exposure group with no electrocoagulation. With electrocoagulation, increased squamous metaplasia was seen in all groups at the most anterior part of the nasal cavity and further back in the high exposure group, basal cell/pseudoepithelial hyperplasia of the respiratory epithelium was seen in the middle and high dose groups anteriorly and further back in the high exposure group, and a thinning of the olfactory epithelium in the high exposure group, and rhinitis in all treatment groups anteriorly and in the high exposure group further back.

A high incidence of nasal tumors (15 squamous cell carcinomas, 1 adenosquamous carcinoma, and 1 adenocarcinoma in 58 animals) was found in rats with receiving electrocoagulation and 10 ppm Formaldehyde for 28 months. In rats that were not electrocoagulated and received 10 ppm Formaldehyde for 28 months, only one squamous cell carcinoma was seen in 26 animals. No nasal tumors appeared in
any control animals. Other nasal tumors appeared scattered among the other treatment levels, in both electrocoagulated and not electrocoagulated animals, but no significant increase was seen. With the exception of one polyploid adenoma seen in 26 animals in the undamaged nose, 3 month high exposure, the other ten tumors scattered throughout the treatment groups were squamous cell carcinomas.

The authors stated that their conclusions were that 1 ppm Formaldehyde did not visibly affect the nasal epithelium after 28 month exposure, electrocoagulated nasal mucosa was more susceptible to Formaldehyde-induced cytotoxic damage, and that, while nasal tumors did not develop in intact nasal mucosa, nasal tumors did develop in electrocoagulated nasal mucosa (Woutersen et al., 1989).

A report from the Chemical Industry Institute of Toxicology (CIIT) authored by Wolf et al. (1995) described the immunohistochemical localization of p53, proliferating cell nuclear antigen (PCNA), and transforming growth factor (TGF)α proteins in formaldehyde-induced rat nasal squamous cell tumors. Based on an earlier CIIT report in which 5/11 Formaldehyde-induced squamous cell carcinomas of the nasal cavity had point mutations in the p53 gene, this study was undertaken to determine if immunohistochemical techniques for retrospective examination for PCNA (as a marker for cell proliferation) and for the growth factor, TGF-α.

Male Fischer 344 rats were exposed to 15 ppm Formaldehyde gas 6 h/day, 5 days/week. Rats were monitored weekly for signs and evidence of a nasal mass. Such animals were killed and the nasal passages dissected, tumor fragments were collected for PCR analysis for p53 analysis, cell lines derived from the tumors were initiated, and sections of the tumors were prepared for immunohistochemistry analysis.

The pattern of p53 protein staining depended on the morphology of the lesion. No nuclear staining was seen in areas of squamous metaplasia and hyperplasia. Immunohistochemistry of the tumors and cell lines derived therefrom with a polyclonal p53 antibody identified positive reactions in all 6 of the cell lines from tumors in which a p53 point mutation had been demonstrated. Monoclonal p53 antibody picked up only 2 of 6.

The pattern and distribution of PCNA immunostaining was similar to that of p53 protein. Immunostaining for TGF-α was not similar to either. The authors concluded that immunohistochemistry is
a useful tool to identify sites within tumors that may be locations for DNA sequencing for detecting p53 lesions and speculated that it could also identify which lesions are intermediate stages in the progression of cancer (Wolf et al., 1995)

Monticello et al. (1996) correlated Formaldehyde-induced nasal carcinomas with cell proliferation indices at sites of carcinoma induction. They used male F344 rats, 6-7 weeks old, exposed to 0, 0.7, 2, 6, 10, or 15 ppm Formaldehyde, 6 h/day, 5 days/week for up to 24 months in a protocol virtually identical to those previously described. Additional features in this study included DNA labeling with methyl-H\textsuperscript{3}-thymidine in 6 animals per group during the last 5 days of exposure. Rather than reporting tumors at four different levels in the nasal cavity as had been done in previous studies, 30 levels were evaluated to better localize any nasal tumors found.

The authors reported a non-linear dose response for nasal squamous cell carcinomas. No nasal tumors were observed at 2 ppm or less. A minimal increase was seen at 6 ppm (1%) and a sharp increase at 10 ppm (22%) and 15 ppm (47%). All nasal squamous cell carcinomas were mapped.

A unit length labeling index (S-phase nuclei/mm basement membrane as determined by histoautoradiography) was determined and mapped. This index was further refined by weighting the data according to the number of nasal epithelial cells at the site. The authors concluded there was good correlation between the population-weighted labeling index and the regional tumor incidence. They suggested that the population of nasal cells at different sites, the increases in cell proliferation at these cites, coupled with differences in air-flow differences in actual Formaldehyde dose to different sites, and the kinetics of Formaldehyde induced DNA damage, can explain the non-linearity with dose and site specificity of Formaldehyde-induced nasal squamous cell carcinomas in rats (Monticello et al., 1996).

Kamata et al. (1997) reported results of a 28-month inhalation toxicity study of Formaldehyde in rats. Male Fischer 344 rats were exposed to 15, 2, 0.3, 0 ppm and a room control, no exposure group (32 rats per group). The exposure concentrations were selected as a level for which nasal cavity tumors had been reported (15 ppm), a level at which no nasal cavity tumors had been reported (2 ppm) and a level which can be perceived by human olfaction as a relatively high concentration in room air (0.3 ppm). Exposures were done 6 h/day, 5 days/week for 28 months. General observation, body weights,
hematology, clinical chemistry, pathology and histopathology all were performed.

Body weights and absolute liver weights were significantly reduced in the 15 ppm group, but not in the other treatment groups. The incidence of epithelial hyperplasia with squamous metaplasia was increased in the 0.3 ppm group from month 24, in the 2 ppm group from month 18, and in the 15 ppm group from month 6. In the 15 ppm group, there was also an increase in epithelial cell hyperkeratosis and squamous cell carcinomas (Kamata et al., 1997).

**Oral**

Soffritti et al. (1989) reported a 2-year drinking water study using rats. Two protocols were used, both of which exposed rats to Formaldehyde in drinking water. The impurities found in the Formaldehyde used in this study were well characterized and included iron at 0.6 mg/l, lead at 0.1 mg/l, sulphur and chlorine at <5 mg/l, and methanol (stabilizer) at 0.3%.

In the first protocol, Sprague-Dawley rats (7 weeks old) were placed into one of 8 exposure groups. Six Formaldehyde exposure groups (1500, 1000, 500, 100, 50, and 10 mg/l) and the vehicle control group contained 50 animals of each sex. The water control group contained 100 animals of each sex.

In the second protocol, rats of different ages at the start of exposure were treated. Breeding animals (25 weeks old) and offspring (12 day old embryos) were exposed to 2500 mg/l or 0 mg/l Formaldehyde for 104 weeks in a transplacental exposure regimen.

No remarkable effects of Formaldehyde treatment on survival were seen in either protocol. No effect on body weight was noted in male or female rats in the first protocol or in the breeding rats in the second protocol. Body weight decreases were seen in animals first exposed to Formaldehyde in utero.

Formaldehyde did enhance the incidence of hemolymphoreticular neoplasias (leukemias). The authors stated that, In the first protocol, animals at all exposure levels had an increase in leukemias when compared to controls and the increase was dose dependant in the animals exposed to the 4 highest doses. The data are given in Table 5, but do not “appear” consistent with the authors statements, nor is there a description of the statistical analyses done to support the author’s conclusions. At 500 mg/l Formaldehyde and above, there does appear to be an increase in leukemias, but it is not clear that 1000 mg/l and 500
mg/l are different. It would appear that 1500 mg/l is higher.

Leukemias in rats initially exposed during breeding and in utero are shown in Table 6. The authors state there was a slight increase in leukemias in male and female breeders (25 week old at initiation of exposure), but again the data in Table 6 do not appear to support any difference.

Table 5. Leukemias in rats - Formaldehyde exposures initiated at 7 weeks (Soffritti et al. 1989).

<table>
<thead>
<tr>
<th>Group</th>
<th>Conc. (mg/l)</th>
<th>Animals</th>
<th>No. rats with leukemias$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sex</td>
<td>Lymphoblastic leukemias and lymphosarcomas</td>
</tr>
<tr>
<td>1</td>
<td>1500</td>
<td>M 50</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 50</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>M 50</td>
<td>6</td>
</tr>
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<td></td>
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<td>500</td>
<td>M 50</td>
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<td>4</td>
<td>100</td>
<td>M 50</td>
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<td>10</td>
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<td>F 50</td>
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<tr>
<td>7</td>
<td>0$^b$</td>
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<td>1</td>
</tr>
</tbody>
</table>

$^a$ includes all hemolymphoreticular neoplasias

$^b$ 15 mg/l methanol in the drinking water (vehicle control)
Table 6. Leukemias - Formaldehyde exposures initiated during breeding/in utero (Soffritti et al. 1989).

<table>
<thead>
<tr>
<th>Group</th>
<th>Conc. (mg/l)</th>
<th>Animals</th>
<th>No. rats with leukemias*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Age</td>
<td>Sex</td>
</tr>
<tr>
<td>1</td>
<td>2500</td>
<td>25 weeks</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>2500</td>
<td>in utero (12 day old embryos)</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>25 weeks</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>in utero (12 day old embryos)</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>49</td>
</tr>
</tbody>
</table>

* includes all hemolymphoreticular neoplasias  † drinking water alone

The authors stated that Formaldehyde treatment also caused the onset of tumors of the gastrointestinal (g.i.) tract. The types of gastric neoplasias found included papillomas and acanthomas (of the forestomach), adenomas, squamous cell carcinomas, adenocarcinomas, and leiomyosarcomas. The types of intestinal neoplasias found included adenomas, leiomyosarcomas, and adenocarcinomas. The authors categorized each as benign or malignant and presented the data for the first protocol in Table 7 and from the second protocol in Table 8. Leiomyosarcoma was the most frequent malignant tumor and adenomas and adenocarcinomas were the most frequent benign tumors in the treatment groups. The authors stated that leiomyosarcomas were exceptionally rare in their historical controls.

Feron et al. (1990), from the TNO-CIVO Toxicology and Research Institute in the Netherlands, challenged the interpretation of the findings in the above study, asserting that a statistical analysis of the data in the first protocol demonstrated that there was no difference in the incidence of leukemias or g.i. tumors when compared to the vehicle control (15% methanol in drinking water). While these authors found that g.i. tract tumors were significantly increased in the group exposed beginning in utero, and in the high dose group exposed beginning at 7 weeks of age, animals exposed beginning at week 25 had no such increase. In the 7 week old group, there was a noticeable absence of a dose-response in that the 10,
Table 7. G. I. tract neoplasias - Formaldehyde exposures initiated at 7 weeks (Soffritti et al. 1989).

<table>
<thead>
<tr>
<th>Group</th>
<th>Conc. (mg/l)</th>
<th>Animals</th>
<th>No. animals with neoplasias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sex</td>
<td>Benign*</td>
</tr>
<tr>
<td>1</td>
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<td>50</td>
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<td></td>
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<tr>
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<td>M</td>
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<td>F</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>0*</td>
<td>M</td>
<td>50</td>
</tr>
<tr>
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<td></td>
<td>F</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
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<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>100</td>
</tr>
</tbody>
</table>

* Includes forestomach papillomas and acanthomas  
* 15 mg/l methanol in the drinking water (vehicle control)

Table 8. G.I. tract neoplasias - Formaldehyde exposures initiated during breeding/in utero (Soffritti et al. 1989).

<table>
<thead>
<tr>
<th>Group</th>
<th>Conc. (mg/l)</th>
<th>Animal</th>
<th>No. animals with neoplasias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Age</td>
<td>Sex</td>
</tr>
<tr>
<td>1</td>
<td>2500</td>
<td>25 weeks</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>2500</td>
<td>in utero (12 day old embryos)</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>0*</td>
<td>25 weeks</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>0*</td>
<td>in utero (12 day old embryos)</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>49</td>
</tr>
</tbody>
</table>

50, and 1500 ppm groups were significantly increased, but not the 100 or 500 ppm groups. These authors
also commented on the heterogeneity of the both the g.i. tract tumors and the leukemias.

Til et al. (1989), from this same laboratory in the Netherlands, reported the results of their own 2-year drinking water study of Formaldehyde in rats. Formaldehyde to provide target intake levels of 0, 5, 25, and 125 mg/kg was administered to 70 male and 70 female Wistar rats in each of three treatment groups and one control group for up to 24 months. The actual mean Formaldehyde doses administered were 0, 1.2, 15, or 82 mg/kg for the males and 0, 1.8, 21, or 109 mg/kg for the females. Hematology and clinical chemistry were performed on 10 animals/sex/group in weeks 26 and 103. Fasting sugars were done in weeks 27, 52, 78, and 104. Orbital blood in week 28 and abdominal blood in week 53, 79, and 105 were analyzed for enzyme activity, e.g., alkaline phosphatase, and clinical chemistry, e.g., cholesterol. Urinalysis was done after a deprivation period in weeks 27, 52, 78, and 104. Pathology and histopathology (complete selection of organs and tissues) was performed on 10 animals/sex/group killed at week 53 and week 79. The remaining 50 animals/sex/group were killed at week 105.

There were no differences in mortality in any group. The high dose males had decreased body weights from week 1 and females from week 24. Liquid consumption was significantly decreased (40%) in the high dose group in both sexes. There was a trend to lower water consumption in the middle dose group, but it was not significant. Food intake was decreased in high dose males, and to a lesser extent, in high dose females.

No differences in hematology were seen. Urinalysis revealed an increase in density, an increase in mean pH in some groups at some times with a decrease at other times. No significant differences in clinical chemistry values were seen.

Heart, liver, and testes weights showed decreases at some times, consistent with the body weight decreases noted above. Increases in kidney weights in females and brain weights in males and females (week 105 only) were seen in the males at various times. Testes weights increased in week 105.

The incidence of tumors was not very different between the groups, except that the high dose males had fewer tumors than did the control males. Two benign papillomas, one in a low dose male and one in a control female, were the only gastric tumors observed. There was no indication of a treatment-related tumor response.
The authors attributed the urinalysis changes and kidney changes to the reduced water consumption. They established a no observable adverse effect of Formaldehyde in rats at 15 and 21 mg/kg day\(^{-1}\) in male and females, respectively (Til et al., 1989).

Tobe et al. (1989) also performed a drinking water study of Formaldehyde in rats. Formaldehyde at 0.5, 0.1, 0.02, and 0 % was provided to groups of 20 male and 20 female Wistar rats for 24 months. Six rats of each sex from each group were randomly selected and killed at 12 and 18 months. Pathology, hematology, clinical chemistry, and histopathology were performed.

Animals in the high dose group did not fare well. Significantly reduced weight gain was seen along with intake of water and food. The first death in this group was observed on day 9 and all animals had died by 24 months. Oddly, no dose-related significant changes in either absolute or relative weights of any organs was seen. Hematology parameters yielded no treatment-related effects.

Non-neoplastic lesions of the forestomach (erosions, ulcers, basal cell hyperplasia, and hyperplasias of the squamous epithelium, with or without hyperkeratosis) and glandular stomach (erosions, ulcers accompanied by submucosal inflammatory cell infiltrates, and glandular hyperplasia) were observed. These changes were significant in the high dose group, and some were seen in the middle dose group, but no toxicological abnormalities were seen in the 0.02% group.

There were no differences in tumor incidence among groups of either sex. Based on these findings, the authors concluded that the no observable effect level of Formaldehyde in drinking water was 0.02% (10 mg/kg day\(^{-1}\)) in male and female rats (Tobe et al., 1989).

Soffritti et al. (2002) reported results of long-term studies of the carcinogenicity of Formaldehyde and acetaldehyde in rats. The protocol was as described in the previous Soffritti et al. (1989) study (Formaldehyde concentrations in drinking water of 1500, 1000, 500, 100, 50, and 10 mg/l, and two controls, one with 15 mg/l methanol as a vehicle control) except that the \(\chi^2\) test was used to evaluate differences in tumor incidence.

Compared to the vehicle control, the total number of tumor bearing animals was higher in 1500 mg/l males (\(p < 0.05\)), and the total number of tumors normalized to 100 animals was higher in 1500 mg/l males and females, 1000 mg/l females (\(p < 0.05\)), 500 mg/l males (\(p < 0.01\)), and 100 mg/l females (\(p <
0.05). Also presented were tabular data on hemolymphoreticular neoplasias, in which the number of animals with these neoplasias was higher in 1500 mg/l males (p < 0.05) and females (p < 0.01), 1000 mg/l males (p < 0.01) and females (p < 0.01), 500 mg/l males (p < 0.01), and 100 mg/l males (p < 0.05). The authors stated that the number of mammary malignant tumors increased in females treated with 1500 mg/l (p < 0.05), and 1000 and 100 mg/l (p < 0.01). The number of testicular interstitial cell adenomas increased in males treated with 1500 and 1000 mg/l (p < 0.05). Other conclusions were stated, but with no accompanying statistical relevance. The authors failed to cite studies presented earlier with contradictory findings (Soffritti et al., 2002).

**REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**

**Dermal**

Overman (1985) reported the absence of embryotoxic effects of Formaldehyde after percutaneous exposure in hamsters. Golden Syrian hamsters of -100 g were individually bred and the times of onset and completion of mating were noted and the midpoint designated time 0. On days 8, 9, 10, or 11 of gestation, 0.5 ml of Formaldehyde (37%) was applied to the clipped dorsol skin of anesthetized (to prevent licking) animals. Controls received water, but were otherwise treated in an identical fashion. Fetuses were recovered by laparotomy under general anesthesia at gestation day 15. Fetuses were weighed and examined for malformations.

The author reported signs of stress in dams greater than previously seen in tests of other agents tested by this method. No significant effect on maternal weight gain or on fetal weight or length was seen. The percent implantation sites resorbed was 0 in the controls, but 4.2, 8.1, 4.6, and 3.2 with Formaldehyde treatment on gestation days 8, 9, 10, and 11, respectively. Two fetuses from the same litter in the day 8 group were significantly smaller than their littermates, as were 2 fetuses from different litters in the day 10 group. One fetus in the day 10 group had a subcutaneous hemorrhage in the dorsal cervical region. No skeletal malformations were found, nor were any other malformations observed. The author concluded that accidental skin exposure to formaldehyde likely would not adversely affect development (Overman, 1985).

**Inhalation**

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Saillenfait et al. (1989) reported the effects of maternally inhaled Formaldehyde on embryo and fetal development in rats. Pregnant Sprague-Dawley rats were exposed to Formaldehyde (5, 10, 20, or 40 ppm, 6 h/day) from gestation days 6 to 20. Control animals breathed room air. There were 25 rats per group. Dams were killed at term and the fetuses examined.

All dams survived, however, there was a significant decrease in body weight gain in the 40 ppm group. There was a significant increase in body weight gain in the 5 ppm group was not considered treatment related.

There were no significant differences in the implantations, resorptions, dead and live fetuses, incidence of pregnancy, or fetal sex ratio. There were significant dose related decreases in fetal body weights in male fetuses at 20 ppm (p < 0.05) and in both sexes at 40 ppm (p < 0.01). No significant increases in external, visceral, or skeletal abnormalities were seen. A non-significant increase in unossified vertebrae occurred in the 40 ppm group. The authors concluded that Formaldehyde exposure to rats under these conditions was not teratogenic, but is slightly fetotoxic at concentration levels that are not maternally toxic (Saillenfait et al., 1989).

Martin (1990) reported a teratology study of inhaled formaldehyde in the rat. An initial range-finding study involved 30 mated Sprague-Dawley rats. The rats were treated with 0, 2, 5, 10, or 16 ppm Formaldehyde from gestation day 6 to 15, 6 h/day. No maternal or fetal deaths occurred during this study.

In the main study design, groups of 25 rats were treated with Formaldehyde at 2, 5, or 10 ppm, 6 h/day from gestation day 6 to 15, with two control groups (one handled the same as the treated animals and one left constantly in the animal room). Group mean values ± SD were calculated for body weights, food consumption, gravid uterine weights, and corrected body weights. Group mean values ± SD for litter size, corporus luteum count, number of implants, and resorptions were determined. Individual and group mean values ± SD for preimplantation and postimplantation losses were calculated. The litter sex ratio and the group sex ratio and the litter mean fetal weights and group mean fetal weights were determined. No tabular data were provided, however.

According to the author, Formaldehyde had no effect on any of the above parameters, except a significantly decreased weight gain and reduced food consumption in dams at the 10 ppm level and a
significant decrease in ossification in the bones of the pelvic girdle in the fetuses at the 5 and 10 ppm levels. The latter finding was linked to larger litter sizes and slightly lower fetal weights in both the 5 and 10 ppm groups and was dismissed as not an adverse effect associated with Formaldehyde exposure (Martin, 1990).

**Intraperitoneal**

Majumder and Kumar (1995) reported that Formaldehyde injections produced inhibitory effects on the reproductive system of male rats. Adult male Wistar rats were given 10 mg/kg intraperitoneally by injection over a period of 30 days. A control group received water injections. On day 31, the animals were killed and the testis, prostate, seminal vesicles, and epididymis were removed and weighed. DNA and protein content was determined from tissue homogenates. The cauda portions of the epididymis were minced separately to obtain a sperm suspension. Sperm count, motility and viability were determined. In addition to the animal study, a sperm suspension of untreated animals was incubated with various concentrations of Formaldehyde at ambient temperature for up to 3 h.

In the in vitro study, no viable sperm were found after 60 minutes after incubation with 5 ng Formaldehyde. Incubation with 500 ng Formaldehyde reduced the time for no viable sperm to 30 minutes. At 2500 ng Formaldehyde, the time was 10 minutes. Sperm motility was eliminated within 10 minutes at 5 ng Formaldehyde and within 30 minutes at 0.125 ng Formaldehyde.

In vivo, there was a significant decrease in tissue DNA content in the testis (p < 0.0001) and the prostate (p < 0.001). The sperm count, viability and motility all decreased (p < 0.0001). The authors speculated that these findings could be attributed to a decrease in Leydig cell population, inhibition of spermatogenesis, and degeneration and calcification of testicular tissue (Majumdar and Kumar, 1995).

**Critical Reviews**

Collins et al. (2001b) reviewed the adverse pregnancy outcomes and Formaldehyde exposures in human and animal studies. The animal portion will be summarized here. Animals studies are reported in rats, mice, dogs, and hamsters using a variety of exposure routes. The authors state that studies using routes of exposure such as subcutaneous, intramuscular, and intraperitoneal injection do not provide data relevant to assessing human risk. Oral studies in rats, they contend, are iffy because rats dosed by
gavage exhibit micronuclei and nuclear abnormalities in the g.i. epithelium, the first site of contact. Inhalation and topical studies were considered relevant. The inhalation studies, mostly, find no increased risk at exposure ranges occurring in the workplace (e.g. the Saillenfait et al. 1989 study described above). Two Russian studies, however, did report long-term, whole-body inhalation studies in which maternal and embryo/fetal effects and female germ cell and bone marrow toxicity in rats. These reports have not been replicated and are typical of many Russian studies in lacking detailed study information.

These authors recount the developmental toxicity from topical application in the form of increased resorptions and birth defects in hamsters in a pilot study and increased resorptions in the main study by Overman (1985) discussed above. These authors, however, point out that these studies were confounded by the stress experienced by the dams.

Overall, the large number of inhalation, ingestion, and topical studies, according to these authors, provide little evidence of Formaldehyde reproductive or developmental toxicity under routes of exposure or levels of exposure relevant to the workplace.

Thrasher and Kilburn (2001) also presented a review of embrotoxicity and teratogenicity of Formaldehyde. Included in this review was a description of several Japanese studies of the uptake of radiolabeled Formaldehyde injected into the tail veins of pregnant mice. The radiolabel was rapidly taken up into the maternal liver, lung, heart, salivary gland, gall bladder, spleen, kidney, bone marrow, nasal mucosa, uterus, placenta, and fetal tissues. The incorporation into placenta, uterus, and fetal tissues was larger than in other maternal organs and elimination of radiolabel was slower than from maternal tissues.

These authors also present the results of the Russian study noted in the previous review which found female germ cell and bone marrow toxicity in rats. Exposures of female rats were 4 h/day, except on non-working days, for 4 months to 0.5 or 1.5 mg/m³ Formaldehyde vapor. The animals were mated with untreated males and the embryos obtained on days 2 and 3 of pregnancy. Bone marrow was taken from the same animals 48 - 72 h after the end of Formaldehyde exposure and prepared for cytogenetic analysis in the normal way. All chromosome aberrations (not including gaps) were scored from 100 metaphases per animal. No significant effect of the low exposure on embryonic development was seen, but at the high
exposure there was blastomere structural damage (e.g., pyknosis of nuclei) and there was an increase in
degenerating embryos \( (p < 0.05) \). In the bone marrow arm, the number of cells with aberrations was
increased at the lower exposure \( (p < 0.05) \) and even more so at the higher exposure \( (p < 0.01) \). The
number of chromatid and chromosome aberrations per 100 metaphases was increased at the high
exposure level \( (p < 0.05) \). The number of chromosomes with aberrations and aneuploidy \(<42\) was
elevated at both exposure levels. The mitotic index was decreased at the lower exposure level and
increased at the higher \( (p < 0.05) \).

This review also considers the Russian studies which found maternal and embryo/fetal effects.
Female rats inhaled 0.012 or 1.0 mg/m\(^3\) Formaldehyde for 10-15 days and were mated with unexposed
males, then exposed again throughout gestation. Exposed animals were killed at parturition. No critical
comments on the study were provided in this review, and the original study’s authors statements of
increased pregnancies, decreased litter size, decreased ascorbic acid content of the fetus and maternal
liver, increase in fetal body weight, increase in fetal organ weights (except for a decrease in lung an liver)
are simply presented.

This review also reports the Russian data on changes in fetal enzyme activity associated with
Formaldehyde exposure, including MDH, SDH, and LDH decreases and GDH increase in mitochondria,
and adenosine triphosphatase increase and inosine diphosphatase and \( \beta \)-glucuronidase decreases in
lysosomes. N-Acetyleneuraminic acid levels increased in maternal and fetal tissues. The changes in
enzyme activity and N-acetyleneuraminic acid correlated with increased fetal mortality. Development of
postnatal behavior was also adversely affected.

Finally, this review article presents a Russian study of the embryotoxic and teratogenic effects of
Formaldehyde (inhalation of 0.5 mg/m\(^3\), 4 h/day during gestation days 1-9) in rats with a background of
induced iron/trace-element disorder. A chelating agent was injected in 25% ethanol to induce iron
deficiency. In animals receiving Formaldehyde alone, post-implantation mortality was increased, along
with hydrophenosis cryptochordism anomalies, and the overall number of embryos with anomalies was
increased. In animals receiving Formaldehyde and the chelating agent, post-implantation mortality was
increased, and cleft palate, hydrophenosis, cryptochordism, and phocomelia (rear limbs) anomalies, breast
bone, digits, and tail cartilages adhesions, and the overall number of embryos with anomalies was increased (Thrasher and Kilburn, 2001).

**CLINICAL ASSESSMENT OF SAFETY**

**Cosmetics Adverse Effects Reports**

de Groot et al. (1988a) patch tested 150 consumers who claimed to have side effects from use of cosmetics and toiletries. Patch testing was done on these self-selected individuals to 1% Formaldehyde in water and there were 2 positive reactions (1.3%).

de Groot et al. (1988b) patch tested 119 patients suffering from cosmetic-related contact dermatitis to determine the ingredients responsible for the reaction. Preservatives were linked to the contact dermatitis in 47 patients, but Formaldehyde was implicated in only 1 patient.

Norton (1991) reported common and uncommon reactions to Formaldehyde-containing nail hardeners. Onycholysis was the most commonly reported and may be inflammatory or non-inflammatory. It is frequently painful and can involve multiple digits simultaneously. A yellow-brown discoloration of the distal portion of the nail plate has been seen by the author and a blue discoloration reported by another. Subungual hemorrhage and hyperkeratosis are seen and the author speculated that Formaldehyde penetration to the nail bed may be possible. As a consequence of the initial symptoms, the nail may eventually be shed. The author also indicated that pterygium inversum unguis has been reported with Formaldehyde in a nail fortifier. It was not clear the extent to which these observations represented a significant body of reports or the authors personal experience, with a smattering of case reports thrown in.

Berne et al. (1996) stated that 191 reports concerning adverse effects of 253 cosmetics and toiletries were received by the Swedish government from 1989 to 1994. While Tosylamide/Formaldehyde Resin was identified in 19/79 individuals as the relevant allergen when they were tested against individual ingredients (the 79 had previously patch tested positive to the product). Formaldehyde was not identified as an allergen in this report.

Baron (2002) reviewed allergies and irritations associated with nail cosmetics. Cases of ectopic dermatitis from nail enamels are reported. The author recommends patch testing with 1 to 2% Formaldehyde in water, presumably because Tosylamide/Formaldehyde Resin present in nail enamel as a

**Human Experimental Studies**

**Ocular Irritation**

Bender et al. (1983) reported on the eye irritation response of humans to Formaldehyde. Exposures were conducted in a 610 ft³ aluminum smog chamber with 7 sets of eye ports. A series of 7-member panel from laboratory staff were screened for a response to Formaldehyde at 1.3 and 2.2 ppm. Panelists who responded (~ half) were included in the study. Formaldehyde was tested at 1.0, 0.9, 0.7, 0.56, 0.35, and 0 ppm.

The authors reported a trend toward earlier response to Formaldehyde with increasing concentration, but only at 1 ppm was the median response time significantly decreased. Only the 1 ppm exposure produced a severity response that was slightly to moderately irritating. All other exposures were less than slightly irritating (Bender et al., 1983).

Yang et al. (2001) conducted a controlled human exposure experiment to determine the extent of ocular irritation caused by Formaldehyde. Formaldehyde gas from plywood was run through modified swim goggles fitted to each of 8 volunteers (4 male, 4 female, 1 male smoker, 1 female smoker). Formaldehyde exposures were 4.31 ± 0.02 ppm, 2.99 ± 0.07 ppm, and 1.65 ± 0.01 ppm. The exposure time was 5 minutes. The Chinese National Criterion limits Formaldehyde to 2.5 ppm over 8 h.

All exposures produced an increase in sensory irritation in the first 2.5 minutes. After that the sensory irritation produced by the lowest Formaldehyde exposure was not different from controls. Sensory irritation produced by the two higher exposures were significantly increased at all times. The eyeblinkning frequency was significantly increased at most times for all exposures. The only non-significant values were for 1.65 ppm at 5 minutes and 2.99 ppm at 3 and 5 minutes. The authors concluded that the study size did not allow a conclusion regarding dose-response (Yang et al., 2001).

**Pulmonary Responses**

Schachter et al. (1987) exposed a group of 15 hospital laboratory technicians (occupationally exposed to Formaldehyde) to 0 and 2 ppm Formaldehyde for 40 minutes in an environmental chamber.
Exposures were repeated on two more occasions with a 10-minute exercise regimen after 5 minutes. A symptom diary was recorded for 24 h after exposure.

Lung function did not alter at any testing time on any exposure day. Peak expiratory flow rate measurements did not indicate any obstruction. Symptoms were mild and transient, with odor and eye irritation the most frequent complaint. The authors concluded that 2 ppm Formaldehyde had no acute or delayed lung function changes at rest or with exercise, and that irritation symptoms were few (Schachter et al., 1987).

Green et al. (1989) reported the acute pulmonary response in healthy, non-smoking adults to inhalation of Formaldehyde and carbon. Non-smoking volunteers (29) were studied. Screening of the volunteers involved a bicycle ergonometer test which 5 individuals had problems with and they were excluded. Each subject was exposed to clean air, Formaldehyde at 3 ppm, activated carbon aerosol at 0.5 mg/m³, and a combination of Formaldehyde and activated carbon aerosol for 2 h. The mean aerodynamic diameter of the activated carbon particles was 1.4 μm, with a geometric standard deviation of 1.8 μm. Exposures were separated by 1 week.

There was no significant effect of Formaldehyde on forced vital capacity (FVC) or forced expiratory volume (FEV), but small significant decrements in forced expiratory flow (FEF) were found at 50 and 110 minutes of exposure, a significant decrease in peak flow was seen at 110 minutes, and on airway conductance at 120 min. A Formaldehyde/activated carbon aerosol interaction effect was significant for FVC at 20 and 50 minutes, FEV at 20 and 50 minutes, and peak flow at 110 minutes.

The authors suggested that Formaldehyde may have absorbed on the activated carbon particles and on inhalation of the particles produced a Formaldehyde exposure deeper than the Formaldehyde gas alone (Green et al., 1989).

Pazdrak et al. (1993) exposed 11 healthy individuals and 9 patients with skin hypersensitivity to Formaldehyde (as a result of occupational exposure) to Formaldehyde at 0.5 mg/m³ for 2 h in a 12 m³ chamber.

No differences were found in responses between the two groups. In all exposed individuals, Formaldehyde produced transient rhinitis symptoms and longer-lasting increases in the number and
proportion of eosinophils, and elevated albumin and protein levels in nasal washings. The authors concluded that exposure to 0.5 mg/m³ Formaldehyde produces a nonspecific inflammatory response (Pazdrak et al. 1993).

**Occupational Studies**

Liebling et al. (1984) reported cancer mortality among workers exposed to Formaldehyde. Deceased employees between 1976 and 1980 were identified through plant records, reports of co-workers, and a systematic review of local newspaper obituaries. A total of 26 decedents were identified as having worked in an area where Formaldehyde exposure was likely. Exposures to several other chemicals also occurred in these areas. Age, race, sex, birthplace, and cause of death were abstracted. Proportionate mortality ratios were calculated to examine how this population compared to the county in which the plant was located and the USA as a whole.

Proportionate mortality ratios were significantly increased for cancer of the colon and rectum and for cancer of the buccal cavity and pharynx when using both the USA and the county mortality profiles in the calculation (Liebling et al., 1984).

Kilburn et al. (1985) studied two groups of male workers to determine the pulmonary and neurobehavioral effects of Formaldehyde exposure. One group worked in phenol-formaldehyde-plastic foam matrix embedding of fiberglass (batt making). The other group worked fixating tissues for histological examination.

Forty-five male fiberglass batt makers were divided into machine operators who managed extrusion, matrix embedding, and oven setting were in the “hot area” group. Workers in other operations within the building were the “cold area” group. Male histology workers were taken as the exposed group and an age-matched control group consisted of male hospital employees (respiratory therapists, gardeners, and attendants). By nature of the occupation, workers exposed to Formaldehyde were also exposed to other chemicals. A questionnaire was self-administered. Spirometry was performed.

Based on reported perceptions of Formaldehyde odor (threshold ~1 ppm) histology workers were divided into two groups: exposure of 1-3 h/day or exposure of ≥4 h/day. Measured Formaldehyde levels in the workplace were between 0.4 and 1.9 ppm. Access was not possible at the fiberglass batt factory, but 5
hot area workers were exposed to 5 ppm Formaldehyde and their perception was noted that the factory exposure was more intense and irritating.

Symptom symptom profiles (e.g., fatigue, chest tightness, palpitations, dry mouth, indigestion, loss of appetite, insomnia, dizziness, and somnolence) were similar between the two Formaldehyde exposure groups, suggesting that the confounding exposure to other chemicals was not an issue. Across the board, however, the batt makers did have a higher frequency of symptoms in the cold area than did the exposed histology workers. Batt makers in the hot area had significant reduction in FEV$_{1.0}$ and diffusing capacity (Kilburn et al., 1985).

Higginson et al. (1988) presented the epidemiology of chronic occupational exposure to Formaldehyde — a report of the ad hoc panel on health effects of Formaldehyde. Contrary to the title, they considered both occupational and environmental studies. Studies of topical sites, which included respiratory tract cancer, were considered separately from non-topical sites which, for example, included brain cancer.

Based on their review the ad hoc panel concluded that there was no human cancer for which there is a convincing relationship with Formaldehyde exposure, and if a relationship did exist, the excess risk must be small (sounds like a lawyer wrote it). They note that, although individual studies of cancer of the nasal sinuses, pharynx, and lung have shown significant association with Formaldehyde exposure, there is no consistency among studies for any of these sites (Higginson et al., 1988).

Gerin et al. (1989) reported the results of a multi-site case-control study in Montreal of cancer risks in men associated with Formaldehyde occupational exposures. Cancer of the esophagus, stomach, colorectum, liver, pancreas, lung, prostate, bladder, kidney, and lymphoid tissue were studied, along with melanoma. Interviews were conducted with 3726 male patients with these cancers and 533 male population controls to obtain detailed lifetime job histories and information on possible confounders. Separate analyses were carried out for each type of cancer using population controls as well as a control series drawn from other cancer sites in the study. Nearly 25% of all men had some occupational exposure to Formaldehyde, although expected exposure levels were low. Even in the small group that was considered long duration, high exposure, the time weighted average concentration was probably below 1
ppm Formaldehyde, according to the authors. There was no significant increased risk of any cancer among men exposed to Formaldehyde. The authors did note that a small increase could not be ruled out given the small size of the highest exposure group.

Holness and Nethercott (1989) studied the health status of 84 funeral service workers and 38 control subjects. A detailed questionnaire was administered to each participant. A physical exam of each patient's skin was made by a dermatologist and Formaldehyde patch tests were done. Pulmonary function tests were done on all participants. A total of 55 of the funeral service workers were active licensed embalmers, 17 were inactive embalmers, and 12 were apprentices. Mean Formaldehyde exposure were measured at 0.36 ppm ± 0.19 with a range from 0.08 to 0.81 ppm.

Nasal and ocular irritation, skin problems in the past, shortness of breath, and muscle or joint pain were all reported at a significantly higher frequency in exposed versus control individuals. No differences in lung function were seen. Comparing the active versus inactive embalmers, eye irritation and skin problems now were significantly higher in the active and FVC was significantly lower in the inactive. In general, apprentice embalmers had more contact dermatitis than licensed embalmers, but none of the apprentices had a positive patch test. In the licensed embalmers group, 4% had positive patch tests. There were no lung function test differences between apprentices and licensed embalmers (Holness and Nethercott, 1989).

Rudzki et al. (1989) reported the results of patch testing of nurses, doctors, and dentists with chemicals found in occupational settings. The study was carried out on 167 doctors, 92 dentists, and 333 nurses. Among the disinfectants patch tested in nurses, Formaldehyde was the most frequent cause of allergic reactions (9.6% of nurses). No allergic reactions to Formaldehyde were reported in dentists. Three doctors reacted to Formaldehyde (1.8%).

Uba et al. (1989) conducted a prospective evaluation of pulmonary function and respiratory symptoms in 103 medical students exposed to Formaldehyde over a 7-month period. Time-weighted Formaldehyde exposures were <1 ppm and peak exposures were <5 ppm. Each participant responded to two different questionnaires. The first was a standard respiratory questionnaire, completed by each subject in September 1984 prior to the first gross anatomy lab and again in April 1985. The second
questionnaire targeted acute symptoms and was administered once after a gross anatomy lab and once after a microanatomy lab — 81 subjects completed this questionnaire twice.

The responses to the first questionnaire indicated that cough was reported significantly more at the end of the course on gross anatomy than before it started. Wheezing, with and without dyspnea, were reported significantly more frequently at the beginning than at the end. It did not appear to matter whether a student had a history of asthma. The pulmonary function tests demonstrated no significant difference before or after the gross anatomy course (Uba et al., 1989).

Chia et al. (1992) studied 150 first-year medical students exposed to Formaldehyde during gross anatomy lab. As a reference group, 189 3rd and 4th year medical students were matched for sex, ethnicity, and age. The mean Formaldehyde exposure levels was 0.50 ppm (range 0.4 - 0.6 ppm) and the mean personal sample was 0.74 ppm (range 0.41 - 1.20 ppm). All participants responded to a questionnaire. A group of 22 participants was selected for pulmonary function testing and tested before and after the gross anatomy lab.

No differences were seen relating to pulmonary function as a result of the gross anatomy lab. Significant increases in exposed individuals of upper respiratory tract and mucous membrane irritation were seen. The authors made a plea that Formaldehyde levels in dissection labs in medical schools be lowered (Chia et al., 1992).

Shaham et al. (1997) examined the DNA-protein cross-links (DPCs) and sister chromatid exchanges (SCEs) in peripheral blood lymphocytes exposed and unexposed workers. DPCs were determined in 12 workers regularly exposed to Formaldehyde and 8 age-matched unexposed workers. SCEs were evaluated in 13 exposed workers and 20 unexposed workers.

DPCs and SCEs were significantly increased in the exposed workers compared to unexposed workers. A further breakdown of the DPC values allowed the authors to separate 6 physicians from 6 technicians in the exposed group. The technicians, who had a higher Formaldehyde exposure, had a significantly higher DPC percentage compared to physicians (Shaham et al., 1997).

Schnuch et al. (1998) reported on contact allergies in healthcare workers in Germany based on data from the Information Network of Departments of Dermatology (IVDK). Data from 31,849 patients were
evaluated. Healthcare workers were further analyzed and the remainder of the patients were the control group. The reactions to 1% Formaldehyde in a patch test was significantly higher in female healthcare workers (3.6%) compared to controls (2.1%).

Standardized relative risks were calculated on the basis of relative risk of subgroups of healthcare workers compared to IVDK total relative risks. Formaldehyde had a significantly increased standardized relative risk for female nurses (2.0), female medical lab workers (2.0), and male and female masseurs (2.1). The authors expressed the view that Formaldehyde has lost its importance as an allergen compared to previous decades (Schnuch et al., 1998).

Burgaz et al. (2001) examined micronuclei frequencies in exfoliated nasal mucosal cell from pathology and anatomy lab workers exposed to Formaldehyde. A questionnaire was used to collect personal data from 23 (12 male, 11 female) staff in pathology or anatomy departments in Turkey, along with 25 males selected from university and hospital staff as controls. Formaldehyde exposure was determined in the work area to be between 2 and 4 ppm, but individual exposures were not determined. Nasal respiratory mucosal cells were scraped from the inner turbinate. Cells were smeared on glass slides, stained, and 3000 cells were read for each subject at 300x. Micronucleated cells were confirmed at 1250x magnification.

There were significantly more micronucleated cells in exposed workers compared to controls. There was no significant difference between smokers and nonsmokers in both groups. Significantly more micronucleated cells, however, were found in exposed smokers compared to control smokers (Burgaz et al. 2001).

Minamoto et al. (2002) studied occupational dermatoses among fiberglass-reinforced plastics factory workers. It was not clear from the description of the chemistry of making these plastics that Formaldehyde was involved. One of 23 workers with a history of skin eczema patch tested positive to Formaldehyde and methyl ethyl ketone peroxide. Overall, the study was subject to selection bias — 11 out of 15 factories were visited; 148 workers completed a questionnaire (out of how many?) and had their exposed skin areas examined; 5 of 11 factories were asked to participate in patch testing because 6 declined further involvement; all the workers in 3 of the 5 declined to be patch tested; in the two factories
that agreed 23/26 workers in one and 6/6 in the other agreed to be patch tested (Minamoto et al., 2002).

**Formaldehyde Effects in Asthmatics**

Sheppard et al. (1984) undertook a study to determine whether exposure to Formaldehyde at indoor air levels could cause bronchoconstriction in subjects with mild asthma. Seven nonsmoking volunteers participated. All were asthmatic, with associated symptoms of allergic rhinitis. Each subject was treated to a series of histamine inhalations to establish a dose-response curve. All subjects had a marked airway hyperresponsiveness to histamine. Formaldehyde was given at 1 ppm for 10 minutes. Pulmonary function was measured before and after each exposure. When it was determined that 1 ppm Formaldehyde inhaled at rest had no effect, Formaldehyde was also given at 1 and 3 ppm during moderate exercise. Even that exposure regimen did not cause significant bronchoconstriction.

Green et al. (1987) measured the acute response to 3 ppm Formaldehyde in exercising healthy nonsmokers and asthmatics. Exposures were done in an environmental chamber. A newspaper ad that did not mention Formaldehyde resulted in the recruitment of 38 nonsmoking individuals; 22 were normal and 16 had a clinical history of asthma. Each subject was exposed to clean air and 3 ppm Formaldehyde for 1 h, with sessions separated by 1 week. Each individual completed symptom questionnaires and pulmonary function tests were performed at 0, 17, 25, 47, and 55 minutes. A 15-minute bicycle ergometer exercise was completed at t=15 and 45 minutes. The asthmatic group engaged in moderate rather than heavy exercise to minimize exercise-induced bronchoconstriction.

The normal group had significant mean pulmonary function decrements at 47 and 55 minutes with 3 ppm Formaldehyde. The asthmatic group had no significant pulmonary function decrements (Green et al., 1987).

This same laboratory also reported (Sauder et al., 1987) on the acute response of 3 ppm Formaldehyde for 3 h in 9 nonsmoking asthmatics. Again, no significant changes in pulmonary function were observed. There was a significant increase in nose/throat irritation at 30 minutes, and in eye irritation at 60 and 180 minutes.

Witek et al. (1987) performed an evaluation of respiratory effects following exposure to 2 ppm Formaldehyde in asthmatics. The protocol was completed by 15 subjects with mild asthma; four exposure
sessions were held on four separate days — Formaldehyde, with and without exercise and clean air, with
and without exercise. Formaldehyde did not induce bronchoconstriction. Complaints were ocular irritation
and nose and throat irritation, which disappeared after the exposure ended.

Górski and Krakowiak (1991) posed the question as to whether Formaldehyde-induced asthma
really exists. A total of 367 workers who had been occupationally exposed to Formaldehyde at
concentrations <0.5 mg/m$^3$, all suffered from respiratory symptoms. Pulmonary function testing was done
and all subjects were provoked with histamine and observed before and after work for three days. Skin
prick tests of common allergens, a Formaldehyde patch test, and a Formaldehyde-specific IgE antibody
assay were performed. There were no significant differences in the respiratory parameters and there were
no reactions to Formaldehyde in patch tests and no Formaldehyde-specific IgE antibodies were found.
The authors doubted that Formaldehyde-induced asthma really exists.

Kieć-Świerczynska et al. (1998) examined the incidence of allergy to Formaldehyde and other
aldehydes in 280 health care workers suffering from skin lesions. Allergy was diagnosed in 64 workers,
39 of whom were allergic to Formaldehyde. Given that the use of Formaldehyde in health care units had
recently been reduced, the authors stated that this observation was difficult to explain. As there were not
many individuals who cross-reacted to other aldehydes, they assumed that this was Formaldehyde
sensitization. They conclude that exposure of health care workers in Poland must result from some
Formaldehyde-based disinfectants which continue to be marketed and are added to other disinfectants.

Krakowiak et al. (1998) measured airway responses to Formaldehyde inhalation in asthmatics with
suspected respiratory Formaldehyde sensitization. Two groups of adults were recruited: 10 workers with
bronchial asthma and 10 healthy control workers. The 10 workers were occupationally exposed to
Formaldehyde gas and solutions and had diagnoses of bronchial asthma probably due to Formaldehyde.
An initial evaluation was made of both the upper and lower respiratory tract, with simultaneous evaluation
of morphological and biochemical changes in nasal washings after placebo exposure. A week later, all
analyses were repeated during Formaldehyde inhalation (mean concentration 0.5 mg/m$^3$ for 2 h).
Formaldehyde exposure produced sneezing, itching, and congestion in all subjects. Symptoms were most
severe immediately after inhalation. None of the parameters determined in the nasal washings was
different between the groups. The authors concluded that Formaldehyde at 0.5 mg/m$^3$ did not induce a specific allergic response in the upper or lower part of the respiratory tract, whether the exposed individual was occupationally exposed to Formaldehyde or not.

**Epidemiology**

Adams and Maibach (1985) reported a study of cosmetic reactions in 713 patients with contact dermatitis seen by 12 dermatologists over a 64 month period. The perception by patients or referring physicians was that the skin reaction was not due to cosmetics was reported in 353 cases (49.5%) and that the reaction was due to cosmetics was reported in 325 (45.6%), with the remainder not responding to the question. The determination that the skin reactions were cosmetic product related was made based on case history and examination, product patch or use tests, ingredient patch tests, or patch tests to product and ingredient. Patch tests were conducted in 87% of the patients. A total of 16 cutaneous reactions were found to Formaldehyde (identified as paraformaldehyde in one case). A total of 23 reactions were noted to Tosylamide/Formaldehyde Resin.

Ritchie and Lehnen (1987) examined the dose-response relationship between Formaldehyde concentration and reported health complaints of nearly 2000 residents living in 394 mobile and 494 conventional homes. Data were developed from a Minnesota Department of Health program in which a free Formaldehyde test would be provided if health screening was done by a family physician. Formaldehyde was tested in those homes were the examining physician made a written request.

For mobile homes with measured Formaldehyde levels $>0.3$ ppm, 90% of individuals reported ocular irritation. At 0.1 to 0.3 ppm, only 20% reported ocular irritation. At $<0.1$ ppm the figure was around 1%. The pattern was similar for nose/throat irritation. For headache, 61% reported the complaint in mobile homes with $>0.3$ ppm Formaldehyde, 28% in homes with 0.1 - 0.3 ppm, and 5% in homes with $<0.1$ ppm. A much less pronounced concentration level effect was seen for skin rash. The data for conventional homes was not distinguishable from that from mobile homes. The authors concluded that these data demonstrate an excessive occurrence of skin rash, eye, nose, and throat irritation, and headache at Formaldehyde concentrations of 0.1 ppm and above (Ritchie and Lehnen, 1987).

Massone et al. (1989) reported 4-day patch test reactions to Formaldehyde in 576 consecutive
patients seen in 1988. Patch tests to Formaldehyde were positive in 15 patients, but 10 of those reactions were negative at day 2 and only appeared at day 4. The authors concluded that a 4-day patch test reading was necessary in order to not underestimate the frequency of reaction.

Holness and Nethercott (1990) reported a study of dermatitis in hairdressers. Of 1440 patients seen at St. Michael's contact dermatitis clinic between 1981 and 1988, 55 were hairdressers. The mean age was 27, and 87% were female. The control group was the rest of the clinic population with contact dermatitis. Of the hairdressers tested, 9.4% had a positive response to Formaldehyde, which was not significantly different from the control population. The authors noted this finding, even though Formaldehyde is present in shampoos. They speculated that the lack of skin sensitization may be attributable to the low concentrations of Formaldehyde in shampoos, the dilution in water during use, and the short residence time prior to rinse-off.

Fowler et al. (1992) conducted a retrospective study of allergic contact dermatitis from Formaldehyde resins in permanent press clothing. A total of 678 patients seen at the University of Louisville Patch Test Clinic and 344 patients seen at the Allergy Section of the Skin and Cancer Clinic at the New York University Medical Center from 1988 to 1990 were included in the study. The authors present several case reports to illustrate particular features of formaldehyde resin allergy. Of the 678 patients (288 male, 390 female) tested in one setting, 13 were positive for formaldehyde resin (7 male, 6 female). In the other setting, 344 patients were tested (98 male, 246 female) and 4 were positive (1 male, 3 female). Of the 17 who reacted, 5 were considered occupational. The 17 individuals positive to formaldehyde resin were tested against 1% Formaldehyde in water. One had a 3+ reaction, 1 had a 2+ reaction, 10 had 1+ reactions, and 4 had no reaction. The authors speculated that exposure to Formaldehyde from permanent press clothing could result from incomplete cross-linking of some resin, making it susceptible to degradation by chlorine bleach to release Formaldehyde, or if acid catalyst remained in the fabric which would hydrolysis under warm, moist conditions to produce Formaldehyde.

Wieslander et al. (1997) studied possible relations between asthma and emissions from newly painted indoor surfaces. A screening questionnaire was mailed to a random sample of 3600 men and women, 20-44 years of age in Uppsala. The response rate was 87% (wow!). All symptomatic responders
(216) were selected for further examination and interview questionnaire. A random sample of 800 persons from the main sample was enhanced by the addition of all persons from the main sample who indicated use of asthma medication, attacks of asthma, or awakening because of shortness of breath were invited to participate. From the enhanced random sample, the response rate was 68% and from the 216 symptomatic individuals, 83%, giving a total of 699 men and women. All 699 were given the interview questionnaire, blood tests, prick tests, and bronchial provocation. Of these, 562 were given a second self-administered questionnaire on characteristics of the building in which they lived and worked and their occupation. Exposure measurements of Formaldehyde and volatile organic compounds (VOCs) were done in a random sample of 62 participants.

Subjects with asthma symptoms had a significantly higher prevalence of any type of home indoor painting, wood painting, and kitchen painting and in the degree of indoor painting. Subjects with symptoms also had a significantly higher prevalence of workplace indoor painting and degree of indoor painting.

Blood eosinophil concentration was significantly increased in subjects living in newly painted dwellings and significantly related to the degree of indoor painting. No link was found for atopy, eosinophilic cation protein, serum IgE, peak expiratory flow variability, or lung function (FEV\textsubscript{1}%).

Exposure measurements were significantly different between painted and not-painted dwellings in only a few cases for a few chemicals. Formaldehyde was significantly less in dwellings with no wood painting. The levels of 2,2,4-trimethyl 1,3-pentanediol diisobutyrate were significantly less in living rooms without the ceiling painted, in dwelling with no wood painting, and when the kitchen was not painted. Butanols were significantly less when the bedroom was not painted. Aliphatic compounds (C\textsubscript{8} - C\textsubscript{11}) were significantly less in the living room without the ceiling painted.

The authors concluded that there was a relation between exposure to recent indoor painting and current asthma and bronchial hyperresponsiveness. They noted that Formaldehyde is a component of acid curing paints that are commonly used for spray painting kitchen wood details in Scandanavia (Wieslander et al., 1997).

Collins et al. (2001a) performed a review and meta-analysis of Formaldehyde exposure and pancreatic cancer. These authors noted that most reviews of Formaldehyde carcinogenicity have focused
on the respiratory tract. Even though respiratory tract cancers may have been the focus, there are 14 epidemiology studies of workers exposed to Formaldehyde where pancreatic cancer rates were reported, and these studies were considered in a meta-analysis. There was a small increase in the meta relative risk (mRR) at 1.1 with a 95% confidence interval of 1.0 to 1.3. This increase, however, was limited to embalmers with a mRR of 1.3 (95% c.i. of 1.0 to 1.6) and pathologists and anatomists with a mRR of 1.3 (95% c.i. of 1.0 to 1.7). There was no increase in the mRR for industrial workers (mRR 0.9, 95% c.i. of 0.8 to 1.1) who had the highest exposures to Formaldehyde. They concluded that a small increase in pancreatic cancer risk from Formaldehyde exposure could not be ruled out, the absence of an elevated mRR in industrial workers and the absence of any mechanism by which the effect would occur, suggests that the mRRs seen in embalmers and pathologists and anatomists is due to a diagnostic bias or an exposure to something other than Formaldehyde in those professions.

Collins et al. (2001b) conducted a review of adverse pregnancy outcomes in humans and animal studies. The animal data was presented earlier. These authors uses meta-analysis to evaluate spontaneous abortion data from 11 epidemiology studies in which there was some form of exposure assessment, including monitoring, expert assessment, and self-reporting. The authors stated that the small number of studies on birth weight, infertility, and other reproductive outcomes, the limitations in the designs of these studies, and the inconsistent findings make it difficult to reach any conclusions on these endpoints. For spontaneous abortions, an mRR of 1.4 (95% c.i. 0.9 - 2.1) was found. This increased risk, however, was mostly the result of spontaneous abortions observed in laboratory workers, but not hospital staff and, more importantly, was seen only in those studies which relied solely on self-reporting of exposure, indicating a potential recall bias. Examining only those studies where evaluation of work tasks determined exposure, there was no increased risk (mRR 0.7, 95% c.i. 0.5 - 1.0). The authors also report evidence of publication bias as the increased risk was limited to small studies (the large studies showed no increased risk). This suggests that other small studies that found no increased risk may not have been published. Combining the human and animal data, these authors concluded that it was unlikely that Formaldehyde exposure in the workplace would increase the risk of reproductive or developmental toxicity in workers.
Case Reports

Lachapelle et al. (1988) reported ring-shaped allergic patch test reactions to allergens in liquid vehicles. Of 24 positive patch test reactions to Formaldehyde, 7 (29%) were ring-shaped. The authors speculated that a pressure or capillary effect could be the explanation.

de Groot and Gerkens (1989) reported contact urticaria from a chemical textile finish in a 55-year old man. A wheal and flare reaction in an open test was seen to the chemical finisher. The components were said to be, dicyandiamide, Formaldehyde, ammonium chloride, and ethylenediamine. The patient had very small weals in response to a 0.1% - 1% - 3% Formaldehyde open test, but no reaction to the other chemicals.

Charpin et al. (2000) reported a case of hypersensitivity to Formaldehyde in an intensive care nurse. The woman had worked in intensive care for 4 months and complained of sore throat. An inadvertant exposure to high concentrations of formol plus glutaraldehyde occurred and she complained of rhinitis, dry cough, chest tightness, and tracheal burning for 2 weeks. RAST to formol and ethylene dioxide were negative. Patch tests to Formaldehyde, cobalt, and nickel were ++, but glutaraldehyde was negative. Further exploration led to a Zymoplex® prescription she had been taking and Arthrodont toothpaste she had used. The toothpaste and the Zymoplex® capsule contained Formaldehyde.

Haikel et al. (2000) published a case report with the title: anaphylactic shock during endodontic treatment due to allergy to Formaldehyde in a root canal sealant. A 41-year old male was undergoing a root canal without local anesthesia (ouch!), followed by a root canal sealant. The dentist did not use latex gloves. Soon after completion of the procedure the patient complained of hyperthermia, general pruritis, and respiratory oppression. He lost consciousness 30 minutes after the procedure. Emergency services were succesful in reviving him. Patch tests to Formaldehyde were negative, but a prick test to one of the liquid components of the sealant was positive and it contained glycerine and Formaldehyde. Further investigation appeared to suggest that formol in the sealant powder was the culprit.

Kim et al. (2001) reported occupational asthma due to Formaldehyde in a 39-year old Korean-Chinese male who had worked in a factory that produced crease resistant trousers. Six months after beginning this work he developed episodic wheezing, shortness of breath, and chest tightness. He had
sought emergency care for severe attacks of dyspnea. Evaluation of the workplace revealed glyoxal containing Formaldehyde and other chemicals, including polyurethane. Formaldehyde levels were 0.06 ppm, but individual short-term exposure levels were 0.12 - 0.13 ppm. Inhalation challenge to Formaldehyde with a closed-circuit breathing apparatus produced no reaction to 0.1 or 0.2 ppm, but at 0.5 ppm a reduction in FEV₁ was seen. On the basis of these results (?) The patient was confirmed as having Formaldehyde-induced asthma. Formaldehyde-specific IgE antibodies were not detected in the patient. Prick and intradermal tests to Formaldehyde-human serum albumin conjugate were negative.

Thrasher and Kilburn (2001) in their review of embryotoxicity and teratogenicity of Formaldehyde mention 2 cases of human birth defects in Formaldehyde-contaminated homes. One case was anencephalic at 2.76 ppm. The other defect at 0.54 ppm was not characterized.

Tas et al. (2002) reported a case of IgE-mediated urticaria from Formaldehyde in a dental root canal compound. A 54-year old male patient developed generalized pruritis and disseminated urticaria on the chest and arms 15 minutes after dental root canal treatment. Emergency therapy led to a quick recovery. The patient's personal history revealed a similar reaction after a previous root canal that used a compound containing guajicol and Formaldehyde. Formaldehyde from the standard series was negative in patch testing. Prick and scratch tests using 1% aqueous Formaldehyde were positive. Formaldehyde-specific IgE was 5 kU/l. Formaldehyde from the root canal compound (not clear how this was obtained) was positive in prick and patch testing.

REVIEW ARTICLES/RISK ASSESSMENTS

Review articles have appeared in the literature regularly since the original CIR safety assessment was published. Obviously, the most recent of these will contain the most information, but it may also be useful to see the progression over the past ~20 years.

Berstein et al. (1984) presented an overview of the toxicology, epidemiology, monitoring, and control of Formaldehyde inhalation exposure. The authors noted an absence of definitive epidemiologic studies (definitive epidemiology is probably an oxymoron). That absence of information aside, Formaldehyde was described as an immunologically mediated skin sensitizer, and as an agent that can cause or exacerbate respiratory distress in individuals with pre-existing bronchial hyperreactivity.
Formaldehyde is an effective alkylating agent and is mutagenic in several assays and carcinogenic (squamous cell carcinomas) in the nasal cavity of the rat at levels less than 10x lower than currently identified occupational and nonoccupational environments. They noted that Formaldehyde likely has acute irritant effects at exposure levels below the then established occupational safety limit of 3 ppm.

Solomons and Cochrane (1984) reviewed the acute and chronic effects of Formaldehyde on health. They concluded that the acute effects were well understood to include effects on the eyes, upper and lower respiratory tract, skin, and central nervous system, all of which stem from the recognition of Formaldehyde as a primary irritant with a clear dose-response. Sensitization as a mechanism of action was mentioned as a competing view. The authors stated that the question of Formaldehyde-induced chronic pulmonary disease is unresolved, but that it is not embryotoxic or teratogenic. While positive mutagenicity studies were available, the authors cited the International Agency for Research on Cancer conclusion that data were not available to assess the genotoxic effect on humans. These authors were not comfortable that the methodological deficiencies in animal carcinogenicity studies could be overcome and a conclusion reached, but combined with the mutagenicity data, were suggestive of a cancer risk. And they were even more skeptical that the human carcinogenicity data was useful.

Hooper and Gold (1986) ranked the carcinogenic hazards of occupational exposures using exposure-potency index values for nine industrial chemicals, including Formaldehyde. The exposure-potency index is the ratio of the dose level to which workers are permitted to be exposed (effective dose in mg/kg day\(^{-1}\)) to the cancer-causing dose (in mg/kg day\(^{-1}\)) in animals. The ratio for Formaldehyde was 28 when the national permissible exposure level was used and 17 when the California permissible level was used in the calculation of the ratio. For comparison purposes, Ethylene Oxide had a ratio of 0.8, indicating that the permissible exposure level and the level at which it is carcinogenic in animals are very close to equal.

Purchase and Paddle (1989) addressed the question: does Formaldehyde cause nasopharyngeal cancer in man? They review the animal data in support of Formaldehyde-induced nasal cancer and conclude there is ample demonstration that high concentrations of Formaldehyde cause irreversible damage to the nasal epithelium that, in some cases, leads to cancer. Regarding mechanism of action,
they conclude that the amount of Formaldehyde reaching a tissue is likely to be the most important determinant of the response of that tissue. They concluded that there was a lack of consistency in the findings in cohort and case-control human studies. Sinonasal and nasopharyngeal cancer appear to be the endpoints in question, with pharyngeal cancer a possible endpoint. The authors stated that, if man had the same sensitivity to Formaldehyde as the rat, stronger evidence of cancer should have been seen in the epidemiology studies conducted to date. This led them to conclude that man is less susceptible to Formaldehyde and that, at low occupational and domestic exposures, the risk of cancer is so low as to be undetectable.

Feron et al. (1991) considered the occurrence, carcinogenic potential, mechanism of action and risk assessment of aldehydes, including Formaldehyde. They concluded that the evidence supports Formaldehyde as a weak mutagen with an effect primarily at cytotoxic doses. They also found clear evidence that Formaldehyde is carcinogenic based on inhalation and oral studies. They dismissed a, then recent, study reporting gastrointestinal and hemolymphoreticular tumors because of the inadequacy of the report. The authors’ assessment of the risk posed by Formaldehyde was predicated on the need for recurrent tissue damage and repair, accompanied by exposure to high, cytotoxic concentrations in order to induce nasal cancers (citing their own work). If the nasal tissue is not sufficiently injured, exposure to non-cytotoxic levels of Formaldehyde was presumed to present a negligible cancer risk. They recommended that Formaldehyde exposures be minimized to prevent exposure to high, cytotoxic concentrations.

Restani and Galli (1991) reviewed the oral toxicity of Formaldehyde and its derivatives. They note that ingested Formaldehyde is rapidly taken up, metabolized to formic acid, and excreted. They reviewed acute, short-term and chronic exposure studies and focused on the question of carcinogenesis. They expressed the view the Formaldehyde is an animal carcinogen and speculated that cross-links with DNA, RNA, and protein could be the mechanism of action. They stated that ingestion represents a minor route of Formaldehyde exposure because of the dilution factor and that Formaldehyde binds to macromolecules present in the food. Considering that Formaldehyde is a normal metabolite in mammals and assuming a threshold of toxicity, these authors applied a safety factor of 100 and derived an acceptable daily intake of 0.1 - 0.2 mg/kg.
Holcátová and Bencko (1997) reported the Czech and Slovak experience with the allergic effects of Formaldehyde in the indoor environment. They cite a threshold for contact dermatitis between 30 and 55 ppm Formaldehyde. They conclude that, despite problems with interpreting genotoxic and immunologic findings in children exposed to Formaldehyde, the trend in the Czech Republic likely will be a lowering of the occupational maximum allowable concentration which is currently at 0.5 mg m\(^{-3}\) for 8 hours and 1.0 mg m\(^{-3}\) for 30 minutes. In favor of this trend, the state, is the smell threshold for Formaldehyde that is below 0.1 mg m\(^{-3}\) in not a negligible portion of the population.

Paustenbach et al. (1997) recommended an occupational exposure limit for Formaldehyde based on irritation based on the findings of the Industrial Health Foundation panel. The panel concluded that eye irritation to Formaldehyde does not occur until at least 1 ppm, and ear, nose and throat irritation does not occur for most persons until 2 - 3 ppm. The no-effect level for ocular irritation was said to be 0.3 ppm. They set this value as the occupational exposure limit (0.3 ppm as an 8-h time weighted average) and set 1 ppm as the concentration not to be exceeded to avoid irritation. They criticized the then current ACGIH TLV of 0.3 ppm as a ceiling value that was too restrictive. The panel concluded that indoor environments (where 24 h/day exposures could occur) kept below 0.1 ppm would prevent irritation in virtually everyone. The panel concluded that there was no group of individuals who were hypersensitive, nor was there evidence of sensitization following inhalation. The panel also found sufficient evidence to suggest that asthmatics react no differently to Formaldehyde exposure at levels under 3 ppm. The panel agreed with other groups who had concluded that the human cancer risk from Formaldehyde was negligible.

Feron et al. (2001) reviewed the health risks associated with inhaled nasal toxicants, including Formaldehyde. This review focuses more on the study of nasal toxicants than on the specific effects of individual chemicals. Consistent with the thinking in the previous review article from this group (Feron et al., 1991), they argue that Formaldehyde is only weakly genotoxic and the effects of cell proliferation or inflammation would dominate at high exposures and the weakly genotoxic effect would predominate at low exposures. Consequently, the risk for humans for nasal cancer is negligible for Formaldehyde exposures that do not cause nasal lesions. They agree with the Chemical Industry Institute of Technology finding that exposures to the clearly noncytotoxic level of 0.1 ppm Formaldehyde would result in an increased lifetime
risk in nonsmokers of $4.1 \times 10^{-9}$.

Bender (2002) considered the use of noncancer endpoints as a basis for establishing a reference concentration for Formaldehyde. A reference concentration is an estimate of daily exposure to humans that is likely to be without appreciable risk of deleterious effects during a lifetime. He concluded that inhalation chamber studies support a conclusion that some individuals begin to sense eye, nose, or throat irritation at 0.5 ppm and that 5 - 20% report eye irritation at 0.5 to 1 ppm. Mild, reversible pulmonary function deficits can occur in sensitized individuals at levels approaching 2 ppm. Nonetheless, the author concluded that a specific no observable adverse effect level or lowest observable adverse effect level for Formaldehyde could be established. Ranges used to estimate exposure levels that cause sensory irritation are the best that can be done, and although safety factors may be applied to account for sensitive subpopulations, human sensory irritation does not provide a strong basis for a reference concentration for Formaldehyde.
REFERENCES


A safety assessment of Formaldehyde was published in 1984 (Elder 1984) with the conclusion that this ingredient is safe in cosmetic products to the great majority of consumers, but, because of skin sensitivity of some individuals to this agent, the formulation and manufacture of a cosmetic product should be such as to ensure use at the minimal effective concentration of formaldehyde, not to exceed 0.2 percent measured as free formaldehyde. The CIR Expert Panel also noted in this earlier safety assessment that it cannot be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized. An extensive number of new studies, along with updated information regarding types and concentrations of use, were considered by the CIR Expert Panel. The Panel determined to not reopen this safety assessment.

Data reported to the FDA by industry in 1981 indicated that Formaldehyde was used in a total of 805 cosmetic products, but that figure decreased to 120 reported uses in 2002. The maximum use concentration reported to FDA in 1981 was in the >5 - 10% range. Data from an industry use concentration survey in 2003 indicate a maximum use concentration of 0.08%. Table 9 presents the recent and historical frequency of use and concentration of use data as a function of product category.

The discussion section in the original safety assessment acknowledged that Formaldehyde can be a skin irritant and sensitizer in clinical tests, and a developmental toxin, a genotoxin, and a neoplastic agent in experimental animal studies. The new clinical studies 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 was also confirmed in the new studies. These studies demonstrate that there is a threshold effect; that is, high doses are required before any effect is seen. Again, the limit on the amount of free Formaldehyde established by the CIR Expert Panel precludes any risk as a result of use of cosmetic products containing Formaldehyde.
Table 1. Frequency of use and concentration of use of Formaldehyde in cosmetic products.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>Number of products with Formaldehyde (total products in category)</td>
<td>Concentration of use</td>
</tr>
<tr>
<td><strong>BABY PRODUCTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby shampoos</td>
<td>7 (35)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Baby lotions, oils, powders and creams</td>
<td>1 (56)</td>
<td>&gt;0.1 - 1%</td>
</tr>
<tr>
<td><strong>BATH PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bath oils, tablets and salts</td>
<td>10 (237)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Bubble baths</td>
<td>109 (475)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Other bath preparations</td>
<td>24 (132)</td>
<td>≤0.1 - 5%</td>
</tr>
<tr>
<td><strong>EYE MAKEUP PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mascara</td>
<td>1 (397)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Other eye makeup preparations</td>
<td>3 (230)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td><strong>FRAGRANCE PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sachets</td>
<td>2 (119)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Other fragrance preparations</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>NON-COLORING HAIR PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair conditioners</td>
<td>95 (478)</td>
<td>≤0.1 - 5%</td>
</tr>
<tr>
<td>Permanent waves</td>
<td>11 (474)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Rinses (non-coloring)</td>
<td>32 (158)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Shampoos (non-coloring)</td>
<td>316 (909)</td>
<td>≤0.1 - 5%</td>
</tr>
<tr>
<td>Tonics, dressings, and other hair grooming aids</td>
<td>21 (290)</td>
<td>≤0.1 - 10%</td>
</tr>
<tr>
<td>Wave sets</td>
<td>37 (180)</td>
<td>≤0.1 - 10%</td>
</tr>
<tr>
<td>Other hair preparations</td>
<td>13 (177)</td>
<td>≤0.1 - 5%</td>
</tr>
<tr>
<td><strong>HAIR COLORING PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair dyes and colors (with caution statement)</td>
<td>5 (811)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Shampoos (coloring)</td>
<td>3 (16)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td><strong>MAKEUP PREPARATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face powders</td>
<td>1 (555)</td>
<td>&gt;0.1 - 1%</td>
</tr>
<tr>
<td>Foundations</td>
<td>2 (740)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Leg and body paints</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Makeup bases</td>
<td>3 (831)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>Other makeup preparations</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>NAIL CARE PRODUCTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle softeners</td>
<td>1 (32)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Nail creams and lotions</td>
<td>1 (25)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Other manicuring preparations</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>ORAL HYGIENE PRODUCTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dentifrices</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mouthwashes and breath fresheners</td>
<td>2 (53)</td>
<td>≤0.1 - 1%</td>
</tr>
<tr>
<td>--------------------------------------</td>
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</tr>
<tr>
<td></td>
<td>Number of products with Formaldehyde (total products in category)</td>
<td>Concentration of use</td>
</tr>
<tr>
<td>PERSONAL CLEANLINESS PRODUCTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bath soaps and detergents</td>
<td>5 (148)</td>
<td>0.1 - 1%</td>
</tr>
<tr>
<td>Deodorants</td>
<td>7 (239)</td>
<td>&gt;0.1 - 1%</td>
</tr>
<tr>
<td>Feminine hygiene deodorants</td>
<td>1 (21)</td>
<td>&gt;1 - 5%</td>
</tr>
<tr>
<td>Other personal cleanliness products</td>
<td>1 (227)</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>SHAVING PREPARATIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aftershave lotions</td>
<td>1 (282)</td>
<td>&gt;0.1 - 1%</td>
</tr>
<tr>
<td>Shaving creams</td>
<td>2 (114)</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>Other shaving preparations</td>
<td>1 (29)</td>
<td>&gt;1 - 5%</td>
</tr>
<tr>
<td>SKIN CARE PREPARATIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin cleansing creams, lotions, liquids, and pads</td>
<td>13 (680)</td>
<td>0.1 - 1%</td>
</tr>
<tr>
<td>Face and neck skin care preparations</td>
<td>47 (832)*</td>
<td>0.1 - 1%</td>
</tr>
<tr>
<td>Body and hand skin care preparations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot powders and sprays</td>
<td>1 (17)</td>
<td>&gt;0.1 - 1%</td>
</tr>
<tr>
<td>Skin moisturizers</td>
<td>11 (747)</td>
<td>0.1 - 1%</td>
</tr>
<tr>
<td>Night skin care preparations</td>
<td>5 (219)</td>
<td>0.1 - 1%</td>
</tr>
<tr>
<td>Paste masks (mud packs)</td>
<td>3 (171)</td>
<td>0.1 - 1%</td>
</tr>
<tr>
<td>Skin fresheners</td>
<td>1 (260)</td>
<td>&gt;0.1 - 1%</td>
</tr>
<tr>
<td>Other skin care preparations</td>
<td>4 (349)</td>
<td>&gt;0.1 - 1%</td>
</tr>
<tr>
<td>SUNTAN PREPARATIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suntan gels, creams and liquids</td>
<td>2 (164)</td>
<td>0.1 - 1%</td>
</tr>
</tbody>
</table>

*product sold only in Europe and no longer marketed
REFERENCES


Ushio, H., K. Nohara, and H. Fujimaki. 1999. Effect of environmental pollutants on the production of pro-


HUMAN UMBILICAL EXTRACT
Memorandum

To: CIR Expert Panel

From: Director, CIR

Subject: Human Umbilical Extract – new data

Date: November 18, 2010

In 2002, CIR published its final safety assessment of ingredients derived from human and animal placentas and umbilical cords. These ingredients included Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract.

The available data were considered insufficient to support safety. Even while announcing this decision, the CIR Expert Panel did alert the industry that none of these ingredients as used in cosmetics should deliver any metabolic/endocrine activity and that any current or future use should be free of detectable pathogenic viruses or infectious agents.

Several of these insufficient data ingredients, including Human Umbilical Extract, have been placed on the “Insufficient Data – No current uses” list with the expectation that if there were to be usage in the future, sufficient data to support safety would be provided.

We have received correspondence from Cosmetikare Laboratories, LLC indicating that they intend to use Human Umbilical Extract in cosmetic products. They have provided a number of pieces of information to support the safety of this ingredient.

In the attached table, we have listed the data needs that led to the original insufficient data finding (along with the two additional caveats adopted by the Panel), and, where possible, have matched the pieces of data provided by the company.

Do these additional data provide a basis for reopening this safety assessment to change the conclusion for Human Umbilical Extract?

An element of potential confusion exists in that the INCI name “Human Umbilical Extract” (which has the circular definition of “extract from human umbilical cord”) appears to be synonymous with “umbilical cord blood serum.” Cord blood would appear to be commonly understood to come from the umbilical cord and serum is clearly a derivative of blood, but the question could be asked: Is blood serum what is usually considered under the single word: “extract?” It may be that this ingredient is not actually Human Umbilical Extract, but the circular definition in the International Cosmetic Ingredient Dictionary and Handbook offers little help in resolving the question.
Final Report on the Safety Assessment of Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract

Various proteins, lipids, or other extracts from human or other animal placentas are described as cosmetic ingredients. Human Placental Protein comprises protein derived from human placentas. Placental Protein is derived from animal placentas. Similarly, Human Placental Lipids and Placental Lipids are the lipid fractions from the same source materials. Hydrolyzed Human Placental Protein and Hydrolyzed Placental Protein are produced from the respective protein extracts by acid, enzyme, or other hydrolysis methods. Human Placental Enzymes and Placental Enzymes are enzymes obtained by aqueous extraction of human or other animal placental material. Human Umbilical Extract and Umbilical Extract are unspecified extracts of material from human or other animal umbilical cords. Different materials called Human Placental Extracts and Placental Extracts, assumed to contain estrogens or other biologically active substances, are not recognized as cosmetic ingredients, even though the use of these ingredients in cosmetics have been reported to the Food and Drug Administration (FDA). Human-derived ingredients are prohibited from use under the provisions of the European Union cosmetics directive based on concerns about transmission of human spongiform encephalopathies and viral diseases, for example, human immunodeficiency virus (HIV). Umbilical Extract has precedent for unrestricted use in Japan, except for certain products. Most of these ingredients are described as hair-conditioning agents and miscellaneous skin-conditioning agents, although the umbilical extracts function as biological additives in cosmetics. Of the human-derived ingredients, only Human Placental Protein is currently reported to be used. Animal-derived placental proteins, hydrolyzed proteins, lipids, and enzymes were all currently reported to be used. No current uses of the umbilical extracts were reported. Most of the available data relates to placental derivatives that appear to have estrogenic or other biological activity. The one clinical study that appears to utilize proteinaceous material is related.

Received 14 December 2001; accepted 6 March 2002.

1Reviewed by the Cosmetic Ingredient Review Expert Panel. Bindu Nair and Amy R. Elmore, former Cosmetic Ingredient Review staff members, prepared this report. Address correspondence to F. Alan Andresen, Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

INTRODUCTION

This report is a compilation of data concerning Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract. Ingredients designated "human," for example Human Placental Protein, are derived from human sources. Ingredients not designated "human," for example Placental Protein, are derived from bovine and other animal sources.

The “Cosmetic Product-Related Regulatory Requirements and Health Hazard Issues” section of FDA’s Cosmetic Handbook has stated the following regarding placental-derived ingredients (both human and animal):

Products containing Estrogenic Hormones, Placental Extract or Vitamins

In addition to being considered misbranded drugs, products claiming to contain placental extract may also be deemed to be misbranded...
cosmetics if the extract has been prepared from placentas from which the hormones and other biologically active substances have been removed and the extracted substance consists principally of protein. The FDA recommends that this substance be identified by a name other than “placental extract” which describes its composition more accurately because consumers associate the name “placental extract” with a therapeutic use or some biological activity (FDA 1994).

Human placental extract and placental extract are not recognized as cosmetic ingredients in the Cosmetic, Toiletry, and Fragrance Association (CTFA) International Cosmetic Ingredient Dictionary and Handbook (Wenninger and McEwen 1997). However, in January 1998 “human placental extract” and “placental extract” were reported to be used in 14 and 31 cosmetic formulations, respectively (see Use section).

The Cosmetic Ingredient Review (CIR) Expert Panel expects that cosmetic grade Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract do not contain hormones or other biologically active components. Similarly, the Panel expects that ingredients identified as human placental “extract” or placental “extract” are also free of biological activity.

The published literature contains numerous articles concerning human placental extract. Three preparation techniques are noted and are detailed in the Chemistry—Method of Production section of this report. Because limited information was found regarding the cosmetic ingredients, articles concerning presumed biologically active human placental extract and placental extract are contained in this report. How the composition of these tested extracts compares to those reported to be used in cosmetic formulations is not known.

CHEMISTRY

Definition

No definitions were found for cosmetic-grade human placental extract or placental extract.

Human Placental Protein

This ingredient is the protein derived from human placenta obtained from normal afterbirth (Wenninger and McEwen 1997). Younoszai and Haworth (1969) reported that term placentas are comprised of 12.0% ± 0.12% protein by wet weight, or 78.5% ± 0.85% by dry weight.

Hydrolyzed Human Placental Protein

This ingredient is the hydrolysate of Human Placental Protein (q.v.) derived by acid, enzyme, or other method of hydrolysis (Wenninger and McEwen 1997). The CAS number 73049-73-7 refers to several hydrolyzed proteins in the CTFA International Cosmetic Ingredient Handbook. Further, the published literature identifies the CAS number as that of tryptone, which is defined as “a peptone produced by proteolytic digestion with trypsin” (Taylor 1988). Because it is not specific to Hydrolyzed Human Placental Protein, this CAS number was not used to obtain published articles.

Human Placental Enzymes

These ingredients are the enzymes derived from human placentas obtained from normal afterbirth (Wenninger and McEwen 1997).

Human Placental Lipids

These ingredients are the lipids derived from human placentas obtained from normal afterbirth (Wenninger and McEwen 1997).

Younoszai and Haworth (1969) reported that term placentas are comprised of ~0.4% lipids by wet weight, or ~2.9% by dry weight. The English abstract of a French article described the lipid content of blood-free placentas to have the following composition: 62% phospholipids, 13% to 18% free fatty acids, and 16% to 18% nonesterified cholesterol. Arachidonic acid accounted for 19.5% of total fatty acids (free and esterified). The 62% phospholipid content was itself composed of 40% diacyl phosphatidylcholine, 25% sphingomyelin, 10% ethanolamine plasmalogen, 7% diacyl phosphatidylethanolamine, 4% phosphatidylserine, 3% phosphatidylinositol, and 9% lysophospholipids. The investigators reported that the industrial process used to extract blood from the placenta did not induce either fatty acid oxidation or phospholipid hydrolysis (Chirouze, Entressangeles, and Helme 1987).

Human Umbilical Extract

This ingredient is an extract of human umbilical cord (Wenninger and McEwen 1997).

Placental Protein

Placental Protein is a mixture of proteins derived from animal placentas (Wenninger and McEwen 1997).

Hydrolyzed Placental Protein

This ingredient is the hydrolysate of Placental Protein (q.v.) derived by acid, enzyme, or other method of hydrolysis (Wenninger and McEwen 1997). Like its human-derived counterpart, Hydrolyzed Placental Protein is also identified by the CAS number 73049-73-7.

Placental Enzymes

Placental Enzymes is a mixture of enzymes obtained from an aqueous extraction of animal placentas (Wenninger and McEwen 1997).

Placental Lipids

Placental Lipids is a mixture of lipids derived from animal placentas (Wenninger and McEwen 1997).

Umbilical Extract

Umbilical Extract is an extract of animal umbilical cords (Wenninger and McEwen 1997).
Method of Preparation

**Human Placental Extract**

Two placental extracts (one human the other bovine) were described as used in "dermocosmetology." These extracts were obtained using the Filatov technique (not explained) under conditions "favoring the development of biogenic stimulants." The extracts were purified by filtration and were sterilized by autoclaving at 120°C. They were supplied as pale yellow liquids with a characteristic odor (CTFA 1998).

Several studies cited in this report tested human placental extract that was prepared in one of two ways. Fresh human placentas from normal deliveries were collected, washed to remove blood, homogenized with buffer, filtered until clear, and the preservative, benzyl alcohol added. Each milliliter of extract was derived from 0.1 g of fresh human placenta. Known constituents of the extract include human placental lactogen (HPL), corticotropin-releasing factor (CRF), fibrin-stabilizing factor (FSF), and lactoferrin (Banerjee, Bishayee, and Chatterjee 1993). Studies cited in this report that used this extraction method identified the human placental extract as HPE.

Other studies tested a human placental extract fraction (EAP). Placentas were collected at delivery and immediately frozen at −20°C. Pools of 500 to 600 placentas were mechanically ground, and then stirred until thawed in an 8% (v/v) ethanol/water solution. Placental blood was separated from the tissue by means of a press. The tissue pulp was extracted with acid and the extract was neutralized and precipitated with 15% ethanol. The supernatant was recovered by centrifugation, concentrated by ultrafiltration, and dialyzed against 0.9% NaCl solution with 10,000 Da cut-off membranes. One liter of the fraction corresponded to ~21 kg of placental tissue pulp (Klein, Chiodino, and Yamasaki 1991).

Contaminants

**Human Placental Extract**

One source reported that two Filatov-type placental extracts (human and bovine) used for "dermocosmetology" were devoid of estrogenic activity. The extracts were subcutaneously administered (20 ml/kg) to 11 female Sprague-Dawley rats (3 weeks old). Five hours after dosing, the rats were killed and the uterus was removed, weighed, dehydrated, and reweighed. The difference between fresh and dry weight was used to calculate the water content of the uterus. An increase in this parameter was evidence of estrogenic activity. No significant increase was noted compared to nontreated controls (CTFA 1998).

Beyssac, Martini, and Cotte (1986) detected estriol at a calculated maximum of 100 µg/l in various human placental extract preparations that were defined as "used in the cosmetics industry." In addition, a survey concerning use of hormone/placenta-containing hair preparations by children measured an estriol content of 1.9% (w/w) in one placenta-containing hair preparation (not distinguished as human or animal in origin). The investigator of the survey suggested that use of these products by children could cause sexual maturation at an earlier age (Tiwary 1997).

**USE**

**Cosmetic**

The human and animal placental-derived protein, hydrolyzed protein, enzyme, and lipid ingredients all function in cosmetic formulations as hair-conditioning agents and skin-conditioning agents—miscellaneous. Human Umbilical Extract and Umbilical Extract are used as biological additives (Wenninger and McEwen 1997).

As of January 1998, Human Placental Protein was reported used in 30 cosmetic formulations. Two uses of "human placental extract, liquid" and 12 uses of "human placental extract, lyophilized" were reported. Placental Protein (identified as animal or bovine) was used in five formulations; Hydrolyzed Placental Protein was used in seven formulations; Placental Enzymes was used in seven formulations; and Placental Lipo, bovine was used in one formulation. "Placental extract" was used in 31 formulations (FDA 1998) (Table 1). Where available, current concentration of use data (CTFA 1999) are also shown in Table 1. Historical concentration of use data are also given in Table 1.

One source recommended use of human and bovine placental extract at concentrations between 5% and 20% (CTFA 1998).


> Whereas cells, tissues or products of human origin are liable to transmit the Creutzfeldt-Jakob disease, human spongiform encephalopathy, and certain virus diseases; whereas it is therefore necessary, given the current state of scientific knowledge, to prohibit their use in cosmetic products.

Umbilical Extract, identified as umbilical cord extract, is listed in the Japanese Comprehensive Licensing Standards of Cosmetics by Category (CLS) (Rempe and Santucci 1997). Umbilical Extract, which conforms to the specification of the Japanese Cosmetic Ingredients Codex (JCIC), has precedent for use without restriction in various CLS categories except eyeliner, lip, oral, or bath preparations for which there are no precedent for use.

**GENERAL BIOLOGY**

**Anti-Inflammatory Activity**

**Human Placental Extract** (presumed active, see Introduction)

Banerjee et al. (1990) reported a reduction in carrageenin-induced inflammation in rats that had been given an
### TABLE 1
Frequency and concentration of use (FDA 1998)

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<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td><strong>Human Placental Protein</strong></td>
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</tr>
<tr>
<td>Hair conditioners (636)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shampoos—noncoloring (860)</td>
<td>5</td>
<td></td>
<td></td>
</tr>
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<td>Tonics, dressings, and other hair-grooming aids (549)</td>
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<td>Night skin care (188)</td>
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<td><strong>1998 total for Placenta Enzymes</strong></td>
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(Continued on next page)
TABLE 1
Frequency and concentration of use (FDA 1998) (Continued)

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<td>0%–0.1%</td>
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<td>Tonics, dressings, and other hair-grooming aids (549)</td>
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<td>Hair shampoo—coloring (24)</td>
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<tr>
<td>Moisturizing (769)</td>
<td>11</td>
<td>0%–5%</td>
<td>—</td>
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<td>Night skin care (188)</td>
<td>2</td>
<td>0.1%–5%</td>
<td>—</td>
</tr>
<tr>
<td>Paste masks—mud packs (255)</td>
<td>1</td>
<td>1%–5%</td>
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</tr>
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<td>Other skin care preparations (692)</td>
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<td>1998 total for Placental Extract</td>
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*Ingredient used in liquid form in these product types.

Intraperitoneal (IP) dose of HPE. Maximal suppression was noted with a dose of 0.3 ml/100 g body weight, given 1/2 hour before or after carrageenan administration.

A dose-dependent inhibition of increased hepatic succinic dehydrogenase (SDH) activity (in response to carrageenan-induced edema) was noted in rats that had been pretreated with a subcutaneous (SC) dose of 1 to 5 ml/kg of HPE. The extract had little or no effect on the hepatic SDH activity of normal rats (Banerjee et al. 1994b).

A study that investigated the biochemical mechanism for the anti-inflammatory action reported a significant reduction in glucose-6-phosphate dehydrogenase activity in the liver, kidneys, and brain of rats following IP administration of a commercial HPE (0.4 ml/100 g body weight). The investigators cautioned that this enzyme was key in the production of NADPH and that inhibition can result in decreased amounts of reduced glutathione that was involved in free-radical scavenging. Enzyme inhibition also could alter steroid synthesis (Banerjee et al. 1992).

Clinical/Therapeutic Application

Vitiligo—Human Placental Extract (presumed active, see Introduction)

Several studies have reported the use of HPE in treatment of vitiligo (skin disorder marked by loss of pigmentation). "Remarkable improvement" in 20.6% and "moderate improvement" in 50% of 34 patients with vitiligo was noted after topical treatment (Sharma et al. 1988). Another study tested the repigmentation claims of a commercially available topical HPE. Following manufacturer’s instructions, the extract was applied to affected areas three times a day, and the treated area was exposed to infrared light following the third application. Of 16 patients with vitiligo, 69% had no significant repigmentation, 19% had scattered repigmentation, and 12% had obvious repigmentation of some lesions. Five had complete repigmentation of some lesions within 3 to 16 months, and two (one a child, and the other a man who had been recently diagnosed) had an "obvious reduction in vitiliginous areas" (Suite and Quamina 1991).

Pal et al. (1995) conducted a guinea pig study to determine the chemical agent responsible for repigmentation. Human placentas were chopped, blended, extracted with ethanol, and then filtered. The 60% hydroalcoholic extract was topically applied around the nipples covering the areola zones of immature male white guinea pigs daily for 60 days followed by 15 minutes of infrared exposure. Clear pigmentation and hypertrophy was noted to varying degrees. The extract was chemically analyzed and glycosphingolipids, known modulators of B and T cells, were considered to induce melanocytes resulting in skin pigmentation.

Chorioretinal Dystrophy—Human Placental Extract (presumed active, see Introduction)

Thirty-four panelists with chorioretinal dystrophy (myopic or senile) of different degrees of anatomofunctional alteration received daily intramuscular doses of 3 ml human placental extract (equivalent to 1.80 g of fresh organ) for 20 days. The preparation method of the extract was not reported. Varying improvement was noted in visual acuity, the luminous sense, the visual field and the electrophysiological activity of the retina.
The investigators considered the “efficacy” of the extract was due to the “high proteic value, to polypeptide with a low molecular weight and to free amino-acids, particularly alanine, leucine, lysine, (and) valine” (Girotto and Malvinerni 1982).

**Neurological Activity**

*Human Placental Extract* (presumed active, see Introduction)

In response to claims that HPE increased the grasping, learning, and retention capacity of children of slow-learners, Banerjee, Bishayee, and Chatterjee (1995) investigated the effect of the extract on rat monoaminergic neurotransmitters and brain monoamine oxidase (MAO) activity. Subchronic IP administration (once daily for 5, 10, 15, or 20 days) of human placental extract (2 to 4 ml/kg/day) increased brain concentration of monoamines and decreased MAO activity in rats.

**Cellular Effects**

*Human Placental Extract* (presumed active, see Introduction)

Ikawa, Aida, and Saito (1975) reported that addition of 2% to 3% of a commercial human placental extract preparation to the clonal culture lines of Friend leukemia cells resulted in hemoglobin production after 4 to 6 days.

O’Keefe and Chiu (1988) reported that incubation with placental extract (100 to 200 μg) resulted in a 50-fold increase of thymidine incorporation by keratinocytes.

Kimoto et al. (1987) reported that a placental extract with demonstrated antimutagenic activity in the Ames assay “greatly diminished” Adriamycin-mediated toxicity. The placental extract was prepared by homogenization of full-term human placentas, incubation with pronase, centrifugation, lyophilizing the supernatant, dissolving the resulting powder, and eluting fractions via a Sephadex column. Fractions were tested in the Ames assay. The fraction demonstrating antimutagenicity was used in in vitro studies with Adriamycin. These in vitro studies noted that the fraction diminished superoxide production by Adriamycin-incubated liver microsomes and reduced the effects of aeration on Adriamycin semiquinone radical generation.

**ANIMAL TOXICOLOGY**

**Short-Term Oral Toxicity**

*Dried Human Placenta* (presumed active, see Introduction)

A group of 10 male weanling rats were fed a diet in which the 10% protein allotment consisted of human placenta that had been dried and powdered. The control group was fed casein. Diets also had 15% fat and were complete with regard to vitamins and minerals. Rats were killed after either 4 or 8 weeks of feeding, and the liver and testes were removed. No changes were noted in the testes. Fatty changes in the liver of placenta-fed rats was noted at microscopic examination. The lesions consisted of diffuse cytoplasmic vacuolation of hepatocytes; the lesion was severe in cells of the periportal and adjacent mid-zones and mild in cells of the central zone. A “striking amount of stainable lipids” was noted when stained with oil red 0. The lesions were similar in rats killed at either 4 or 8 weeks of feeding. Mild-to-moderate vacuolation was also noted in controls rats, but little-to-no stainable fat was detected (Banji and Krishnamurthi 1970).

In order to elucidate the cause of the lesions, a second study was conducted in which six male rats were fed a 20% placenta-protein diet. The diet contained 0.4 g cholesterol, 0.02 mg estradiol, and 0.5 mg progesterone/100 g diet and controls were fed a casein diet containing a comparable amount of one or more of these hormones. Animals were killed after 4 weeks, and the liver and testes were removed for microscopic examination. The liver was also analyzed for lipids and proteins. No changes were noted in the testes. Livers of test animals were mottled in appearance with no significant change in weight. Total lipids, triglycerides, and total cholesterol concentrations were markedly greater in the liver of rats fed the placenta diet compared to the control group. No changes in phospholipid and protein concentration were noted. Similar changes in hepatic lipids were noted in rats that were fed casein supplemented with cholesterol, but not in those that were fed casein supplemented with estrogen and/or progesterone. At microscopic examination, the livers of rats fed the placenta diet and those fed casein supplemented with cholesterol (with or without hormones) had mild cytoplasmic vacuolation of cells of the periportal and adjacent midzone lobules. In oil red 0–stained sections mild accumulation of fat as multiple small and fine round globules was observed in the cytoplasm without nuclear displacement (Banji and Krishnamurthi 1970).

In an earlier study, 20 rats were fed ad libitum for 112 days 5.0% acetone-dried human placental powder or 0.30% Human Placental Lipids (identified as placental lipid extract). Hepatic malic oxidase (MO) activity (p < .05) and testicular oxygen uptake (p < .01) were significantly increased. Hepatic succinoxidase (SO) activity was comparable between treated and control rats. In the second part of the study, immature male rats were fed raw human placenta for 83 days. Significant increases in MO (p < .05) and SO (p < .01) activities, increased testicular oxygen uptake, and atrophy of the testes were noted. The atrophy was attributed to the sex hormones contained in the placenta, but the investigators did not consider hormones to have induced the increased oxygen uptake (Gershbein and Malik 1967).

**Parenteral Toxicity—Acute**

*Human Placental Protein*

Some published literature refers to human placental protein(s) that have hormone-like effects. These proteins are different from the cosmetic ingredient. Florini et al. (1966) reported a “human placental protein” that when injected into hypophysectomized rats and mice, had adabolic effects such as those noted after dosing with human growth hormone. The protein also reacted to antisera for human growth hormone.
Using the extraction technique of Florini et al. (1966), Riggi et al. (1966) reported that intramuscular (IM) administration of "purified human placental protein" into fasted rabbits (25 or 50 mg/kg), and monkeys (50 or 100 mg/kg) produced significant increases in plasma free fatty acid concentrations. Plasma lactescence associated with hypertriglyceridermia and hyperglycemia developed in rabbits following daily SC dosing with 75 mg/kg for 25 days. In mice, hepatic lipodosis developed after injections with 16 mg of Human Placental Protein daily for 7 days. All of the described effects were similar to those observed following porcine growth hormone administration.

Human Placental Extract (presumed active, see Introduction)

Banerjee, Bishayee, and Chatterjee (1993) reported that a single IP dose of HPE (4 ml/kg) to rats caused a significant enhancement of lipid peroxidation with a decline in both hepatic and blood glutathione (GSH) concentrations. A dose-dependent increase in glutathione S-transferase (GST) activity and dose-dependent inhibition of catalase, glutathione peroxidase, and glutathione reductase activities were noted. The extract was considered hepatotoxic because it increased serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, serum lactate dehydrogenase activities, and blood methemoglobin concentration. The magnitude of the increase of serum enzymes was "much less" than that induced by carbon tetrachloride.

In a subsequent study by Bishayee, Banerjee, and Chatterjee (1995), rats were given a single IP injection of HPE (4 ml/kg) and some were killed at 2, 6, 24, 45, 72, and 96 hours post treatment. Livers were removed, homogenized, and centrifuged, and the fractions were analyzed for cytochrome and enzymic activity. The vehicle control received 1.5% (v/v) Benzyl Alcohol in buffer. Maximal induction of hepatic microsomal cytochrome P-450 and cytochrome b$_5$ activities was noted beginning at 24 hours post dosing. Cytosolic GST activity was also significantly increased beginning at 48 hours post dosing. A reduction in microsomal UDP-glucuronosyltransferase activity was also observed. All activity returned to zero-time values 96 hours after treatment.

Parenteral Toxicity—Short-Term

Human Placental Extract (presumed active, see Introduction)

Dose-dependent increases in hepatic cytochrome parameters and GST activity were noted in rats following repeated IP dosing (30 days) with 1, 2, or 4 ml/kg HPE. The cytochrome changes were significant with the 2 ml/kg dose ($p<.05$); more pronounced increases were noted in the 4 ml/kg dose group where the change in cytochrome P-450 activity was 130% ($p<.01$) and the increase in cytochrome b$_5$ activity was 88% ($p<.05$) greater than the control. Microsomal NADPH cytochrome c reductase activity was not affected by either acute or repeated treatment. The investigators cautioned that human placental extract had "substantial ability to alter the patterns of drugs metabolizing enzyme systems in mammals," and that prolonged administration could induce some forms of hepatic neoplasms (Bishayee, Banerjee, and Chatterjee 1995).

Similar findings were reported in an earlier study in which HPE (1 to 4 ml/kg) was injected IP into rats for 15 days. Significant increases were noted in the activities of serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase, glutamic dehydrogenase, and sorbitol dehydrogenase. Activities of other enzymes were also increased. A marked depletion of cytochrome P-450 and reduction of hepatic glycogen and protein concentrations were noted with a concurrent rise in hepatic lipid peroxides. The investigators considered that the active components of the extract, 19-hydroxyprogesterone and corticotropin-releasing factor, were responsible for the alterations (Banerjee et al. 1994a).

Dermal Irritation

Human and Animal Placental Extract (presumed active, see Introduction)

Two Filatov-type placental extracts (human and bovine) used for "dermocosmetology" were each applied (0.5 ml) under gauze to rabbit skin. Both extracts were nonirritating. No further details were given (CTFA 1998).

Ocular Irritation

Human and Animal Placental Extract (presumed active, see Introduction)

Two Filatov-type placental extracts (human and bovine) used for "dermocosmetology" were each instilled (0.5 ml) into one conjunctival sac of six rabbits. The human placental extract was "very slightly irritating" and the bovine placental extract was nonirritating. No further details were given (CTFA 1998).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In Vitro

Animal Placental Extract (presumed active, see Introduction)

Mammalian embryonic development was studied by Huxham et al. (1982) using a postimplantation rat-embryo culture. Wistar rat conceptuses were explanted on day 9.5 and cultured for 2 days with homogenate preparations from normal rat placenta or decidua. Conceptuses were examined for heart beat, vitelline circulation, yolk-sac diameter, and the achievement of allantoic fusion with the ectoplacental cone. Abnormal embryos (neural-tube defects, severe reduction in embryonic size) were produced with 2.5 to 4 mg/ml of the placental homogenate and 1.2 to 4 mg/ml of the decidual homogenate. Abnormalities were not induced by either solutions of bovine serum albumin or protein preparations of rat lung tissue.
GENOTOXICITY

Human Umbilical Extract

Immunizing mice with an extract of human umbilical cord significantly decreased the number of micronuclei in bone marrow cells for 5 days. The extract was described as having no clastogenic property. The investigators hypothesized that the antimutagenic effect was related to interferon induction by the extract (Mkrtdchyan and Nersessian 1993).

PROTECTIVE ACTIVITY

Human Placental Extract

Klein et al. (1991) reported that in vitro studies, EAP inhibited growth of Ha-ras–transformed BALB/c 3Tc cells and human squamous lung carcinoma A-2182 cells. The fraction did not alter anchorage-dependent growth of these cells, but a slight mitogenic activity was noted in nontransformed cells. No significant cytotoxicity was noted. The fraction did contain transforming growth factor β, but the investigators did not consider that the growth factor was solely responsible for the observed growth suppression.

In a subsequent study, Klein, Chiudino, and Yamasaki (1993) reported that EAP suppressed growth of only the most highly tumorigenic cells in soft agar medium; growth of non– and low–tumorigenic counterparts was not affected or was stimulated, respectively, by the extract. Cells of both the colorectal and esophageal cell lines that had the greatest percentage of colonies in soft agar had their colony-forming efficiency decreased by the presence of 100 μg/ml EAP. In contrast, cells that did not give any colonies in soft agar did not grow in either the absence or presence of EAP. Growth of cells with an intermediate colony-forming efficiency was stimulated (by 150% in colorectal cells, and 200% in esophageal cells) in the presence of EAP. Similar findings were noted with murine BALB/c 3T3-1-1 cells that had been transfected or infected with various oncogenes. Further, “EAP did not significantly affect the doubling time of anchorage-dependent cell growth, suggesting that the extract specifically suppresses tumorigenic characteristics of cells such as their ability to grow in soft agar medium.” Transforming growth factor β was most effective on less tumorigenic cells.

Placental Extract (presumed active, see Introduction)

Mochizuki and Kada (1982) investigated the antimutagenic action of extracts prepared from the placentas of a human, monkey, dog, rat, and mouse. Placentas were washed with potassium chloride, homogenized without buffer, and centrifuged. The supernatant was treated with pronase followed by overnight dialysis in distilled water. Each solution was heated and loaded onto ion-exchange resin columns and eluted with water. Fractions were collected and evaporated under vacuum; the residue was dissolved in water and filtered using millipore filters. Bacteria (E. coli B/r WP2 trp+) were mutated by either radiation or incubation with MNNG and then combined with an extract and plated. The extracts were also tested alone and were not mutagenic. The number of mutant colonies induced by UV irradiation, γ-ray, and MNNG were “decreased markedly in the presence of the placental extracts without significant effects on survival.” The data were not analyzed for statistical significance.

Human Umbilical Extract

Vaccination of rats and mice with an extract of human umbilical cord resulted in a significant inhibition of growth, decreased tumor incidence, and partial resorption of ascitic fluid of transplantable tumors such as Ehrlich’s ascites tumor, sarcoma 37, and Zajdel’s hepatoma. The inhibition was not noted when sarcoma 180 was transplanted. Vaccination also interfered with dimethyl benzanthracene and benzo(a)pyrene-induced carcinogenesis by reducing tumor incidence and increasing the latent period and slowing cancer progression (Mkrtdchyan et al. 1990).

CLINICAL ASSESSMENT OF SAFETY

Patch Testing

Human Placental Protein

A patch testing reference book by DeGroot (1994) noted that the published literature does not contain recommended test concentrations for Human Placental Protein. As a guide to the clinician, DeGroot reported the findings of an unpublished (and at the time, ongoing) study by members of the Dutch Contact Dermatitis Group. No irritant reaction was noted in 1 to 20 patients (exact number tested with ingredient not specified) suffering from or suspected to suffer from cosmetic product contact allergy after being patch tested with 30% Human Placental Protein aqua.
Animal Placental Extract (presumed active, see Introduction)

von den Driesch et al. (1993) reported contact dermatitis of the hand in a cosmetician up to 3 hours after external application of calf placenta extracts. She had previously worked as a hairdresser but developed a delayed-type allergy to p-phenylenediamine. The placenta extract contained mesodermin, collagen, and hyaluronic acid. The cosmetician and 10 healthy volunteers were tested with the extract and the solvent via the prick and scratch-chamber method. The cosmetician reacted in both tests. In the scratch-chamber test, the dissolved extract produced an eczematous reaction at day 1 and the undissolved extract caused a reaction after 2 days; the dissolved antigens were considered to have had greater penetration.

SUMMARY

The ingredients Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, and Human Placental Lipids are derived from human placentas obtained from normal afterbirth. Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, and Placental Lipids are mixtures derived from animal placentas. Human Umbilical Extract and Umbilical Extract are obtained from human and animal umbilical cords, respectively.

The human and animal placental-derived ingredients function in cosmetic formulations as hair- and skin-conditioning agents. The umbilical cord extracts are reported to function as biological additives.

As of January 1998, Human Placental Protein was reported to be used in 30 cosmetic formulations, Placental Protein (identified as animal or bovine) was used in five formulations, Hydrolyzed Placental Protein was used in seven formulations, Placental Enzymes was used in seven formulations, and Placental Lipids, bovine was used in one formulation. In addition, human placental “extract” and placental “extract” were reported to be used in 14 and 31 cosmetic formulations, respectively. These two extracts are not recognized as cosmetic ingredients. Virtually all of the available safety test data related to extracts. These extracts are presumed to be biologically active, for example containing hormones.

Animal and clinical studies testing biologically active human placental “extract” have reported anti-inflammatory activity, improvement in the treatment of vitiligo and chorioretinal dystrophy, and neurological and cellular effects.

Oral- and parenteral-dose rat studies tested biologically active extracts and protein preparations and found changes in hepatic enzyme activities. Placental “extracts” (both human and animal) were negative in dermal and ocular irritation studies using rabbits. In in vitro studies, placental “extracts” demonstrated antimitogenic action in bacteria and had anticarcinogenic activity against some tumor cell lines.

Human umbilical extract was negative for mutagenicity in a micronuclei assay, and inhibited growth of tumors transplanted in rats and mice.

Some studies stated that protein was tested. However, the study design appeared to test the effects of hormonal activity. The one exception is a clinical study that reported no irritant reaction to Human Placental Protein following patch testing of patients with cosmetic product contact allergy.

DISCUSSION

The CIR Expert Panel faced many issues with this group of ingredients. First was the reported use of substances identified as “human placental extract” and “placental extract.” These names are not recognized in the CTFA International Cosmetic Ingredient Dictionary and Handbook. Further, FDA warned that cosmetics claiming to contain these ingredients may be misbranded and recommended using nomenclature other than “extract.” The CIR Expert Panel advised industry that cosmetic formulations should not be identified as containing “human placental extract” or “placental extract” so as to comply with FDA guidelines.

The Expert Panel expected that the CTFA-recognized ingredients—Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract—will not deliver any metabolic/endocrine activity (e.g., hormones, growth factors).

The Panel was also concerned with the dangers inherent in using human or animal-derived ingredients, namely the transmission of infectious agents. The CIR Expert Panel stressed that these ingredients must be free of detectable pathogenic viruses or infectious agents (e.g., HIV, Bovine Spongiform Encephalopathy (BSE), or Creutzfeld-Jakob disease prions). Suppliers and users of these ingredients must accept responsibility for assuring that these ingredients are risk-free. Tests to assure the absence of a pathogenic agent in the ingredients, or controls to assure derivation from pathogen-free sources are two approaches that should be considered.

With the above conditions met, the CIR Expert Panel noted that additional data still were needed to assess the safety of the cosmetic ingredients. The vast majority of studies cited in this report tested biologically active “extracts” and other preparations. Thus, the Panel was unable to apply results of these studies to the safety assessment of the cosmetic-grade ingredients.

Section 1, paragraph (p) of the CIR Procedures states that “A lack of information about an ingredient shall not be sufficient to justify a determination of safety.” In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on these ingredients were not sufficient for determination whether the ingredient, under relevant conditions of use, was either safe or unsafe. The Panel released a Notice of Insufficient Data on March 20, 1998, outlining the data needed to assess the safety of these ingredients. Comments concerning a human and bovine placental extract were received during the 90-day public comment period. However, additional data needed to make a safety assessment are:
1. Skin sensitization at concentration of use
2. Gross pathology and histopathology in skin and other major organ systems associated with repeated exposures, and dermal reproductive and developmental toxicity data
3. Photosensitization
4. One genotoxicity assay in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using NTP methods may be needed
5. Ocular toxicity, if available

(*To be done on all ingredients unless chemical analysis data shows similarity among ingredients.)

CONCLUSION

The CIR Expert Panel concludes that the available data are insufficient to support the safety of Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract for use in cosmetic products. If these ingredients are used, they should not deliver any metabolic/endocrine activity, and they must be free of detectable pathogenic viruses or infectious agents.

REFERENCES


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Available for review: Director, Cosmetic Ingredient Review 1101 17th Street, NW Suite 310, Washington, DC 20036-4702, USA.


October 11, 2010

F. Alan Andersen, PhD, Director, Cosmetic Ingredient Review, Members of The CIR Expert Panel.

Dr. Andersen, thank you and the Expert panel for the promptness of your reply.

On behalf of Rimar, LLC: Mark R. Engelman, M.D. is prepared to respond to questions and comments from the CIR Expert Panel. 602-840-6961

Dr. Engelman may also be identified on the search engine: Google.com

Re: for the attention of the CIR Expert Panel meeting December 13–14, 2010: the attached data/documents/information and the outline following, is presented in support of the use of “Human Umbilical Extract” in cosmetic products by Rimar, LLC, Lighter & Livelier, LLC and Cosmetikare Laboratories, LLC

Al Needleman BSc Pharm. Executive Director Lighter & Livelier, LLC, Las Vegas, NV Executive Director Cosmetikare Laboratories, LLC, Las Vegas, NV Executive Director Research and Development, Rimar, LLC, Scottsdale, AZ.

The following opinion: “Rational for utilizing umbilical plasma components as additives for skin treatments” was requested as a professional review of our proprietary research data, from Avi Treves, PhD Director of Research, Sheba Medical Center (Tel Hashomer) Israel, Cancer Research Center.

The encouraging opinion formed the basis for the eventual addition of Human Umbilical Extract (Cord Serum Complex) to our clinically successful topical cosmetics.

“During the adult life, skin regeneration is enabled by continuous development of its various layers and secretion of the extra cellular matrix components. Several types of progenitor cells are involved in this process, including fibroblasts, keratinocytes, hair pulp stem cells, melanoblasts and progenitors of sebaceous and sweat glands. The proliferative potential of the various skin stem and progenitor cells decreases with age and in several pathological conditions. Thus, maintaining the functional capacity and stimulating the secretory activity of the building blocks of skin layers, may enhance skin regeneration, viability and elasticity.

The umbilical cord plasma contains numerous growth factors, cytokines, hormones and chemokines that support and enhance the viability, function and proliferation of stem and progenitor cells. Cord blood is enriched in hematopoietic stem cells, as well as mesenchymal, endothelial and pluripotent stem cells. Additional cells with regenerative capacity were also reported in cord blood, and their detailed lineage composition is still being studied. Thus, the sera and plasma of the umbilical cord blood are natural supportive nutrient environment for many types of stem, progenitor and regenerative cells. Indeed, cord blood serum was reported to replace fetal calf serum or other sources of sera in tissue culture media for ex vivo growing of several types of human cells, including fibroblasts and keratinocytes. Taking together, although the direct interaction between skin stem cells or skin tissue with cord plasma components was not studied yet, it is suggested that such interaction may lead to similar stimulatory effect, and eventually to enhanced skin
Regeneration*. The combination of umbilical cord plasma components together with other known skin care reagents, may have a synergistic effect and further improve the regenerative activity of the combined preparation**.

Note: * and ** proven in the human invasive clinical trial (biopsies) attachment: “Histogeometric Analysis of the effects of Product A versus Product B on Human Skin”


Additional information regarding the intense safety procedures instituted for the collection, storage, shipment and use in our cosmetic products...

Al,

We have the clonogenic assays in hand and can look at the CFU activity of the plasma + or – exogenous cytokines. Storage of the TNC in LN2 is not a problem.

Test specifics:

Aerobic and fungi culture
Anaerobic culture

HBs Ag
HCV Ag
Anti HBc
Anti HTLV I/II (Human T cell Lymphotropic Virus)
Anti HIV 1&2
Anti HCV
Syphilis Test (Treponema Pallidum)
Anti CMV_IgG and IgM
HIV antigen p 24

As far as estimating residual reagent, it would be very dependent upon the collection volume and hematocrit of the CBU. The bag sets are designed to accommodate a 150mL cord blood volume. Anything below this volume (which would be the majority of samples) would have more PrepaCyte remaining. Please let me know if you have any available time during your evenings over the next several days. BioE is located in Minneapolis which is one hour behind us here in Florida. I will arrange a call based on your and BioE’s availability.

Best regards,

Donald L. Hudspeth, BSCLS, MT(ASCP)

General Manager and
Additional sample tests prior to the shipment of processed and tested Human Umbilical Extract (Cord Serum Complex)

2034377 (CB-001) no bacterial growth observed
2036083 (CB-002) no bacterial growth observed
2039027 – CMV positive (CB-003) no bacterial growth observed
2036079 (CB-004) no bacterial growth observed
2012531 (CB-005) no bacterial growth observed
2034240 (CB-006) no bacterial growth observed
2036033 – CMV positive (CB-007) Not done (sample lost in transit)

Skin sensitization at concentration of use: Attached Clinical Trial, 48 hours occluded patch test, 50 Participants. Results: “under the conditions of this study, test material, Anti Aging Face Cream with Cord Serum Complex (Human Umbilical Extract) NBP0073, Batch 0013 did not indicate a potential for dermal irritation”. Clinical Trial performed by Consumer Product Testing Company, Inc., Fairfield, N.J. (973) 808-7111. Clinical trial attached.

Conclusive evidence of safety and efficacy for the use of Human Umbilical Extract in Rimar cosmetics: Robert M. Lavker, PhD, Northwestern University, department of dermatology, was tasked with generating a clinical trial protocol based on participant tissue biopsies of skin areas following the application of the cosmetic product containing Human Umbilical Extract (Cord Serum Complex) versus the cosmetic product without Human Umbilical Extract. The detailed study, including histological slides and focused conclusions of this 26 day clinical trial are attached.

A detailed document outlining the pharmacodynamics of the ingredients used in the various cosmetic products, and separate human ingredient studies are included in the document: “Anti Aging with Cord Serum Complex Development Document” attached and “UnderEye Recovery Cream with Cord Serum Complex” document attached.

Julio Garcia, MD
Cosmetic & Plastic Surgeon
Engaged to perform a study on 30 participants, a power point presentation, photos and his comments are attached. Dr Garcia is a well known cosmetic surgeon with extensive practices in the U.S. (Las Vegas, NV) and in India as a teaching and practicing cosmetic surgeon.
The attached patent application has been accepted by the USPTO and is presented here for your review. Please note the first sale date of cosmetics containing Human Umbilical Extract is within the body of the patent*.

UNITED STATES PATENT APPLICATION FOR:

TOPICAL COMPOSITION COMPRISING
UMBILICAL CORD BLOOD SERUM

INVENTORS:
Alvin Needleman

Attorney Docket No.: 838611-0002


There are no reports of adverse reactions to any of the cosmetic products, no products have been returned for credit as a result of an irritability (merchant account charge records are available for review). A list of trial participants can be provided for your review.

All the information and data can be supplied in hard copy, as needed.

Thank you for the opportunity to present supportive documentation for the use of “Human Umbilical Extract”

Regards

Al Needleman
Cosmetikare Laboratories, LLC
1001 Meadowleah Street
Las Vegas, NV 89145-8642
From: Alan Andersen [mailto:andersena@cir-safety.org]
Sent: Friday, October 01, 2010 8:23 AM
To: Al Needleman  
Cc: Halyna Breslawec
Subject: Re: Human Umbilical Extract

Mr. Needleman - thank you for letting us know that Cosmetikare Laboratories, LLC intends to include human umbilical extract in several cosmetic formulas. We would appreciate receiving the data from the clinical testing you described and the experiential findings from the field trials.

The CIR Expert Panel next meets on December 13-14. We could include a discussion of new data for human umbilical extract at that meeting if we received information before October 25th. Otherwise, the next meeting would be March 3-4, 2011 and we would need a submission by January 17th.

Let me remind you that the CIR Expert Panel had asked for data regarding 1) skin sensitization at concentration of use, 2) gross pathology and histopathology in skin and other major organ systems associated with repeated exposures, and dermal reproductive and developmental toxicity data, 3) photosensitization, 4) one genotoxicity assay in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using NTP methods may be needed, and 5) Ocular toxicity, if available. It is not immediately clear how the data you have would address those issues, but that may be clear once we see the data.

In addition, I am certain the CIR Expert Panel would readress the issue of function of human umbilical extract in cosmetics as it is currently "not reported" in the International Cosmetic Ingredient Dictionary and Handbook. The Panel has a low tolerance for uncertainty in the data when the reason for putting the ingredient in cosmetics isn't clear.

You mentioned that FDA standards for blood collection are followed. You may need to be more specific in terms of the waiting period between use of the extract and any test done to determine HIV infection in a donor in which sufficient antibody has not manifested to be detectable, if antibody tests are being used by Cryobanks Laboratories.

If you have any questions, let me know.

F. Alan Andersen, PhD  
Director, Cosmetic Ingredient Review  
1101 17th Street, NW, Suite 412  
Washington, DC 20036  
ph 202.331.0651  
fax 202.331.0088  
andersena@cir-safety.org

>>> "Al Needleman" <alneedleman@cox.net> 9/28/2010 6:36 PM >>>
CIR, Hello,

A recent review of the "Zero use ingredients with insufficient Data" listed an ingredient we expect to include in several cosmetic formulas: (2) Human Umbilical Extract.

The safety and function of the ingredient was proven in 2010, in an (invasive) clinical trial at Northwestern University, the department of dermatology. Punch biopsies were taken from several participants in three areas of application: (1) the product containing the human umbilical extract ingredient (2) the product without the human umbilical extract ingredient and (3) an area where no cosmetics were applied. The results of the trial proved the validity of the ingredient. The data is available for review. Products containing the ingredient have been in field trials for 36 months with approximately 2500 users, there are no reports of irritability or interaction.

The ingredient is produced using applicable FDA standards for blood collection, processing and testing by Cryobanks...
laboratories, Tampa, Fl.

I look forward to your reply as soon as possible.
Thank you

Al Needleman
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702-243-7423
Mobile: 702-203-2196
Fax: 702-838-7424

This e-mail is intended only for the addressee(s) and may contain confidential information. If you are not the intended recipient, you are hereby notified that any use of this information or dissemination, distribution or copying of this e-mail is strictly prohibited. If you have received this e-mail in error, please notify the sender immediately by return e-mail and delete the original message. Thank you.
Anti Aging Face Cream with Cord Serum Complex™, Formula Composition, Efficacy, Safety, Pharmacodynamics, Rational and Clinical studies of participants aged 39 to 74. **Anti-wrinkling and lifting efficacy assessed by profilometry and photography T-30 as compared to T-0. Effect on muscle relaxation using in vitro nerve muscle co-culture.**

Skin is made up of three main layers: the epidermis, the dermis and the hypodermis. The epidermis is the only layer we can see with our eyes and as we age, remarkable changes occur which are hidden from our view. For instance, the skin gradually thins over time, especially around the eyes. Elastin and collagen, located in the dermis keep the skin resilient and moist, but with ageing these fibers break down to create lines and wrinkles. Exposure to ultraviolet radiation accelerates this process. The best way to reduce fine lines and wrinkles is to limit our exposure to the sun and ultraviolet radiation. Regular, twice daily, application of the Anti aging face cream with cord serum complex restores collagen, elastin, and nutritional communication between cells at the DEJ (Dermal Epidermal Junction) modulates muscle neural sensitivity and raises HA.

The Anti Aging Face Cream is the future in botox-like (non-invasive) skin care. Formulated to restore “age-lost adhesion” between the dermis and the epidermis… reducing fine lines, wrinkles and sagging.

**The Anti Aging formulas are (site) focused on successful repair-restructure & enhancement of the physical, neural and chemical properties of the 3 main skin layers, sub-dermal musculature & the DEJ**

**Keywords:** DEJ; elastin; collagen; profilometry; lamina; co-culture; charge coupled; bio-peptides, HA (Hyaluronic acid)
The anti aging formulas generate a high percentage of active energy at the DEJ, a prime area of “separation”; restoring smoothness, elasticity, adhesion and reducing fine lines and wrinkles (up to 45%) in 30 days or less.

General overview: Anti Aging actives: Composed of charge coupled Bio-peptides, marine micro algae, antioxidants, neurotransmitter modulators, sub dermal muscle control, tissue lubricant technology, damaged DNA replacement, topical-cellular immune system stimulation, accelerated active absorption and molecules from cord serum complex (Human Umbilical extract); comprising enzymes, hormones, proteins, vitamins, antioxidants, amino acids and minerals.

Collagen molecules and soft Keratin are responsible for skin strength and elasticity. Collagen degradation leads to wrinkles that accompany aging.

Anti Aging Formula Ingredients effecting ABC and ABCD repair damage and restore collagen.

Support for the Lamina Layers (B & C) and their protein network contributes toward cell attachment and differentiation, cell shape and movement, maintenance of tissue phenotype, and promotion of tissue survival.

Ingredients supporting B & C maintain and restore lamina layer integrity.

Integrins (A) play an important role in cell signaling by connecting to the extracellular matrix molecules (E) causing a signal to be relayed into the cell through protein kinases attached to the intracellular end of the Integrin molecule (B).

Ingredients supporting A & B maintain the functional properties of the Integrins. (Cell surface receptors, interact and mediate various intracellular signals)

Summary of Clinically validated ingredient results: 26% increase in skin smoothness in 26 days. 17% increase in skin tone. 55% decrease in skin fatigue. 30% reduction in the depth of wrinkles after 30 days. 44.9% reduction in surface occupied deep wrinkles. 18.5% reduction in main wrinkle average volume. 14.4% reduction in skin roughness. 16.6% reduction in skin complexity. 19.5% increase in elasticity. 2.6 times increase in sensitivity to neurotransmitter signaled muscle contraction.

Formula ingredients, not in the order of prominence (Patent pending): Acetyl Hexapeptide-3, Palmitoyl Oligopeptide, Palmitoyl Tetrapeptide-7, Lipopeptide (NATAH Ester), Undaria pinatifida, Stevia rebaudiana-Bertoni, Juglans regia leaf extract, Juglans regia shell extract, Centella asiatica extract, Pyrus germanica extract, Cord serum complex, Myristamidopropyl...
(cont.) PG Dimonium Cl Phosphate,
Dimethyl Isosorbide, Lecithin, Purified water, emulsion matrix.

Clinical validation:
Peptide (short polymers formed from the linking of alpha amino acids) reinforced cosmeceuticals in the Anti Aging formulas.

Peptide cosmeceuticals are one of the new popular options to treat aging skin. There are three main categories of cosmeceutical peptides: signal peptides, neurotransmitter-affecting peptides and carrier peptides. The evidence to support their use has been scientifically well validated enhancing their use in Anti aging cosmetics and their practical use in dermatology.

Botulinum neurotoxins (BoNTs) represent a revolution in cosmetic science because of their remarkable and long-lasting anti-wrinkle activity. However, their high neurotoxicity seriously limits their use. Thus, there is a need to design and validate non-toxic molecules that mimic the action of BoNTs.

Peptide: Affecting Neurotransmitters

Acetyl Hexapeptide-3 (revised as -8) was identified as a result of a rational design program. Noteworthy, skin topography analysis of an oil/water (O/W) emulsion of this hexapeptide solution on healthy women volunteers reduced wrinkle’s depth up to 30% upon 30 days treatment.

Analysis of the mechanism of action showed that the hexapeptide (Acetyl Hexapeptide-3) significantly inhibited neurotransmitter release with a potency similar to that of BoNT. Although, as expected, it displayed much lower efficacy than the injected neurotoxin. Inhibition of neurotransmitter release was due to the interference of the Hexapeptide with the formation and/or stability of the protein complex that is required to drive Ca^{2+}-dependent exocytosis, (cellular direction of secretory vesicles) namely the vesicular fusion (known as SNARE=soluble NSF attachment receptor) complex.

Notably, the hexapeptide did not exhibit in vivo oral toxicity or primary irritation at high doses.

Taken together, these findings demonstrate that this hexapeptide is a non-toxic, anti-wrinkle molecule that emulates the action of currently used BoNTs. Therefore, the inclusion of this hexapeptide represents a biosafe alternative to BoNTs in cosmetics.

Clinical results of skin topography using silicon impressions taken from the lateral preorbital area in healthy women volunteers and analyzed by confocal microscopy.

Reduction of wrinkle depth (below)

The top row illustrates the use of a placebo cream, while the bottom row illustrates the use of the molecule at the inclusion rate. The three periods from left to right illustrates the measurements taken at day 0, day 15 and day 30.
Peptide: messengers of cutaneous restructuring and repair.

Palmitoyl Oligopeptide & Palmitoyl Tetrapeptide-7.

The peptides are two matrikines (extracellular matrix-derived peptides which regulate cell activity) they act in synergy to restore and maintain the skin’s youthful appearance. Activating the neosynthesis of extracellular matrix molecules providing visible anti-wrinkle efficacy. As messenger molecules the matrikines are capable of regulating cell activities. They interact with specific receptors to activate certain genes involved in the extracellular matrix renewal and cell proliferation. (Since, with age these mechanisms become progressively weaker).

Clinical study: two panels of twenty three volunteers aged 39 to 74 applied a cream containing Palmitoyl Oligopeptide and Palmitoyl Tetrapeptide-7 to one half of their face against a placebo on the other half...twice a day for 30 days. Anti-wrinkling and lifting efficacy was assessed by profilometry and photography.

Results:

Visible and measurable proof of Anti-wrinkle efficacy.

Surface occupied by deep wrinkles (>200µm) – reduced 44.9%.

Main wrinkle density – reduced by 37%

Main wrinkle average depth – reduced by 15.1%

Main wrinkle average volume – reduced by 18.5%

Roughness – reduced by 14.4%

Lifting effect (complexity) - reduced by 16.6%

Elasticity – increased by 9%

Tone – increased by 19.5%

Gene activation (+): Collagen-1 (50%), Fibronectin (60%), Hyaluronic Acid (45%)

The synthesis of matrix molecules: In order to determine the ability of fibroblasts incubated for 72 hours with the bio-peptides, at 3 strengths, to identify the levels of stimulation of extracellular matrix components. Check validity

Photographic results below at ideal concentration.

Lipo-dipeptide, a messenger of tranquility and muscle relaxation.

Relieves skin/muscle tension to help prevent the onset of wrinkles and expression lines.

N-Acetyl-Tyrosyl-Arginyl-Hexadecyl Ester helps promote the release of pro-endorphins to provide a tranquilizing effect on the skin; relieves tension and inhibits the muscle contractions responsible for the appearance of wrinkles and expression lines.

In Vitro validation:

NEUROTRANSMITTERS FOR
RELAXATION; β-endorphin and met-enkephalin are neuropeptides involved in the “down-regulation” of nerve and muscle activity… human keratinocytes were incubated for 24 hours. The RT-PCR technique was used to measure the increase in gene expression for Proopiomelanocortin (the precursor for β-endorphin and met-enkephalin). Results: 63% increase in muscle relaxation.

NEUROTRANSMITTER FOR CONTRACTION; calcitonin gene related peptide (CGRP) is a neuropeptide involved in the stimulation of muscle activity and sensitization. Human nerve culture was incubated in reagents and the amount of CGRP released was measured. Results: a decrease in contraction of 3.1% as measured against standardized capsaicin.

EFFECT ON MUSCLE RELAXATION; using an in vitro model of nerve-muscle co-culture containing axons and a neuromuscular junction. Results: the lipo-peptide progressively decreased muscle contraction frequency and within two hours totally inhibited contractions.

Botanicals:

Stevia rebaudiana Bertoni (SrB); moisturizing, smoothing, fine line reduction, anti-oxidant, tonifying. Related activities of active molecules: Diterpenglycosides = moisturizing, smoothing, conditioning. Flavonoids = anti-irritant, free radical scavenger. Clinical Test; SKIN MOISTURIZER AND SMOOTHNESS: Two groups of twenty participants each. Twenty participants applied Stevia rebaudiana (SrB) Bertoni (1% aqueous gel) twice daily on the forearm for 28 days. Second group – of twenty participants applied a, physically and visually similar, placebo in the same manner. Results: skin hydration increased 20% after 28 days. Skin smoothness increased 24% after 14 days. Skin smoothness increased to 26% after 28 days. SrB supports the skin’s moisture barrier, with a moisturizing effect 3 times greater than glycerin.

(a) Juglans Regia Leaf Extract, (b) Juglans Regia Shell Extract, (c) Centella Asiatica Extract, (d) Pyrus Germanica Extract. In order to increase safety and efficacy, this botanical combination is based on 2 facts: the synergy between plants, the whole is more than the sum of its parts. Each plant imparts its own special qualities to the RMI formula, each one necessary for the firming effect.

(a) and (b): firming, free radical scavenging, tonifying. (c) Anti-stress, strengthening, stimulation of collagen synthesis. (d) Bio-protective, astringent, smoothing, restore skin moisture. (Tightens sagging skin).

Clinical study: TONIFYING AND ANTI-FATIGUE EFFECT; two groups of participants 10 volunteers per group. Twice daily application of an active cream to the neck for 28 days. Placebo group inactive cream same application for 28 days. Results: immediate reaction 17% increase in tonicity. 28 days: 55% reduction in Cutaneous fatigue vs. the placebo (untreated skin). FIRMING EFFECT; two groups of participants 10 volunteers per group. Twice daily application of an active cream to the neck and forearms’ for 28 days. Placebo group inactive cream application for 28 days. Results: forearms – Elasticity (reversible skin deformability) increased 7%, forearms – Viscoelasticity (elastic/plastic ratio during suction) decreased 10%. Firmness-neck (elastic deformity ratio) increased 12%.

Algae:
Undaria pinnatifida L. maintains skin firmness, elasticity and smoothness, supports skin regeneration, defends against environmental stress. Active molecules; sulphated polysaccharides = Anti-hyaluronidase/anti-elastase effect, moisturizing, anti-oxidant, stimulation of fibroblasts. Proteins = conditioning, Oligoenticments = skin moisture balance, Vitamins = cell protection and regeneration, anti-oxidant.

Clinical study: volunteers participated in a dose related study of hyaluronidase inhibition. At 2% Undaria p. demonstrated 36% inhibition of hyaluronidase at 5% more than 50% inhibition. Inhibition of Hyaluronidase prevents deterioration of the extracellular matrix. This is a dominant factor in defending skin against aging and the diminution of dermal thickness. Sulphated polyfucose (polysaccharide) protects both the algae body walls and human tissues from losing integrity and stability.

PHOSPHOLIPID COMPLEX:

Myristamidopropyl PG-Dimonium Chloride Phosphate; reduces reliance or preservatives, provides sensorial benefit (feel), substantive conditioning, co-emulsifier.

Clinical and safety trials: Eye irritation None, using Red Blood Hemolysis assay. Eye irritation (2) None, using Isolated Eye test to screen for eye irritation. Skin irritation None, using Skin Integrity Function Test. Skin irritation (2) None, using 48 hour occluded skin patch tests.

DERMAL ABSORPTION, SITE DELIVERY:

Dimethyl Isosorbide (DMI), focuses the delivery of active ingredients where they are most needed. Formula benefits; enhances formulation and API (Active Pharmaceutical Ingredients) stability, improves formulation aesthetics, reduces cellular irritation, stable to hydrolysis, reduces gelatin cross linking, improves spreading. Clinical and safety studies conclusion: non-irritating as provided by testing under National Industrial Chemicals Notification and Assessment Scheme-Full Public Report. File # STD/1052, 05.12.2004. Super refined DMI (Dimethyl Isosorbide) is produced to the highest purity standards for advanced performance. Super Refining is a proprietary refining process that removes polar impurities, preventing adverse interactions with Active Pharmaceutical Ingredients (APIs). By minimizing peroxide and formaldehyde levels.

Absorption Ingredient summary: enhances formulation and API stability, improves epidermal penetration, improves formulation aesthetics, reduces cellular irritation, stable to hydrolysis, reduces gelatin cross linking.

CORD SERUM COMPLEX:

*The Energy of Creation™

Cord Serum Complex (FDA compliant, “Human Umbilical extract”): a proprietary composition containing enzymes, hormones, antioxidants, antigens, vitamins, amino acids, proteins and minerals. Derived from pooled cord blood plasma, obtained by regulatory approved methods of umbilical cord blood processing at accredited cord blood banks.

The Human Umbilical extract (plasma) is obtained following removal of all cellular components and further processed and freeze stored.

The Human Umbilical extract is pre-tested for microbial and fungal contamination and the mother's blood is pre-tested for
infectious viral contaminants as required for regular Umbilical extract and cord blood storing for clinical utilization.

Cord blood (Umbilical extract) is known to contain higher numbers of more clinically effective stem cells… for clinical indications and regenerative treatments.

The serum components (Human Umbilical extract) supports the viability and function of these stem cells. Cord serum components have been identified in research as potential promoters of the proliferation of skin progenitor cells such as keratinocytes and fibroblasts, and stimulate their function in regenerating skin tissue. Cord Serum Complex provides robust elasticity, smoothness, environmental and free radical damage repair, and enhances the product functional longevity.

Additional clinical evaluations: 48 hour occluded patch test. Consumer Product Testing, Fairfield, New Jersey. Six week clinical trials, Las Vegas, NV, under the direction of Dr. Julio Garcia

Note: Second generation RMI research and product development includes the new area of stem cell exudates (Paracrines and Paracrine signaling).

Al Needleman
Certificate of Analysis
The name printed at the end of this document is an electronic signature

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<td>Anaerobic culture</td>
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/Al Needleman/ (electronic signature)
FINAL REPORT

CLIENT: Lighter & Livelier, LLC…RMI, Inc.
1001 Meadowleah Street
Las Vegas, NV 89145

ATTENTION: Al Needleman

TEST: 48 Hour Patch Test
Protocol No.: 1.02

TEST MATERIAL: Anti Aging Face Cream with Cord Serum Complex NBP0073
Batch 0013

EXPERIMENT REFERENCE NUMBER: C08-5584.01

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor any member of its staff, may be used in connection with the advertising, or sale of any product or process without written authorization.

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

Study No.: C08-5584.01

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of clinical laboratory studies. These studies have been performed with adherence to the applicable ICH Guideline E6 for Good Clinical Practice and requirements provided for in 21 CFR parts 50 and 56 and in accordance to standard operating procedures and applicable protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study. All data pertinent to this study will be stored in the Consumer Product Testing Company archive, unless specified otherwise, in writing by the Sponsor.

Quality Assurance personnel involved:

[Signature]

Quality Assurance

12/12/05

Date

The representative signature of the Quality Assurance Unit signifies that this study has been performed in accordance with standard operating procedures and the applicable study protocol as well as any government regulations regarding such procedures and protocols.
Objective: To determine by epidermal contact the primary irritation potential of a test material.

Participants: Fifty-four (54) subjects, male and female, ranging in age from 20 to 79 years, who qualified were selected for this evaluation. Fifty-three (53) subjects completed this study. The remaining subject discontinued her participation for personal reasons unrelated to the use of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16+ and over.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor’s care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: Anti Aging Face Cream with Cord Serum Complex NBP0073 Batch 0013

Study Schedule:

<table>
<thead>
<tr>
<th>Panel #</th>
<th>Initiation Date</th>
<th>Completion Date</th>
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<tbody>
<tr>
<td>20080489</td>
<td>December 2, 2008</td>
<td>December 5, 2008</td>
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Methodology: Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. When secured to the appropriate treatment site, this dressing formed an occlusive patch.

*With parental or guardian consent
Methodology (continued):

The test material remained in contact with the skin for a total of forty-eight hours. This site was then evaluated for gross changes. Absence of any visible skin change was assigned a zero value. The test site was re-evaluated at seventy-two hours.

Evaluation Criteria (Erythema and additional Dermal Sequelae):

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<th>Score</th>
<th>Description</th>
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<td>No visible skin reaction</td>
<td>E</td>
</tr>
<tr>
<td>0.5/+</td>
<td>Barely perceptible</td>
<td>D</td>
</tr>
<tr>
<td>1</td>
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<td>S</td>
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<tr>
<td>4</td>
<td>Severe</td>
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Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Results:

The results of each participant are appended (Table 1).

Observations remained negative throughout the test interval.

Subject demographics are presented in Table 2.

Summary:

Under the conditions of this study, test material, Anti Aging Face Cream with Cord Serum Complex NBP0073 Batch 0013, did not indicate a potential for dermal irritation.
Table 1
Panel #20080489

Individual Results

Anti Aging Face Cream with Cord Serum Complex NBP0073 Batch 0013

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**Table 1**
(continued)
Panel #20080489

**Individual Results**

Anti Aging Face Cream with Cord Serum Complex NBP0073 Batch 0013
### Table 1
Panel #20080489

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Table 2
(continued)
Panel #20080489

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L&L Cord Extract Blind Study
Study Design

• No artificial damage allowed to skin prior to study
• No Botox or laser/chemical peel for 3 months prior
• Only products allowed were blind creams and previous facial skin cleanser
• All other products ceased
Study Design

• 6 week trial
• Photos taken before and at completion
• Evaluation by a plastic surgeon and random female in same office with no formal medical training
• All photos were rated prior to code being broken
Study Design

- Investigator given blinded samples of product, control and cord extract
- Ingredients of creams were also not divulged to investigator
- Patient chose which container would be used on which side
- 30 patients identified to participate
Study Design

- Rating System of wrinkles (static only)
- No change
- 25% reduction
- 50% reduction
- 75% reduction
- Complete effacement of wrinkle
Demographics

- Age range 29-73
- Avg. Age 56.6
- Female:Male ratio 27:2
Clinical results

- Plastic Surgeon (PS) found 53% of participants showed a response on either side
- PS found 47% did not respond
- Office member (OM) found 45% of participants showed a response on either side
- OM found 55% did not respond
Clinical Results

- PS found 28% showed results on control side
- OM found 10% showed results on control side
Clinical Results

• PS found 35% of patients responded on cord extract side
• OM found 35% of patients responded on cord extract side
Clinical Results

• Of those patients that showed a visible response
• PS saw a 31% degree of wrinkle reduction in the controls and a 43% degree of reduction on the cord extract side
• OM saw a 33% degree of reduction in the controls and a 40% reduction on the cord extract side
Clinical Results

- One participant did not complete the study
- (moved)
- No adverse or allergic reactions
Summary

• Positive response rate in 35% of participants using cord extract
• Positive response in 19% of participants using control
• Of those that responded, the control side showed a 32% improvement and the cord extract side showed a 41% improvement
Summary

- Unsolicited comments on patient perception made during last photo session
- “Skin feels smoother”
- “Skin feels milky soft”
- “The surface seems softer”
Summary

- Solicited question
- 10 of 29 said they would buy the cream
- 16 of 29 said they would probably buy the cream
- 3 said they would not buy the cream
- Patients not shown their pictures until study completed
- Patient perception of change not evaluated as had been done in previous study
Demonstrative Slides

• Show before on left and after on right
• All photos show side that received cord extract
Histogeometric Analysis of the Effects of Product A versus B on Human Skin

Introduction

The marketplace is filled with products that purport to have “anti-aging” or “skin rejuvenation” properties. Most of these claims are supported by non-invasive measurements such as clinical photography, and skin surface evaluation using replicas to assess the depth of fine-line wrinkles and other surface phenomena. Some products make claims based on in vitro studies where keratinocytes or fibroblasts are cultured in the presence of the “active” ingredients, and various parameters (i.e., proliferation, migration, protein synthesis) are assayed. These types of studies can provide valuable insights into the mechanism(s) by which certain agents might be working; however, in vitro findings often do not translate into the in vivo situation. A more stringent test is to apply the product to human skin in a manner similar to its anticipated use and evaluate its effects on the epidermis and dermis using a combination of morphology, histochemistry and immunohistochemistry.

The present study was designed to test the effects that a topical cream containing cord serum complex, had on human skin. As a control, the topical cream minus the cord serum complex was also evaluated and both products were compared with an untreated site.

Methods

Six healthy volunteers were enrolled in the study. Product A was applied to the right aspect of the upper inner arm (R site), product B was applied to the left aspect of the upper inner arm (L site) and the volar forearm was chosen as the untreated site. Both products were applied daily for 26 days. The volar forearm received no product but was rubbed gently, daily, in an effort to simulate the manner in which both products were applied to the skin.

At the end of the treatment period, a 2-3 mm punch biopsy specimen was obtained from each treated site and the untreated site. Each specimen was immediately placed in 10% buffered formalin and processed for paraffin sectioning. All sections were five-micrometers in thickness and all slides were stained simultaneously as a group for each histochemical determination. The entire biopsy specimen was photographed with a high resolution digital camera system (Axiocam, Zeiss Corporation) mounted on a Zeiss Axioplan 2 light microscope at a magnification of 20x. All slides were photographed under the identical white balance light settings and exposure time to insure consistency in micrographs. Micrographs were subsequently analyzed using computer assisted image analysis software (Axiovision, Zeiss Corporation). All measurements were made from at least four areas of the biopsy specimen, except for two biopsies, which had smaller amounts of tissues and thus three measurements were made.

Five-micrometer paraffin sections were stained with hematoxylin-eosin (H&E) for overall morphologic evaluation and viable epidermal thickness determinations (VET; Fig. 1; Table 1). For estimation of viable epidermal thickness, care was taken to cut the sections perpendicular to the surface. The VET includes the area from the dermoepidermal interface to the lowermost...
portion of the stratum corneum (seen as a bright red zone; Fig. 1). For estimation of elastic fibers, van Geison’s stain was employed, which stains elastic fibers blue-black to black, collagen pale red, other tissue elements yellow, and nuclei blue to black (Fig. 2). For pro-collagen, anti-type I collagen (EMD Bioscience Inc.) antibody was used. This antibody to type I collagen was made against the triple helical portion and it is able to stain procollagen I. Immunohistochemical analysis of the paraffin sections was carried out using the DAB kit, which produces a brown reaction product (Fig. 3). For glycosaminoglycans (GAGs), Hale’s colloidal iron was used since Hale’s stainable material (blue) represents, for the most part, GAGs and is commonly used as an indicator of changes in ground substance (Fig. 4).

The quantification of stainable material was determined using a custom designed software program, integrated into the Axiovision image analysis system (Zeiss Corporation). The analysis is conducted in the following manner: (i) the reaction product (i.e., blue-black – elastin; brown – pro-collagen; blue – GAGs) is detected from a histogram and only objects with that color are outlined on the micrograph. The total area occupied by the outlined areas is measured; (ii) the entire area of the dermis is outlined and measured; and (iii) area of reaction product divided by total area = the percentage of material deposited.

It should be noted that all photomicrographs were taken and analyses performed in a double blind manner, and only after the data was tabulated was the investigator informed about the identity of the R and L sites.

Results

Morphology

The epidermis did not appear to be morphologically altered in any of the subjects at the two treatment as well as the untreated site (Fig. 1). In most instances, the undulating nature of the dermoepidermal interface was maintained. The granular layer was prominent in all specimens and there was little evidence of apoptosis (sunburn cells) within the epidermis. The “basketweave” architecture of the stratum cornea, characteristic of formalin-fixed human skin was maintained in all subjects in all sites.

For the most part, the fibrous components of the dermis (i.e., collagen, elastin) did not appear altered on the H&E sections from any of the treatment regimens or the untreated sites of the six subjects (Fig. 1). In some cases, the dermis from the untreated site appeared more compact (Fig. 1). In a few subjects, occasional areas of blue-gray staining material, usually associated with elastosis, were noted; however, frank signs of photodamage were not seen in any of the subjects. Importantly, there did not appear to be an unusual amount of inflammatory cells in biopsies from any of the treatment sites or in the untreated site from any of the subjects. Some increased cellularity was noted around portions of hair follicles present in some of the sections but this was not deemed significant. Vascular profiles appeared normal and there was no evidence of increased vascularity, vasodilatation and/or extravasation of red blood cells.
**Viable Epidermal Thickness (VET)**

There was no consistent trend seen in the VET measurements (Tables 1 and 2). Subjects 1, 2, and 6 had similar VET values for the untreated, R and L sites. Subject 3 had a thinner VET measurement for the untreated site compared with the R and L sites. Subject 4 had a thinner VET for the R site compared with the untreated and L site, whereas Subject 5 had a thinner VET for the L site when compared with the R and untreated sites. Given the lack of inflammation, which usually is responsible for epidermal thickening, it is not surprising that VET was not affected by either R or L treatment.

**Elastin**

The overall area of the dermis occupied by elastin appeared to be greater in the untreated sites from all six subjects when compared with either the R or L treatment site (Fig. 2, Tables 1 and 2). This finding in no way implies that either of the treatments had a negative impact on elastin fiber deposition, synthesis and/or destruction. It most likely represents inherent differences in elastin content between the volar forearm and the upper inner arm.

With respect to treatment sites, there was no obvious trend (Tables 1 and 2). Subjects 1 and 4 had significantly more elastin-stained material in the L site when compared with the R site. In contrast, subject 3 had significantly more elastin-stained material in the R site compared with the L site. There was no significant difference in elastin-stained material when the R and L sites were compared in Subjects 2, 5, and 6. When all six subjects were compared there was no change in elastin-stained material (Table 1). This is not surprising since elastin is one of the more stable components of the dermis with an extremely long turnover time. New elastin deposition is most often seen during tissue regeneration following a wound. Thus the lack of evidence for skin perturbation due to either of the treatments (Fig. 1) could account for the failure to detect a change in elastin.

**Procollagen**

Five of the six subjects showed an increase in the immunostaining for procollagen when the R site was compared with the L site (Fig. 3, Tables 1 and 2). Of these 5 subjects, one (#4) was significant at the P<0.05 level and two (#2 and #3) were highly significant (P<0.01 level). While an overall increase in procollagen immunostained material was detected for the R site versus the L site in Subjects 1 and 5, the difference was not statistically significant. The R and L treatment did not affect the procollagen-stainable material in Subject 6. When all six subjects were combined there was a greater amount of immunostaining for procollagen in the R site versus the L site; however due to subject to subject variability, this difference was not significant.

With respect to the untreated site, 4 subjects had less immunostained material corresponding to procollagen when compared with the R site; two subjects had more immunostained material. Due to potential differences in the dermis between the treated and untreated sites it is difficult to meaningfully interpret these changes.
GAGs

Four of the six subjects had significantly (P<0.01) increased Hale’s-stainable material in the R site when compared with the L site (Fig. 4, Tables 1 and 2). Subjects 4 and 6 had increases in Hale’s-stainable material in the L site versus the R site; however, this difference was not statistically significant. When all six subjects were combined there was a greater amount of Hale’s stainable material in the R site versus the L site; however due to subject to subject variability, this difference was not significant.

The untreated site showed the greatest subject to subject variability in Hale’s-stainable material. Nevertheless 4 subjects had greater Hale’s-stainable material in the R site when compared to the untreated site. As mentioned previously, due to regional differences it is difficult to draw meaningful comparisons between the treated and untreated sites.

Conclusions

There are several conclusions to be drawn from this small pilot study. The active and vehicle-only formulations did not have any deleterious effects on the skin that were discernable at the light microscopic level. This is important because any changes seen in the other parameters were not confounded by and/or secondary to an inflammatory response. The assumption is that any changes are the result of the cord serum complex. The R site, which received the cord serum complex daily for 26 days, had increased amounts of stained material corresponding to procollagen and GAGs compared to the vehicle-treated (L) site. Cord serum complex had no discernable effect on VET or elastin.

Since ground substance (GAGs) is the dermal component that is most rapidly turned over, it is not surprising that changes were seen. Furthermore, ground substance is well known for its water-holding capacity, and it is this increase in water binding that could be partially responsible for the disappearance of fine-line wrinkling after use of this product. The increase in procollagen-stained material was also significant. In contrast to the GAGs, collagen is slowly turned over. This suggests that some of the changes in skin quality that have been reported following use of the cord serum complex facial cream may be somewhat more long lasting than the GAG-induced changes because collagen fibers are a more stable dermal component than GAGs. Taken together these findings suggest that facial cream with serum cord complex stimulates the synthesis of GAGs and procollagen, which in part is responsible for the clinical changes seen after use of this product.

It is important to remember that conclusions drawn from histochemical staining experiments need to be tempered by the fact that despite precautions taken to eliminate and/or minimize the subtle differences in section thickness and staining inconsistency, such events can occur, which will confound the results. The conclusions drawn thus far can be markedly strengthened by determining the change in levels of these proteins biochemically using immunoblotting techniques. For example, following biopsy, the epidermis can be separated from the dermis and a dermal protein lysate can be obtained. This lysate can be analyzed for the amount of procollagen and certain GAG components (e.g., hyaluronate, heparin sulfate) by Western blot techniques. If increases in the levels of procollagen and hyaluronate are observed, this would
dramatically strengthen the histochemical data. Alternatively, the study can be repeated with a larger cohort of subjects. If this study were to be conducted, the procollagen and GAG components should be analyzed. H&E sections should also be obtained for an overall morphological assessment.

Robert M. Lavker, Ph.D.

June 14, 2010
Figure 1
elastin - Van Geison’s stain

Figure 2
Pro-collagen - Type 1 collagen stain

Figure 3
GAGs - Hales Colloidal Iron stain

Figure 4
### Table 1 - Histogeometric Analyses

<table>
<thead>
<tr>
<th>VET (microns)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Σ</th>
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<tr>
<td>NT</td>
<td>126 ± 7</td>
<td>99 ± 7</td>
<td>98 ± 8</td>
<td>115 ± 4</td>
<td>128 ± 16</td>
<td>114 ± 3</td>
<td>113 ± 12</td>
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<tr>
<td>R</td>
<td>132 ± 7</td>
<td>94 ± 4</td>
<td>120 ± 15</td>
<td>91 ± 2</td>
<td>118 ± 20</td>
<td>119 ± 3</td>
<td>96 ± 37</td>
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<tr>
<td>L</td>
<td>126 ± 5</td>
<td>91 ± 8</td>
<td>115 ± 10</td>
<td>108 ± 4</td>
<td>87 ± 9</td>
<td>113 ± 5</td>
<td>107 ± 14</td>
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</table>

<table>
<thead>
<tr>
<th>Elastin (% dermis)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>NT</td>
<td>14.0 ± 1</td>
<td>10.4 ± 1.1</td>
<td>12.5 ± 0.7</td>
<td>12.2 ± 2.4</td>
<td>13.0 ± 2.0</td>
<td>7.6 ± 2.0</td>
<td>11.6 ± 2.0</td>
</tr>
<tr>
<td>R</td>
<td>8.0 ± 1.5</td>
<td>9.8 ± 0.3</td>
<td>8.3 ± 1.6**</td>
<td>5.8 ± 0.4</td>
<td>8.2 ± 1.1</td>
<td>5.0 ± 0.4</td>
<td>7.5 ± 1.6</td>
</tr>
<tr>
<td>L</td>
<td>11.8 ± 0.7**</td>
<td>8.0 ± 1.7</td>
<td>3.3 ± 0.3</td>
<td>8.6 ± 0.8**</td>
<td>9.5 ± 1.1</td>
<td>5.3 ± 1.1</td>
<td>7.8 ± 2.8</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Procollagen (% upper dermis)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td>NT</td>
<td>8.1 ± 0.6</td>
<td>9.9 ± 1.7</td>
<td>4.7 ± 1.8</td>
<td>16.2 ± 1.8</td>
<td>9.1 ± 1.5</td>
<td>16.4 ± 2.9</td>
<td>10.7 ± 4.3</td>
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<tr>
<td>R</td>
<td>9.2 ± 0.6</td>
<td>13.3 ± 1.6**</td>
<td>9.9 ± 1.7**</td>
<td>12.3 ± 2.3*</td>
<td>19.2 ± 5.4</td>
<td>10.0 ± 2.4</td>
<td>12.3 ± 3.4</td>
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<tr>
<td>L</td>
<td>9.1 ± 1.1</td>
<td>7.8 ± 1.3</td>
<td>5.2 ± 2.1</td>
<td>7.9 ± 1.8</td>
<td>15.8 ± 3.4</td>
<td>11.5 ± 1.4</td>
<td>9.6 ± 3.4</td>
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</table>

<table>
<thead>
<tr>
<th>GAGs (% dermis)</th>
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<tbody>
<tr>
<td>NT</td>
<td>1.8 ± 0.8</td>
<td>24.3 ± 2.8</td>
<td>22.6 ± 2.4</td>
<td>13 ± 3.8</td>
<td>14.7 ± 2.2</td>
<td>5.2 ± 0.9</td>
<td>13.6 ± 8</td>
</tr>
<tr>
<td>R</td>
<td>22 ± 5.0**</td>
<td>11.4 ± 0.9**</td>
<td>20.1 ± 2.2**</td>
<td>15 ± 2.7</td>
<td>23 ± 3.0**</td>
<td>10.8 ± 3.9</td>
<td>17.4 ± 4.9</td>
</tr>
<tr>
<td>L</td>
<td>4.4 ± 2.3</td>
<td>6.5 ± 1.5</td>
<td>8.3 ± 2.7</td>
<td>18 ± 5.5</td>
<td>14.3 ± 1.5</td>
<td>13.9 ± 3.9</td>
<td>10.9 ± 4.8</td>
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</tbody>
</table>

*P <0.05; **P < 0.01
## Table 2 - Percentage Change

<table>
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<tr>
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<th>1</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R vs L</td>
<td>4.5↑</td>
<td>3↑</td>
<td>4.2↑</td>
<td>15.7↓</td>
<td>26.3↑</td>
<td>5.0↑</td>
</tr>
<tr>
<td>R vs NT</td>
<td>4.5↑</td>
<td>5.1↓</td>
<td>18.3↑</td>
<td>20.8↓</td>
<td>7.8↓</td>
<td>4.2↓</td>
</tr>
<tr>
<td>L vs NT</td>
<td>0</td>
<td>8.1↓</td>
<td>14.8↑</td>
<td>6.1↓</td>
<td>32.0↓</td>
<td>0.9↓</td>
</tr>
</tbody>
</table>

|        |      |      |      |      |      |      |
| **elastin** |      |      |      |      |      |      |
| R vs L  | 32.2↓| 18.4↑| 60.2↑| 32.6↓| 13.7↓| 5.7↓ |
| R vs NT | 42.9↓| 5.8↓ | 33.6↓| 52.5↓| 36.9↓| 34.2↓|
| L vs NT | 15.7↓| 23.1↓| 73.6↓| 29.5↓| 26.9↓| 30.3↓|

|        |      |      |      |      |      |      |
| **procollagen** |      |      |      |      |      |      |
| R vs L  | 1.1↑ | 41.4↑| 47.5↑| 35.8↑| 17.7↑| 13.0↓|
| R vs NT | 12.0↑| 25.6↑| 52.5↑| 24.0↓| 52.6↑| 39.0↓|
| L vs NT | 11.0↑| 21.2↓| 9.6↑ | 51.2↓| 42.4↑| 6.1↓ |

|        |      |      |      |      |      |      |
| **GAGs** |      |      |      |      |      |      |
| R vs L  | 80.0↑| 43.0↑| 58.7↑| 16.7↓| 37.8↑| 22.3↑|
| R vs NT | 91.8↑| 53.1↓| 11.1↓| 13.3↑| 36.0↑| 51.9↑|
| L vs NT | 59.0↑| 73.3↓| 63.3↓| 27.8↑| 2.7↓ | 62.6↑|
UNITED STATES PATENT APPLICATION FOR:

TOPICAL COMPOSITION COMPRISING UMBILICAL CORD BLOOD SERUM

INVENTORS:

Alvin Needleman

Attorney Docket No.: 838611-0002
TOPICAL COMPOSITION COMPRISING
UMBILICAL CORD BLOOD SERUM

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is based on and claims priority to U.S. Provisional Application Serial No. 61/278,040, filed on October 2, 2009 which is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

BACKGROUND OF THE INVENTION

[0003] 1. FIELD OF THE INVENTION

[0004] The present invention relates to the field of cosmetics. It relates more particularly to novel cosmetic compositions comprising umbilical cord blood serum, and to novel uses of such compositions in the field of cosmetics, especially as an anti-aging and anti-wrinkle formulation.

[0005] 2. DESCRIPTION OF RELATED ART

[0006] The gradual development of facial wrinkles, whether fine surface lines or deeper creases and folds, is an early sign of accumulated skin damage and skin aging, which may be intrinsic and/or caused or accelerated by external factors. For example, premature aging and wrinkling of the skin may be accelerated by excessive exposure to the sun and other damaging elements, overactive facial expression muscles, frequent use of tobacco products, poor nutrition, or skin disorders. Fine surface wrinkles that progress to deeper creases, deepening facial expression due to repeated skin folding, and deep folds which develop with one's maturity are visible changes which may combine to portray a less desirable appearance.
Various attempts at anti-aging skin care compositions have used botanicals, antioxidants, and biopeptides, among other things. Several invasive techniques are available in which substances are injected or implanted in the area of the skin which either temporarily weaken the muscles or act as skin volume fillers. However, invasive techniques are often risky and require the supervision or assistance of a physician, which can be inconvenient and costly, and non-invasive treatments have historically met with only minimal success. Regardless of the cause of facial creases or folds, safe and effective treatments for reduction or elimination of these problems have been exceedingly difficult to achieve. Thus, there remains a need for new and improved topical skin care compositions that are useful as an anti-aging composition.

**BRIEF SUMMARY OF THE INVENTION**

The present invention is directed to novel skin care composition comprising an effective amount of umbilical cord blood serum for topical application to the human skin. The compositions are useful for imparting an so-called "anti-aging" benefits to the skin.

In one aspect, the umbilical cord blood serum is present in an amount from about 0.0001 wt% to about 90 wt% of the composition, more preferably between about 0.01 wt% to about 25 wt%, and still more preferably about 0.01 wt % to about 10 wt%.

In still another aspect, the composition is formulated with other cosmetic actives and excipients. For example, in one exemplary aspect, the skin care composition comprises a peptide selected from the group consisting of the tyr-arg, acetyl hexapeptide-3, palmitoyl oligopeptide, palmitoyl tetrapeptide-7, and mixtures thereof. In another exemplary aspect, the skin care composition comprises a biological additive selected from the group consisting of *Juglans regia, Centella asiatica, Pyrus germanica* extract, and mixtures thereof. In another exemplary aspect, the skin care composition comprises a biological additive selected from the
group consisting of *Undaria pinnatifida* and *Stevia rebaudiana Bertoni*, and mixtures thereof. In still another exemplary aspect, the skin care composition comprises one or more phospholipids, such as one selected from the group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, diphosphatidyl glycerol, and mixtures thereof. In still another exemplary aspect, the skin care composition comprises one or more preservatives, such as methyl and/or propyl paraben. In yet another exemplary aspect, the skin care composition comprises one or more penetration enhancers, such as dimethyl isosorbide and diethyl-glycol-monoethylether. In still another exemplary aspect, the skin care composition comprises one or more neutralizing agents, such as triethanolamine. In yet another exemplary aspect, the skin care composition comprises one or more hyaluronans. In still a further exemplary embodiment, the skin care composition comprises one or more skin-conditioning emollients, such as those selected from the group jojoba oil, almond oil, capric/caprylic triglyceride and mixtures thereof. In yet another exemplary aspect, the skin care composition comprises one or more surfactants, such as myristamidopropyl PG-dimonium chloride phosphate. In yet another aspect, the skin care composition comprises one or more spreading agents, such as PPG-3 benzyl ether myristate is used as a spreading agent. In another aspect, the skin care composition comprises one or more gelling agents, such as carbomer.

[0011] In another aspect, the skin care composition comprises about 0.1 to about 0.35 wt% umbilical cord blood serum, about 3 to 5 wt% palmitoyl oligopeptide, about 3 to about 5 wt% palmitoyl tetrapeptide-7. In still yet another aspect, the skin care composition comprises about 2 to about 3 wt% of the dipeptide tyr-arg and about 0.5 to 1.5 wt% acetyl hexapeptide-3. Yet in another aspect, he skin care composition about 0.5 to about 1.2 wt% phosphatidyl choline, about 0.5 to about 1.5 wt % hyaluronans, and 0.01 to 0.085 of a preservative selected from the
The present invention is also directed to a method for imparting an anti-aging benefit to human skin comprising: topically applying to the skin of an individual in need thereof any of the foregoing compositions. In a preferred aspect, application of the composition to the skin results in improved procollagen and glycosaminoglycans content.

Additional aspects of the invention, together with the advantages and novel features appurtenant thereto, will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned from the practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

**DETAILED DESCRIPTION OF PREFERRED EMBODIMENT**

The present invention is directed to novel skin care compositions for topical application to the human skin comprising an effective amount of umbilical cord blood serum. The umbilical cord blood serum is present in an effective amount to treat, reverse, ameliorate, and/or repair signs of skin damage or skin aging. Such benefits may include without limitation, the following: (a) treatment, reduction, and/or prevention of fine lines or wrinkles, (b) improvement in skin thickness, plumpness, and/or tautness; (c) improvement in skin suppleness and/or softness; (d) improvement in skin tone, radiance, and/or clarity; (e) improvement in procollagen and/or collagen production; (f) improvement in maintenance and remodeling of elastin; (g) improvement in skin texture and/or promotion of retexturization; (h) improvement in
skin barrier repair and/or function; (i) improvement in appearance of skin contours; (j) restoration of skin luster and/or brightness; (k) replenishment of essential nutrients and/or constituents in the skin; (l) improvement of skin appearance decreased by aging; (m) improvement in skin moisturization and/or hydration; (n) increase in and/or preventing loss of skin elasticity and/or resiliency; (o) treatment, reduction, and/or prevention of skin sagging; and/or (p) treatment, reduction, and/or prevention of discoloration of skin.

[0015] In practice, the compositions of the invention are applied to skin in need of treatment. That is, the composition is applied to skin which suffers from a deficiency or loss in any of the foregoing attributes or which would otherwise benefit from improvement in any of the foregoing skin attributes. As such, in certain preferred embodiments the compositions and methods of the invention are directed to the prevention, treatment, and/or amelioration of fine lines and/or wrinkles in the skin. In one exemplary preferred case, the compositions are applied to skin in need of treatment, by which is meant skin having wrinkles and/or fine lines. Preferably, the compositions are applied directly to the fine lines and/or wrinkles (which may be the entire face and/or neck area, or a portion thereof). The compositions and methods are suitable for treating fine lines and/or wrinkles on any surface of the skin, including without limitation, the skin of the face, neck, and/or hands.

[0016] The compositions of the present invention may be applied as needed to the skin. The composition can be applied periodically, e.g., daily, twice daily, weekly, or several times a week. The composition is generally applied for a duration of one week to indefinitely, such often will be applied for a period of 1, 2, 3, 4, 5, 6 or more months. The duration of application can also be applied for an indefinite time period, if desired. It will be appreciated that the results discussed herein will depend upon the amount frequency, and duration of application, with
highest amounts and more frequent applications providing accordingly faster results. The skin-care compositions daily preferably for at least four weeks, and more preferably at least eight weeks, by which an effect upon the appearance of skin should be observed. Application may be continued as long as desired to maintain the condition of the skin.

[0017] In addition, it is also contemplated that the compositions of the present invention may be applied to normal healthy skin, and may improve the brilliance, smoothness, radiance, and/or elasticity of the normal skin. Thus, in another aspect, the compositions are applied to the skin of the face, neck, and/or hands of a patient having normal skin. It is anticipated that the surface characteristics of the unwrinkled, unsagging skin may be improved.

[0018] The compositions according to the invention can be formulated in a variety of forms for topical application. Typically, the compositions will comprise from about 0.0001 wt% to about 90 wt% of umbilical cord blood serum, and preferably will comprise from about 0.001 wt% to about 25 wt%, more preferably from about 0.01 wt% to about 10 wt%, and still more preferably about 0.05 wt% to about 5 wt% of umbilical cord blood serum. Within the more preferred range, the composition may comprise umbilical cord blood serum within a 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1.0 wt% of the total composition. As an example, the compositions will comprise an effective amount of umbilical cord blood serum, by which is meant an amount sufficient improve procollagen and/or glycosaminoglycans, for example as discussed in the Examples. An "effective amount" in the context of umbilical cord blood serum includes one that provides a particular anti-aging benefit to the skin and refers to the amount required to provide a clinically measurable improvement in the particular manifestation of skin aging when applied for a time sufficient to provide a clinically measurable improvement in the particular manifestation of skin aging.
The composition of the present invention may be formulated in a variety of product forms, such as, for example, a lotion, cream, serum, spray, aerosol, cake, ointment, essence, emulsion, gel, paste, patch, pencil, towelette, mask, stick, foam, concentrate, and the like, particularly for topical administration. Preferably the composition is formulated as a lotion, cream, ointment, serum, or gel.

The compositions of the present invention may include a cosmetically acceptable vehicle. Such vehicles may take the form of any known in the art suitable for application to skin and may include water (e.g., deionized water); vegetable oils; mineral oils; esters such as octyl palmitate, isopropyl myristate and isopropyl palmitate; ethers such as dicapryl ether and dimethyl isosorbide; alcohols such as ethanol and isopropanol; fatty alcohols such as cetyl alcohol, cetearyl alcohol, stearyl alcohol and biphenyl alcohol; isoparaffins such as isoctane, isododecane and is hexadecane; silicone oils such as cyclomethicone, dimethicone, dimethicone cross-polymer, polysiloxanes and their derivatives, preferably organomodified derivatives; hydrocarbon oils such as mineral oil, petrolatum, isoeicosane and polyisobutene; polyols such as propylene glycol, glycerin, butylene glycol, pentylene glycol and hexylene glycol; waxes such as beeswax and botanical waxes; or any combinations or mixtures of the foregoing.

The composition may optionally comprise other cosmetic actives and excipients, obvious to those skilled in the art including, but not limited to, fillers, emulsifying agents, antioxidants, surfactants, film formers, chelating agents, gelling agents, thickeners, emollients, humectants, moisturizers, vitamins, minerals, viscosity and/or rheology modifiers, sunscreens, keratolytics, depigmenting agents, retinoids, hormonal compounds, alpha-hydroxy acids, alpha-keto acids, anti-mycobacterial agents, antifungal agents, antimicrobials, antivirals, analgesics, lipidic compounds, anti-allergenic agents, H1 or H2 antihistamines, anti-inflammatory agents,
anti-irritants, antineoplastics, immune system boosting agents, immune system suppressing agents, anti-acne agents, anesthetics, antiseptics, insect repellents, skin cooling compounds, skin protectants, skin penetration enhancers, exfollients, lubricants, fragrances, colorants, depigmenting agents, hypopigmenting agents, preservatives, stabilizers, pharmaceutical agents, photostabilizing agents, neutralizers, and mixtures thereof. In addition to the foregoing, the cosmetic compositions of the invention may contain any other compound for the treatment of skin disorders.

[0022] As used herein, "topical application" means directly laying on or spreading on outer skin.

[0023] As used herein, "cosmetically acceptable" means that drugs, medicaments, botanicals, chemicals, or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, incompatibility, instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio. Cosmetically acceptable vehicles must be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the human or lower animal being treated.

[0024] As used herein, "comprising" means that other steps and other ingredients which do not affect the end result can be added. This term encompasses the terms "consisting of" and "consisting essentially of."

[0025] The term "sagging" as used herein means the laxity, slackness, or the like condition of skin that occurs as a result of loss of, damage to, alterations to, and/or abnormalities in dermal elastin and includes the age-related loss of adhesive plaque at the dermal-epidermal junction.
The terms "smoothing" and "softening" as used herein mean altering the surface of the keratinous tissue such that its tactile feel is improved. "Signs of skin aging" include, but are not limited to, all outwardly visible or tactilely perceptible manifestations as well as any other macro or micro effects due to skin aging. Such signs may be induced or caused by intrinsic factors or extrinsic factors, e.g., chronological aging and/or environmental damage. These signs may result from processes that include, but are not limited to, the development of textural discontinuities such as wrinkles and coarse deep wrinkles, skin lines, crevices, bumps, large pores (e.g., associated with adnexal structures such as sweat gland ducts, sebaceous glands, or hair follicles), or unevenness or roughness, loss of skin elasticity (loss and/or inactivation of functional skin elastin), sagging (including puffiness in the eye area and jowls), loss of skin firmness, loss of skin tightness, loss of skin recoil from deformation, discoloration (including under eye circles), blotching, sallowness, hyperpigmented skin regions such as age spots and freckles, keratoses, abnormal differentiation, hyperkeratinization, elastosis, collagen breakdown, and other histological changes in the stratum corneum, dermis, epidermis, the skin vascular system (e.g., telangiectasia or spider vessels), and underlying tissues, especially those proximate to the skin.

The term "umbilical cord blood" or "cord blood" generally refers to blood obtained from a neonate or fetus, most preferably a neonate and preferably refers to blood which is obtained from the umbilical cord or placenta of newborns. The use of cord or placental blood is advantageous because it can be obtained relatively easily and without trauma to the donor. Cord blood is preferably obtained by direct drainage from the umbilical vein of a discarded placenta.
The term "umbilical cord blood serum" or "cord serum" generally refers to umbilical cord blood in which the cells have been removed so that the cord serum is substantially free of whole cells.

The term "wrinkle" or "wrinkling" includes both fine wrinkling and coarse wrinkling. Fine wrinkling or fine lines refers to superficial lines and wrinkles on the skin surface. Coarse wrinkling refers to deep furrows, particularly deep lines/wrinkles on the face and around the eyes, including of expression lines such as frown lines and wrinkles, forehead lines and wrinkles, crow's feet lines and wrinkles, nasolabial fold and marionette lines and wrinkles. Forehead lines and wrinkles refer to superficial lines and/or deep furrows on skin of the forehead. Crow's feet lines and wrinkles refer to superficial lines and/or deep furrows on skin around the eye area. Marionette lines and wrinkles refer to superficial lines and/or deep furrows on skin around the mouth. Wrinkles can be assessed for number, length, and depth of the lines.

Umbilical cord blood serum

The umbilical cord blood serum used in the compositions of the present invention is typically prepared in the following manner. First, umbilical cord blood is collected at the time to birth from pre-screened mothers for infectious disease causing organisms, such as HIV 1 and 2, Hbs and HCV and sexually transmitted diseases. The collection is made after the baby is separated from the clamped cord, and therefore there is no harm to the baby. Blood is collected from an umbilical vein using the conventional blood bag containing no anticoagulants. The needle of the bag is inserted into the vein and blood is allowed to flow into the blood bag. A good collection can average 40 ml and may exceed 100 ml. This blood is now allowed to clot at room temperature and transported to the processing area, which is a cGMP clean room. The
clotting process is allowed to take place from 8-16 hours. The blood is then centrifuged at 1000 g in a blood bag centrifuge and the clear serum is collected into sterile containers. The cord serum is tested for sterility by microbiological assays for aerobic or anaerobic microorganisms. The complement is inactivated by keeping the cord serum at about 56 °C for 1/2 hour. The serum is then aliquoted into 50 ml sterile vials and capped and frozen at about -70 °C for future use in the compositions of the present invention. Suitable cord serum is commercially available from Cryobanks Laboratories (Allamonte Springs, FL).

[0032] Other Optional Components

[0033] The cosmetic compositions of the present invention preferably include one or more bioactive peptides, including but not limited to, dipeptides, tripeptides, tetrapeptides, pentapeptides, and hexapeptides, and derivatives thereof. The peptides are provided in the compositions of the present invention in amounts that are safe and effective. As used herein, "peptides" refers to both the naturally occurring peptides and synthesized peptides. Also useful herein are naturally occurring and commercially available compositions that contain peptides. The peptides used in the present invention may include neuropeptides as well as so-called charge-coupled peptides.

[0034] Suitable dipeptides for use herein include carnosine (beta-ala-his) and tyr-arg. Suitable tripeptides for use herein include gly-his-lys, arg-lys-arg, and his-gly-gly. Preferred tripeptides and derivatives thereof include palmitoyl oligopeptide (palmitoyl-gly-his-lys); Peptide CK (arg-lys-arg); Peptide CK+ (ac-arg-lys-arg-NH₂); and a copper derivative of his-gly-gly sold commercially as lamin, from Sigma (St. Louis, Mo.). Suitable tetrapeptides for use herein include Peptide E, arg-ser-arg-lys (SEQ ID NO: 1); palmitoyl tetrapeptide-3/7 (palmitoyl-gly-gln-pro-arg (SEQ ID NO: 2)). Suitable pentapeptides for use herein include lys-thr-thr-lys-
ser (SEQ ID NO: 3), palmitoyl-lys-thr-thr-lys-ser (SEQ ID NO: 4). Suitable hexapeptides include acetyl hexapeptide-3 (Ac-Glu-Glu-Met-Gln-Arg-Arg (SEQ ID NO: 5)). When included in the present compositions, peptides are typically present in amounts of from about 0.01 wt% to about 10 wt%, or from about 0.1 wt% to about 6.0 wt%, or from about 1.0 to about 0.5%, by weight of the composition.

[0035] In one aspect, the cosmetic compositions of the present invention include one or more dipeptides. The preferred dipeptide comprises tyr-arg. In a preferred aspect, the tyrosine-arginine dipeptide is acetylated to make it more lipophilic, more stable, and bio-available on a cutaneous level. Acetyl tyrosine-arginine-1 cetyl ester stimulates the synthesis of the messenger neuropeptides of muscular relaxation and inhibits the synthesis of the messenger mediators of muscular contraction. The compositions of the invention preferably comprise about 0.001 to 5.0 wt% of the dipeptide, especially tyr-arg. In a preferred aspect, the compositions comprise about 2.0 to about 3.0 wt% of the dipeptide.

[0036] In one aspect, the cosmetic composition of the present invention includes one or more tripeptides and tetrapeptides. In a preferred embodiment, the cosmetic composition includes the commercially available product known as MATRIXL 3000® (Sederma Corp., France), which includes both palmitoyl oligopeptide and palmitoyl tetrapeptide-7. The compositions of the invention preferably comprise about palmitoyl oligopeptide and palmitoyl tetrapeptide-7 in a combined amount of about 0.001 to about 10 wt%. In a preferred aspect, the compositions comprise about 3.0 to about 5.0 wt% palmitoyl oligopeptide and palmitoyl tetrapeptide-7.

[0037] In one aspect, the cosmetic compositions of the present invention include one or more hexapeptides. The preferred hexapeptide used in the compositions of the invention is
acetyl hexapeptide-3. The peptide may be purchased from Lipotec under the tradename Argireline® in either the powder or solution form. The powder form appears as a white to off-white powder. The compositions of the invention preferably comprise about 0.001 to about 5.0 wt% acetyl hexapeptide-3. In a preferred aspect, the compositions comprise about 0.5 to about 1.5 wt% acetyl hexapeptide-3.

[0038] In one aspect, the cosmetic compositions of the present invention include one or more biological additives, such as botanicals or herbals. As used herein, the term "biological additive" indicates any compound obtained from a natural source, including plants, animals, bacteria and yeast, which has a medicinal or otherwise beneficial effect when topically applied to the skin. Examples of biological additives include oil of *Melaleuca alternifolia*, oil of *Lavandula angustifolia*, *Carica papaya* extract, *Echinacea angustifolia* extract, *Mimosa tenuiflora* extract, *Hydrocotyl (centella) asiatica* extract, *gingko biloba* extract, oil of *Melaleuca alternifolia* (tea tree oil), *Matricaria chamomila* (chamomile) extract, *Hypericum perforatum* extract, *Aloe barbedensis* extract, and the like. The biological sources for "biological additive" may also include, but are not limited to the following: Aloe Vera, *Aloe Barbedensis*; Arnica, *Arnica Montana*; Bladderwrack (seaweed), *Fucus Vesciculosus*; Seaweed, *Undaria pinnatifida*; Birch, *Betula Alba (Pendula)*; Chamomile, *Matricaria Chamomila (Chamomila Recutita)*; Marsh Mallow, *Althea Officinalis*; Meadow Sweet, *Spirea Ulmaria (Filipendula)*; Mint/Lemon Balm, *Melissa Officinalis*; *Mimosa Tenuiflora*; Myrrh Tincture, *Commiphor Myrrha*; Neem, *Melia Azadirachta*; Nettle (stinging), *Urtica Dioica*; Papaya, *Carica Papaya*; Propolis (bee glue), *Propolis Cera*; Raspberry, *Rubis Idaeus*; Red Poppy, *Papaver Rhoeas*; Rose Hip (dog rose), *Rosa Carima*; Rosemary, *Rosemarinus Officinalis*; Sage, *Salvia Officinalis*; St. Johns Wort, *Hypericum Perforatum*; Strawberry, *Fragaria Vesca*; Thea Sinensis (green tea), *Camelia
Sinensis; Walnut, Juglans Regia; Witchhazel (dist/extr), Hamamelis Virginiana; Yarrow, Achillea Millefolium; Wild Yam, Dioscorea Villosa; Hawthorn, Crataegus Monogina/Oxyantha; Herma (black/rod), Lawsoma Ehemus; Hops, Humulus Lupulus; Horse Chestnut, Aesculus Hippocastanum; Horse Tail, Equisitum Arvense; Ivy, Hedera Helix; Linden/Lime Tree Blossoms, Tilia Argentea Cordata; Madder, Rubia Tinctorum; Marigold, Calendula Officinalis; Centella Asiatica, Centella Asiatica Urban (hydrocotyl Asiatica); Carrot (roots), Daucus Carota; Comfrey (Allantoin), Symphytum Officinale; Coneflower (Echinacea), Echinacea Angustifolia; Cucumber, Cucumis Sativus (Fruces Cucumis); Fenugreek, Trigonella Foenum Greacum; Gingko, Gingko Biloba; Ginseng, Panax Ginseng; Great Burdock, Radix Bardanea/Arctium Lappa; Tea Tree Oil, Oil of Melaleuca Alternifolia; Colts Foot, Tussilago Farfara; Clover, Trifolium Pratense; Speedwell, Veronica Officinalis; Medlar, Pyrus Germanica.

In another aspect, the biological additive may be those selected from the group consisting of plants such as Angelica keiskei, adzuki bean, avocado, hydrangea, Gynostemma pentaphyllum, Aruteka, Arnica, almond, aloe, apricot, nettle, iris, fennel, turmeric, Eijitsu, Scutellariae radix, Amur cork tree, goldthread, barley, gumbo, Saint-John's-wort, dead nettle, ononisu, watercress, persimmon, the root of kudzu, Valeriana fauriei, birch, cattail, chamomile, chamomilla, oats, licorice, raspberry, kiwi, cucumber, apricot, coconut, cape jasmine, Sasa albo-marginata, a walnut, cinnamon, mulberry, gunjo, gentian, cranesbill, burdock, sesame, wheat, rice, Camellia sasangua, saffron, hawthorn, Japanese pepper tree, mushroom, Rehmannia clutinosa, prop root, beefsteak plant, Japanese linden, Filipendula multijuga, peony, ginger, calamus, white birch, Japanese honeysuckle, field horsetail, Stevia rebaudiana Bertoni, western ivy, western hawthorn, elder, needle juniper, milfoil, mint, sage, common mallow, Cnidium officinale, Japanese green gentian, soybean, daiso, thyme, tea plant, clove, dried orange peel,

[0040] In one exemplary aspect, the biological additive comprises PHYTOTAL FM (Croda Singapore) in an amount of about 0.5 to about 6.0 wt% of the composition, preferably about 3.5 to about 5.5 wt% of the composition. PHYTOTAL FM comprises glycerin, butylene glycol, *juglans regia* leaf extract, *Juglans regia* shell extract, *Centella asiatica* extract, *Pyrus germanica* extract, lecithin. In another exemplary aspect, the cosmetic compositions of the present invention comprise about 0.1 to about 6.0 wt%, preferably about 2.0 to about 4.0 wt% seaweed, *Undaria pinnatifida*. In still another exemplary aspect, the cosmetic compositions of the present invention comprise *Stevia rebaudiana Bertoni* in an amount of about 0.1 to about 6.0 wt%, with about 2.0 to about 4.0 wt% being most preferred.

[0041] In general, these extracts can be obtained by grinding the whole of the respective plants or one or more of their parts (hereinafter referred to as "stocks" such as leaves, bark, roots, branches, seeds or fruits or nuts, and flowers or blossoms after drying them or without drying them, and then extracting them either with a solvent or by means of an extractor such as a Soxhlet's extractor at ordinary temperature or an elevated temperature. No particular limitation
is imposed on the solvent used here. However, examples thereof include known solvents, such as water, primary alcohols such as methyl alcohol and ethyl alcohol, liquid polyhydric alcohols such as propylene glycol and 1,3-butylene glycol, lower alkyl esters such as ethyl acetate, hydrocarbons such as benzene and hexane, ethyl ether, and acetone. These solvents may be used either singly or in any combination thereof.

[0042] The cosmetic compositions also preferably includes one or more lipids, preferably one or more phospholipids. Examples of three classes of phospholipids are phosphoglycerides, lysophosphoglycerides, and and sphingomyelins. Examples of phosphoglycerides include phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, diposphatidyl glycerol, and mixtures thereof. The most preferred phosphoglycerides include phosphatidyl choline and lecithin, particularly soybean lecithin, which comprises a mixture of some of the above examples of specific phosphoglycerides. Examples of lysophosphoglycerides includes: lysophosphatidyl choline, lysophosphatidyl ethanolamine, lysophosphatidyl serine, lysophosphatidyl inositol, and mixtures thereof. The lipids (e.g. phosphadityl choline) preferably comprise about 0.1 to about 6.0 wt% of the cosmetic composition, with about 0.5 to about 1.2 wt% being most preferred.

[0043] The cosmetic compositions of the present invention may also comprise one or more preservatives. Suitable traditional preservatives for compositions of this invention are alkyl esters of para-hydroxybenzoic acid. Other preservatives include hydantoin derivatives such as 1,3-bis(hydroxymethyl)-5,5-dimethylhydantoin, propionate salts, and a variety of quaternary ammonium compounds such as benzalkonium chloride, quaternium 15, benzethonium chloride, and methylbenzethonium chloride. Cosmetic chemists are familiar with appropriate preservatives and routinely choose them to satisfy the preservative challenge test and to provide
product stability. Particularly preferred preservatives are disodium EDTA, phenoxyethanol, methyl paraben, propyl paraben, imidazolidinyl urea, sodium dehydroacetate and benzyl alcohol. The preservatives should be selected having regard for the use of the composition and possible incompatibilities between the preservatives and other ingredients in the emulsion. The preservatives preferably are employed in amounts ranging from about 0.001 wt% to about 5 wt%, more preferably from about 0.01 wt% to about 2.5 wt%, and most preferably from about 0.01 wt% to about 1 wt%, by weight of the composition.

[0044] The cosmetic compositions of the present invention may also comprise one or more penetration enhancers. As used herein, a penetration enhancer is a material capable of aiding the penetration of the active agents into the skin. Examples of penetration enhancers include, but are not limited to, dimethyl isosorbide and diethyl-glycol-monoethylether. The penetration enhances typically comprise about 0.5 to about 5 wt% of the composition, preferably about 1.0 to 3.0 wt%.

[0045] In still another aspect, the compositions of the present invention may further comprise one or more neutralizing gents or pH adjusters, which may be used to adjust the pH of the compositions. The term "neutralizing agent," as used herein, refers to a material that may be used to modify the pH of the present compositions, for example, from an acidic pH to a more basic pH, or from a basic pH to a more acidic pH. Components of the present compositions, such as certain of the thickening agents, may be acidic, and may be preferably neutralized to achieve the desired thickening effect. Accordingly, the neutralizing agents are preferably those materials which may be used to modify the pH of the present compositions from an acidic pH to a more basic pH.
A wide variety of neutralizing agents are known to those skilled in the art and may be used in the practice of the present invention. Exemplary neutralizing agents include, for example, ammonium hydroxide, arginine, 2-amino-2-methyl-1-propanol (AMP-95 (Angus)), dimethanolamine, dibutanolamine, diisobutanolamine, tributanolamine, triisobutanolamine, tri-sec-butanolamine, tripropylamine, ethanolamine, diethanolamine, triethanolamine, PEG-15 cocamine, diisopropanolamine, methylethanolamine, diisopropylamine, dipropyleneetriamine, tromethamine, isopropylamine ethylene diamine, triisopropanolamine, tetrahydroxypropyl ethylenediamine, trimethamine, 2-aminobutanol, aminoethyl propanediol, aminomethyl propanediol, aminomethyl propanol, sodium hydroxide, potassium hydroxide and mixtures thereof. Most preferably, the neutralizing agent triethanolamine. The amount of neutralizing agent in the cosmetic composition is preferably about 1.0 to about 6.0 wt% with about 0.75 to about 1.5 wt% being most preferred.

In one aspect, the cosmetic compositions of the present invention include one or more hyaluronans. Preferably, the hyaluronan is present in the form of hyaluronic acid or salt thereof or a homologue, analogue, derivative, complex, ester, fragment and subunit of hyaluronic acid. More preferably, the hyaluronic acid in the form of sodium hyaluronate and is of medical grade and has an average molecular weight of about 700 kilodaltons. The compositions of the invention preferably comprise about 0.01 to about 4.0 wt% hyaluronan (in the form of sodium hyaluronate). In a preferred aspect, the compositions comprise about 0.5 to about 1.5 wt% hyaluronan (in the form of sodium hyaluronate).

In one aspect, the cosmetic compositions of the present invention include one or more skin-conditioning emollients. The emollient functions as a softener to help the composition give a desirable feel on the skin. Useful emollients include, but are not limited to fatty bodies...
liquid at ambient temperature, such as esters, mineral oils, animal oils, vegetable oil, synthetic oils, and silicone oils. Examples of useful esters include, but are not limited to, isononyl isononanoate, octyl palmitate, cetyl lactate, pentaerythrityl tetraoctanoate, tridecyl octanoate, tridecyl behenate, isopropyl jojobate and jojoba alcohols, butyloctyl salicylate, polyglyceryl-3 diisostearate, squalane, tridecyl trimellitate, tridecyl stearate, and neopentylglycol dicaprylate/dicaprate. Examples of useful oils include, but are not limited to, petrolatum oil, liquid lanolin, arara oil, sesame oil, macadamia oil, almond oil, jojoba oil, silicone oils such as phenyl trimethicone and dimethicone, and synthetic triglycerides such as capric/caprylic triglyceride and hydrogenated cocoglycerides. The emollient(s) can be present in the present invention in an amount about 0.1 wt% to about 6 wt%. In an exemplary aspect, the cosmetic composition comprises about 0.1 to about 6 wt%, and more preferably about 0.75 to 1.5 capric/caprylic triglycerides. In another aspect, the cosmetic composition comprises about 0.1 to 6 wt%, and more preferably about 0.3 wt% to about 0.7 wt% almond oil. In an another aspect, the cosmetic composition comprises about 0.1 to about 6 wt%, and more preferably about 0.75 wt% to about 1.5 wt% jojoba oil.

[0049] In still another aspect, the cosmetic compositions may contain one or more surfactants. Exemplary surfactants are disclosed in Harrison et al. U.S. Patent No. 6,642,194, which is incorporated by reference. A preferred surfactant is myristamidopropyl PG-dimonium chloride phosphate and it has cationic properties and is also a preservative booster. Another preferred surfactant are the betaines, preferably alkylamidoalkylbetaines, such cocoamidopropylbetaine.

[0050] Other agents that may form part of the cosmetically acceptable vehicle of the cosmetic formulation include carbomers, propylene glycol, butylene glycol, dipropylene glycol,
glycerin, glycereth-18 ethylhexanoate, glycereth-18, betaine, diglycerin, glycol, inositol, meadowfoamamidopropyl betaine, ethyl alcohol, isopropyl alcohol, polyethylene glycol with varied molecular weights, sorbitol, xylitol, urea, tripropylene glycol, sodium PCA, glycereth-7 glycolate, diglycereth-7 malate, 2,3-butanediol, propanediol, xylose, almond oil PEG-6 esters, apricot kernel oil PEG-6 esters, argan oil PEG-8 esters, argan oil polyglyceryl-6 esters. Other vehicle agents include PEG-3 dimethicone, PEG/PPG-20/23 dimethicone; PEG-8 dimethicone, cyclomethicone, dimethicone, cetyl dimethicone, caprylyl methicone, ethyl trisiloxane, trimethylsiloxyamodimethicone, stearyl dimethicone, silicones with polypropylene glycol functionality such as PPG-12 dimethicone, silicones with polyethylene glycol functionality such as PEG-8 trisiloxane, PEG-10 dimethicone and silicones which combine both functionalities in varying ratios such as PEG/PPG-5/3 trisiloxane, PEG/PPG-8/26 dimethicone, PEG/PPG-20/15 dimethicone, bis-PEG-4 dimethicone, bis-PEG-12 dimethicone, bis-PEG/PPG-14/14 dimethicone, bis-PEG/PPG-18/6 dimethicone, bis-PEG/PPG-20/20 dimethicone, butylene glycol behenate, butylene glycol diisononanoate, butylene glycol laurate, butylene glycol myristate, butylene glycol oleate, butylene glycol palmitate, butylene glycol stearate, butyl isostearate, butyl myristate, butyloctyl behenate, butyloctyl benzoate, butyloctyl cetearate, butyloctyl palmitate, butyl oleate, butyl stearate C14-15 alcohols, C18-28 alkyl acetate, C12-15 alkyl benzoate, C16-17 alkyl benzoate, C30-45 alkyl cetearyl dimethicone crosspolymer, C32 alkyl dimethicone, C30-45 alkyl dimethicone/polycyclohexene oxide crosspolymer, C12-13 alkyl ethylhexanoate, C12-15 alkyl ethylhexanoate, C14-18 alkyl ethylhexanoate, C12-13 alkyl lactate, C12-15 alkyl lactate, C20-24 alkyl methicone, C24-28 alkyl methicone, calodium capense nut oil, calophyllum tacamahaca seed oil, cetearyl dimethicone/vinyl dimethicone crosspolymer, cetearyl ethylhexanoate, cetearyl isononanoate, cetearyl nonanoate, cetearyl
palmitate, cetrimonium laureth-12 succinate, cetyl acetate, cetyl caprylate, cetyl C12-15 pareth-8 carboxylate, cetyl dimethicone, cetrimonium dimethicone/bis-vinyl dimethicone crosspolymer, cetyl dimethyl octanoate, cetyl esters, cetyl ethylhexanoate, cetyl glyceryl ether, cetyl glycol, cetyl glycol isostearate, cetyl isononanoate, cetyl lactate, cetyl laurate, cetyl oleate, cetyloxy dimethicone, C12-15 pareth-3 benzoate, C 12-15 pareth-9 hydrogenated tallowate, C11-15 pareth-3 oleate, C 12-15 pareth-12 oleate, C 11-15 pareth-3 stearate, C11-15 pareth-12 stearate, dibutyl adipate, dibutyldecyl IPDI, dibutyloctyl IPDI, dibutyloctyl malate, dibutyloctyl sebacate, dibutyl sebacate, Ddi-C12-15 alkyl adipate, di-C12-15 alkyl fumarate, di-C12-13 alkyl malate, di-C12-15 alkyl maleate, di-C12-13 alkyl tartrate, -C14-15 alkyl tartrate, dicaprylyl carbonate, dicaprylyl ether, dicaprylyl maleate, dicetyl adipate, dicocoyl pentaerythrityl distearyl citrate, diethyl adipate, isobutyl myristate, isobutyl palmitate, isobutyl pelargonate, isobutyl stearate, isobutyl tallowate, isocetyl alcohol, isocetyl ethylhexanoate, isocetyl isodecanoate, isocetyl isostearate, isocetyl laurate, isocetyl linoleyl stearate, isocetyl palmitate, isocetyl stearate, lanolin, lanolin oil, lanolin wax, lauryl lactate, neopentyl glycol diheptanoate, neopentyl glycol diisononanoate, neopentyl glycol dilaurate, octyldodecyl ethylhexanoate, octyldodecyl lactate, octyldodecyl neodecanoate, octyldodecyl neopentanoate, PPG-3 benzyl ether myristate, PPG-1 -ceteth-1, PPG- 1 -ceteth-5, PPG-1-ceteth-10, PPG-1-ceteth-20, sunflower oil, safflower oil, mineral oil, almond oil, and jojoba oil diisoamyl malate, diethylhexyl malate, dibutyloctyl malate, dimethyl capramide, diethylhexyl 2,6 naphthalate, N,N-dimethyldesamide, diisopropyl adipate, phenethyl benzoate, octocrylene, PEG-7 methyl ester, and combinations thereof. In one aspect, PPG-3 benzyl ether myristate is used as a spreading agent. In another aspect, a carbomer is used as a gelling agent or rheology modifier.
The invention provides a method for treating aging skin by topically applying a cosmetic composition comprising umbilical cord blood serum, preferably in a cosmetically acceptable vehicle, over the affected area for a period of time sufficient to reduce, ameliorate, reverse, or prevent dermatological signs of aging. This method is particularly useful for treating signs of skin photoaging and intrinsic aging.

Generally, the improvement in the condition and/or aesthetic appearance involves the regulation of wrinkles and/or surface enhancement, such as radiance and glow. In one aspect, improvement in the condition and/or aesthetic appearance involves is selected from the group consisting of: reducing dermatological signs of chronological aging, photo-aging, hormonal aging, and/or actinic aging; preventing and/or reducing the appearance of lines and/or wrinkles; reducing the noticeability of facial lines and wrinkles, facial wrinkles on the cheeks, forehead, perpendicular wrinkles between the eyes, horizontal wrinkles above the eyes, and around the mouth, marionette lines, and particularly deep wrinkles or creases; preventing, reducing, and/or diminishing the appearance and/or depth of lines and/or wrinkles; improving the appearance of suborbital lines and/or periorbital lines; reducing the appearance of crow's feet; rejuvenating and/or revitalizing skin, particularly aging skin; reducing skin fragility; preventing and/or reversing of loss of glycosaminoglycans and/or collagen; ameliorating the effects of estrogen imbalance; preventing skin atrophy; preventing, reducing, and/or treating hyperpigmentation; minimizing skin discoloration; improving skin tone, radiance, clarity and/or tautness; preventing, reducing, and/or ameliorating skin sagging; improving skin firmness, plumpness, suppleness and/or softness; improving procollagen and/or collagen production; improving skin texture and/or promoting retexturization; improving skin barrier repair and/or function; improving the appearance of skin contours; restoring skin luster and/or brightness;
minimizing dermatological signs of fatigue and/or stress; resisting environmental stress; replenishing ingredients in the skin decreased by aging and/or menopause; improving communication among skin cells; increasing cell proliferation and/or multiplication; increasing skin cell metabolism decreased by aging and/or menopause; retarding cellular aging; improving skin moisturization; enhancing skin thickness; increasing skin elasticity and/or resiliency; enhancing exfoliation; improving microcirculation; decreasing and/or preventing cellulite formation; and any combinations thereof.

[0053] The composition will typically be applied to the skin one, two, or three times daily for as long as is necessary to achieve desired anti-aging results. The treatment regiment may comprise daily application for at least one week, at least two weeks, at least four weeks, at least eight weeks, or at least twelve weeks. Chronic treatment regimens are also contemplated.

[0054] A composition comprising umbilical cord blood serum is topically applied to an "individual in need thereof," by which is meant an individual that stands to benefits from reducing visible signs of skin damage or aging. In a specific embodiment, the umbilical cord blood serum is provided in a pharmaceutically, physiologically, cosmetically, and dermatologically-acceptable vehicle, diluent, or carrier, where the composition is topically applied to an affected area of skin and left to remain on the affected area in an amount effective for improving the condition and aesthetic appearance of skin.

[0055] In one embodiment, methods for treating fine lines and wrinkles comprise topically applying the inventive compositions comprising umbilical cord blood serum to the skin of an individual in need thereof, e.g., topically application directly to the fine line and/or wrinkle in an amount and for a time sufficient to reduce the severity of the fine lines and/or wrinkles or to prevent or inhibit the formation of new fine lines and/or wrinkles. The effect of a composition
on the formation or appearance of fine lines and wrinkles can be evaluated qualitatively, e.g., by visual inspection, or quantitatively, e.g., by microscopic or computer assisted measurements of wrinkle morphology (e.g., the number, depth, length, area, volume and/or width of wrinkles per unit area of skin). This embodiment includes treatment of wrinkles on the skin of the hands, arms, legs, neck, chest, and face, including the forehead.

[0056] It is also contemplated that the compositions of the invention will be useful for treating thin skin by topically applying the composition to thin skin of an individual in need thereof. "Thin skin" is intended to include skin that is thinned due to chronological aging, menopause, or photo-damage. In some embodiments, the treatment is for thin skin in men, whereas other embodiments treat thin skin in women, pre-menopausal or post-menopausal, as it is believed that skin thins differently with age in men and women, and in particular in women at different stages of life.

[0057] The method of the invention may be employed prophylactically to forestall aging including in patients that have not manifested signs of skin aging, most commonly in individuals under 25 years of age. The method may also reverse or treat signs of aging once manifested as is common in patients over 25 years of age.

[0058] The following examples are directed to various exemplary embodiments of the skin care compositions and their use in accordance with the present invention.

**Example 1: Cosmetic composition**

[0059] In this example, an exemplary cosmetic composition was prepared in accordance with the present invention. The cosmetic composition is ideally suited to be used as an anti-aging cream. Table 1 provides a list of the various ingredients.

**Table 1: Formulation Containing Cord Serum**
<table>
<thead>
<tr>
<th>Formulation Components</th>
<th>Range %</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl Hexapeptide-3 (Argireline)</td>
<td>0.5-1.5</td>
<td>A</td>
</tr>
<tr>
<td>Dipeptide Tyrosil Arginine (Calmomensine, Hydroxyethylcellulose, Laureth-3 Acetyl Dipeptide, Cetyl Ester)</td>
<td>2.0-3.0</td>
<td>A</td>
</tr>
<tr>
<td>Human Umbilical Cord Serum</td>
<td>0.1-0.35</td>
<td>A</td>
</tr>
<tr>
<td>Juglans Regia leaves &amp; shell extract, Centella leaves, Pyrus Germanica leaves, Lecithin (PHYTOTAL FM)</td>
<td>3.5-5.5</td>
<td>A</td>
</tr>
<tr>
<td>Palmitoyl Oligopeptide and Palmitoyl Tetrapeptide-7 (MATRIXL 3000)</td>
<td>3.0-5.0</td>
<td>A</td>
</tr>
<tr>
<td>Phosphatidyl choline</td>
<td>0.5-1.2</td>
<td>A</td>
</tr>
<tr>
<td>Stevia Rebaudiana Bertoni (Phytesence Stevia)</td>
<td>1.0-2.5</td>
<td>A</td>
</tr>
<tr>
<td>Undaria Pinnatifidia (Phytesence Wakami)</td>
<td>2.0-4.0</td>
<td>A</td>
</tr>
<tr>
<td>Deionized water qs</td>
<td>35-45ml</td>
<td>B</td>
</tr>
<tr>
<td>Dimethyl Isosorbide (Arlasolve DMI-PC)</td>
<td>1.0-3.0</td>
<td>B</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.01-0.085</td>
<td>B</td>
</tr>
<tr>
<td>Propyl Paraben</td>
<td>0.01-0.085</td>
<td>B</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>0.75-1.5</td>
<td>B</td>
</tr>
<tr>
<td>Hyaluronic Acid (Sodium hyaluronate)</td>
<td>0.5-1.5</td>
<td>C</td>
</tr>
<tr>
<td>Myristamidopropyl PG-Dimonium Chloride Phosphate (Arlasilk Phospholipis PTM)</td>
<td>0.3-0.6</td>
<td>C</td>
</tr>
<tr>
<td>PPG-3 Benzyl Ether Myristate (Crodamol STS)</td>
<td>2.0-3.8</td>
<td>C</td>
</tr>
<tr>
<td>Almond Oil (Cropure Almond)</td>
<td>0.3-0.7</td>
<td>D</td>
</tr>
<tr>
<td>Caprylic/Capri triglycerides (Crodamol GTCC)</td>
<td>0.75-1.5</td>
<td>D</td>
</tr>
<tr>
<td>Jojoba Oil</td>
<td>0.75-1.5</td>
<td>D</td>
</tr>
<tr>
<td>Carbomer 924 (Optasense G-34)</td>
<td>0.75-1.5</td>
<td>D</td>
</tr>
</tbody>
</table>

[0060] The components of Part A were mixed together and heated to about 70 to 80 °C. The mixture was allowed to cool to about 50 °C. The Part A mixture was then homogenized at increasing rates. In this example, the mixtures was homogenized for about 60 seconds at about 11,000 RPM, about 60 seconds at about 13,000 RPM, about 60 seconds at about 19,00 RPM, about 60 seconds at about 22,000 RPM, and about 60 seconds at about 24,000 RPM.

[0061] Separately, the components of Part B were mixed together and heated to about 70 to 80 °C. After cooling to about 50 °C, the mixture from part A was added to Part B with continuous mixing. The Part A/B mixture was then allowed to cool to about 40 °C.
[0062] Separately, the components of Part C were mixed together with sufficient deionized water to solubilize the components of Part C.

[0063] Lastly, the Part A/B mixture, Part C mixture, and Part D components were all mixed together at about 75 to 80°C. The mixing was continued to provide a uniform texture as the product cooled. In the laboratory, an IKA Turrax homogenizer T-25 Basic S1 equipped with an IKA S25N-10G dispersing tool was used. The dispersing tool is immersed in the completed formula, and the homogenizer is operated for about 5 to 8 minutes at about 24,000 rpm. The intense shearing reduces particles size to about 50 to 1000 nanometers.

**Example 2: Histogeometric Analysis**

[0064] This example was designed to test the effects that the topical cream containing cord serum of Example 1 had on human skin. As a control, the topical cream minus the cord serum was also evaluated and both products were compared with an untreated site.

[0065] **Methods**

[0066] Six healthy volunteers were enrolled in the study. Product A was applied to the right aspect of the upper inner arm (R site), product B was applied to the left aspect of the upper inner arm (L site) and the volar forearm was chosen as the untreated site. Both products were applied daily for 26 days. The volar forearm received no product but was rubbed gently, daily, in an effort to simulate the manner in which both products were applied to the skin.

[0067] At the end of the treatment period, a 2-3 mm punch biopsy specimen was obtained from each treated site and the untreated site. Each specimen was immediately placed in 10% buffered formalin and processed for paraffin sectioning. All sections were five-micrometers in thickness and all slides were stained simultaneously as a group for each histochemical determination. The entire biopsy specimen was photographed with a high
resolution digital camera system (Axiocam, Zeiss Corporation) mounted on a Zeiss Axioplan 2 light microscope at a magnification of 20x. All slides were photographed under the identical white balance light settings and exposure time to insure consistency in micrographs. Micrographs were subsequently analyzed using computer assisted image analysis software (Axiovision, Zeiss Corporation). All measurements were made from at least four areas of the biopsy specimen, except for two biopsies, which had smaller amounts of tissues and thus three measurements were made.

[0068] Five-micrometer paraffin sections were stained with hematoxylin-eosin ("H&E") for overall morphologic evaluation and viable epidermal thickness determinations (VET; Table 2). For estimation of viable epidermal thickness, care was taken to cut the sections perpendicular to the surface. The VET includes the area from the dermoepidermal interface to the lowermost portion of the stratum corneum. For estimation of elastic fibers, van Geison’s stain was employed, which stains elastic fibers blue-black to black, collagen pale red, other tissue elements yellow, and nuclei blue to black. For pro-collagen, anti-type I collagen (EMD Bioscience Inc.) antibody was used. This antibody to type I collagen was made against the triple helical portion and it is able to stain procollagen I. Immunohistochemical analysis of the paraffin sections was carried out using the DAB kit, which produces a brown reaction product. For glycosaminoglycans ("GAGs"), Hale’s colloidal iron was used since Hale’s stainable material (blue) represents, for the most part, GAGs and is commonly used as an indicator of changes in ground substance.

[0069] The quantification of stainable material was determined using a custom designed software program, integrated into the Axiovision image analysis system (Zeiss Corporation). The analysis is conducted in the following manner: (i) the reaction product (i.e., blue-black –
elastin; brown – pro-collagen; blue – GAGs) is detected from a histogram and only objects with that color are outlined on the micrograph. The total area occupied by the outlined areas is measured; (ii) the entire area of the dermis is outlined and measured; and (iii) area of reaction product divided by total area = the percentage of material deposited.

It should be noted that all photomicrographs were taken and analyses performed in a double blind manner, and only after the data was tabulated was the investigator informed about the identity of the R and L sites.

Results

Morphology

The epidermis did not appear to be morphologically altered in any of the subjects at the two treatment as well as the untreated site. In most instances, the undulating nature of the dermoepidermal interface was maintained. The granular layer was prominent in all specimens and there was little evidence of apoptosis (sunburn cells) within the epidermis. The “basketweave” architecture of the stratum cornea, characteristic of formalin-fixed human skin was maintained in all subjects in all sites.

For the most part, the fibrous components of the dermis (i.e., collagen, elastin) did not appear altered on the H&E sections from any of the treatment regimens or the untreated sites of the six subjects. In some cases, the dermis from the untreated site appeared more compact. In a few subjects, occasional areas of blue-gray staining material, usually associated with elastosis, were noted; however, frank signs of photodamage were not seen in any of the subjects. Importantly, there did not appear to be an unusual amount of inflammatory cells in biopsies from any of the treatment sites or in the untreated site from any of the subjects. Some increased cellularity was noted around portions of hair follicles present in some of the sections but this was
not deemed significant. Vascular profiles appeared normal and there was no evidence of increased vascularity, vasodilatation and/or extravasation of red blood cells.

[0075] **Viable Epidermal Thickness (VET)**

[0076] There was no consistent trend seen in the VET measurements (Tables 2 and 3). Subjects 1, 2, and 6 had similar VET values for the untreated, R and L sites. Subject 3 had a thinner VET measurement for the untreated site compared with the R and L sites. Subject 4 had a thinner VET for the R site compared with the untreated and L site, whereas Subject 5 had a thinner VET for the L site when compared with the R and untreated sites. Given the lack of inflammation, which usually is responsible for epidermal thickening, it is not surprising that VET was not affected by either R or L treatment.

[0077] **Elastin**

[0078] The overall area of the dermis occupied by elastin appeared to be greater in the untreated sites from all six subjects when compared with either the R or L treatment site (Tables 2 and 3). This finding in no way implies that either of the treatments had a negative impact on elastin fiber deposition, synthesis and/or destruction. It most likely represents inherent differences in elastin content between the volar forearm and the upper inner arm.

[0079] With respect to treatment sites, there was no obvious trend (Tables 2 and 3). Subjects 1 and 4 had significantly more elastin-stained material in the L site when compared with the R site. In contrast, subject 3 had significantly more elastin-stained material in the R site compared with the L site. There was no significant difference in elastin-stained material when the R and L sites were compared in Subjects 2, 5, and 6. When all six subjects were compared there was no change in elastin-stained material (Table 2). This is not surprising since elastin is one of the more stable components of the dermis with an extremely long turnover time. New
elastin deposition is most often seen during tissue regeneration following a wound. Thus the lack of evidence for skin perturbation due to either of the treatments could account for the failure to detect a change in elastin.

Procollagen

Five of the six subjects showed an increase in the immunostaining for procollagen when the R site was compared with the L site (Tables 2 and 3). Of these 5 subjects, one (#4) was significant at the P<0.05 level and two (#2 and #3) were highly significant (P<0.01 level). While an overall increase in procollagen immunostained material was detected for the R site versus the L site in Subjects 1 and 5, the difference was not statistically significant. The R and L treatment did not affect the procollagen-stainable material in Subject 6. When all six subjects were combined there was a greater amount of immunostaining for procollagen in the R site versus the L site; however due to subject to subject variability, this difference was not significant.

With respect to the untreated site, 4 subjects had less immunostained material corresponding to procollagen when compared with the R site; two subjects had more immunostained material. Due to potential differences in the dermis between the treated and untreated sites it is difficult to meaningfully interpret these changes.

GAGs

Four of the six subjects had significantly (P<0.01) increased Hale’s-stainable material in the R site when compared with the L site (Tables 2 and 3). Subjects 4 and 6 had increases in Hale’s-stainable material in the L site versus the R site; however, this difference was not statistically significant. When all six subjects were combined there was a greater amount of Hale’s stainable material in the R site versus the L site; however due to subject to subject variability, this difference was not significant.
The untreated site showed the greatest subject to subject variability in Hale’s-stainable material. Nevertheless 4 subjects had greater Hale’s-stainable material in the R site when compared to the untreated site. As mentioned previously, due to regional differences it is difficult to draw meaningful comparisons between the treated and untreated sites.

Conclusions

There are several conclusions to be drawn from this small pilot study. The active and vehicle-only formulations did not have any deleterious effects on the skin that were discernable at the light microscopic level. This is important because any changes seen in the other parameters were not confounded by and/or secondary to an inflammatory response. The assumption is that any changes are the result of the cord serum complex. The R site, which received the cord serum complex daily for 26 days, had increased amounts of stained material corresponding to procollagen and GAGs compared to the vehicle-treated (L) site. Cord serum complex had no discernable effect on VET or elastin.

Since ground substance (GAGs) is the dermal component that is most rapidly turned over, it is not surprising that changes were seen. Furthermore, ground substance is well known for its water-holding capacity, and it is this increase in water binding that could be partially responsible for the disappearance of fine-line wrinkling after use of this product. The increase in procollagen-stained material was also significant. In contrast to the GAGs, collagen is slowly turned over. This suggests that some of the changes in skin quality that have been reported following use of the cord serum complex facial cream may be somewhat more long lasting than the GAG-induced changes because collagen fibers are a more stabile dermal component than GAGs. Taken together these findings suggest that facial cream with serum cord
complex stimulates the synthesis of GAGs and procollagen, which in part is responsible for the clinical changes seen after use of this product.

Table 2 - Histogeometric Analyses

<table>
<thead>
<tr>
<th>VET (microns)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>126 ± 7</td>
<td>99 ± 7</td>
<td>98 ± 8</td>
<td>115 ± 4</td>
<td>128 ± 16</td>
<td>114 ± 3</td>
<td>113 ± 12</td>
</tr>
<tr>
<td>R</td>
<td>132 ± 7</td>
<td>94 ± 4</td>
<td>120 ± 15</td>
<td>91 ± 2</td>
<td>118 ± 20</td>
<td>119 ± 3</td>
<td>96 ± 37</td>
</tr>
<tr>
<td>L</td>
<td>126 ± 5</td>
<td>91 ± 8</td>
<td>115 ± 10</td>
<td>108 ± 4</td>
<td>87 ± 9</td>
<td>113 ± 5</td>
<td>107 ± 14</td>
</tr>
</tbody>
</table>

| Elastin (% dermis) | NT  | 14.0 ± 1 | 10.4 ± 1.1 | 12.5 ± 0.7 | 12.2 ± 2.4 | 13.0 ± 2.0 | 7.6 ± 2.0 | 11.6 ± 2.0 |
|                   | R  | 8.0 ± 1.5 | 9.8 ± 0.3 | 8.3 ± 1.6** | 5.8 ± 0.4  | 8.2 ± 1.1  | 5.0 ± 0.4 | 7.5 ± 1.6 |
|                   | L  | 11.8 ± 0.7** | 8.0 ± 1.7 | 3.3 ± 0.3 | 8.6 ± 0.8** | 9.5 ± 1.1 | 5.3 ± 1.1 | 7.8 ± 2.8 |

| Procollagen (% upper dermis) | NT  | 8.1 ± 0.6 | 9.9 ± 1.7 | 4.7 ± 1.8 | 16.2 ± 1.8 | 9.1 ± 1.5 | 16.4 ± 2.9 | 10.7 ± 4.3 |
|                              | R  | 9.2 ± 0.6 | 13.3 ± 1.6** | 9.9 ± 1.7** | 12.3 ± 2.3* | 19.2 ± 5.4 | 10.0 ± 2.4 | 12.3 ± 3.4 |
|                              | L  | 9.1 ± 1.1 | 7.8 ± 1.3 | 5.2 ± 2.1 | 7.9 ± 1.8 | 15.8 ± 3.4 | 11.5 ± 1.4 | 9.6 ± 3.4 |

| GAGs (% dermis) | NT  | 1.8 ± 0.8 | 24.3 ± 2.8 | 22.6 ± 2.4 | 13 ± 3.8 | 14.7 ± 2.2 | 5.2 ± 0.9 | 13.6 ± 8 |
|                | R  | 22 ± 5.0** | 11.4 ± 0.9** | 20.1 ± 2.2** | 15 ± 2.7 | 23 ± 3.0** | 10.8 ± 3.9 | 17.4 ± 4.9 |
|                | L  | 4.4 ± 2.3 | 6.5 ± 1.5 | 8.3 ± 2.7 | 18 ± 5.5 | 14.3 ± 1.5 | 13.9 ± 3.9 | 10.9 ± 4.8 |

*P <0.05; **P <0.01

Table 3 - Percentage Change

<table>
<thead>
<tr>
<th>VET</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>R vs L</td>
<td>4.5 †</td>
<td>3 †</td>
<td>4.2 †</td>
<td>15.7 †</td>
<td>26.3 †</td>
<td>5.0 †</td>
</tr>
<tr>
<td>R vs NT</td>
<td>4.5 †</td>
<td>5.1 †</td>
<td>18.3 †</td>
<td>20.8 †</td>
<td>7.8 †</td>
<td>4.2 †</td>
</tr>
<tr>
<td>L vs NT</td>
<td>0</td>
<td>8.1 †</td>
<td>14.8 †</td>
<td>6.1 †</td>
<td>32.0 †</td>
<td>0.9 †</td>
</tr>
</tbody>
</table>

elastin

| R vs L | 32.2 † | 18.4 † | 60.2 † | 32.6 † | 13.7 † | 5.7 † |
| R vs NT | 42.9 † | 5.8 † | 33.6 † | 52.5 † | 36.9 † | 34.2 † |
| L vs NT | 15.7 † | 23.1 † | 73.6 † | 29.5 † | 26.9 † | 30.3 † |

procollagen

| R vs L | 1.1 † | 41.4 † | 47.5 † | 35.8 † | 17.7 † | 13.0 † |
| R vs NT | 12.0 † | 25.6 † | 52.5 † | 24.0 † | 52.6 † | 39.0 † |
| L vs NT | 11.0 † | 21.2 † | 9.6 † | 51.2 † | 42.4 † | 6.1 † |

GAGs

| R vs L | 80.0 † | 43.0 † | 58.7 † | 16.7 † | 37.8 † | 22.3 † |
[0089] From the foregoing it will be seen that this invention is one well adapted to attain all ends and objectives herein-above set forth, together with the other advantages which are obvious and which are inherent to the invention. Since many possible embodiments may be made of the invention without departing from the scope thereof, it is to be understood that all matters herein set forth are to be interpreted as illustrative, and not in a limiting sense. While specific embodiments have been shown and discussed, various modifications may of course be made, and the invention is not limited to the specific forms or arrangement of parts and steps described herein, except insofar as such limitations are included in the following claims. Further, it will be understood that certain features and subcombinations are of utility and may be employed without reference to other features and subcombinations. This is contemplated by and is within the scope of the claims.
CLAIMS

What is claimed and desired to be secured by Letters Patent is as follows:

1. A skin care composition for topical application to the human skin for imparting an anti-aging benefit to the skin comprising an effective amount of umbilical cord blood serum.

2. The skin care composition of claim 1, wherein said umbilical cord blood serum is present in an amount from about 0.0001 wt% to about 90 wt%.

3. The skin care composition of claim 1, wherein said umbilical cord blood serum is present in an amount from about 0.01 wt% to about 25 wt%.

4. The skin care composition of claim 1, wherein said umbilical cord blood serum is present in an amount from about 0.01 wt % to about 10 wt%.

5. The skin care composition of claim 1 further comprising a peptide selected from the group consisting of tyr-arg, acetyl hexapeptide-3, palmitoyl oligopeptide, palmitoyl tetrapeptide-7, and mixtures thereof.

6. The skin care composition of claim 1 further comprising a biological additive selected from the group consisting of Juglans regia, Centella asiatica, Pyrus germanica extract, and mixtures thereof.

7. The skin care composition of claim 1 further comprising a biological additive selected from the group consisting of Undaria pinnatifida and Stevia rebaudiana Bertoni, and mixtures thereof.

8. The skin care composition of claim 1 further comprising one or more phospholipids.

9. The skin care composition of claim 8 wherein said phospholipid is selected from the group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, diphosphatidyl glycerol, and mixtures thereof.
10. The skin care composition of claim 1 further comprising one or more preservatives.

11. The skin care composition of claim 10 wherein said preservative is selected from the group consisting of one or more parabens.

12. The skin care composition of claim 1 further comprising one or more penetration enhancers.

13. The skin care composition of claim 12 wherein said penetration enhancer is selected from the group consisting of dimethyl isosorbide and diethyl-glycol-monoethylether.

14. The skin care composition of claim 1 further comprising one or more neutralizing agents selected from the group consisting of ammonium hydroxide, arginine, 2-amino-2-methyl-1-propanol, dimethanolamine, dibutanolamine, diisobutanolamine, tributanolamine, triisobutanolamine, tri-sec-butanolamine, tripropylamine, ethanolamine, diethanolamine, triethanolamine, PEG-15 cocamine, diisopropanolamine, methylethanolamine, diisopropylamine, dipropylentriamine, tromethamine, isopropylamine ethylene diamine, triisopropanolamine, tetrahydroxypropyl ethylenediamine, trimethamine, 2-aminobutanol, aminoethyl propanediol, aminomethyl propanediol, aminomethyl propanol, sodium hydroxide, potassium hydroxide and mixtures thereof.

15. The skin care composition of claim 1 further comprising one or more hyaluronans.

16. The skin care composition of claim 1 further comprising one or more skin-conditioning emollients selected from the group jojoba oil, almond oil, capric/caprylic triglyceride and mixtures thereof.

17. The skin care composition of claim 1 comprising about 0.1 to about 0.35 wt% umbilical cord blood serum, about 3 to 5 wt% palmitoyl oligopeptide, about 3 to about 5 wt% palmitoyl tetrapeptide-7.
18. The skin care composition of claim 17 further comprising about 2 to about 3 wt% of the dipeptide tyr-arg and about 0.5 to 1.5 wt% acetyl hexapeptide-3.

19. The skin care composition of claim 18 further comprising about 0.5 to about 1.2 wt% phosphatidyl choline, about 0.5 to about 1.5 wt % hyaluronans, and 0.01 to 0.085 of a preservative selected from the group consisting of methyl paraben and propyl paraben, or mixtures thereof.

20. The skin care composition of claim 19 further comprising **juglans regia**, **centella asiatica**, **pyrus germanica**, **Undaria pinnatifida**, and **Stevia rebaudiana Bertoni**.

21. A method for imparting an anti-aging benefit to human skin comprising: topically applying to the skin of an individual in need thereof a composition of claim 1 to impart the anti-aging benefit to the skin.

22. The method according to claim 21, wherein said umbilical cord blood serum is present in an amount sufficient to increase procollagen or glycosaminoglycans.

23. The method according to claim 21, wherein said umbilical cord blood serum is present in an amount from about 0.0001 wt% to about 90 wt%.

24. The method according to claim 21, wherein said umbilical cord blood serum is present in an amount from about 0.01 wt% to about 25 wt%.

25. The method according to claim 21, wherein said umbilical cord blood serum is present in an amount from about 0.01 wt % to about 10 wt% .
26. The method according to claim 21, wherein said anti-aging benefit is selected from the group consisting of: (a) treatment, reduction, and/or prevention of fine lines or wrinkles, (b) reduction of skin pore size, (c) improvement in skin thickness, plumpness, and/or tautness; (d) improvement in skin suppleness and/or softness; (e) improvement in skin tone, radiance, and/or clarity; (f) improvement in procollagen and/or collagen production; (g) improvement in maintenance and remodeling of elastin; (h) improvement in skin texture and/or promotion of retexturization; (i) improvement in skin barrier repair and/or function; (j) improvement in appearance of skin contours; (k) restoration of skin luster and/or brightness; (l) replenishment of essential nutrients and/or constituents in the skin; (m) improvement of skin appearance decreased by menopause; (n) improvement in skin moisturization; (o) increase in and/or preventing loss of skin elasticity; (p) treatment, reduction, and/or prevention of skin sagging; or (q) treatment, reduction, and/or prevention of discoloration of skin.

27. The method according to claim 26, wherein said anti-aging benefit is treatment, reduction, and/or prevention of fine lines or wrinkles.

28. The method according to claim 26, wherein said anti-aging benefit is treatment, reduction, and/or prevention of skin sagging.

29. The method according to claim 26, wherein said anti-aging benefit is treatment, reduction, and/or prevention of discoloration of skin.

30. The method according to claim 26, wherein said anti-aging benefit is increase in and/or preventing loss of skin elasticity.
TOPOCAL COMPOSITION COMPRISING
UMBILICAL CORD BLOOD SERUM

ABSTRACT OF THE DISCLOSURE

Cosmetic compositions comprising umbilical cord blood serum and methods of using such compositions to impart anti-aging benefits to the skin are disclosed.
Part I: removing dark circles

Under-eye circles are created when red blood cells leak from capillaries in the eye area, releasing hemoglobin when they explode in nearby skin tissue. The iron-rich hemoglobin degrades into the yellow pigment—bilirubin—as well as other various colored pigments, resulting in a bruised appearance to the skin under the eyes and sometimes on the eyelids. It still is not clear why the capillaries leak, although inflammation—due to stress, genetics, allergies and lack of sleep—is believed to be a key trigger.

Under eye recovery cream with cord serum complex is a combination of proprietary components/systems that binds to the iron released by the red blood cells, trapping the molecules before bilirubin can be formed and making the iron soluble enough to be eliminated from the skin. (Haloxyl) and stimulates a natural enzyme that clears bilirubin that already has been formed. Working with these molecules are components providing support to the fragile connective tissue around the eyes. When it begins to lose its tone and elasticity, the irritation that leads to dark circles can increase.

Studies show a reduction in the production of the key inflammatory enzyme prostaglandin E2 (PGE2) in epidermal keratinocytes by 93% and in dermal fibroblasts by 86% when these cells are exposed to ultraviolet B (UVB) irradiation—long believed by researchers to be a significant contributor to aging around the eye area. In a two-month clinical study of 22 females whose average age was 32.7 years, application of the cream, two times a day showed an average 63% decrease in under-eye darkening.

Part II: relieving puffiness

Helps reduce puffiness and bags under the eyes. Puffy eyes are due to fluid build-up caused by poor drainage, fragile capillaries, and loss of elasticity. Fluid leaks into surrounding tissues and produces "bags". The Recovery Cream targets all three conditions by improving drainage, reducing capillary fragility, and reducing irritation and skin slackening while increasing skin firmness and elasticity (Eyeliss)

Part III: Instant and gradual skin brightening

Following the application of the Under Eye Regeneration serum with CSC we notice an even complexion with less visible spotting. Luminosity and skin appearance: lines, pores and blemishes are improved and the yellow or red skin color is brighter. Daily use of the UnderEye serum results in significant progressive and durable reduction of the quantity of melanin in the skin leading to a clearer skin by 16%. Pores are less apparent and lines are optically erased.

Part IV: Light Manipulation
The appearance of fine lines and wrinkles on the skin’s surface is a result of uneven textures. The UnderEye serum through the addition of microscopic clear lenses (Chronosphere opticals) to the formula is able to manipulate light striking the skin surface in the eye area creating an illusion on the adjacent skin areas reducing the darkness and shadows caused by fine lines and wrinkles on the skin. The clarity of the microlenses allows the product to be used on all skin tones.

**Part V: Reduction of fine lines and wrinkles**

Charged coupled biopeptides, neuropeptides, micro algae’s and botanicals reduce the depth of fine lines and wrinkles, additional-synergistic support is provided by the Cord Serum Complex (Human Umbilical Extract) see attachment “Histogeometric Analysis of the effects of Product A versus product B on human skin.”

Note: Clinical data and trial information available