
Safety Assessment of Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride) as Used in Cosmetics

Status: Draft Tentative Report for Panel Review
Release Date: August 18, 2017
Panel Date: September 11-12, 2017

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Interim Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Ivan Boyer, Ph.D., Toxicologist.

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1620 L STREET, NW, SUITE 1200 ♦ WASHINGTON, DC 20036-4702 ♦ PH 202.331.0651 ♦ FAX 202.331.0088 ♦ CIRINFO@CIR-SAFETY.ORG



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Memorandum

To: CIR Expert Panel Members and Liaisons

From: Wilbur Johnson, Jr.
Senior Scientific Analyst

Ivan J. Boyer, Ph.D., D.A.B.T.

Date: August 18, 2017

Subject: Draft Tentative Report on Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride)

Given the inclusion of two chemical names in the title of this safety assessment, the report introduction contains a fair amount of detail relating to the use of the INCI name Polyaminopropyl Biguanide to represent the chemical polyhexamethylene biguanide hydrochloride throughout the report text. The Panel should determine whether further revision of this section of the report is warranted.

- I. An Insufficient Data Announcement with the following data requests was issued at the June 12-13, 2017 Expert Panel meeting (this was the second IDA issued for this ingredient):
- a) Calculation of a margin of safety (MOS) for Polyaminopropyl Biguanide inhalation exposure, using toxicity data from a short-term (28-day) rat inhalation-exposure study and use concentration data on Polyaminopropyl Biguanide in hair sprays, both of which were included in the CIR safety assessment.
 - b) Further clarification of urticarial reactions reported in SCCS reports on Polyaminopropyl Biguanide.
 - c) Raw data sheets (i.e., individual scores obtained during the induction and challenge phases) on subjects evaluated in the HRIPT on a product containing 0.2% Polyaminopropyl Biguanide submitted (HRIPT with raw data sheets) by the Council on May 2, 2017.
 - d) A dermal sensitization quantitative risk assessment (QRA) for Polyaminopropyl Biguanide.

Additionally, industry was encouraged to provide any available HRIPT data that could yield a more refined no-expected-sensitization-induction-level (NESIL); the current NESIL of $25\mu\text{g}/\text{cm}^2$ was considered likely to be overly conservative for use in the QRA. Furthermore, at the meeting, the Council informed the Panel that they would provide CIR with a corrected HRIPT summary and a corrected concentration of use table.

In response to this IDA:

- a) The latest survey information submitted by the Council on July 18 (*polyam092017data1.pdf* and *polyam092017data2.pdf*) indicates that Polyaminopropyl Biguanide is no longer being used in any cosmetic sprays. Nevertheless, MOSs for Polyaminopropyl Biguanide inhalation were calculated by the CIR staff using the ConsExpo Web Model, and are presented under the Risk Assessment subheading in the Chronic Toxicity Studies section (Inhalation) of the safety assessment report. The MOS was 200 for propellant hair sprays and 11 for pump hair sprays using this model. Exposure concentrations that would yield an MOS of 100 for propellant and pump hair sprays and propellant deodorant sprays were also estimated using the model. The Panel should determine whether the safety assessment report presents the modelling effort adequately, and whether an MOS of 100 would likely be sufficiently protective for this ingredient if it were used in cosmetic spray products.
- b) Given the Panel's concern about contact urticaria, the 3 case reports in the published literature that were identified as relevant (Kautz et al., 2010; Creyten et al., 2014; Goossens, 2016) are summarized under the Contact Urticaria subheading in the section on Case Reports.

The Panel should also determine whether case reports relating to anaphylaxis should be added to the Case Reports, Contact Urticaria section of the safety assessment report. The Panel should develop language for the report Discussion that addresses the occurrence of contact urticaria and anaphylactic reactions in case reports on Polyaminopropyl Biguanide.

- c) The updated use data corrected the previously reported highest maximum use concentration of 0.5% in suntan products; the highest maximum use concentration in a leave-on product is now 0.2% in eye lotions. A corrected summary of the HRIPT on a leave-on product containing 0.5% Polyaminopropyl Biguanide (provided by the Council on 6-15-2017) was also received. It was determined that the product tested in this study was actually a leave-on product that contained 0.1% Polyaminopropyl Biguanide. The HRIPT report on a product containing 0.2% Polyaminopropyl Biguanide is attached for the Panel's evaluation (*polyam092017data3.pdf*); this report, which was submitted by the Council in May 2017, presents raw data sheets for the HRIPT. The corrected HRIPT summary is also attached (*polyam092017data4.pdf*).
- d) To date, a dermal sensitization QRA has not been received from the Council, and the same is true for any additional available HRIPT data that might yield a more refined NESIL.

The Panel should decide if all of the needs defined in the IDA have been met, or are otherwise deemed moot.

- II. Comments from Council were received and addressed. In addition, comments relating to the inhalation toxicity of polyhexamethylene guanidine phosphate (PHMG) were received from Women's Voices for the Earth (WVE; *polyam092017data5.pdf*) and are attached. In these comments, the "discrepancy of professional opinion" with respect to how similar PHMG and Polyaminopropyl Biguanide are to each other was noted, and the following publications were provided:

- A review by Kim et al. (2016) on the lung toxicity of PHMG used in the past as a humidifier disinfectant in Korea
- A risk assessment by Lee et al. (2012) of PHMG as used as a humidifier disinfectant
- A refined risk assessment by Lee et al. (2014) of PHMG as used as a humidifier disinfectant

The **Lee et al., 2013** publication (*polyam092017data6.pdf*) is attached for the Panel's review. However, because of copyright restrictions, the 2 other publications (**Lee et al., 2012; Lee et al., 2013**) will be distributed as handouts at the Panel meeting.

The papers cited by WVE applied a no observed adverse effect concentration (NOAEC) from a 28-day inhalation-exposure study of Polyaminopropyl Biguanide (0.024 mg/m³) to assess the risks associated with inhalation exposure to PHMG, because of:

- The absence of data on which to base a NOAEC for PHMG
- The similarities of the chemical structures; toxic effects on the lungs, eyes, skin, and acute LD₅₀s reported in animal studies

No assessment factor (aka uncertainty factor) was applied to address the uncertainty associated with using toxicity data from an analog (i.e., Polyaminopropyl Biguanide) to estimate the risks associated with exposures to PHMG. However, the authors did apply an assessment factor of 600 (i.e., 10 for inter-species extrapolation x 6 for short-term to chronic exposure extrapolation x 10 for inter-individual uncertainty = 600).

The refined risk assessment, published in 2014, estimated an 8-h time-weighted average (TWA) PHMG concentration of 0.06 mg/m³ for the humidifier use scenario, which is 27 times greater than the 0.0022 mg/m³ inhalation exposure concentration estimated for 0.053% Polyaminopropyl Biguanide in a pump hair spray. The exposure duration for PHMG in the humidifier use scenario (8 h) is 96 times greater than the exposure duration/event assumed for Polyaminopropyl Biguanide in the consumer spray scenarios (5 min).

In light of the concern purported in WVE's comments about the extent to which Polyaminopropyl Biguanide is similar to PHMG, the Panel should consider this issue and determine whether or not the publications relating to PHMG-induced lung injury that are summarized in the Other Clinical Reports section of the Draft Tentative Report are relevant to this safety assessment.

- III. The safety assessment report has also been revised (See section on Case Reports) to include 2 case reports (Bervoets and Aerts, 2015; Pastor-Nieto, 2017), and the summary of the case report by Kautz et al., 2010 has been revised to include additional details.
- IV. Also, a Risk Assessment subheading has been added to the section on Sensitization. Sensitization data that, according to one source, have been used in a risk assessment suggesting that Polyaminopropyl Biguanide may not be a relevant contact allergen are included under this subheading. These data are also included in Table 15 of the safety assessment report, and were previously reviewed by the Panel.
- V. It should be noted that 2 different sources for the results of an Alderley Park mouse developmental toxicity study are included in Table 12 of the safety assessment report. Different values for the maternal NOAEL and the developmental NOAEL are presented, although the same primary reference for the study is listed in both sources. The Council has requested the primary reference for this study.
- VI. The Panel expressed concern about the irritation and sensitization potential of Polyaminopropyl Biguanide and discussed the likely recommendation that products containing Polyaminopropyl Biguanide be formulated to be non-irritating and non-sensitizing using the QRA or a similar risk assessment method. As noted above, no QRA has yet been submitted to the CIR. The Panel also discussed the possibility of using a NESIL of 25 µg/cm² based on negative HRIPT data.

After reviewing the available data, the Panel should determine whether a Tentative Report with a safe as used, safe with qualifications, insufficient data, or unsafe conclusion should be issued at this meeting. With respect specifically to the potential for incidental inhalation exposure, the Panel should determine whether a safe conclusion with inhalation specific qualifications is warranted.

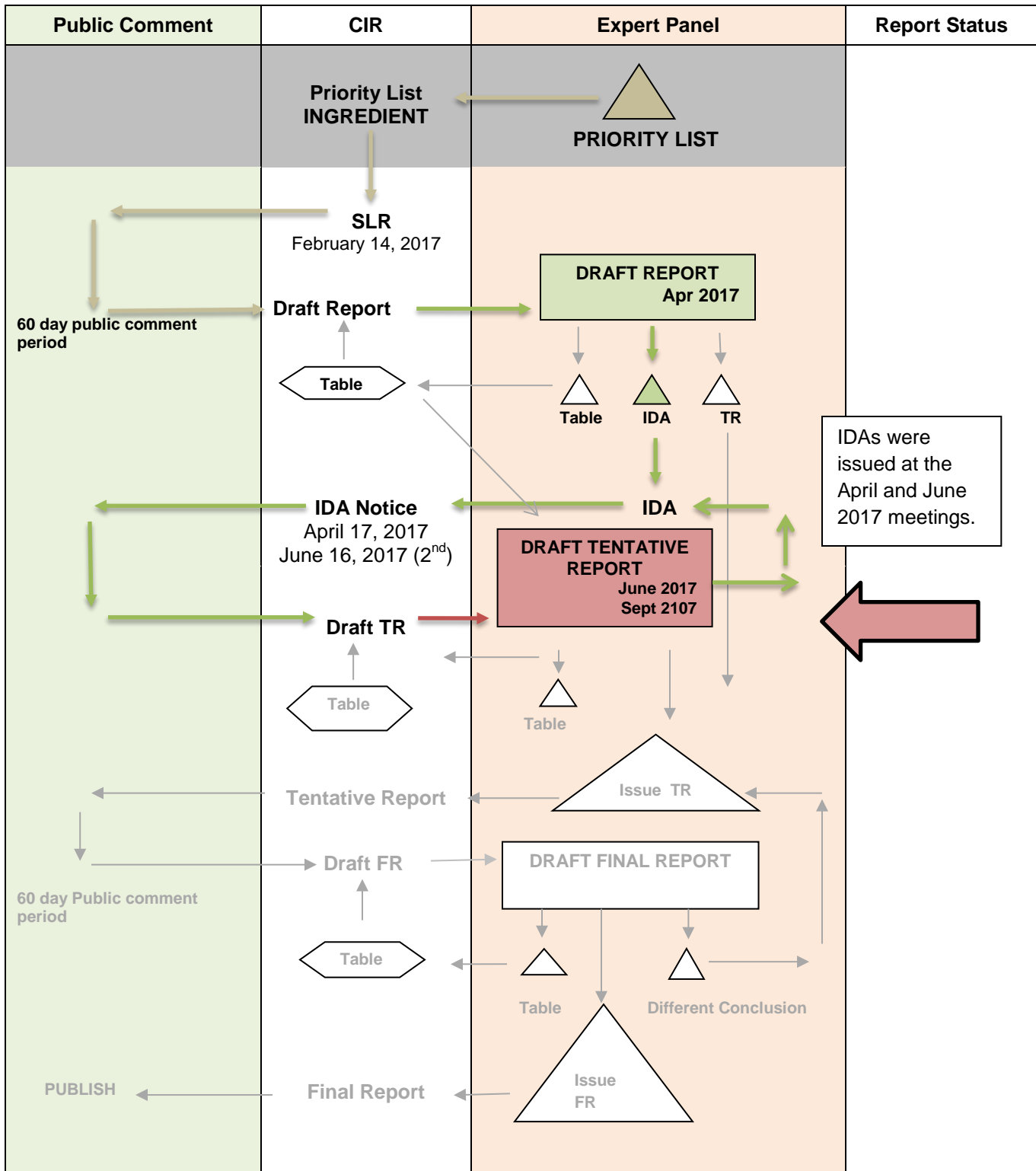
Also included in this package for the Panel's review are:

- the Draft Tentative Report (*polyam092017rep.docx*)
- the CIR report history (*polyam092017hist.docx*)
- Flow chart (*polyam092017flow.docx*)
- Literature search strategy (*polyam092017strat.docx*)
- Ingredient data profile (*polyam092017prof.docx*)
- 2017 FDA VCRP data (*polyam092017FDA.xlsx*)
- Minutes from the April 10-11, 2017 and June 12-13, 2017 Expert Panel meetings (*polyam092017min.docx*)
- the published CIR Final Report on Cocamidopropyl Betaine (*polyam092017prev.docx*)
- comments that were received from the Council (*polyam092017pcpc.pdf*).

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Polyaminopropyl Biguanide (i.e., polyhexamethylene biguanide hydrochloride)

MEETING Sept 2017



CIR History of:

Polyaminopropyl Biguanide

A Scientific Literature Review (SLR) on Polyaminopropyl Biguanide was issued on February 13, 2017.

Draft Report, Teams/Panel: April 10-11, 2017

The following ingredient data that were submitted by the Council have been added to the Draft Report: Use concentration data, Supplier comments on the identity of Polyaminopropyl Biguanide, and a Cosmetics Europe Dossier on the safety of Polyaminopropyl Biguanide. Comments that were received from the Council (*polyam042017pcpc*) have also been incorporated.

An Insufficient Data Announcement (IDA) with the following data requests was issued:

- (1) Skin sensitization data to determine the no-effect-level (i.e., threshold) for Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride)-induced sensitization
- (2) Data needed to evaluate anaphylactic reactions to Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride) in case studies
- (3) Data from Korean studies on lung injury/mortalities attributable to exposure to a disinfectant (polyhexamethylene guanidine phosphate) used in humidifiers

Draft Tentative Report, Teams/Panel: June 12-13, 2017

In response to the IDA that was issued, the following data were received from the Council: (1) Data summaries from the Cosmetics Europe Consortium (relating to skin sensitization potential) and (2) Human repeated insult patch test (HRIPT) on a neck cream containing 0.2% Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride). The studies summarized in the Cosmetics Europe Consortium data submission are not new data, and were included in the Draft Report that was reviewed at the April 2017 Panel meeting.

Regarding item #2 of the IDA, the primary references (in published literature) for the 2 case studies (referenced in Draft Tentative Report) relating to anaphylactic reactions to the hospital disinfectant Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride) after surgical wound exposure were received. Regarding item #3 of the IDA, the 3 Korean studies relating to (polyhexamethylene guanidine phosphate/polyhexamethylene guanidine inhalation exposure-related lung injury/mortalities previously provided by the Council are summarized in the report text (enclosed in borders).

An Insufficient Data Announcement (IDA) with the following data requests was issued at the June 12-13, 2017 Expert Panel meeting:

- Calculation of a margin of safety (MOS) for Polyaminopropyl Biguanide inhalation exposure, using exposure data from the short-term (28 days) rat inhalation toxicity study and current use concentration data on Polyaminopropyl Biguanide in hair sprays, both included in the CIR safety assessment.
- Further clarification of urticaria reactions reported in SCCS reports on Polyaminopropyl Biguanide.
- Raw data sheets (i.e., individual scores during induction and challenge phases) on subjects evaluated in the HRIPT on a product containing 0.2% Polyaminopropyl Biguanide, that was provided by the Council.
- A dermal sensitization quantitative risk assessment (QRA) for Polyaminopropyl Biguanide.

Additionally, industry is encouraged to provide any available HRIPT data that can yield a more refined no-expected-sensitization- induction-level (NESIL); the current NESIL, at 25µg/cm², is likely to be overly conservative for use in the QRA.

Furthermore, at the meeting, the Council informed the Panel that they will provide CIR with a corrected HRIPT summary and a corrected concentration of use table.

Draft Tentative Report, Teams/Panel: September 11-12, 2017

Responses to the IDA were received. The MOS calculation for Polyaminopropyl Biguanide inhalation was completed by the CIR staff, and is included under the Risk Assessment Subheading in the Short-Term Toxicity Studies section of the report. Given the Panel's concern relating to contact urticaria, the 3 case reports in the published literature that have been identified as relevant

to an evaluation of contact urticaria potential (Kautz et al., 2010; Creyten et al., 2014; Goossens, 2016) have been placed under the Contact Urticaria subheading in the section on Case Reports. Because the raw data sheets from the HRIPT on a product containing 0.2% Polyaminopropyl Biguanide were included in a previous Council data submission, this study is available for the Panel's further evaluation. More recent use concentration data were received from the Council, and these data are also available for the Panel's evaluation. A corrected summary of the HRIPT on a leave-on product containing 0.5% Polyaminopropyl Biguanide (previously provided by the Council) was also received. It was determined that the product tested in this study was actually a leave-on product that contained 0.1% Polyaminopropyl Biguanide, and the corrected HRIPT summary is available for the Panel's evaluation.

To date, a dermal sensitization QRA has not been received from the Council, and the same is true for any additional available HRIPT data that can yield a more refined NESIL.

Comments relating to the inhalation toxicity of polyhexamethylene guanidine phosphate (PHMG) that were received from Women's Voices For The Earth (WVE) are available for the Panel's evaluation. In these comments, the "discrepancy of professional opinion" with respect to how similar PHMG and Polyaminopropyl Biguanide are was noted and CIR was made aware of the following 3 publications: a review article on PHMG-induced lung toxicity (Kim et al., 2016) and 2 inhalation risk assessments on PHMG (Lee et al., 2012; Lee et al., 2013).

The Panel expressed concern over the irritation and sensitization potential of Polyaminopropyl Biguanide and discussed the likely recommendation that products containing Polyaminopropyl Biguanide be formulated to be non-irritating and non-sensitizing using the QRA or a similar risk assessment method. It was suggested by the Panel that the discussion and conclusion in the published CIR Safety Assessment on Cocamidopropyl Betaine serve as the basis for developing appropriate language relating to the QRA and NESIL for these sections of the Polyaminopropyl Biguanide safety assessment. A decision on specific language for the discussion was not made at the Panel meeting; however, the Panel discussed the possibility of using a NESIL of 25 $\mu\text{g}/\text{cm}^2$ because this is the dose of Polyaminopropyl Biguanide that was applied to the skin in the negative HRIPT on a leave-on product containing 0.1% Polyaminopropyl Biguanide. Additionally, the Panel needs to develop language for the report discussion that addresses the occurrence of contact urticaria and anaphylactic reactions in case reports on Polyaminopropyl Biguanide.

Polyaminopropyl Biguanide Data Profile for September 11 th - 13 th 2017 Panel – Wilbur Johnson																															
				Dermal Penetration	Nail Penetration	Penetration Enhancement	ADME				Acute Toxicity			Short-Term Toxicity	Sub-Chronic Toxicity	Chronic Toxicity	DART		Genotoxicity	Carcinogenicity	Other Relevant Studies	Dermal Irritation*	Dermal Sensitization*/ Phototoxicity*		Ocular Irritation *	Clinical Studies	Case Reports		Epidemiology Studies		
				In Vivo-Human	In Vivo-Human	In Vitro-Animal	Animal-Dermal	Animal-Oral	Animal-IV	Human-Oral	Animal-Dermal	Animal-Oral	Animal-Inhalation	Animal	Animal	Animal	In Vitro	In Vivo	In Vitro/In Vivo	In Vivo/In Vivo	In Vitro	In Vivo-Animal	Animal/Human	Animal	Human	In Vitro	Animal/Human	Human-Dermal/Oral	Human-Dermal	Human-Oral	Human
Polyaminopropyl Biguanide				X				X			X	X	X	X	X	X		X	X/X	X/X			X/X	X/X	X/X		X/X	X/0	X		

X = data; 0 = no data*

[Polyaminopropyl Biguanide (11/09/2016 & 11/14/2016; Updated on 3/7/2017; Updated on 8/14/17

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	ECETOC
Polyaminopropyl Biguanide	133029-32-0 32289-58-0	1/1	18/162	3/126	3/11	No	Yes	No Dossier	No	No	No	No	No	No	No	No	No	No
Polyhexamethylene Biguanide	28757-47-3	1/1	8/84	13/370	4/99	no	Yes	No Dossier	No	No	Yes	Yes	1/19	1/4	0/2	No	No	No

Search Strategy

[document search strategy used for SciFinder, PubMed, and Toxnet]

[identify total # of hits /# hits that were useful or examined for usefulness]

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - <http://www.personalcarecouncil.org/science-safety/line-infobase>
SciFinder (usually a combined search for all ingredients in report; list # of this/# useful) - <https://scifinder.cas.org/scifinder>
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - <http://www.ncbi.nlm.nih.gov/pubmed>
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – <https://toxnet.nlm.nih.gov/> (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm> (CFR); then, list of all databases: <http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm>; then, <http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true> (EAFUS); <http://www.fda.gov/food/ingredientpackaginglabeling/gras/default.htm> (GRAS); <http://www.fda.gov/food/ingredientpackaginglabeling/gras/scogs/ucm2006852.htm> (SCOGS database); <http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives> (indirect food additives list); <http://www.fda.gov/Drugs/InformationOnDrugs/default.htm> (drug approvals and database); <http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf> (OTC ingredient list); <http://www.accessdata.fda.gov/scripts/cder/iig/> (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - <http://ec.europa.eu/growth/tools-databases/cosing/>
ECHA (European Chemicals Agency – REACH dossiers) – <http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1>
IUCLID (International Uniform Chemical Information Database) - <https://iuclid6.echa.europa.eu/search>
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- <http://webnet.oecd.org/hpv/ui/Search.aspx>
HPVIS (EPA High-Production Volume Info Systems) - <https://ofmext.epa.gov/hpvis/HPVISlogin>
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- <https://www.nicnas.gov.au/>
NTIS (National Technical Information Service) - <http://www.ntis.gov/>
NTP (National Toxicology Program) - <http://ntp.niehs.nih.gov/>
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/> (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - <http://www.ecetoc.org/>

Botanical Websites, if applicable

Dr. Duke's <https://phytochem.nal.usda.gov/phytochem/search>
Taxonomy database - <http://www.ncbi.nlm.nih.gov/taxonomy>
GRIN (U.S. National Plant Germplasm System) - <https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx>
Sigma Aldrich plant profiler <http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html>

Fragrance Websites, if applicable

IFRA (International Fragrance Association) – <http://www.ifraorg.org/>
RIFM (the Research Institute for Fragrance Materials) should be contacted

Day 1 of the April 10-11, 2017 CIR Expert Panel Meeting – Dr. Belsito's Team

Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride)

DR. BELSITO: Okay; okey doke. So, then, we're moving on to polyaminopropyl biguanide. There's a lot of data there. I wonder if it all comes from use, in pills, and

(inaudible). So this is our first time we're looking at this preservative. I think it's important to look at because Europe is going to regulate it, and we should be on board too. So, the original opinion was a limit of .3, and then, I think, there were some people who wanted to get rid of it completely, but the SCCS came in with a revised opinion in Europe; I'm talking about a .1. There's been some confusion about its name. The ingredient is polyaminopropyl biguanide, correct, Bart?

DR. HELDRETH: Yes.

DR. BELSITO: That's the cosmetic ingredient; and the chemical is polyhexamethylene biguanide?

DR. HELDRETH: Yes; the hydrochloride salt.

DR. BELSITO: Right, hydrochloride salt; but it's the same thing. So, we've been asked to look at all that data and decide where we are with it. There was a ton of data on this, and I don't know if we just want to go through our comments on the report first. But on page 11, Wilbur, at the bottom of the page, the paragraph, what is wINCI monograph?

DR. HELDRETH: So, that is the council provides info base that we used to look through the dictionary, but there's also a publicly available one that anybody can get access to if they pay the fee, and that's called wINCI.

DR. LIEBLER: I thought it was a typo when I struck it out.

MR. ANSELL: No, wINCI, like Wikipedia.

DR. LIEBLER: So, we need to save the wINCI, okay; I can see that.

DR. BELSITO: And then I had a question for everyone about the impurities on PDF, page 12; any of those jump out to you? I mean, there are a lot of things like hexans and things, cyanos?

GROUP: No.

DR. KLAASSEN: In regard to the chemistry here in the -- way in the beginning, it talks about this problem in South Korea and it says --

DR. BELSITO: What page are you on, Curt?

DR. KLAASSEN: Actually, the absolute first page, kind of the preface, the memoranda --

DR. SNYDER: The memo from Wilbur.

DR. LIEBLER: He's looking at the memo from Wilbur?

DR. KLAASSEN: Yeah; and it says in here that this compound that we're looking at is the hydrochloride, and then it says that the phosphates are different chemicals. How true is that? I mean, yes it is; but, biologically are they that different?

DR. HELDRETH: That was, actually, our point was to put that question to all of you. That data on the phosphate was submitted to us in regard to this report. We weren't sure of the relevance of it; so, Wilbur was posing that question to you all to decide if that data was useful for looking at the hydrochloride salt.

DR. KLAASSEN: I would think that it would be; that's why I'm bringing it up.

DR. LIEBLER: Right; I agree. I mean, the chemical biological driver here is going to be the organic piece, and either the chloride or phosphate, just, you know, counter anions in salts; and so, I would think that unless there's some unanticipated difference in the absorption or distribution of these molecules -- which I don't think there would be -- because they disassociate -- then I think that the data from the PHMG phosphate should be considered in our report.

DR. KLAASSEN: And the scientific significance of this phosphate in Korea is huge in that it's been added to water vapor-type things, and there've been a number of children died from it in the last couple of years; and, in fact, there is a toxicologist that's in prison right now because someone interpreted what he wrote in a manuscript as different from what he really said.

Nobody can get him out of prison; so, I think as people look at this -- not that it's identical as far as -- but I think we need to put this story in here because somebody's going to look at this and -- like we're not aware of it.

DR. BELSITO: What happened? This guy was interpreted as advocating that this material be added to water?

DR. KLAASSEN: Well, no. It had been --

DR. BELSITO: Because it's used as like a pool and - -

DR. SNYDER: Bactericide.

DR. BELSITO: Yeah, bactericide to replace percelphates that people are allergic to; Babaquel is, I think, the trade name.

DR. KLAASSEN: And this was added -- I don't know the whole story here. I wrote a letter for him to try to help to get him out of prison -- but in South Korea, they were adding it, you know, like you have for children, a vaporizer?

DR. BELSITO: Oh, yeah, a steam vaporizer for like asthma, or whatever.

DR. KLAASSEN: Yeah; something like that; and they were putting this chemical in there and children were dying; and then they had people testify and things, and somehow since he had published something and he said, you know, under these conditions, well they didn't -- the court doesn't understand science, and meanwhile he's sitting in prison -- I don't know the whole story. But, I mean, it has gone so far that there have been numerous children that have died.

DR. LIEBLER: I'd like to see the papers. I haven't seen those; but it doesn't make much sense because this molecule is not going to be volatile unless it somehow gets into droplets that are -- I don't know --

DR. BOYER: Like a vaporizer.

DR. LIEBLER: Yeah, but, so, I'd like to see the --

DR. KLAASSEN: Yeah; no; I agree.

DR. LIEBLER: -- because I'm not sure if we'd be able to draw a conclusion that this substance, per se, is

(inaudible); I'd like to look at the data.

DR. KLAASSEN: That's all I'm saying is I think we should look at this because someone else might look at it through those eyes, and if we didn't mention it -- and maybe you know more about than I do.

DR. BOYER: No, I don't; but it could, I'm speculating, I'm imaging it's these cool midst vaporizers that basically aerosolize the water, so you're not getting that kind of, just deletion of high heat.

DR. BELSITO: Right; I see, sort of more aggressive at forming midst droplets, yeah.

DR. BOYER: Right.

DR. LIEBLER: Well, I think the argument that these are chemically dissimilar doesn't wash; and so, we should consider the data on this as well as part of this report.

DR. JOHNSON: One question that I have, are you saying that polyhexamethylene guanidine and polyaminopropyl biguanide are one in the same, because that name is slightly different?

DR. BELSITO: Chemically, not.

DR. JOHNSON: Not?

DR. BELSITO: The problem is this polyaminopropyl biguanide is the inky, right; and it's chemically wrong, but it's the inky name, so it's what we have. So, but the name of this phosphate is what it is, apparently; and so it is -- even though it has a different name than the inky name, it's the same structure, except for the counter anion.

DR. JOHNSON: So polyhexamethylene guanidine and polyhexamethylene biguanide are, from a biological standpoint, they are the same?

DR. LIEBLER: Right.

DR. BELSITO: They are. I mean, that's the introduction; so, it's the dictionary misnamed this chemical; and this is what the chemical structure really should be called, but that's not what we're going to call it because it's not what it's called in the dictionary.

DR. JOHNSON: Yeah, I was just -- the guanidine versus the biguanide, but

they're basically the same?

DR. BELSITO: Yeah.

DR. JOHNSON: Okay.

DR. LIEBLER: Yeah, I would like to see the papers, and I would like to see the identity of that, of the compound that's implicated in this apparent toxicology confirmed.

DR. BELSITO: Okay.

DR. ANSELL: Yeah, one of the papers says that the disinfected views were actually a combination of a variety of materials. Two of the other papers said something different; but we do think the three papers should be --

DR. LIEBLER: Yeah, I agree.

DR. BELSITO: But if the reports are that iffy about exactly what was in the materials, I think we could mention it and not spend a lot of time; and then dismiss it, and say these reports, you know, add nothing. Let's look at them.

DR. LIEBLER: Let's just see the papers before we draw any conclusions.

DR. KLAASSEN: Right. I don't think it probably will affect the conclusion --

DR. BELSITO: Right.

DR. KLAASSEN: -- what I was suggesting, there probably needs to be a short paragraph about this.

DR. BELSITO: Okay; so, on page 17 of the PDF under carcinogenicity studies there's a dermal that shocked me at first, but I guess it's a non-genotoxic mechanism because if it was genotox, it would automatically be banned in Europe. So, I was wondering what you thought about this, Paul?

DR. SNYDER: Yeah; I read through that pretty carefully, and it's all at near maximum tolerated doses and it has (inaudible) toxicity and secondary changes, so I don't think it's --

DR. BELSITO: Relevant?

DR. SNYDER: -- yeah, I think we've captured it appropriately.

DR. JOHNSON: Which study is this; I'm sorry.

DR. BELSITO: The dermal carcinogenicity study on page 17. I don't think we need to delete it, but we will need for Paul to suggest some comments in the discussion as to why. There is further information later on, obviously, in the genotox section that this affect is not genotoxic. We need an explanation as to why we thought it happened and why it doesn't bother us using this material in cosmetics. So, Paul, you'll think about?

DR. SNYDER: Yes.

DR. ANSELL: Yeah; that study is discussed in the dossier.

DR. BELSITO: Yeah; okay. So, then on other relevant study effects on lung cells. This is going to be coming up under the respiratory boilerplate and the large letter we got from Missoula, Montana -- I forget what is the --

DR. LIEBLER: WVE.

DR. BELSITO: -- the Women's for --

DR. SNYDER: Women's Voices for the Earth.

DR. BELSITO: Right. Going down to the last paragraph on page 17, effects on lung cells and reactive oxygen generation, and F-count could be activation. It's used in aerosol products, and you don't necessarily have to get down to the alveoli and begin activating all those immune substances, which could go along with the vaporizers and the Korean issues, which I wasn't even aware of until now.

DR. SNYDER: I have a question. Why did the SEC revise their acceptable level in 2016 from .3 to .1?

DR. BELSITO: We're going to discuss that under sensitization. We don't have a noel for sensitization.

DR. SNYDER: Okay; so, it was all sensitization; it wasn't anything else?

DR. BELSITO: I think so.

DR. SNYDER: That's what I was worried about; okay. I had a sensitization question mark.

DR. BELSITO: My understanding was that they, actually, there was a movement to ban it because of the carcinogenicity study, and then the SEC has, actually, if you

read the entire report, you see they actually talk about the importance of biocides; and then they went through all the data, they looked at it pretty thoroughly, and they came up with the conclusion that the issue is sensitization.

DR. SNYDER: And they banned it in the spray -- aerosol use.

DR. BELSITO: I don't think so.

DR. SNYDER: I thought it was -- was it banned in aerosol use in cosmetics, and .1 percent for all other uses, or is that (inaudible)?

DR. BELSITO: I think we'll have to look up the year. It's in the report.

DR. JOHNSON: They expressed the need for inhalation toxicity data.

DR. BELSITO: Okay; so maybe they --

DR. JOHNSON: The evaluation (inaudible).

DR. BELSITO: Maybe that's what it is. So, it could be based upon this too. I didn't --

DR. JOHNSON: Well, also, they completed two additional skin penetration studies; you know, one at 0.3 percent and one at 0.1 percent, and seemed as though they were leaning in the direction of 0.1 percent based upon skin penetration data.

DR. BELSITO: I got the sense from my read of it -- and we'll get to it when we talk about sensitization. So, what they did is they sensitized people at 2 percent; and then they took those people who were sensitized and they tested them; and at .5 percent they still got a significant number of people reacting strongly; and then at .1 percent they had, I think, two people with very weak reactions; and they said, okay, if we take sensitized people and we can barely elicit a reaction at .1 percent, then .1 percent should be okay not to induce new sensitization. But we do not have a shown level at which you cannot induce new sensitization. So, that will be a question moving forward. First of all, you know, if we go safe as used, it's up to .5 in this country, right?

DR. SNYDER: Yes.

DR. BELSITO: So, I mean, that's going to be a rather high dose; and then if we don't go safe as used, where do we go?

DR. ANSELL: We actually don't think it is .5. We think that report was 20 percent active.

DR. SNYDER: So, it's .1?

DR. ANSELL: Yes.

DR. SNYDER: So, it's 20 percent applied (inaudible)?

DR. ANSELL: Yeah; when we get to that level of detail.

DR. BELSITO: Okay; when we get there. So, we're dealing with lung right now. I need some comments.

DR. LIEBLER: Yeah. I thought I was going to comment on your question about effect on lung cells; so, we're back to the bottom of PDF 17. So, this is another one of these studies that I hate when they show up in our reports because basically you take some cultured cells, you dump some chemical on them, and then you measure something that there is an assay for and you, you know, the NF-kappa B is the major transcriptional regulator for a whole battery of genes involved in inflammation.

DR. JOHNSON: (Inaudible)

DR. LIEBLER: And there are many things, many, many things -- that trigger activation of the NF-B, and of kappa B, and its downstream genes. And, I think, you know this is 10 to 80 mg/ml of this material on lung cells. You know, I haven't looked at that paper; but, you know, I'm not sure that I would draw any significant inference from it. I mean, I think, it's -- if the conclusion is that this compound induces inflammatory responses by the NF-B signaling pathway, well, just about everything that causes inflammation activates this pathway. So, that's not news; and whether that says that this compound is uniquely toxic or pro-inflammatory, I think, is way too much for stretch based on just one experiment like that.

DR. BELSITO: And I'm just concerned when we clear the rescuable part that this type of inflammatory response will occur if it gets in the epiglottis; if it gets in the upper airway; if it gets in the lower airway, but not the alveoli; and how do we say, okay, I mean -- we can't -- in the aerosols -- and then that gets back to I didn't catch that in the SEC report, I concentrated mainly on the skin part, and skimmed the rest; but if the SECS is still asking for

respiratory data, then how do we clear its aerosol uses because we don't have inhalation toxicity here?

DR. LIEBLER: Right.

DR. BELSITO: This is what we have.

DR. LIEBLER: I would think that if we have a question about respiratory, we need respiratory data. Particularly, in light of this Korean thing; if there's an issue there that we can attach to this chemical. So, but I don't think this study that's cited here on the 85 part anion cells really sheds much light one way or another.

DR. BELSITO: Okay. So, I think that looking at -- I mean, we probably won't clear this at this meeting -- we need all of the data on the Korean studies, and --

DR. LIEBLER: Right.

DR. BELSITO: -- we need to go out and probably try to get some inhalation data on this.

DR. SNYDER: We have some.

DR. JOHNSON: Acute-A, no long term.

DR. SNYDER: Table 8 and 9 is --

DR. BELSITO: But we don't have anything long term, right?

DR. LIEBLER: I understand. I'm just trying to see how long --

DR. BELSITO: Four weeks, no?

DR. JOHNSON: Yeah; just acute and short term toxicity information, tox data.

DR. KLAASSEN: When they did this four-hour exposure, they did have dark red lungs for observing the

(inaudible) which doesn't -- which shows something can go on there; not a very high concentration.

DR. LIEBLER: Well, these are all at near 20 percent.

DR. KLAASSEN: Yeah.

DR. JOHNSON: Did you want the comments relating to the effect on lung cells to be addressed in the discussion?

DR. BELSITO: I don't think we're even there yet, Wilbur. Let's wait for the discussion once we get through all our other points. I think that a lot of that is going to depend upon what we see in the Korean study and if we can get any additional inhalation data because -- what was the longest we had again?

DR. LIEBLER: What I was looking at here -- looks like --

DR. BELSITO: Four weeks, no?

DR. LIEBLER: I don't believe we have that.

DR. KLAASSEN: Inhalation?

DR. LIEBLER: 28 days.

DR. KLAASSEN: Yeah; there is a 28 days in which they determine a no-observed adverse effect concentration of 0.025 mg/m².

DR. BELSITO: To bring some area of expertise, but it sounds like a fairly high amount, no?

DR. SNYDER: Yeah; and it looks like it was eliciting irritation to because there was (inaudible), and that's typical upper respiratory response to irritation over a period of time.

DR. BELSITO: So, we know that a very high amount used in an aerosol product could over the long term create issues; but we don't know, even a low (inaudible), which is a problem, no?

DR. SNYDER: Well, this was a 20 percent (inaudible) solution and they targeted the paradyamic size to a rescuable size too, so; and if our formulations aren't rescuable, so, I think, it's going to be complicated.

DR. BELSITO: But, again, my point is that, you know, we're talking about an inflammatory response. It doesn't really need to get down to the (inaudible). We're not talking about something that's going to get absorbed and go through the system. We're talking about something that would cause an inflammatory response in the airway.

DR. SNYDER: So, this was in the larynx region; so --

DR. BELSITO: Yeah.

DR. ANSELL: Also, not certain -- I only see one generic listing for an aerosol

application and the use concentration is 0.0002.

DR. SNYDER: I have the max spray use as.27.

DR. ANSELL: Yeah, per pump. All good questions.

DR. SNYDER: Yeah; so, I think we just have to flesh it out a little more.

DR. BELSITO: Okay; moving along. So, for respiratory we need the -- I wish I was as (inaudible) with you Dan in how to deal with these comments here. Okay, so, we want the Korean studies, and if there's any other inhalation data out there, would be nice. Anything else, there; and then at some point, we'll have to deal with it in the discussion.

DR. SNYDER: Yeah, we'll have to probably get --

DR. BELSITO: And we have 0.27 in a pump spray, right?

DR. SNYDER: Yes;.5 percent for all others.

DR. BELSITO: Right; okay. So, then we have a dermal study with a non-genotoxic affect. Do we need to ask Ivan to look at margins of exposure because of that --

DR. SNYDER: No, I think we can explain --

DR. BELSITO: -- or do we mention it at all in the discussions?

DR. SNYDER: -- well, I think we have to because if you read it, it appears to be this affect, but it was due to the persistent side of toxicity, it had nothing to do with the chemical's effect on endothelial cells.

DR. BELSITO: Okay; so we don't need --

DR. SNYDER: No. We'll just make sure we have wording in there to address it.

DR. BELSITO: Okay. You'll work on that wording with Tom Slaga?

DR. SNYDER: Yes. And we did get sensitization data, 20 percent in the oil in wave 2.

DR. BELSITO: We got a lot of sensitization data, but, you know, we don't have a noel for sensitization. You know, we know that it sensitizes at 2 percent. That's the lowest concentration; and then we know that if you take up people who are sensitized and you take them back and you patch test them, you can get reactions down to.1 percent. So, or.2 elicitation; can be listed as concentration beginning at.2, I believe. So, I mean, I think that's the basis as to why the Europeans went at.1. They say, okay, you can sensitize at 2 percent; you can elicit at.2; and so, let's go to.1 because everyone agrees that you sensitize at a concentration higher than you elicit. But, we don't have -- I mean, it's not like we used to seeing at Riffen where we have nestles and HRIPTs, and we have part, you know, EC3's and we have hard data that are then confirmed in an HRIPT; we just have data that sensitizes and then we call back a bunch of patients to patch test. I think it's okay, but it's not very scientifically robust.

DR. ANSELL: I think when you look at the dossier there's going to be more relevance. They do have a conclusion that 1 percent did not induce.

DR. BELSITO: I didn't see that -- in the dossier? What page?

DR. ANSELL: It's page 79 of the PDF.

DR. SNYDER: Under what?

DR. BELSITO: It's the SCCS opinion that was added to this.

DR. ANSELL: No; this is the submission to the SCC. This is the cosmetic dossier --

DR. BELSITO: Okay.

DR. ANSELL: -- that was provided. I think this was a way to --

DR. BELSITO: Yeah, I do know. Oh, it's wave 2?

DR. ANSELL: I'm not too sure?

DR. BELSITO: Yeah; it's in the actual one, here.

DR. LIEBLER: Page 79.

DR. BELSITO: Skin and mucus membrane irritation sensitization.

DR. ANSELL: So, it's the summary of reliable tox data.

DR. BELSITO: So, it says that in guinea pig maximization Buehler, threshold concentration for induction to sensitization in guinea pigs is demonstrated to be above 1 percent. But, I didn't see that data.

DR. SNYDER: Above 1 percent, what does that mean?

DR. BELSITO: That means it was 2 percent; but it doesn't say that they did 1

percent and it was negative.

DR. SNYDER: Right; and it was negative, exactly.

DR. BELSITO: I didn't read that as that. The only data I saw was that they did 2 percent, it was positive; and 2 percent is above 1 percent, but, you know, I mean, it's like - -

DR. SNYDER: Well, I mean, we can take the same approach they did, simply the maximum concentration is .5 percent, so we're not anywhere below that, so.

DR. BELSITO: I don't think .5 is safe.

DR. JOHNSON: It's actually 0.2, now.

DR. ANSELL: I think the use of concentration is .1.

DR. JOHNSON: 0.2 is the highest; I mean, based upon what Carol gave us today.

DR. ANSELL: I think what Carol gave you was that the --

DR. HELDRETH: 20 percent of the .5?

DR. ANSELL: --.5 was 20 percent active; so, that would be a fifth; it'll be .1.

DR. JOHNSON: But she had some ranges that, you know, based upon that calculation --

DR. ANSELL: Okay; well, I mean, this is the first time. These are all good questions.

DR. BELSITO: Well, here's what was on our table today, and there's an eye lotion at .2. And, you know, that's a question that's going to come up repeatedly that's so very confusing with these things that aren't supplied at 100 percent, and what are these concentrations we're getting? Are they concentrations of the active, or are they concentrations of the cold product? And then there will be another question I will pose tomorrow, Jay, and this really concerns me and so as does a lot of patch testing. So, when -- if this chemical -- there is a chemical; I'm forgetting which one it is. I think it's the polyurethane sitters supplied in methylisothiazolinone as a preservative in what's given to the manufacturer to make. Do they have to label methylisothiazolinone, or are they labelling only the active? Do you know the answer to that question?

DR. ANSELL: For the raw material, or for the finished (inaudible)?

DR. BELSITO: The raw material comes to them and they're buying chemical X, but chemical X has BHA in it as an antioxidant and methylisothiazolinone is little known as a preservative. Do they have to label the BHA and the methylisothiazolinone?

DR. ANSELL: They don't have to as long as it is not used, as long as it's not effective. If they put into their concentration of the preservative, then it would appear there. But non-functional additives that would come in that way are not required to be labeled.

DR. BELSITO: So, the answer is no; they wouldn't have to necessarily label them.

DR. ANSELL: No; they wouldn't have to.

DR. BELSITO: That's what I thought. Okay; back to this. So, I'm okay going with point one; I'm not okay going with point 2; and even that point one is a rather non-scientific. It's out of the approach that Europe is taking with what's called the minimal elicitation threshold 10 for nickel and chromium and other things that they've restricted. They take people who were sensitized; they bring them back; they patch test them; and they look at how low can you and still have 10 percent of that sensitive population reacting; and at that level, we think it's okay in the general population. I mean, to use that theory, I guess .1 is fine, but I don't see a no-affect level for sensitivity; and I don't think we can fudge this one and say when formulated to be nonsensitizing because it's not going to be added to anything else that we can't control that would cause issues.

DR. SNYDER: I have a moderate to strong sensitizer as low as .2 percent, and so that's not a very big difference to .1 percent. So, I think we need to see if there's any additional data out there. We can see if we have a no-affect level for sensitization.

DR. BELSITO: It would certainly be nice. I mean, again, this is the first time we're looking. We're already asking for some additional inhalation studies or data; we're asking to look at the Korean reports; and I think we can ask for additional sensitization data that would indicate a level which it does not in desensitization.

DR. SNYDER: I think, let's ask, and then we'll know. I think we'll be more scientifically sound than just arbitrarily saying.

DR. ANSELL: Right.

DR. BELSITO: Okay. So, it's no longer used at .3 in eye products. It's been corrected. It's now .2. So, we know that 20 percent can be irritating, and .04 percent is not irritating around the eye, but we don't have anything in between. So, do we want to ask for additional ocular irritation studies at the reported concentration of .2? I mean, we didn't used to ask for them because they're done in animals, but now, you know, there are OECD guidelines for in vitro ocular irritations, so I don't see why we have any concerns about asking for them.

DR. LIEBLER: Yeah, I think even if we had the animal model irritation data at the use concentration, we would probably be able to roll that into a stronger weight of evidence; so, I think we should ask for it.

DR. BELSITO: Okay.

DR. SNYDER: So, I think that probably goes along with the lung things. I think the lung thing is all irritation too. So, we want to --

DR. BELSITO: Now, but if you say the lung thing is all non-exposure of irritation from induced inflammatory side effects, you're right.

DR. SNYDER: I think it's all related irritation. So, I think --

DR. LIEBLER: There are a lot of toxic chemicals that are not what you would think of as inflammatory chemicals that can activate NF-kappa B. I mean, I just remember, and Curt does too, I'm sure, at the SOT meeting there was like an NF-kappa B activation era in the mid-nineties where every kappa-toxic chemical that got thrown into any kind of model, it was just a new thing you could measure. So, this study reminded me of that.

DR. SNYDER: It's that there's no biological context?

DR. LIEBLER: Right. I think it's just an observation at this point, but we do have inhalation data that suggests that it is irritating. So, how can we obviate the irritation ocular in the lungs? So, we would probably want to know at what point what concentration we

DR. BELSITO: Mm-hmm; okay.

DR. LIEBLER: -- we don't foresee those affects, so we put those together.

DR. BELSITO: Yeah. So, then, I just wanted to make a comment about, you know, that all of the patch testing studies were done in Europe where this is -- now with Jay's comment, it may not have been used at such a different concentration -- but it may have also, so, none of those patch test study data are coming out of the U.S. Was anyone bothered by the anaphylactic issues with use on damaged skin? I could not get those reports or read them. Can you tell us more about those, Wilbur?

DR. JOHNSON: What page?

DR. BELSITO: Page 20 of the --

DR. SNYDER: Under case reports.

DR. BELSITO: -- two case reports, surgical wound dressing, .2 percent polyaminopropyl biguanide deaths from severe anaphylactic reactions.

DR. JOHNSON: Actually, those studies were in the SCCS report and, I think, that was an unpublished study; but that's, you know, basically all the information that I was able to capture from the SCCS report.

DR. SNYDER: A hospital disinfectant would have lots of other things in it that could be an issue. I'm not certain about the wipes.

DR. BELSITO: That's what I mean, but, you know, it's there looking like it was the biguanide that caused that.

DR. SNYDER: I think we look at those and say that they just have a table that listed everything that was in there and said, any of these were potential.

DR. LIEBLER: One of the references is cited as this NECNAS --

DR. JOHNSON: Yes, I'm sorry, not the SCCS, but the NECNAS report, yes.

DR. LIEBLER: And then there are two publications -- third reference is 35 and 36, that you cite for those. The two cases of anaphylaxis and then the paragraph right after it that also refers to anaphylaxis.

DR. BELSITO: Yeah, it's a German -- that I couldn't access and Columbia Library doesn't subscribe to Allergy either, so I couldn't look at either of those reports.

DR. JOHNSON: If that report is that important then we could, you know, perhaps have that special ordered.

DR. BELSITO: If it's in German, then you'll have to get it translated; but I just don't like the idea of us just - - first of all, you know, as I tell my students, you can read something in an article and you can say that they quoted the paper and that's what they thought the paper said, but unless you actually got the paper and read it, that's not what you report. So, we've, you know, limited polyethylene -- well, I call them burn patients because of renal damage -- I mean, is this an issue where we need to consider -- of course, then we got rid of that -- but, I mean, is this an issue where we need to consider limiting the use of this chemical on individuals who have, you know, severely damaged skin? I don't no.

DR. SNYDER: You want wait for Matt?

DR. LIEBLER: So, we should get copies of both papers, 35 and 36. So, one is in this Swiss journal -- and I wouldn't be surprised if there's an English version of it -- and then the other's an Allergy study; so, that shouldn't be an issue.

DR. BELSITO: Yeah; I know. Okay, so far in the discussion, we're going to have the respiratory boilerplate which is going to have to deal with the inflammatory findings in the lung; we're going to have to deal with the sensitization issue and, hopefully, have that resolved by data that we're going to ask for along with respiratory data.

DR. JOHNSON: Any concentration limit for sensitization?

DR. BELSITO: We're not even going with a conclusion here, Wilbur.

DR. JOHNSON: Oh, no, I don't mean -- I'm just saying with respect to the sensitization data --

DR. BELSITO: Whatever concentration industry wants us to approve up to.

DR. JOHNSON: Okay.

DR. BELSITO: I mean, you know, they need to give us a no-affect level, or a level that they want to use that doesn't have enough (inaudible).

DR. SNYDER: Right now it's.2, based it on eye lotion.

DR. BELSITO: Yeah, right now it's.2. So, if that eye lotion wants.2, they better show us data on.2; --

DR. JOHNSON: Okay.

DR. BELSITO: -- and, particularly, if they want.2, they'd better show us data on the ocular irritation on.2. We want the references 35, 36 on the anaphylaxis. So, we're going insufficient. We would like additional inhalation data at use concentrations; we'd like sensitization at whatever concentration of use they want to use; ocular irritation at whatever concentration they want to use; and we want to review references 35 and 36 in the current report.

DR. SNYDER: So, what dermal absorption data did we have?

DR. JOHNSON: It's in the table on skin penetration.

DR. LIEBLER: Yeah, there are a number of animal studies that doesn't appear to be not very significant to our --

DR. SNYDER: I wouldn't expect it to be. It's a 4,000 molecular weight on average.

DR. LIEBLER: Okay.

DR. BELSITO: So, anything else -- inhalation, sensitization, ocular irritation, and get us references, 35 and 36? And so far in the discussion, we're going to be talking about, obviously, inhalation sensitization; but we're going to also talk about the generous.

DR. JOHNSON: Are there any concerns relating to reproductive and developmental toxicity?

DR. BELSITO: I didn't have any, Paul?

DR. SNYDER: No; I didn't see any. Did you have anything specific in mind, Wilbur?

DR. JOHNSON: Yeah. I know there was a teratogenicity study involving rats and the chemical was classified as teratogenic at an intraperitoneal dose of 10 mg/kg per day.

DR. BELSITO: You okay with that?

DR. LIEBLER: Yeah, that's fine. (Inaudible).

DR. BELSITO: Okay; anything else?

DR. KLAASSEN: Not to be the devil's advocate -- how do we know Tulcid isn't teratogenic?

DR. SNYDER: Well, we have other studies; there a number of studies in Table

12, right?

SPEAKER: Mm-hmm.

DR. JOHNSON: And that value was also a no-observed adverse effect level in mice?

DR. KLAASSEN: Right.

DR. JOHNSON: 10 mg/kg per day?

DR. KLAASSEN: Okay; fine; now we're okay.

DR. SNYDER: Any of them are oral?

DR. JOHNSON: That's why I'm looking. I thought they were all dietary.

DR. LIEBLER: I think the other thing is the IP administration. I think this material doesn't get absorbed very well, if much at all.

DR. SNYDER: No; probably had a raise in pertinetis.

DR. BELSITO: Okay; so, to repeat, so insufficient, we want inhalation data, and then we also want the Korean studies that talked about these tests; we want sensitization on ocular irritation on concentration of use; and we want to review the two reports on anaphylaxis, the current references 35, 36. Anything else?

DR. HELDRETH: I have one thing -- what we had brought up about the identity of the polyhexamethylene guanidine phosphate that's in the Korean papers, do we want to have some further clarification that is really the biguanide and it's not some mono-guanide?

DR. LIEBLER: That's one of the things I'd like to review when I see those papers.

DR. HELDRETH: Because looking through those papers, they all just say polyhexamethylene guanidine phosphate; and even tracking down through the references that they cite, can't find anything that gives you a structure or tells you the CAT's number or anything to verify that they really meant the biguanide.

DR. LIEBLER: So, you could have the guanide, I guess.

DR. HELDRETH: And that was our major rationale, not so much the phosphate salt issue, but that we weren't sure that this really is the ingredient under review.

DR. LIEBLER: Well, we should review the papers and we'll take a look at that, and if that issue can't be resolved, we'll just need to consider that when we consider the importance of those reports to our conclusion, so; but let's see the papers anyway.

DR. KLAASSEN: We could also contact the author.

DR. LIEBLER: Yeah; right; because these are very recent publications.

DR. KLAASSEN: Yeah; these are recent papers.

DR. LIEBLER: Contact the author. I hope it's not the guy in jail.

DR. SNYDER: I hope he doesn't use his one phone call.

DR. KLAASSEN: Rather keep his phone call for his wife.

DR. LIEBLER: (Inaudible).

DR. BELSITO: Oh, God.

DR. KLAASSEN: They've had their fair amount of troubles this year.

DR. JOHNSON: One last question, Dr. Belsito, you said the discussion should have some language relating to the tumor formation that was observed --

DR. BELSITO: Right.

DR. JOHNSON: -- and why we're --

DR. SNYDER: Why we're not concerned; I can insert something in there for you.

DR. JOHNSON: Okay.

DR. KLAASSEN: I mean, if you have a bile side, you're going to expect some toxicities, by definition.

DR. BELSITO: Let me make sure I save this so I don't --

DR. KLAASSEN: In fact, this is pesticide is the reason why there's a fair amount of data.

Day 1 of the April 10-11, 2017 CIR Expert Panel Meeting – Dr. Marks' Team

Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride)

DR. MARKS: So this is a first review of the polyaminopropyl biguanide. And we don't have to ask if ingredients are okay since there's a solo ingredient, so it's okay. But Wilbur, I'll ask you to clarify the chemical names in a minute perhaps. So, Tom, Ron, and Ron, that might be part of what you're asking. Do you have needs for this single ingredient? And this is the first time we've reviewed it.

DR. SLAGA: To me, there's sufficient data.

DR. HILL: I think so too actually.

DR. MARKS: Sufficient. Ron Shank, you're looking with a smirk, I can see. I have okay, but I would set a limit based on sensitization.

DR. SHANK: Well, a limit but, there's data there.

DR. MARKS: Sufficient data. Well, I'm not sure on the sensitization.

DR. SHANK: No.

DR. MARKS: Oh, you want to hear what I say?

DR. SHANK: No, I want to know, you want to set a limit on what chemical?

DR. MARKS: Oh yeah, well that gets into what chemical. The polyaminopropyl biguanide. And you're not quite sure which chemical this is.

DR. SHANK: Well, I found this very hard to read.

DR. MARKS: Okay.

DR. SHANK: And it's not the writer's fault. Let me explain. If understand this correctly, the title compound is polyaminopropyl biguanide. But that's not the chemical that's being used. But that's the name that's being used. Really? Okay? Now let me go farther. You read the search, how it was searched, and both compounds were searched. So what am I reading here? It says will use only the name polyaminopropyl biguanide. No matter what it is. So are all of the data for the PHMB? But it's not called that. It's called PAPB. So I think either we should table this until the dictionary is corrected. But then, all of that labeling and history, the wrong compounds. I think this is very confusing. And I really don't know what I'm reading. On the other hand, dermal penetration of whatever it is, seems to be small. And what stays on the skin seems to be in the epidermis. And it's not a reservoir for circulation. So it's probably okay. But I don't know what it is. Sorry.

DR. MARKS: No, you don't have to apologize. That was my first comment. Wilbur, clarify the chemical names. So you suggest tabling it. Til we clarify what chemical we're really dealing with. Is that? Will you be able to do that Wilbur?

DR. HELDRETH: I can clarify it now.

DR. MARKS: Oh, you can.

DR. GILL: And I would just add that the issue about tabling it, this was added by the panel as a priority, because it is a preservative. And the concern that the European decision might impact that. So if Bart can clarify it for you.

DR. HELDRETH: All right. Much of what Dr. Shank presumed is correct. This ingredient, the ingredient name is polyaminopropyl biguanide. But if you use that as a chemical name, none of that is in the ingredient. And, my understanding, talking with the INCI Committee, and there interaction with the suppliers, nobody's ever been using the chemical polyaminopropyl biguanide as an ingredient. In every case it's been polyhexamethylene biguanide hydrochloride. And so it's that one ingredient that we're reviewing. And that's the chemical that whoever included tox data on in the report. polyhexamethylene biguanide hydrochloride. It's right there at the beginning of the chemistry section.

DR. SHANK: Okay. So can the title be changed? So it says polyaminopropyl biguanide parentheses?

DR. HELDRETH: Sure

DR. SHANK: And the other one? Or the other way around? And then explain it in the introduction, as you did, very nicely. But then, everything in the report is referred to, not everything, but a lot of the report, refers to a polyaminopropyl biguanide. But it ain't that.

DR. HELDRETH: I agree. It's extremely confusing. What we've been trying to

do, over the past couple of years, at least, is try to only use the INCI name throughout our documents. And stick with just that one. Instead of using other technical names, or trade names. But just use that strictly. And much still to do on that. I push that we stick with that process and just use the INCI name throughout the report. We can certainly make a title change and put more introductory language in the report, and make those changes. But it is truthful that it's all this PHMB HCL. Is all we're really looking at.

DR. GILL: Is there a plan to change that name?

DR. HELDRETH: I have not heard that there's a plan to change that name. You do have to remember that part of the rationale for not changing a name in the INCI dictionary is because other countries use older versions of our dictionary and if you go and change those names in a country that has very difficult registration systems for bringing a new ingredient, you may severely impact their ability to do business in that country. So making a change on an ingredient like this, that's quite old, and in significant use numbers, could have a profound effect. So, the INCI folks did help us out by making the monograph of the definition in the dictionary more clear that it's just specifically this ingredient. So, if I had to guess, I wouldn't suspect that the name is going to change anytime soon.

DR. MARKS: And again, the chemical name is polyhexal methyl biguanide

DR. HELDRETH: polyhexalmethylene

DR. MARKS: Methylene. Okay.

DR. HELDRETH: Kind of a weird way of saying hexane.

DR. MARKS: Yeah

DR. HELDRETH: But, hexal methylene biguanide hydrochloride. Is our understanding is, from the suppliers, that it's always hydrochloride.

DR. SHANK: Then I would suggest having the title polyamino propyl biguanide. And then in parentheses the hexylmene biguanide.

DR. HELDRETH: We can do that.

MR. JOHNSON: If I might just add, if you look at PDG page 18, under cytotoxicity. The section titled Cytotoxicity and Antimicrobial Activity. Polyhexalmethylene biguanide and polyaminopropyl biguanide are compared. I think that's the only instance in which you actually have data on polyaminopropyl biguanide.

DR. MARKS: Now I'm confused. When you say you have data on both. I thought they were the same.

DR. HELDRETH: So what Wilbur's trying to say here is that, in the paragraph on cytotoxicity, the authors are comparing the toxicities of the chemical names.

DR. MARKS: Okay

DR. HELDRETH: Polyaminopropyl biguanide and polyhexalmethylene biguanide. Only the polyhexalmethylene biguanide though is an ingredient. So herein Wilbur laid out those instances where they use the chemical, polyaminopropyl biguanide by calling it PABP. Give it a little bit different of a moniker so that it's as less confusing as can be.

DR. MARKS: Yeah. So, can we say then all the tox data we have in this report is on polyhexalmethylene biguanide, aka INCI name polyaminopropyl biguanide? Because I think, Ron Shank, that was your initial concern is, what were we testing when we read this data.

DR. HELDRETH: That is true except for this instance where they compare.

DR. MARKS: Except for the instance, okay. Okay. With that in mind, now that we've clarified the chemical names. And unless Lillian or Wilma, you have any concerns, I like the title. It includes both names in it. Then the introduction would also clarify that.

DR. BERGFELD: And your discussion perhaps.

DR. MARKS: Yeah, and discussion. But I think right up front, it hopefully will minimize the confusion that could occur. So, with that in mind, as I recall now, we had a fair amount of discussion since Tom. Tom, you were fine with the safety of this?

DR. SLAGA: I didn't say that. It's a very toxic chemical.

DR. MARKS: Oh, I know. It's an irritant and a sensitizer.

DR. SLAGA: You don't get cancer, but you have three feet going up in the air.
(laughter)

DR. MARKS: So now we'll come to the next. Now that we've clarified what the ingredient is we're really looking at here. Now the question is concerns.

DR. EISENMANN: Well, speaking up, if I give you updated concentrations of use. I'll look more careful, talk to the company that had the highest concentrations reported. And they were reporting a concentration of 20% solution. So 0.1% is the maximum that company is using. I have one company still reporting 0.2% in an eye lotion. And that's now the highest concentration. So it's gotten to be more consistent with the European conclusion.

DR. HILL: So they said 0.5%, but really only 20% of that was the ingredient?

DR. EISENMANN: Correct. So I gave you updated concentration of use information this morning.

DR. MARKS: SO what is the highest now?

DR. EISENMANN: 0.2

DR. MARKS: Okay, so we go from 0.1 to 0.2. Is the highest concentration?

DR. EISENMANN: Went from 0.5 to 0.2.

DR. MARKS: Yes.

DR. EISENMANN: And the European limit it 0.1

MR. JOHNSON: Is that official now, Carol?

DR. EISENMANN: Yes. Well, unless I hear something else changes. But I confirmed the 0.2 so.

MR. JOHNSON: I mean the European limit

DR. EISENMANN: Oh, the opinion. No, the opinion's not. They're still working on it.

MR. JOHNSON: Okay.

DR. EISENMANN: The counter period is over. They have not finalized it yet.

MR. JOHNSON: All right.

DR. MARKS: Well concerning sensitization there it a Bueller testing which set a sensitization threshold of 1%. There's an HRIPT that showed no irritation at 2% but it could sensitize at the concentration. But that's ten times higher than the use concentration and 0.2 is below the Bueller threshold at 1%. So I thought it was okay with that. But I can't speak to these limbs going up in the air, Tom.

DR. SLAGA: Well those are high doses.

DR. MARKS: High doses, okay. So, Ron, Ron, and Tom. A tentative report with a conclusion of? Or do we have insufficient data? Is it safe or not safe?

DR. SHANK: It has a broad toxicity profile. And you can argue dosage, which is a good argument. I don't understand the molecular weight. Ranges from less than 500 to more than 1,000. That's quite a range.

DR. HILL: It's a polymer, so what you have to get is the nature of hexamethylene diamide. So that in itself is a complex substance when you actually have a bottle of that. Because the simplest form it can take is sort of a cage like structure where you have multiple interconnected six member rings with three nitrogens in it. So then if you take that and react it with anything, stuff comes apart, rearranges and so forth. And so when you do that, which is what they're doing here. And they're reacting it with a compound that is also a mixture, which is the sodium dicyanamide, which is also a mixture. The equilibrium, that's a nice little figure in there, those are very different compounds. Then you're getting a complex mixture with a range of molecular weights. And in fact, while we're on the subject, where it says impurities. If you read those compounds that are listed before you get to the trace metals, those are really the monomers and dimers that you would expect to get in the process of doing that chemistry. So I guess you could regard them as impurities, but I don't. I regard those as just part and parcel to this polymeric substance. Because on the low end, with the 500 molecular weight, that's probably dimers, maybe trimers, but I think dimers with the calculation. So you've got a complex mixture and it's been tested however and evaluated as such. And the only ambiguity in here is the place where you've got a poly, the propyl, where you've got two amides on the end and just three carbons in between instead of six. That would be giving us a very different substance. So the issue there is any toxicology studies that were actually done on that propyl, in the middle, we should ditch those. They shouldn't even be used for read across here. Because I don't think they relate.

DR. HELDRETH: We only have one in there and it's for comparison.

DR. HILL: Okay. As long as we're very explicitly clear, because of the confusion and nomenclature, then it would be bad to take it out, it would be better to leave it in.

But I just want to make sure everyone is clear in reading that. What the story is because of this name mess-up. Which as far as I can tell is just because somebody put the brackets on the wrong place in the polymer and named it.

DR. HELDRETH: I think that's the case, but unfortunately there is actual, the chemical name.

DR. HILL: I know. I know. I got that. And it's good that we pointed that out in the context.

DR. HELDRETH: There was some global confusion about this. I mean, you'll notice even in the SCCS report, it's got CAS numbers that will take you to polyaminopropyl biguanide, the chemical name, as well.

DR. HILL: But as far as staying on the skin, these guanide residues are what amounts to a permanent positive charge. Comparable to a quaternary ammonium. PKs are up around 12.5, 13. So they're always going to have a positive charge. That means for them to get through this intact skin, except when we have something like a mucous membrane, is not easy. So that's the good news in terms of surface type applications. Now, inhale a little into the nasal passages, put it on mucous membranes, that's a different story.

DR. MARKS: They get through the skin to sensitize.

DR. HILL: Yes. I would say they get into the skin.

DR. MARKS: So, Ron Shank, do you have needs? So it's either a tentative report with a conclusion

DR. SHANK: I don't have needs. The dermal penetration is very small, so. Dermal application is okay. Wilbur asked should we include the Korean data, where this was used as a preservative in some spray.

DR. HELDRETH: Using a humidifier.

DR. SHANK: Korean study where humans were exposed to

DR. HELDRETH: It's a humidifier additive.

DR. SHANK: Humidifier additives. And developed lung injury. So I would say it should not be used, there was no concentration given, that I can remember.

DR. HELDRETH: Part of our rationale for proposing, is this relevant or not, is again, with more nomenclature issues. In all three of the publications that were provided, they use the term polyhexamethylene guanide phosphate. Which would suggest not the biguanide, but a monoguanide polymer. Now that may just be a nomenclature issue, and they really meant the biguanide. But, looking through all three papers, and chasing down the citations that are in those papers, there's no way to make that clear. So we don't know if they're talking about the same chemical or not. And that's why whoever put this in a memo to you, are these relevant, we don't know.

DR. HILL: Although I don't know how you get a polymer if they only had one group on there. Effectively that's what you're seeing anyway. Starting with the hexamethylene diamide. I get your point though. I guess what I'm saying is, you're not starting with something that has a guanide already on it. You're reacting an amine with the cyanamide. Generating the guanide while in situ in such a way that you're getting polymers. And then the interesting thing is, cyanamines on the other end.

DR. MARKS: Ron, so how, would the inhalation

DR. SHANK: Presumably having this as a disinfectant in a humidifier, the exposure would be over a significant amount of time. Whereas used in an aerosol, cosmetic aerosol, would be very short exposure. But that's a lot of unknowns. So, topical application seems to be all right. But I don't know about aerosol products. So if we can't really have the information, I guess the way out is to say that's insufficient for products that can be inhaled. So they'd have to provide inhalation data.

DR. MARKS: So I guess the question then in my mind, that would be a way of handling this, and obviously in the discussion, you have to point out the chemical difference there. But we could either put a insufficient data announcement and then ask for, or we could do a tentative report, safe for topical, insufficient for inhaled products. And I think it just depends on how we want to handle it. Do we want to press forward with a tentative report? Or do want to just, usually when we ask for more data we do an insufficient data announcement.

DR. SHANK: Safe for dermal application of an inhalation product?

DR. MARKS: No, no. I thought you said safe for topical.

DR. SHANK: Only

DR. MARKS: Yes

DR. SHANK: Not inhalation

DR. MARKS: Yeah. Insufficient for inhalation.

DR. SHANK: Yes

DR. MARKS: If I wasn't clear, that's what I meant.

DR. SHANK: Okay

DR. MARKS: But, do you want to do this as an insufficient data announcement pointing out for insufficient? Yes.

DR. GILL: Well if part of it is insufficient, since this is the first time, it will be an insufficient data announcement.

DR. MARKS: Okay.

MR. JOHNSON: I'd just like to add that the safety assessment does contain acute and a short term inhalation toxicity data.

DR. SHANK: Sorry, where is that?

DR. HILL: But it's only acute and short term. That's the bothersome thing there.

MR. JOHNSON: Okay.

DR. HILL: So sensitization, that's probably, I guess you'd pick that up. But since this is being put out there as having carcinogenic effects, if you don't have chronic, I think you're missing something. In my humble opinion. I don't know what these guys think.

DR. MARKS: I just lost, damn.

DR. HILL: Of course that insufficiency is consistent with the European's take on this. Which is they think there's not enough information to make them comfortable for safety in spray products, is what it says, what I got.

DR. SHANK: Okay, the animal inhalation toxicity data, say what the exposure concentration was in milligrams per cubic meter. But nothing about the aerodynamic properties. If that information is available it should be stated.

MR. JOHNSON: It wasn't stated. These data are taken from the SCCS report. And that specific information is not included.

DR. MARKS: Okay. So tomorrow, I presume we're gonna, I will second an insufficient data announcement for this ingredient.

DR. SHANK: Well, what do we do with the inhalation data that's in there? If we ask for inhalation data and we already have it?

DR. MARKS: But not for chronic is what I understood. There was acute and sub-acute, but not chronic.

DR. SHANK: 28 days inhalation.

DR. MARKS: That's enough for you? Ron?

DR. SHANK: Yes. Yes.

DR. EISENMANN: In the dossier that we got later, it does give the particle size.

DR. SHANK: And what was it?

DR. EISENMANN: 0.32 to 1.3 micrometers.

DR. SHANK: Okay

DR. EISENMANN: And, depends on the concentration, so the 0.257 milligram per meter cube is 0.48 to 5.06. And the 2.47, the highest concentration was 0.67 to 1.67.

DR. SHANK: Point, zero point?

DR. EISENMANN: Yes.

DR. SHANK: Respirable?

DR. EISENMANN: mm hmm

DR. SHANK: For 28 days.

DR. MARKS: You feel comfortable?

DR. HILL: You wouldn't see any carcinogenic effects.

DR. SHANK: No carcinogenic, but you would get the lung injury. Presumably. So. That would have to be in the discussion. To counter the Korean data.

DR. MARKS: So, how do you want to move forward, Ron? You would put

tentative report? Or an insufficient? It sound like you said we have enough inhalation data now to come to a conclusion.

DR. SHANK: Yes. Tentative.

DR. MARKS: Tentative report. And the conclusion is? Safe?

DR. SHANK: Safe.

DR. MARKS: No restrictions?

DR. SHANK: Well, concentration.

DR. MARKS: Yes. The 0.2%, which is the use concentration. So we don't have to put that in the conclusion.

DR. SHANK: Okay.

DR. HILL: But, what do we have in spray products? Do we know whether there's a pump hairspray that could be used every day for years and years and years?

DR. BERGFELD: Body lotion with 0.2.

DR. SHANK: The use in sprays says it's not, it may be sprays and it may not.

DR. HILL: That's what I thought it said.

DR. BERGFELD: The concentration (inaudible)

DR. SHANK: 0.5%

DR. BERGFELD: So 0.5 is not (inaudible)

DR. HILL: Yeah. It's the 0.2.

DR. HILL: 0.5 in sprays right now?

DR. BERGFELD: Correct.

MR. JOHNSON: In hair sprays it's up to 0.004% in aerosol sprays. And 0.052% in pump sprays.

DR. MARKS: Okay. So I'll be, for our team, I'll be seconding presumably a motion that's issue a tentative report with a safe conclusion. And from a discussion point of view, we'll include the chemical and INCI name in both the abstract, the introduction and the discussion to clarify the nomenclature. Does that summarize it, do you think?

DR. SLAGA: Great.

DR. MARKS: Oh, title. Yes. Thank you. I have to include the title there, thank you. Somehow I deleted all my notes and I had to go back.

DR. HILL: I'm sorry. I've got a question. I'm looking at the use table. And it has hairsprays, pump spray, up to 0.27%. Is that a mistake?

MR. JOHNSON: We received new data this morning

DR. HILL: But that's not there anymore?

DR. EISENMANN: That was one of the concentrations that they were reporting concentration of the mixture rather than

DR. HILL: Okay. So divide by five.

DR. MARKS: Okay. Wilbur.

MR. JOHNSON: Are there any concerns relating to reproductive and development of toxicity? Genotoxicity or carcinogenicity that would need to be addressed in the discussion?

DR. MARKS: I didn't hear any comments from Ron, Ron, or Tom. Specifically do you have any concerns

DR. SLAGA: No

DR. SHANK: The in vitro utegenicity assays really aren't valid because it's antimicrobial. So those in vitro studies usually are complicated by cytotoxicity. And the reproductions, developmental changes we're seeing only at very high doses.

DR. MARKS: Okay. Good. That answers that, Wilbur.

MR. JOHNSON: Yes. Thank you.

DR. MARKS: No, thank Ron Shank. Okay. Any other comments? Well we managed to stretch this one ingredient out to a robust discussion. Okay. Well I think for all of us because of the nomenclature issue.

Day 2 of the April 10-11, 2017 CIR Expert Panel Meeting – Full Panel

Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride)

Moving onto the next one, which is a preservative, Dr. Belsito, the polyaminopropyl biguanide, I guess it's pronounced?

DR. BELSITO: Yeah. Interest pointing it's the INCI name, but it's not the chemical name. But we will stay with the INCI name. I really had asked that this be moved up as a priority ingredient, because it's increasingly being used as preservatives. And the EU is rapidly moving. And has actually set out a revised opinion to limit this to .1 percent in preservatives. So I was very interested in the U.S. getting their opinion in about this. So, having said that, we looked at this, and the issue is, I had two issues with this. First of all, we know that at two percent, it induces sensitization. And in those individuals in whom it induces sensitization at two percent, that sensitization can be elicited in patch testing down to .1 percent in a very small number of individuals. Very weak reactions. What we don't have is a no effect level for sensitization. Wilbur was kind enough to send me over, and I was trying to --. So we know there's a hazard. We don't know how to assess the risk of the hazard. I asked Wilbur to send me the Gerbrick article, and it simply states, that there was a positive LLNA. And that there was a positive guinea pig maximization in the 4 biguanide. What it doesn't give me is an EC3 value. It gives me any sense for how potentially sensitizing this is. Nor is there a reference in there specifically. So, I think the positive LLNA exists somewhere maybe in P&G's files. Or one of the other company files of the co-authors on this paper that included, I believe, David Basketer, who was with Unilever at the time. So, someplace out there, there must be an LLNA. But it is not in the published literature. I spent over a half hour trying to search for it. So, at this point, I'm not comfortable signing off on this at any concentration, even .1, without knowing the sensitization capacity of this material. And the second issue, minor, but still there, were the reports of anaphylaxis when this was used in wound dressings. And I was wondering if this is an issue similar to the pegs, where it's simply, you know, damaged skin and severely damaged skin. Or what was going on. And it was a late request, late Saturday night, I think, to Lillian, to get those reports. Those two reports I've not yet had a chance to review. So, I think it's insufficient for a no effect level for sensitization. And I would like to review the two papers that talked about anaphylactic reactions in wound dressings.

DR. MARKS: So, that would be an insufficient data announcement.

DR. BERGFELD: Announcement.

DR. BELSITO: Yes.

DR. BERGFELD: Yeah. It's a new one.

DR. MARKS: Very interesting Don. I had a little bit of a different take. But certainly our team can support that. I was somewhat reassured by the sensitization data in this report. That, if I interpreted things correctly, the Bueller sensitization threshold is one percent.

DR. BELSITO: But we didn't see that data. It's just --

DR. MARKS: Yeah.

DR. BELSITO: -- summarized in the SCCS opinion.

DR. MARKS: Okay. Yeah. Any rate. So, I'll second that insufficient data announcement.

DR. BELSITO: There was one final request. I believe it came from Dan. And that had to do with inhalation studies. Some Korean studies. Do you want to comment on that?

DR. LIEBLER: Well, only that --

DR. BELSITO: Or to Curt?

DR. LIEBLER: -- there was a question about, in the memo, about whether we wanted to see that. That the council had brought this up. And Curt commented on these studies as being an important significant tox problem in Korea. And, you know, I felt that we needed to see this to verify, if possible, that the chemical substance studied there was the same as what we're evaluating. So there's -- it's not entirely clear that's the case. And then to evaluate the toxicology, and figure out to what extent that's relevant to our assessment. If Curt has further comment.

DR. SHANK: I think it's relevant. It should be in the report.

DR. BERGFELD: Curt, do you want to make a comment?

DR. KLAASSEN: Yeah. For those that don't know, there had been a number of children that died in Korea in the last few years from humidifiers in the homes. And, they'd been adding a similar compound. And that's what we're trying to figure out. If it is exactly the same or not. But, if it is or not, it should be included in here, so people know the story. It's kind of a national disaster in South Korea at the present time in the last couple of years. In fact, a relatively well known toxicologist is sitting in prison now as a result of this.

DR. BERGFELD: Well, I believe that the whole panel agrees that we can wait and do an insufficient data announcement to make sure that we have everything. Does anyone else want to comment?

DR. SHANK: Yes.

DR. MARKS: Yes.

DR. BERGFELD: Go ahead.

DR. MARKS: No. Do you want to?

DR. BERGFELD: Ron was first.

DR. MARKS: Okay. Ron was first?

DR. BERGFELD: Mm-hmm.

DR. MARKS: He hit the button before I did.

DR. BERGFELD: You did. You did.

DR. MARKS: Yeah. I think. Go ahead Ron. You're probably going to say the same thing I did.

DR. SHANK: You have to be quick. I'd like to change the name of the document. And in parenthesis add, what is it?

(Polyhexamethylene biguanide hydrochloride). Because that's actually what we're reviewing. And we're not reviewing the amino propyl biguanide. But that has to stay in the title because that's the name in the dictionary. But I think the title should clearly show that what we're reviewing chemically is the hexamethylene compound.

DR. BERGFELD: Jim.

DR. LIEBLER: I agree.

DR. MARKS: Good. Because I was going to say the same thing Ron, at your request. Because I remember yesterday, Dr. Shank said, this is a confusing paper to read because the different names. And so not only include the chemical and INCI name in the title. But, actually throughout the report in the abstract in the introduction and also in the discussion. So it's clear that we're dealing with a chemical. PHMB hydrochloride.

DR. HILL: Particularly important, because there is a polyaminopropyl biguanide that has a separate identity. A three carbon instead of six carbon-bridge.

DR. BERGFELD: Okay. I'm going back. Dr. Belsito, do you want to list the request that your --?

DR. BELSITO: So what we need, the data need is for threshold for induction of sensitization. And then the additional requests are for the papers that exist on the anaphylactic reactions to wound dressings. And the papers that deal with these reactions in Korea.

DR. BERGFELD: Okay.

DR. BELSITO: The respiratory reactions. That data is out there, so it's not in data request. It's a request that we actually see the hard documents.

DR. BERGFELD: All right. Beth.

DR. JONAS: Yes. I just wanted to make sure and to just clarify that the ingredient of concern in Korea, is actually a different ingredient. I want to make sure everybody's aware of that. And that's on the record. And the other per your data request, we have requested the LLNA data. And hope to get it. Of course, we're still in that 60 day combat period, and so our members still have time to respond.

DR. BELSITO: Right.

DR. BERGFELD: Thank you.

DR. BELSITO: I mean, it exists someplace, because it's in Gerbrick's paper.

DR. JONAS: It's out there somewhere.

DR. BELSITO: It's just not published.

DR. JONAS: Yes.

DR. BERGFELD: So, it seems reasonable that this would come to the June meeting then.

DR. BELSITO: Do we have time?

DR. BERGFELD: I don't know. I'm asking.

DR. JONAS: We always request it. It's just whether people will provide the information.

DR. EISENMANN: I've requested it, but, you know, I'm a little concerned that it didn't show up in the European dossier. So, whether or not it's an internal study that was done a long time ago, and they have concerns about it. And that's why they didn't --. I don't know. So, I'm trying to get an answer one way or another. Either get the study or --.

DR. BELSITO: From Frank? Or from whom?

DR. EISENMANN: From one of those companies. Yes.

DR. BELSITO: Well, I mean, but Frank was the first author on this paper. Frank Gerbrick. So, I mean, he's been with P&G forever. So.

DR. EISENMANN: As far as I understand, no, it's not from Frank.

DR. BELSITO: Okay. But he should know where he got that information for the paper. He's an author.

DR. EISENMANN: Well, no we've gotten who we're supposed to be asking.

DR. BELSITO: I see.

DR. EISENMANN: We've asked them.

DR. BELSITO: Okay.

DR. EISENMANN: But so far, they have not come up with it. And so I either want them to come up with a study. Or the reason why they're not coming up with a study.

DR. BELSITO: I see. Okay.

DR. BERGFELD: All right. Well, we'll try for June, and we'll see how that goes. All right. Thank you.

Day 1 of the June 12-13, 2017 CIR Expert Panel Meeting – Dr. Belsito's Team

So now the next one, polyaminopropyl biguanide. So this is an up and coming cosmetic preservative and at the April meeting we issued an insufficient data announcement with the following request. Skin sensitization data to determine a no effect level for polyaminopropyl biguanide. Data needed to evaluate the anaphylactic reactions to this in case studies and data from the Korean papers on lung injury mortality as attributable to material that we were not certain whether it was structurally related to a polyaminopropyl which is actually polyhexamethylene guanidine that we are reviewing.

So we got the Korean data including some last minute handouts because of copy right laws that could not be sent to us. We got a lot of data on in the report and then some additional data in wave 2 on HRIPT and what we didn't really get was a lot of data on the anaphylactic reactions.

MR. JOHNSON: Just the two case reports.

DR. BELSITO: Right.

MR. JOHNSON: That were provided, yes.

DR. BELSITO: So I guess the first question is the deaths, the lung disease, pulmonary disease linked to this material in humidifiers. Where are we with that? We have got all the information. It's not my area of expertise.

DR. LIEBLER: Well, I think that was determined that that was another substance that the ingredient that we are reviewing is not one of the substances that was present in the humidifier solution and that the focus on that was -- and I just got these papers or this paper. But my understanding is that the focus was on another ingredient that was superficially structurally related. In fact I actually have a little bit of language for the draft discussion on that. But it's a different substance.

DR. BELSITO: Do you want to share your language with us?

DR. LIEBLER: Yes, sure. So this regards PDF page and it is the, let's see, one, two, three, four, fifth

paragraph regarding the issue of inhalation exposure. And the final sentence is the relevance of the finding to polyaminopropyl biguanide as a cosmetic ingredient will be determined after these studies. And I just struck that sentence and I substitute the panel noted that these structures are significantly different particularly in the biguanide in the cosmetic ingredient versus guanidine in the inhaled toxicant. The toxicity of the guanidine compound was considered not to be relevant to the assessment of the polyaminopropyl biguanide.

DR. BELSITO: So you've deleted the last sentence.

DR. LIEBLER: Right.

DR. BELSITO: In that paragraph.

DR. LIEBLER: The last sentence of that paragraph.

DR. BELSITO: And you've word smithed it to point out that it's a different chemical.

DR. LIEBLER: Correct.

DR. BELSITO: Okay. So who is reporting on this?

DR. LIEBLER: Jim.

DR. BELSITO: Jim. But if they don't point that out I will refer to you to word smith.

DR. LIEBLER: Sure. Yes.

DR. BELSITO: Okay. The anaphylactic issue. I don't know what to make of this personally because what you're finding is that this is increasingly being used in particularly in eye medications and contact lens solutions and things like that and there have not been any reports of, you know, significant urticarial reactions to them. You know, I'm not even sure that it warrants a damage skim at this point unless we want to actually ask for specific information like we did with PEG's.

DR. LIEBLER: You know, assessing a case report like this is definitely outside of my own expertise. One thing that occurred to me is that the guanidine compound that was apparently the source of the trouble with the inhalers in South Korea is chemically somewhat similar to the biguanide that we are analyzing and it's always possible that that compound, the

guanidine could be a contaminant of a, of the ingredient that we are evaluating. Depending on how the, you know, the material was generated and how it was purified or whether it was purified, et cetera. And I don't know if that could be related to the effect and this was simply a potential chemical explanation for what they were seeing but I'm not sure that clinically you could accept the conclusion, Don, of what's reported in this report?

DR. BELSITO: Yes, I mean --

DR. LIEBLER: That the anaphylaxis is due to this compound?

DR. BELSITO: Yes, I mean it doesn't say what else is in the compound and we know that chlorhexidine is a frequently used, you know, hospital disinfectant and has been reported to cause anaphylaxis. I mean, the FDA just recently put out an announcement on that so, you know, I'm not overwhelmed by the data. I guess the question becomes, you know, how do, you know, we explain it. I mean, if you look at the, so if you look at the reports here, okay, first guy gets angioedema and pruritus after using a wet wipe and he's patch test negative but he's prick test positive but there's no control. There are a lot of things you can prick into the skin that aren't IgE mediated that cause you to develop a wheal and flare. So that I can't make a lot of sense of.

And then the next guy has contact dermatitis and we will talk about that. And then the two cases of severe anaphylaxis that were reported after a hospital disinfectant and they don't give you any other information, you know, as to why they believe that it's polyaminopropyl biguanide. And again, the same thing with the grade three anaphylaxis with a using a new brand of wet toilet paper. So I'm not -- the literature is out there. I think we need to or I can go into it further and craft some language for the discussion as to why it, you know, the conclusions that they were due to this material are not appropriate. But it is there and it is from cosmetic use. Its --

DR. LIEBLER: And they attribute it to this disinfectant called lavasept which the text of this article or this report simply says it contains polyhexanide biguanide and polyethylene glycol and (inaudible) lactate. So in this little two pager that Wilbur just gave us, I'm just reading this for the first time but the patient case one, patient under anesthesia with bupivacaine presented an anaphylactic shock while the medullary cavity of the femur was being washed with lavasept. Now I don't know if anything else about this situation would be potentially able to cause anaphylaxis and again I ask my clinician colleagues about that because I have no idea.

DR. SNYDER: The one question I had was that there, in this introduction it says that urticarial reactions to lavasept appear to be rare but have been reported to the Swiss Center of Pharmacovigilance. So do we have access to that data to see if there, that there are urticarial reactions that are above? Because this seems to be an expansion that not only urticarial reactions but then these two case reports on anaphylaxis.

DR. LIEBLER: Both of these patients were under general anesthesia.

DR. KLAASSEN: Yes, right. I mean, thank goodness they were I guess.

DR. LIEBLER: I mean, it sounds like they were using this stuff by the gallon.

DR. KLAASSEN: Inside of the body.

DR. LIEBLER: Yes. Right.

DR. BELSITO: And, I mean, they are coming to the conclusion only because of the structural relationship of the biguanide to chlorhexidine which is known to cause urticarial reactions. You know, on the other hand we have approved chlorhexidine for use in cosmetic products.

DR. SNYDER: But and then it also does go to the in context that this damaged skin.

DR. BELSITO: Right. Mucosal. I mean, more than damaged skin.

DR. KLAASSEN: Right. This was inside the body.

DR. BELSITO: Yes.

DR. LIEBLER: They were pouring it on the bone.

DR. KLAASSEN: Yes, both cases.

DR. LIEBLER: Right.

DR. KLAASSEN: Like an IV administration.

DR. LIEBLER: So I don't know, I mean, maybe you can consider those factors in crafting some language here but it just seems like the exposures are so dissimilar. The only thing that gives me any pause is, you know, anaphylaxis, you know, I understand in some cases

can be caused by exposure to a very small amount of a substance so you can't rule out the possibility that a small amount could present a risk to the right person. But it seems like the overall safety profile of this stuff doesn't point you in that direction at all.

DR. BELSITO: Yes, I mean, the first patient was, you know, clearly multi allergenic individual, you know, cat dander, grass, cereals, corn, hazel, birch, walnut were IgE, you know, were all positive. His skin prick tests were negative to everything. It was his intradermal that was positive at a 1 to 10 dilution or ten to the minus four micrograms per ML of polyhexanide.

And the same thing with the other, I mean, if this were even on damaged skin, okay, you know, skin prick test is where you're putting it, you know, you're not even scratching the skin. You're putting it down underneath the epidermis and then the intradermals were positive. I mean, that, I mean, if this were an allergic reaction the skin prick test should have been positive. You know, particularly since they are claiming the intradermal reaction occurred at such a low dose. I mean, the studies just don't make sense to me.

DR. LIEBLER: Um-hum.

MR. JOHNSON: I have one question. The chemical structure is on page two and my question is is this the chemical structure for polyaminopropyl biguanide or polyhexamethylene biguanide hydrochloride?

DR. BELSITO: Dan?

DR. LIEBLER: Which one?

DR. BELSITO: The top one is chlorohexidine. The bottom one is polyhexanide. Is that the chemical we are looking at?

DR. LIEBLER: I am paging down to the table in our report, hang on just a moment.

MR. JOHNSON: And I'm saying that because according to the dictionary polyaminopropyl biguanide is not the cosmetic ingredient for polyhexamethylene biguanide hydrochloride. It's actually the cosmetic ingredient.

DR. BELSITO: Right.

DR. HELDRETH: Yes, they've drawn hexamethylene. It even says hexamethylene in the name below the structure on that page.

DR. LIEBLER: Yes, the structure is the same.

MR. JOHNSON: So that means polyhexamethylene biguanide --

DR. BELSITO: That's what we are looking at.

MR. JOHNSON: Right, okay. Thank you.

DR. LIEBLER: Yes. Speaking of structures, I would suggest going back to the Korean vaporizer episode, the bottom of PDF page 38 the bottom where you say the chemistry of PHMG which is the abbreviation, the acronym for the ingredient implicated in that toxicity. I suggest you actually show the structure there just to show that it's different.

DR. KLAASSEN: Yes, I agree.

MR. JOHNSON: What page are we on?

DR. LIEBLER: PDF 38 at the bottom, very last sentence. So right around there you could, you know, put figure X and show the structure of PHMG. You've already got the structure of the other one elsewhere in the report but it would be clear that they are different.

DR. KLAASSEN: Preferably put both of them there.

DR. LIEBLER: Yes you could put them both their just side by side.

DR. KLAASSEN: So us dummies don't have to go back and compare it.

DR. LIEBLER: Right. Right.

DR. KLAASSEN: On the computer it's not so easy to do.

DR. BELSITO: So where would you do that, Don? So beginning in 2006 that's the paragraph you are talking about?

DR. LIEBLER: Yes. Somewhere around that paragraph. But the two structures are ingredient.

DR. BELSITO: So maybe right after the third sentence which says these disinfectants contain put a comma which is chemically dissimilar, see figure whatever.

DR. LIEBLER: Sure, yes.

DR. BELSITO: So that last line, Wilbur, on page 38 where it says dodecyl

dimethyl ammonium chloride comma which is chemically dissimilar to the material under review and then see figure.

DR. LIEBLER: Also I would say chemically it's similar.

DR. BELSITO: Which is --

DR. LIEBLER: But its --

DR. BELSITO: -- toxicologically?

DR. LIEBLER: Right. Yes. And I, that's why I have that other line in the discussion to explain the dissimilarity is significant enough. I mean, chemically they're similar but they're dissimilar enough to have different biological effects.

DR. BELSITO: Which is so then we can just say which is not the material under review and deal with that in the discussion and --

DR. LIEBLER: Exactly.

DR. BELSITO: And so which is not the material under review see figure.

DR. LIEBLER: Yes, so you could edit the last line of that paragraph on PDF 38, the chemistry of PHMG comma and I will put it into mine, Wilbur, which is not the material under review.

DR. BELSITO: Okay.

DR. LIEBLER: Is similar, right.

DR. BELSITO: Okay. So where are we with the anaphylaxis?

MS. BURNETT: Chlorhexidine.

DR. BELSITO: Yes. Yes. The FDA has recently put out an announcement on chlorhexidine. Right. So I guess we are going to note those in the discussion, state that what? The negative skin prick testing and positive intradermal testing is a little bit unusual for an IgE mediated process? That -- the other reports were uncontrolled?

DR. LIEBLER: And damaged skin.

DR. BELSITO: And could be damaged skin but then are we saying that it should be used on damaged skin? I mean, you know, because we also can't say it can't be used on mucosal surfaces because right now its biggest use are eye drops. And Europe has said now they are allowing it to point three, is that right? It was point.

MR. JOHNSON: No point one.

DR. BELSITO: It was point one. But I thought they just upped it to point three now at the recent --

MR. JOHNSON: No its 0.1, I mean, they've lowered it to 0.1 in the final monogram.

DR. BELSITO: All right. I thought they upped it.

MR. JOHNSON: It was 0.3 and they lowered it to 0.1.

DR. LIEBLER: PDF 30 --

DR. BELSITO: Yes, I see it.

DR. LIEBLER: Third paragraph.

DR. BELSITO: So they said unsafe at point three and they have now said safe at point one. Right. Okay. I mean, but we have this in our reports, I think we need to address it. I --

DR. KLAASSEN: Well, I think the point that this wasn't placed on the skin but it was placed in essence on the bone and on the gut, you know, while they were doing surgery. I mean, that's, I don't know how relevant that is to in fact I don't think it's relevant at all to dermatology.

DR. BELSITO: Well, except one case report was following the use of a wet wipe. So a male patient with a history of angioedema and pruritus after using a wet wipe.

DR. KLAASSEN: Maybe we can be asking for more data.

DR. BELSITO: And then a female patient after using a wet wipe. I mean, its 14, 17 and 38 are the references, right? I thought I looked at those. Let me make sure they may have been ones I couldn't get, I don't know. 14, 17, -- it was 14, 17.

DR. SNYDER: 14, 17 and 38.

DR. BELSITO: Yes so NICNAS I didn't see obviously.

DR. LIEBLER: Did we get 14?

DR. BELSITO: 14 is from contact dermatitis.

DR. LIEBLER: Right. Did we get that?

DR. BELSITO: And I don't know that I could get allergy --

DR. LIEBLER: Okay.

MR. JOHNSON: From the Korean studies and the anaphylactic reaction report.

DR. BELSITO: But they are 14, 17 and 38 all dealt with anaphylaxis.

DR. LIEBLER: Is contact urticaria syndrome or urticaria syndrome anaphylaxis?

DR. BELSITO: It can result in anaphylaxis, yes, latex is a good example.

DR. LIEBLER: But as in described in that reference. I just, I mean, I honestly if I was reading contact dermatitis I would be lucky to just know it's right side up.

DR. BELSITO: Right.

DR. LIEBLER: So I defer to you guys to tell me if it, if this is relevant.

DR. BELSITO: Let me get to the Columbia website. E-resources. So while I'm trying to get that data from the Columbia library here's an issue that I have with this material. First of all, Wilbur, with the new data that we have on the HRIPT, you've misstated the dose because it was a dilution so in that HRIPT at the new data we got on wave two, the NESIL, the dose that did not create an issue was let me pop this up. Was a point, you stated it was 125 micrograms per square centimeter. That's not true. Or, I'm sorry --

MR. JOHNSON: 0.125 micrograms or, I mean, milligrams per square centimeter.

DR. BELSITO: But in the report it was not correct. Where are we? So many comments on this.

DR. SNYDER: It says summary of an HRIPT (inaudible) point five percent --

DR. BELSITO: Yes so this is wave two.

DR. SNYDER: Yes, page seven of the wave two documents.

DR. BELSITO: Right. But in Wilbur's summary he says that a dose sensitivity of 25 milligrams per centimeter squared in the summary. If that were to come into the report its actually 125 micrograms per centimeter squared.

DR. SNYDER: Okay.

MR. JOHNSON: Now see I'm looking at PDF page 8. The actual data.

DR. BELSITO: I'm looking at wave two.

MR. JOHNSON: Yes, these are the wave two data --

DR. BELSITO: Yes, the actual data is correct. But your summary of it at the beginning in your letter dated June is incorrect.

MR. JOHNSON: Okay.

DR. BELSITO: So if you were to take your summary and put it into the text it would be incorrect because if you were looking at what you summarized for the human sensitization data you would think that the results of the HRIPT were negative at 25 milligrams per centimeter squared.

MR. JOHNSON: Yes.

DR. BELSITO: You said the product point one gram under two by two centimeter occlusive patch was applied for at a dose density of 25 milligrams per centimeters squared. That was not the, the does density was 250 micrograms per centimeter. 125 micrographs per centimeter squared.

MR. JOHNSON: Well, I'm looking at the --

DR. BELSITO: I'm looking at your introduction, Wilbur.

MR. JOHNSON: Yes. I'm looking at the actual data.

DR. BELSITO: I'm looking at the actual data too.

MR. JOHNSON: Okay.

DR. BELSITO: I'm just saying your conclusion of the actual data in your letter of June 2 is incorrect.

MR. JOHNSON: Okay.

DR. BELSITO: So you need to correct that because it should not hopefully if you cut and paste what you summarized as the dermal irritation sensitization that value will be wrong.

MR. JOHNSON: Okay.

DR. BELSITO: And I point that out only because very interestingly since we last looked at this I had a woman with severe eyelid dermatitis who I tested to her CVS saline solution for sensitive eyes which contains .0003 percent of the material under polyaminopropyl biguanide. The other constituents were boric acid, potassium chloride, sodium chloride and EDTA. She had a three plus reaction to the cleansing solution and I have no explanation other than polyaminopropyl biguanide. I just got the material. She is clear using contact lens solutions without it and doesn't want to come back in for confirmatory patch testing.

I point that out only because I think that this is a preservative where we have to use the QRA and not just simply say safe as used. Because just to point out that for instance if this, if we assume that the NESIL for this is 125 micrograms per centimeter squared I couldn't exactly find one in the RIFM database that's the same. Isoeugenol is 250 micrograms per centimeter squared and it ranges from point 01 in lip products and point 02 percent in intimate wipes up to 1.25 percent rinse off products.

So I think this is a conclusion that we need to craft like we did the cocamidopropyl betaine solution and the stearamidopropyl conclusions that we don't endorse the QRA but you need to use some type of approach to what you are using. Otherwise I think this will end up being the next methylisothiazolinone on the market. I'm very concerned about it and I think and we are losing so many preservatives that I don't want to see this one lost.

DR. ANSELL: Yes, we would support the inclusion of that in the I don't know the discussion or somewhere in the report.

DR. BELSITO: Yes.

DR. ANSELL: That safe when formulated based on a QRA similar language.

DR. BELSITO: Or some other --

DR. ANSELL: Yes.

DR. BELSITO: -- toxicological approach to where it's used and how it's used. Now just an across the board statement of -- I just throw that out. I'm just trying to get to the contact dermatitis for the urticaria. I can't get allergy here. So I don't know if you have that report, Wilbur? Its Columbia Library doesn't prescribe to it.

MR. JOHNSON: Which number is it in the reference manual?

DR. BELSITO: I don't know. Can someone tell me the contact dermatitis one is 38 or 14?

DR. LIEBLER: Let me look here. 14.

MR. JOHNSON: 14.

DR. BELSITO: No, the allergy one is 38. Isn't it? Wilbur needs to get the non-contact dermatitis.

DR. BERGFELD: 38 is allergy.

DR. BELSITO: Yes. So 38.

DR. HELDRETH: For Wilbur's summary where he had the megas per cubic centimeter dose density I'm looking at the

data and I see where he got it from. I think they have called two things in the raw data dose density. Whereas one of them is intended to mean the density of the entire amount of formulation that was applied.

DR. BELSITO: Right.

DR. HELDRETH: So I think that's where the error came into there.

MR. JOHNSON: It was in the report 0.125 value relates to polyaminopropyl biguanide.

DR. HELDRETH: Yes.

DR. KLAASSEN: I mean, the last sentence of the sensitization paragraph actually is eight references in regard to human skin sensitization. And they say that begins at 0.2 percent active ingredient. That's pretty important.

DR. BERGFELD: Was that skin?

DR. KLAASSEN: Yes.

DR. BERGFELD: Not rabbit?

DR. KLAASSEN: Humans. The last sentence, it's on page 37. There's a sensitization paragraph.

DR. BELSITO: So what was the article on anaphylaxis from contact dermatitis?
What volume, what was the reference?
MR. JOHNSON: Let's see, so there's reference 38.
DR. BELSITO: No reference 38 was allergy this is reference 14.
DR. LIEBLER: Yes it's a volume 71 so year 2014.
DR. BELSITO: Yes.
DR. LIEBLER: Volume 71 issue 5, page 307.
DR. BELSITO: Yes, here it is okay. So this is a report that came out of Holland. How do I reverse this here? Hey Dan, I just flipped this whole thing sideways. How do I get it back up? Okay.
MR. JOHNSON: Oh yes, I have it right here. What's his email address? Okay.
Let me attach this to you and send this to you.
DR. BELSITO: Okay.
MR. JOHNSON: I have the Creighton's publication, we are going to send it to you.
DR. BELSITO: The allergy one?
MR. JOHNSON: Yes, the Creighton.
DR. BELSITO: I have it.
MR. JOHNSON: You have that one?
DR. BELSITO: Yes. It's the allergy one I need, Wilbur. I don't have access. I have access to contact dermatitis.
MR. JOHNSON: 38. Oh, okay, 38.
DR. BELSITO: Right. So basically this was a 39 year old woman and she did have strong immediate positive prick test to the wipes and to the ingredients. So these were prick tests after 15 minutes and then they did a flow assisted basophil activation test which I don't believe is FDA approved and it was positive to polyaminopropyl biguanide. And that test was positive, was performed in three healthy controls who had been exposed but not, did not develop symptoms and was negative. But that was the basophil activation test. And it doesn't look like they did any controls for the skin prick testing on polyaminopropyl biguanide.
MR. JOHNSON: But this is it.
DR. BELSITO: So they didn't do positive controls, so they said the problem was cleared by not using wet wipes with polyaminopropyl biguanide. And then if you go into contact dermatitis since I was searching for this there's a review of contact urticaria with polyaminopropyl biguanide. I don't know that, if you saw that, Wilbur?
MR. JOHNSON: Which one is that?
DR. BELSITO: I'm just popping it up again because I was just thrown out of the library for being a bad student. It says contact urticarial syndrome by polyaminopropyl biguanide wipes. This is another reference from 2000, wait a minute, is this the same one? Yes. Sorry, it's the same one. There is an article in 2016 polyhexamethylene biguanide and wound care products are non-negligible cause of peri-ulcer dermatitis. So that gets us to some damaged skin that probably should be brought in and then the one that I was referring to is a 2016 cosmetic components causing contact urticarial, a review and update and I suspect it's by N. Gussen (phonetic) so it probably just adds polyaminopropyl biguanide to the list of materials. I'll pop it up now, see if it's even relevant to review. But it would be nice to look at that one done in sterile wound care.
Yes basically just ads, just to review adding her finding that it can cause contact urticaria there is no additional data there. So I don't know it doesn't really seem to be an issue. There have been a couple of case reports not conclusively documented. One used only basophil analysis in controls not skin prick testing. So I'm not sure where to go with the urticarial issue.
DR. KLAASSEN: This reference number 31 from the title it says the biocide polyhexamethylene biguanide remains an uncommon contact allergen, recent multi center surveillance data and contact dermatitis.
DR. BELSITO: Yes, I agree.
DR. KLAASSEN: That might be a useful reference.
DR. BELSITO: But that's for contact dermatitis and I think part of the issue is and the reason why I wanted this brought forth is that as the number of cosmetic preservatives gets

limited in Europe, you know, they are now limiting, further limiting parabens, they've banned methyl dibromoglucurteral (phonetic) nitride. They've essentially banned methylisothiazolinone except in the MCMI mixture. This material is going to get increasingly used. It's not a common sensitizer because it's not been a common preservative until recently. But you're seeing it coming into more and more cosmetic products.

And I think that it's just like methylisothiazolinone. When we reviewed it in 2005 not only did we have the HRIPT data wrong but we weren't thinking of how these materials are used and we said across the board 100 parts per million. Well 100 parts per million wasn't an issue for, you know, wash off products but when you started putting it in baby wipes it caused this huge epidemic. I would hate to see this material get banned in Europe because we got it wrong and it caused epidemics. I mean, if they do the QRA I think it will be fine or some other means of risk assessment for contact dermatitis.

But that doesn't address the urticaria angioedema issue which is extraordinary rarely reported and I don't think, I would like to see the allergy paper but and N. Gussen is a wonderful researcher but, I mean, the skin prick tests were not controlled in the basophil activation tests as far as I know are not FDA approved or scientifically approved by any regulatory body to be used as a surrogate so the fact that three negative controls were negative with the basophil activation test doesn't, I mean, I would have liked to see them skin pricked tested with the material. Did you send the allergy paper, Wilbur?

MR. JOHNSON: Well, actually I don't have that but I can order it and it can be here by tomorrow morning if not before the end of the day.

DR. BELSITO: Okay. Let me try one other avenue to get into Columbia Library on that. I might be able to get it. What's the reference for the allergy paper which is 38?

MR. JOHNSON: That's, yes, that's --

DR. ANSELL: 2010.

MR. JOHNSON: Yes. Volume 65 issue 8.

DR. BELSITO: Okay. Who is the author?

MR. JOHNSON: Kautz, that's K-a-u-t-z and Schumann, that's S-c-h-u-m-a-n-n.

DR. BELSITO: No results. Oh, I misspelled it. It would help if I spelled correctly, huh. Allergy --

DR. LIEBLER: Vanderbilt versus Columbia.

DR. BELSITO: Allergy and clinical immunology is what I'm getting.

MR. JOHNSON: Its number eight. Issue eight.

DR. BELSITO: It's just allergy, right?

DR. LIEBLER: Yes, I've got it.

MR. JOHNSON: Just allergy. Angioedema and oh that's not the right word. 1068 I'm looking for --

DR. BELSITO: Keeps shunting me to allergy and clinical immunology.

DR. LIEBLER: Here we go. I've got the reference.

DR. SNYDER: Email it to everybody.

DR. LIEBLER: I am going to download it. Okay.

DR. SNYDER: And, Scott, he is going to email it.

DR. BELSITO: Yes, I keep getting shunted back over to allergy and clinical immunology where it doesn't exist. I thought I had it. And you have the NICNAS data reference --

MR. JOHNSON: Yes, sir.

DR. BELSITO: You're sending that to me?

MR. JOHNSON: Yes.

DR. BELSITO: And you're going to send me the allergy paper.

DR. LIEBLER: Here it comes. It has been sent.

DR. BELSITO: Okay. So, I mean, I think I will see if I can draft something to address the urticaria issues and I'm fine with safe as used when formulated to be non-sensitizing and then in the discussion, you know, state that they can use various ways of assessing sensitizing capacity QRA or other similar methodologies.

DR. LIEBLER: Okay, I'm good with that.

DR. BELSITO: I got it. Thank you, Dan.

DR. LIEBLER: Sure.

DR. BELSITO: And you'll send me the NICNAS, the other?

MR. JOHNSON: Yes.

DR. BELSITO: Okay. Anything else on polyaminopropyl biguanide? Now that I've lost my page. Oh yes, so Ron Shanks comment and I understand where he is coming from but this is not polyaminopropyl biguanide. It's actually polyhexamethylene biguanide. Putting that in parenthesis has throughout this document made it extremely, extremely confusing for me to understand what you're saying. And in some places I actually think that you got it wrong by using the comment twice and I was just wondering could we do something like polyaminopropyl biguanide, I mean, it's not trademarked, that's not the trademarked name but could we come up with some super script INCI instead of putting in parenthesis polyhexamethylene biguanide because when I was reading it it's like which one are you talking about here, you know, I mean, is it the material we are reviewing, is it the material that is, you know, the actual polyaminopropyl biguanide?

DR. LIEBLER: Well, you've got the convention that we use in our reports of capitalizing the names of the INCI names of the ingredients we review. So and you clearly state in the second paragraph or the first paragraph of the introduction, the discrepancy between the actual the INCI name and the correct chemical name and what you have been doing is putting the correct chemical name in parentheses after the INCI name but you could simply state right up front that the INCI name is what it is, its capitalized throughout the report and that refers to this chemical substance as shown in table one and leave it at that. And then not have to drag the parenthesis and then the long chemical, correct chemical name in throughout the report.

DR. BELSITO: Yes.

DR. LIEBLER: And maybe that would satisfy Ron and --

DR. BELSITO: I mean, I agree we need to distinguish but for instance, I mean, it just results in screw ups. On page 38 where you described cytotoxicity, Wilbur.

MR. JOHNSON: Yes.

DR. BELSITO: Basically you say however the last paragraph or the last sentence in the paragraph, however, concentrations greater than point 25 percent polyaminopropyl biguanide were highly cytotoxic to cells of both cell lines after 24 hours. When compared directly polyaminopropyl biguanide consistently resulted in significantly higher survival rates than polyhexamethylene biguanide. And irrespective of the concentration so it really starts getting, you know, very, very confusing there because, you know, polyhexamethylene biguanide is what we are reviewing and so then you should put in parenthesis before that polyaminopropyl, you know, biguanide parenthesis polyhexamethylene. I mean, it was just, it was mind blowing for me to try and read and take a pause each time and decide, okay, what are we comparing? So I like Dan's idea of throughout the text when its capitalized and bold it's the material we are reviewing and when it's not capitalized and not bold its actually polyaminopropyl biguanide.

DR. LIEBLER: Yes, I mean, I don't, I don't even think bold is necessary. It's capitalized according to our convention in the reports. We don't really need to add the bold. You just say in the first paragraph in the introduction the capitalized name is the INCI name and that's the name we would use to refer to this substance. The correct chemical name is blah, blah, blah and you put that in the first paragraph and its done and it's also in table one. And then that takes care of it.

DR. BELSITO: Okay. Okay, yes. We will see what Ron says about that. Then on PDF page 28 I again I thought that it was like really too exhaustive going through the INCI name and yada, yada, yada. I essentially got rid of with that first paragraph in the introduction accordingly and just dropped the whole thing. I thought it was just too much. I think that, you know, indeed the cosmetic -- indeed the chemical polyaminopropyl biguanide is not a cosmetic ingredient. In this report when capitalized polyaminopropyl biguanide refers to the cosmetic ingredient which is actually polyhexanide hexamethylene, whatever. Get rid of all of that and then the whole thing about the SCCCS, I don't think we need to define to the world what the SCCCS is. So the following paragraph I got rid of the whole thing, I mean, you can tell us what their opinion was but you don't need to tell us what they were incorporated to do or what their mission is.

Then I had a question for Paul some place. So it was with the hepatic and the hemangio sarcomas in the so on page 41 of the PDF under the carcinogenicity oh that's where I

noted it on the summary but it's in the carcinogenicity section. What did you think of those studies?

DR. SNYDER: Yes, that's all secondary to cytotoxicity's so that's not, it's not relevant to the --

DR. BELSITO: Is it even important enough that we bring it up in discussion?

DR. SNYDER: Well, I think we should bring it up because it is data. But I think I thought it was appropriate when we discussed this before that it's related to --

MR. JOHNSON: It's in the discussion.

DR. SNYDER: Yes. It's in the discussion.

DR. BELSITO: You're happy with that?

DR. SNYDER: Yes, yes.

DR. BELSITO: And then on page 42 if of the PDF I think and this is in the summary that you have it backwards, Wilbur, because I thought the polyaminopropyl biguanide cosmetic consistently you said resulted in a higher survival rate that is less cytotoxicity than the polyaminopropyl biguanide. Oh. What you didn't, what you got wrong is you added polyhexamethylene biguanide to the first polyaminopropyl biguanide so it should simply say this is on page 42 PDF the second paragraph. Polyaminopropyl biguanide, get rid of polyhexamethylene consistently results in significantly higher survival rate, less cytotoxicity than polyaminopropyl biguanide in parenthesis polyhexamethylene biguanide irrespective of the concentrations because if the cosmetic material was more cytotoxic than the polyaminopropyl biguanide.

DR. LIEBLER: So, Wilbur, I have added at the first paragraph of the discussion to simplify and it and to explain we are just using the INCI name to refer to this ingredient.

MR. JOHNSON: Sure. Now what about the conclusion which will you just have polyaminopropyl biguanide in the conclusion?

DR. LIEBLER: Correct.

MR. JOHNSON: Okay.

DR. LIEBLER: Again because the conclusion will refer to the INCI name of the ingredient.

DR. KLAASSEN: In regard to the topic of epigenetic effects on page 36, we have two or three paragraphs, two paragraphs there. I don't think they should be called epigenetic effects. There's kind only one sentence in that, in those two paragraphs that really have to do with what we now called epigenetic effects. And that is the DNA methylation and modification of DNA basis. I guess in fact it goes on and this is really I don't know what we should call this or where we should place it. It's really kind of talking about what kind of molecular effects of --

DR. LIEBLER: Its cytotoxicity.

DR. KLAASSEN: Okay.

DR. LIEBLER: It is, I mean, there is some mechanistic aspects to it but basically its cytotoxicity studies so.

DR. KLAASSEN: But we shouldn't call it epigenetic effects.

DR. LIEBLER: No. You're right, Curt, because that connotes a very specific, it used to mean non DNA damage effects but now it connotes something much more molecularly specific and well defined.

MR. JOHNSON: So move those to the cytotoxic section in the report?

DR. LIEBLER: Yes.

MR. JOHNSON: Okay.

DR. KLAASSEN: Yes, what you have written is okay it just has the wrong title.

DR. LIEBLER: Yes. If you just remove that heading, epigenetic effects because it's right under the cytotoxicity section anyway.

DR. BELSITO: What page from the PDF is that, Curt?

DR. KLAASSEN: 36.

DR. SNYDER: It's probably better under the title other cellular effects because it's more than just cytotoxicity but.

DR. LIEBLER: Well, I looked at it as cytotoxicity with some mechanistic insight thrown in so it goes under the setting toxicity basket.

DR. KLAASSEN: You just had it there.

DR. BELSITO: So just get rid of that heading.

DR. LIEBLER: Yes.

DR. ANSELL: That's not a sound you want. Not hearing that sound. With all the construction over here they could have just been offloading containers or something but then the air conditioning just went off.

DR. KLAASSEN: It's going to come back on tomorrow afternoon.

DR. BELSITO: What's that?

DR. KLAASSEN: Air conditioner is off. It's going to come back on tomorrow afternoon.

DR. BELSITO: Okay. Anything else?

DR. BERGFELD: Could you repeat your conclusion then or what you're going to?

DR. BELSITO: Safe as used when formulated not to be sensitizing.

DR. BERGFELD: Sensitizing.

DR. BELSITO: And the discussions say that you can use QRA whatever types of methods you want but the current NESIL we have based upon the most recent HRIPT in wave two at point five gives us a NESIL of 125 micrograms per centimeter squared.

DR. BERGFELD: Now what about the data on the 0.2 being the high threshold for sensitization?

DR. BELSITO: Well, I mean --

DR. BERGFELD: I know that point five was in there.

DR. BELSITO: Yes. So that's my whole point about QRA. It depends upon where you look at sensitization. I mean, where did my patient who while I haven't confirmed its polyaminopropyl biguanide allergic it looks like she has developed a sensitivity that allowed her to react to a contact lens solution that contained .00003 percent of this material. And yet, you know, she is cleared completely or either the dermatitis has gone away completely switching away from products without polyaminopropyl biguanide have improved it but was she sensitized in a wet wipe, was she sensitized -- where was she sensitized I don't know.

So that's what I'm saying that the point two yes, I mean, you know, if you used you know, 50 parts per millions of methylisothiazolinone in a wet wipe you could get sensitized. If you used it in a shampoo you wouldn't be so that's why I think you need to do QRA. We have shown on the back which is where QRA is based on with an HRIPT that point five it was 207 subjects if I remember off the top of my head. I mean, it was a pretty good study was fine. So I think we can start that as a NESIL but then we need to apply it depending upon where this product is going to be used and how.

DR. BERGFELD: Okay.

MR. JOHNSON: Dr. Belsito, will you please repeat the language for the discussion relating to the QRA and --

DR. BELSITO: I think you can take it from the language where we have used QRA before. Just go into I think it was in the cocamidopropyl betaine report --

MR. JOHNSON: Okay.

DR. BELSITO: Going to that and look or betaine sorry, Christina. Report that we can go in and see exactly what we said, use the same language.

MR. JOHNSON: Okay.

DR. BELSITO: Anything else?

DR. LIEBLER: Nope.

Day 1 of the June 12-13, 2017 CIR Expert Panel Meeting – Dr. Mark's Team

Next is the polyaminopropyl biguanide, aka whatever name --

DR. HILL: PHBG.

DR. SHANK: I like that better.

DR. MARKS: At the April meeting of this year, an insufficient data announcement was issued. There are three data needs skin sensitization data. We need to evaluate the issue of anaphylactic reactions and, also, data from the Korean studies on lung injury and mortality; and we did receive new data.

So, let's first deal with, number one, the skin sensitization. I thought that looked good, and we got Wave 2 with .5 percent maximum leave-on, and a negative HRIPT sensitization threshold of one percent from previous data. So, I thought was okay from that point of view.

DR. HILL: So, explain to me goes on there with the threshold thing. You're looking at a threshold of -- I think, at one point, didn't they say .2 percent or something like that? But then we've got a study up to 5 percent, that's an HRIPT -- sorry, I need some education --

DR. MARKS: That was not enough to sway the thinking last time that's why it went as an insufficient data. In Wave 2, we had a negative HRIPT sensitization study with .5 percent, which is the maximum leave-on. So, that was reassuring to me. To me, I'd check that box.

DR. HILL: Okay.

DR. MARKS: From Wave 2.

DR. EISENMAN: Part of the problem with the original sensitization settings they were all done in aqueous solutions.

DR. HILL: Mm-hmm.

DR. EISENMAN: And these additional studies were done in actually formulations.

DR. MARKS: Yes.

DR. EISENMAN: One thought is to have a conclusion similar to what you did for MI say it's been formulated to the non-sensitizing, which can be too determined based on a QRA. So, if you wanted to base the -- if you didn't want to do an HRIPT, you would do a QRA calculation, probably use the approximately one percent which is approximately 1 mg/cm² and that comes out to a level of about .1 percent in the highest exposure products, which is what the SEC ask conclusion is; but if you wanted to go higher and be sure your formulation was right, you wouldn't have to do a HRIPT.

DR. HILL: HRIPT, which they did. And there's a sun tan product that has .5, you said?

DR. EISENMAN: Yes.

MR. STEINBERG: Is this as the 100 percent active material, or as it's commercially sold; because it's sold as a solution.

DR. EISENMAN: I know; it's sold as a solution. That is part of the problem. I think -- I want to say it's as the commercial preparation, not as the 100 percent.

MR. STEINBERG: Yeah, because that changes your numbers now.

DR. EISENMAN: Right.

MR. STEINBERG: Because, I think, it's 20 percent solution -- is what it's sold as.

DR. EISENMAN: Mm-hmm.

MR. STEINBERG: So, if it's .5, it's actually .1.

DR. HILL: Well, on that other issue, it's not a single compound.

DR. EISENMAN: Right.

MR. STEINBERG: That's true.

DR. HILL: It's a mixture.

MR. STEINBERG: But it's still 80 percent water; it's 20 percent of the mixture.

DR. EISENMAN: But they're supposed to be telling me the concentration of PSO. I would assume its concentrate. That's what they're supposed to be telling me the concentration of a PSM base; so, I would assume it's .5; but they did the calculation themselves and came up with the 0.125 mg/cm² of PHMB, so; but I can go back and check that.

MR. JOHNSON: Ms. Carol, you're talking about commercial preparations of polyhexamethylene biguanide hydrochloride; is that right?

DR. EISENMAN: Right; PHMB.

MR. JOHNSON: Okay.

DR. MARKS: Okay; next issue on the insufficient data announcement was the NFY-degree reactions; and it said we would get the paper but, due to copyright restrictions, there were two case reports, and after surgically-wound exposures, so presumably it's really a significant exposure to me. Two cases wound exposure -- we have no cases from exposure to personal care products. So, again, I found that reassuring; rare in a report. Is Tom, Ron, Ron is that --

DR. SLAGA: I have no problem with that.

DR. MARKS: Okay; and then, the last one was -- so, you have the paper, was there anything more from that, Ron Hill?

DR. HILL: You have it too in the pile they gave us this morning.

DR. MARKS: That was in this morning?

DR. HILL: Yeah.

DR. MARKS: Okay.

DR. MARKS: And then last was the lung injury.

DR. HILL: We got this paper right here.

DR. SLAGA: The Korean one.

DR. HILL: Yeah; that also came to us this morning; and from what we can tell, and come up with a (inaudible) of a different chemical than the ingredient.

DR. MARKS: So, it's a different chemical and, obviously, it's not relevant?

DR. HILL: Yeah, this is guanine instead of biguanide; is that correct?

MR. JOHNSON: Mm-hmm.

DR. HILL: Yes? So, we would presume that to not be relevant, but we don't know.

Let's see -- prevent the growth of micro-organisms, humidifiers disinfectants are placed in the humidifier water tank. These disinfectants contain (inaudible) biguanide chloride (PGH), polyhexamethylene guanidine (PHMG),

(inaudible), so MIT was in there. (Inaudible) would have known that one, and another one; but no PHMB. Yeah; so we think it's not relevant.

This is a serious precautionary tale.

(OFF THE RECORD)

DR. MARKS: So, the lung injury and The Korean's -- Ron Hill, do you -- different chemical, not relevant? We can move forward?

DR. HILL: Yeah, it's pretty clear.

DR. MARKS: Okay, so, I see both Ron Shank's still reading; Tom Slaga, shall we proceed with a -- our team will be moving tomorrow a tentative report with a safe conclusion?

DR. SLAGA: Yes.

MR. JOHNSON: Is it safe for the formulation to be non-sensitizing? I guess safe as used?

DR. MARKS: It's going to be safe as used; we have sensitization data that --

MR. JOHNSON: Okay.

DR. MARKS: -- at the maximum leave-on. It's not a sensitizer.

DR. HILL: And this other paper we got seems to be a different chemical, as well; I believe. It says chlorhexidine. It's a biguanide, but it's not.

DR. HELDRETH: Yeah, in the case study, they looked at both chlorhexidine which is a (inaudible) and polyhexanide which is another name for PHMB.

DR. HILL: And that was the one that was the problem-child, so-to-speak?

DR. HELDRETH: Yes.

DR. MARKS: They used chlorhexidine as a -- that is a reference, another disinfectant, that can cause anaphylactic reactions; but it's got to be extremely rare because that's one of the preferred disinfectants that's still being used. And the other thing that is reassuring to me is that these cases were from 1998; and we don't have any cases since that, so we got almost 20 years without other cases of anaphylactic, particularly from personal care product.

DR. SHANK: How are you going to handle that in the discussion?

DR. MARKS: Just with that -- that it's a rare occurrence, and there haven't been case reports since that one back in 1998; and that was in a wound exposure. I was looking to see if they gave the concentration, and they didn't give the concentration.

MR. STEINBERG: It was used in a drug, as opposed to a cosmetic application.

DR. MARKS: Yes.

DR. HILL: Well, yeah; it's actually the use of

(inaudible) that might have resulted in the sensitization. I don't know if they're still marketing (inaudible) with that same stuff in there or not.

MR. STEINBERG: I don't know.

DR. HILL: I remember (inaudible). I just didn't much like it in the swimming pool.

DR. MARKS: Okay; so, does that sound -- team -- motion tomorrow, a tentative report with a conclusion safe.

DR. SLAGA: With a good construction.

DR. SHANK: And the Korean. The case report was on wounds.

DR. MARKS: Right; wounds, there was a rare occurrence.

DR. SHANK: All right; but what about the inhalation?

DR. HILL: Not the same chemicals.

DR. MARKS: Yeah; different chemical; therefore, not relevant.

DR. SHANK: Well, how do you -- because it just gives the initial.

DR. HILL: No, they're written out on page -- I'll show you where.

DR. HELDRETH: Do you think it would be helpful to add a comparative structure in that section where we say this is a different chemical.

DR. SHANK: I think so; yes.

DR. MARKS: You weren't here when Ron asked for chemical structures. You're going to be busy with chemical structures.

DR. HELDRETH: I like that; that's fun stuff.

DR. MARKS: And earlier a group of ingredients. We went from 25 to safe, to insufficient.

DR. HILL: It's on the second page; the back of the cover.

DR. MARKS: Yeah; I think the other good reason for putting that in there is because -- I know I wouldn't want our chemical here in my humidifier -- just a little too close. Do you want that put in there?

DR. HILL: No; I guess I just said it on the record, but, no.

DR. MARKS: That's obviously not a cosmetic use, but at the same time --

DR. SHANK: And, so, the child interstitial lung disease is going to be handled by saying a cosmetic ingredient was not one of the disinfectants.

DR. MARKS: Correct; any other comments.

DR. SHANK: Okay.

DR. HILL: Of course, if we were going to read them across, they are structurally smaller.

DR. SHANK: Well, can't have it both ways.

DR. HILL: That was my jab against excessive read- across; that's what that was. In case you didn't catch it.

DR. MARKS: Okay. Tomorrow I am going to move for a tentative report with safe -- a conclusion that's safe -- and we will -- I'm not sure we need to discuss the skin sensitization -- that'll be in the summary -- but I think the anaphylactic and the lung injury needs to be in the discussion for sure.

DR. HILL: There was something with the discussion. No, hang on.

MR. JOHNSON: So, that chemical structure isn't similar enough to a cosmetic ingredient to warrant any concern?

DR. MARKS: Correct.

MR. JOHNSON: Okay.

DR. SHANK: Pretty similar.

DR. HILL: Well, we have happily a raft if found there of how many of what I

consider to be new state-of-the-art sensitization studies and formulations we have. So, that's the point.

DR. MARKS: I guess what you're saying Ron, is you'd like to see inhalation studies to -- there would not be any lung injuries.

DR. EISENMAN: Or, it might not but (inaudible) uses are very low,.007 hairspray. So, you might want to call that out and say at that low level, but not higher; or something like that. The SCCS says it should not be used in spray products.

DR. MARKS: Yeah.

DR. SHANK: I think I would agree with that; but to say in the report that one of the many compounds in the disinfectant in this humidifier was not the same chemical, and that's true; but it was close. It just has a few more compounds.

DR. HILL: Yeah; the nitrogen's. The biguanide group is different from the guanidinium group, substantially; but yet.

DR. MARKS: How would you like to handle that, Ron, Ron Shank? I can see just in the end, in the discussion saying, we note the Korean experience, but it's a different chemical and it's not relevant. You are still uncomfortable because, chemically, it is similar.

DR. SHANK: But it's basically to be answered by the chemist, and if it's just not close enough -- if it were part of a series of compounds, would it be included in a read-across? And if the answer is clearly no, then it's

(inaudible).

DR. HILL: I would not include it.

DR. SHANK: Okay.

DR. HILL: But I have no strong basis for saying that because the problem with that kind of read-across is you've got, essentially, two data points. That structure class, which is arguably somewhat similar to that structure class, but yet guanidinium is different than biguanide. It's not a question for the chemist; it's a question for the biologist to look at that endpoint and see if they overlap or not, and that, I don't think, is purview here; but there are no inhalation studies, but we have good state-of-the-art -- and lots of them -- dermal studies; so, if that's a concern and they're in hairsprays, then you go to the concentration as .000-something or other, very low; doesn't mean you couldn't sensitize somebody, but it's very low, and no case reports right now.

MR. JOHNSON: The safety assessment includes acute and short-term inhalation toxicity --

DR. HILL: Yeah.

MR. JOHNSON: -- studies; and I'm wondering whether or not those should be mentioned in the discussion in relation to the humidifier, you know, studies?

DR. SLAGA: If there was no concern there, right?

MR. JOHNSON: Yeah.

DR. HILL: But I'm not an inhalation toxicologist.

DR. SHANK: I would just like the discussion to handle that clearly so that the average consumer who might be interested in this understands that it's not exactly the same compound, even though it killed 80 children; it's not exactly the same -- not the same as insufficient --

DR. SLAGA: Overly dismiss it.

DR. MARKS: No, no; I think your concern is right on, Ron. That's why I didn't move on. So, Carol, in this report, do we have inhalation move, or are you're implying in this report the inhalation --

MR. JOHNSON: Acute and short-term inhalation tox studies.

DR. MARKS: Now, that should be reassuring that they were safe -- the end, there is no toxicity. That would be another reason, Ron, that you can be reassured. It's a different chemical and this chemical has (inaudible).

DR. HILL: So many inhalation problems in those particular exposures.

DR. SHANK: When you have a --

DR. HILL: It's page 32.

DR. SHANK: Oh, I remember that the LC-50 is reported as greater than .36 mg/L. I think that was the highest concentration used and no one died. This is what, a dog -- no, rat. That's kind of misleading when you say the LC-50 was greater than this. No; the LC-50

wasn't determined is the way it should be stated. As tested, concentration was.36.

DR. MARKS: Besides this, it's just looks like it was worded, but the study --

DR. SHANK: So, that's just wording. So, yeah, I think, I'd repeat the reference to this inhalation study in the discussion that the cosmetic ingredient was tested for inhalation toxicity.

DR. MARKS: Yeah, to me that's --

DR. SHANK: That's stronger.

DR. MARKS: Yes; exactly, I agree. So, when you put together that it's a different chemical that caused a lung injury, we have inhalation studies in this report that are okay; then, to me, in low concentration and hair dyes we could mention that, but that's not, to me, as powerful as saying it's a different chemical and the inhalation studies --

DR. HILL: That's the acute one.

DR. SHANK: Yes.

DR. EISENMAN: It's at Table 9 is where the most details are.

DR. HILL: Oh, for the short-term inhalation.

DR. EISENMAN: Yes.

DR. MARKS: What page is that, Carol?

DR. EISENMAN: I don't know the page number

(inaudible).

MR. JOHNSON: I can tell you.

DR. HILL: She said in Table 9.

MR. JOHNSON: It's on page 54. It starts on 54; yeah; so, basically, just two short-term inhalation tox studies.

DR. MARKS: Ron, does that bring that into the discussion -- and does that, I think, support the safe conclusion and answer the issue of what happened in Korea?

DR. SHANK: That's the only data we have.

DR. MARKS: Right; but, I think, is it enough to say it's a different chemical, and our inhalation studies in this report are okay; therefore, we feel this is safe?

DR. SHANK: Yes.

DR. MARKS: Okay.

DR. HILL: So, no act is quite 0.025 mg/m³; so how would that relate to use of a hairspray? What's the concentration in the hairspray?

DR. MARKS: It was very small.

DR. HILL: .00-something percent, wasn't it?

DR. SHANK: I don't recall what the adverse was, but it wasn't --

DR. HILL: Anything above that, you had --

DR. SHANK: -- what the affect was. It certainly wasn't this.

DR. HILL: Well, it wasn't entire concentrations, it was at 12.5 and 26 mg/m³ all the rats died; at 2.75 mg/m³, signs of nasal irritation and dyspnea and moderate pneumonitis; thymus glands with severe depletion of lymphocytes and loss of normal architecture.

DR. SHANK: What's the point?

DR. HILL: That's at 2.75. At 25 mg/m³, one rat died; moderate nasal irritation and tachypnea in this group; and some histopathological affects: slight-to-moderately severe pneumonitis; thymus glands; three male and three female rats with red; patchy loss of cilia in tracheal epithelium of three rats; so, .025 mg/m³ seems to be fine; .25 is problematic.

MR. JOHNSON: Let me add that with respect to use concentrations, it's used at concentrations up to 0.0004 percent in aerosol hairsprays, and up to concentrations of 0.053 percent in pump hairsprays.

DR. HILL: .53 percent, so, yeah; so then you have to do some calculations to find out what that really is in terms of human exposure.

MR. JOHNSON: Mm-hmm; and I noticed that in one of the short-term studies, they're reporting severe nasal irritation and dyspnea.

DR. HILL: In some of the higher doses.

MR. JOHNSON: Yeah.

DR. HILL: We need to do calculations to find out. I mean that sounds like it's such a low concentration it shouldn't be problematic for the aerosol -- pump, you don't end up

breathing much of that, I guess.

DR. SHANK: When figured (inaudible).

DR. HILL: Mm-hmm.

DR. MARKS: So, back to lung injury, are we okay with different chemical at low concentration, hairspray's inhalation studies, in this report, are we okay; and that'll be handled in the discussion -- this supporting the safe conclusion?

Tom is yes; Ron Shank, are you (inaudible)? Do you like that for the discussion -- or I should say, more importantly, do you still like the safe conclusion?

DR. SHANK: I have to go back and look at reference five, does it have a good (inaudible); see if I can remember it.

DR. HILL: Reference five is the SCCS opinion.

MR. JOHNSON: Right.

DR. SHANK: So, we don't have enough information from the actual study?

DR. MARKS: Do you think this can be resolved between now and tomorrow, Ron Shank; or do you think we should --

DR. SHANK: No, because I tried to find the study and I couldn't. So, we don't know anything about the exposure conditions which are extremely important in inhalation studies; and many, many times they're not done correctly, especially in characterizing the particles.

DR. HELDRETH: Do you have the SCCS' summaries on that, already?

DR. SHANK: Just the summary. If I remember correctly, there's no detail. Though this has more detail than what I have. Thank you.

Well, if we have to get down to calculating the eight comparable exposure between the rat studies and what you think might happen in consumer use of sprays, that makes me a little nervous -- or not nervous, but concerned. More animal exposure data won't help. So, you'd either have to calculate a margin of safety, or just say this product ingredient shouldn't be used in inhalable products.

DR. MARKS: It sounds like that's where, Ron, you'd feel the most comfortable not using inhalation --

DR. SHANK: Inhalation -- products that can be inhaled.

DR. MARKS: Even though we have these other things, it's still not quite enough to sway you?

DR. SLAGA: You can say that they're somewhat similar in structure, and that would be a precautionary measure is not to have it in any inhalation-type products.

DR. SHANK: You have a significant number of human deaths associated with this chemical, and either you'd need a high margin of safety for exposure for using the cosmetic spray is a thousand times less than what these children were exposed to -- not children, rats.

DR. MARKS: Which would you prefer to go? At this point, I think we could wait for, as you said, it would be very difficult to calculate a margin of safety.

DR. SHANK: I think so.

DR. MARKS: It seems like the reasonable way to handle it would be insufficient data for use in inhalants.

DR. HILL: Currently, insufficient.

DR. SHANK: So, then you'll have to say what do you need.

DR. MARKS: Yeah; its --

DR. SHANK: We already have inhalation data.

DR. HILL: Inhalation data with particle-size carrier dries in such a way that it would relate to pump sprays and aerosol sprays as currently used, or something along those lines?

DR. SHANK: Yeah; I supposed you'd have to try to compare the exposure between the rat study and what you would expect from humans. Now, you do have the main difference between human exposure is a very short term, maybe repeated. But my assumption is when used as a spray once or twice, and then not again for a day, or at least hours; whereas these animals were exposed for several hours a day.

DR. HILL: Then, again, if you have a hairdresser who's using this spray several times an hour?

DR. SHANK: That's more like the rat then.

DR. HILL: I don't know because we don't have the calculation in the

characterization.

DR. SHANK: So, I guess to be fair to the manufacturers of this it would be to say insufficient if the lack of data is quantitative comparison between expected human exposures compared to the rat exposures in the short-term studies. (Inaudible).

Well, that's probably the way to go -- insufficient data; and what we need is quantitative comparison between expected human exposures compared to the short-term rat studies.

DR. MARKS: Okay; safe, except for an insufficient data for --

DR. SHANK: For (inaudible).

DR. MARKS: -- for inhaled cosmetics.

DR. SHANK: Yes.

DR. MARKS: And that relates, really, to the lung injury concern from these Korean reports; and even though -- but I think this all has to be brought out in the discussion even though it's a different chemical, it's close; even though there's low concentration in hairsprays, we don't know exactly how much is inhaled; even though the inhalation studies in this report are okay, we want to develop a margin of safety from rat studies, making a quantitative comparison between the rat's exposure and expected human exposure, both by the consumer and the beautician who may have much higher

(inaudible) since they may be spraying this, as you mentioned Ron, multiple times during the day, not just one or two. Does that sound reasonable? And, then, Ron, I'll probably ask you to clarify tomorrow, Ron Shank, if you want, but --

DR. SHANK: Okay.

DR. MARKS: -- does that sound -- so, tomorrow I'm going to move that a tentative report be issued that's safe, except for an insufficient data in inhaled cosmetics.

DR. SHANK: I think that's stronger and more logical than to say we dismissed Korean episodes because it's not the same chemical.

DR. MARKS: Yeah; no. If we're ever going to err -- how many deaths were there in Korea?

DR. HILL: 83 children.

DR. SHANK: 84.

DR. MARKS: If we're ever going to err, we better err on the safe side.

MR. STEINBERG: What were they exposed to there?

DR. SHANK: A humidifier.

DR. HILL: The vaporizer.

MR. STEINBERG: Vaporizer with the dimethyl sulfates (phonetic) on it also?

DR. HILL: Well, you know, it's interesting because I don't think of -- you know, when you run a vaporizer, I certainly smell lots of menthol, but I never really thought there's a whole lot of aqueous particles in the air from the humidifier, at least the normal ones; and you have a compound that isn't volatile -- these ones, I guess, maybe the

(inaudible) is a little, but, yeah, that's what I thought too -- so, these would be in water particles. Effectively, they're coming up into the air with the dissolved substances from a humidifier which -- I mean, I don't know what the design of those Korean humidifiers was; but it just stuns me, really.

DR. MARKS: Okay.

MR. JOHNSON: Because, actually, you have polyhexamethylene biguanide phosphate and polyhexamethylene guanidine in those humidifier formulations.

DR. HILL: What was the two (inaudible)?

DR. MARKS: And this is a tentative report, so there can always be in the next

--

(OFF THE RECORD)

DR. MARKS: Okay; I think we're at the point now, let's summarize -- I want to summarize --

MR. STEINBERG: We were just talking; one quick

(inaudible). You saying that it does contain the chlorohexidine ; and chlorohexidine breaks down to chlorobenzene, which is really a bad actor.

DR. SHANK: That's the wound.

MR. STEINBERG: Is that just the wound. It's not in this one? It's not in the humidifier?

DR. HILL: No.

MR. STEINBERG: Okay.

DR. SHANK: (Inaudible) in six different chemicals -- you don't know how much in each one; so you can argue well, why do you pick on this one; why not the others? But you want to be safe.

DR. HILL: Exactly.

MR. STEINBERG: Yeah; well, with those number of fatalities you'd want to be sure.

DR. SHANK: These aren't rats; these are children.

DR. MARKS: Well I think this is, to me, the prudent way to move forward. We can issue a tentative report that's safe, except for insufficient data for inhaled cosmetics; and whoever's making it for inhaled cosmetics come forward with more safety data.

As you mentioned, Ron, the big thing is get a quantitative comparison between rat exposure -- and these studies in this report, which would support the safety of it, but also the expected human exposure.

DR. SHANK: Right.

DR. MARKS: Okay; any other comments? Then, Ron, when we get in the discussion tomorrow --

DR. SLAGA: There's another red flag --

DR. MARKS: Oh.

DR. SLAGA: -- that Wilbur brought up about the nasal, severe nasal irritation; so that's another reason about not being in products that could be inhaled.

DR. HILL: And it might be after all the dust settles, it's still perfectly good in that aerosol spray at .0004 percent, or whatever.

DR. SLAGA: Right; that's fine. I agree with that but --

DR. MARKS: What page is the severe nasal irritation and what (inaudible)?

MR. JOHNSON: Page 55.

DR. MARKS: 55; and the concentration there was --

DR. HILL: There was a dose escalation study, whole range. So, .25 mg/m³, you saw that -- at .025 you didn't see it; at .25 you did see it.

DR. MARKS: .025?

DR. HILL: .025 was clean; .25 mg/m³, you begin to see that irritation.

DR. MARKS: And we have the maximum, well that's leave-on (inaudible).

DR. HILL: This is in mg/m³.

DR. MARKS: Okay; well, another indication of potential inhalant toxicity if you're getting nasal irritation. Okay; any other comments?

DR. HILL: In the dosing, there were 6 hours per day, 5 days a week, for 3 weeks total. So that's --

DR. SHANK: Standard.

DR. MARKS: Okay; any other comments? Well, this should be a robust discussion tomorrow, which will be good.

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DR. MARKS: Okay. At the April meeting this year we issued an insufficient data announcement for polyaminopropyl biguanide, also known as PHMB, which is included throughout the report which is good. We needed sensitization data, and I felt -- our team felt that what we received in wave two met this requirement, but now we have a correction dated June 13th. And could you interpret this for me? It says the INCI name 0.1 percent and the trade name 0.5 percent, and we're basing our sensitization okay that 0.5 percent is the maximum leave-on concentration, and we had a negative HRIPT at this concentration, as well as in the previous data we reviewed that appeared that the threshold for sensitization was 1 percent. So I'm not quite understanding why you have a 0.1 percent for the INCI and a 0.5 percent for the trade name.

DR. BELSITO: Because it was 20 percent. The biguanide was only 20 percent of what was provided.

DR. MARKS: So how does that relate for the HRIPT?

DR. BELSITO: The actual concentration of biguanide was one-fifth of what it was thought to be. So the trade name product was used at 0.5 but only contained 20 percent of the active ingredient.

DR. MARKS: So now we have a HRIPT at only 0.1 percent?

DR. BELSITO: Right.

DR. ANSELL: Well, we still have the other HRIPT at 0.2.

DR. BELSITO: At 0.2.

DR. ANSELL: But you're right. The one that was reported as 0.5 is actually 0.1 active.

DR. MARKS: Okay. So that obviously changes our concerns about the sensitization. We could move forward and limit the concentration to 0.1 if we wanted to.

Second concern in the insufficient data was anaphylactic reactions. There were two cases reported in 1998 from wound exposure, and we -- our team felt this is obviously a rare event. We haven't heard anything since 1998, and there are no reported cases of anaphylaxis in the use of cosmetics, so we thought that we could take care of that issue.

And then lastly, data from Korean studies on lung injury, as well as mortality. So significant problems.

We had quite a bit of discussion about this lung injury issue. Presumably, it was a different chemical, but it was one that was related to the polyhexamethylene biguanide. It's low concentration in hairsprays, but in the present inhalation studies we had in this report it was okay. However, it does cause severe nasal irritation. So that insufficient data we felt was not met. We felt we needed a margin of safety which could be developed from the rat studies with a quantitative comparison between rat exposure in the studies that are in this document and the expected human exposure was both a consumer or beautician. So we didn't feel we would meet that. So we felt that. So we felt that we would move forward with a tentative report; that it would be safe except for insufficient data for inhaled cosmetics. I think with a sensitization issue maybe we need to put a limit on the concentration for the other uses besides inhaled cosmetics.

DR. BERGFELD: A comment from the Belsito Group?

DR. BELSITO: Yes. So first I'll let Dan address the Korean issue because it was my assumption we're dealing with a totally different chemical there.

DR. LIEBLER: Yeah. So the substance associated with the effect in the Korean effects due to the inhalers was polyhexamethylene guanidine, which is, I would say, it's chemically similar, but it's a guanidine as opposed to a biguanide structure, which is different enough to not be the same chemical. It's not, you know, I don't think we can say that that effect would be reasonably predicted to occur with the ingredient that we're reviewing in this report.

DR. MARKS: We had that same discussion. I'll let Ron Shank comment to that and Ron Hill possibly. But we had quite a bit of discussion that it was similar. We couldn't read-across in terms of would it be safe or would it be toxic? So that's why Ron Shank, why don't you go ahead and elucidate more?

DR. SHANK: All right. We discussed this at length. We realize that the chemical associated with the children's deaths in Kora is not the same as the cosmetic ingredient. But we do have inhalation toxicity data for the ingredient. And it is not inactive. The exposures,

especially the short- term inhalation toxicity exposures did produce a variety of adverse effects at relatively low exposures. And that is for the cosmetic ingredient. So I would like, rather than just dismiss the issue of inhalation toxicity by saying the Korean experience was with a different compound, I'd like to see a margin of safety analysis between human exposures to hairsprays and the rat short-term inhalation toxicity studies.

DR. LIEBLER: So you're referring, Ron, specifically, to PDF 32 under the acute inhalation --

DR. HILL: The subchronic.

DR. LIEBLER: So the acute inhalation is the one in rates that referred to the results with dark red lungs observed at necropsy and a dose related depression of respiratory rate reported in a study in which mice exposed --

DR. SHANK: On page 33, PDF page 33, there is short- term or subchronic toxicity studies which were inhalation. And --

DR. BELSITO: It was negative.

DR. SHANK: Pardon me?

DR. BELSITO: It was negative.

DR. SHANK: No, it wasn't. If you could go to --

SPEAKER: Table 9, page 15.

DR. SHANK: Yeah, Table 9.

DR. BELSITO: But there was no observed affect at .025 milligrams per meter cubed.

DR. HILL: .025. You're right. At .025 you're right, they're not, but at .25 percent there was. And so --

DR. BELSITO: Not percent; it's milligrams per meter cubed.

DR. HILL: I mean, sorry, not percent. Yes.

DR. SHANK: It's milligrams per cubic meter. I think with the issues that have recently been

brought up as to how much of these hairsprays are actually inhalable, we've been dealing with that. I would like to see a margin of safety analysis trying to find out what would be a reasonable exposure from the use of hairsprays and compare that.

DR. BELSITO: Look at the exposure. I mean, what are the concentrations of use in hairsprays that are extraordinarily low?

DR. SHANK: Well, yes, the concentrations are low. But how often are the hairsprays -- I don't think we can just dismiss it and say, well, these aren't inhaled and it has nothing to do with --

DR. BELSITO: I don't think we're dismissing it. It would be something we'd bring into the discussion.

DR. SHANK: Right. It's just a calculation. But it should be done by people who know the hairsprays, not by me.

DR. BELSITO: Okay. Well, I mean.

DR. MARKS: And then the other issue was by consumer it might be only one, two times a day, but if you're using it as a beautician, it could be multiple times a day, so there could be a significant more exposure in that setting.

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

DR. HILL: Yeah. I was just going to say in the concentration in the pump spray is higher. It's .053, and I don't know if we have a good handle. I mean, I actually know we have, even from the material that we reviewed for that boilerplate preparation, there was some analysis of potential incidental exposure from pump sprays. So I think what we were looking for is to relate that potential at .053 percent is the information we have here with the way the exposures were done in the rats and say we have a 10,000-fold margin and I think where we landed was children died in Korea. Eighty-some children died in Korea. It's a different chemical so we don't have any reason to believe that this would be a problem with either of these, but we don't have any data to show that it wouldn't or analysis of that. I think that's where we landed. Is that consistent with what our discussion was?

DR. SHANK: Yes.

DR. MARKS: Yes.

DR. BELSITO: I guess I just want to follow up on your last comment, Jim, and just clarify what the purview of this panel is because I know that in RIFM, we're doing QRA. We're not looking at occupational exposures. We're looking at consumer exposures. Is the purview of this panel to look at safety of a "cosmetic ingredient" as used under all circumstances, including by beauticians? Or is it to look at the safety as used by consumer? Because I would think beautician safety in their workplace would be more OSHA and not more us. But I don't know. I just raise the issue because it's something that will go across many other different products.

DR. BERGFELD: Dr. Marks, do you want to comment?

DR. MARKS: I think, haven't we in the past, advised the cosmeticians or beauticians as I recall to protect themselves possibly with the acrylates that we gave advice of how they should use protective?

DR. BELSITO: It was more consumers for home use.

DR. MARKS: Yeah.

DR. HELDRETH: Certainly, the most common purview of this panel is to look at exposure to humans. But if this panel is aware of any perceived hazards or risks to other settings, I think it's worthwhile to make --

DR. LIEBLER: I mean, if there was no issue, if you completely set aside the issue of possible exposure of people who worked in salons, for example, your request for or your suggestion to do this quantitative or margin of exposure calculation doesn't go away; right? So I think it's really a side issue. I mean, I think the question really is more do we do this margin exposure calculation, which I personally think is very reasonable. We have the data. We could do it. And, you know, I don't think we need to decide whether this panel deals with occupational or individual consumer exposures to make that decision.

DR. ANSELL: Yeah. I think we've jumbled a number of issues all together. I mean, we started talking about Korea and you're talking about potential exposure to the material based on data on the material. And then we've thrown in this whole occupational, and I really think we need to detangle the discussions so we fully support the concept that the Korean issue is not relevant to this discussion. We also would support the calculation of a margin of exposure. And I would stay quiet on the occupational issue until it's demonstrated that it's of some relevance to the discussions.

DR. MARKS: Jay, I'd beg to differ just a little bit. I think the Korean incident that was reported is relevant because if that didn't happen we wouldn't be talking about it. It's just that since this chemical that we're reviewing is similar, that gave us pause. And that's why we liked the margin of safety so that we can say with this chemical we know that inhalation toxicity is not a concern. Does that sound proper interpretation?

DR. SHANK: Yeah.

DR. MARKS: That was the alert, really.

DR. SHANK: We have rat inhalation data, quantitative. I think we should use that to show that the margin of safety is sufficient.

DR. LIEBLER: Right. And I think that's the really, in my view, the only really compelling reason to do this. And it's perfectly appropriate diligence for this panel to do it and for CIR staff to assist us with the calculation. But I think that makes sense. You know, whether you buy into the cross structure comparison with the prehistoric --

DR. HILL: There's no data one way or the other.

DR. LIEBLER: You know, it's just a matter of opinion.

DR. MARKS: Right.

DR. LIEBLER: But we do have data. We can do the calculation. This is, you know, an acceptable procedure for a risk evaluation like this. So I think I agree with doing that.

DR. BERGFELD: All right. Bart?

DR. HELDRETH: I just wanted to clarify a little bit about the worker versus the consumer issue. Very much like in the report we just finished, the persulfates, we took this line out of the definition that gives instructions to the hairdresser of how they should use it to be safe, and we kept that in the discussion. I think that would be the appropriate level of concern that the panel could apply here for its concern to hair dressers, but it shouldn't be in the conclusion.

DR. LIEBLER: Correct.

DR. HELDRETH: It should just be for the consumer.

DR. BERGFELD: Correct. Paul, do you have a comment?

DR. SNYDER: I agree.

DR. BERGFELD: So I'll entertain a motion to table.

DR. MARKS: Well, do we want to table or do we move with a tentative report

--

DR. BERGFELD: Well?

DR. MARKS: -- with insufficient data for inhaled cosmetics and then we get the margin of safety. If it's okay, then we go --

DR. BELSITO: There is more because we don't agree with their conclusion.

DR. MARKS: Oh, okay. Well, certainly, we have sensitization we need to clarify, too.

DR. BELSITO: We need to clarify sensitization big time because this is a sensitizer.

DR. MARKS: Yes.

DR. BELSITO: We need big studies. It's a moderate to, in some studies, strong sensitizer, and this is MI, about to happen if we don't regulate it. And I think this is one that we have to do like cocamidopropyl betaine. We don't come out with a single concentration that's acceptable across all product lines. We ask that QRAs or some other type of risk assessment be performed depending upon the product. And right now we don't have a NESIL to put into the QRA -- well, we do. We have a NESIL to put into the QRA which is 25 micrograms per sonometer squared. I'm not sure, Don, if we can use the.2 study. I would like to look at that in depth because that was really quirky. There were a lot of questionable reactions going on during the sensitization and challenge phase that they said were read as negative, but I would really like to look at that before, even if you can calculate the dose per unit area on that study before we sign off on it. So I think that when we do get an appropriate NESIL, it has to be with a conclusion as we did with cocamidopropyl betaine to be clear that we're not saying it can go out at.1 or.2 or whatever the HRIPT allowed but it has to be put to some type of quantitative risk.

Also not so certain that I want to dismiss the urticarial reactions. I looked at this last night. There are more than just two. The initial two were done by Oliveri and those were actually fairly well studied. They were confirmed in skin prick testing to react to the polyaminopropyl biguanide. They were also confirmed by blood testing, IGE levels. And both of those patients pretty clearly historically were sensitized by burn wound dressings. And then you have the one report coming out from Ann Goossens in Brussels or Leuven, and it's really not 100 percent because she tested with the dressing, which also contains polyethylene glycol 4000. She did not do controls but she then did some base fill activation testing and said it was positive in her patient.

And then there's another report of an additional patient again seems to be a burn patient. So the question is whether this is how we handle this. Is this something that we just de facto said, like we did initially for the polyethylene glycol should not be used on damaged skin or to what extent do we pursue it? But it's quite clear that you can get -- oh, and then the other reaction was tracheal during surgery where they were spraying in onto mucosal services. So it's quite clear that you can get severe, life-threatening anaphylaxis because the patient subsequently reacted to wet wipes with anaphylactic reactions from sensitization. But the question is how were they sensitized? And it's not 100 percent clear, but it suggests they were sensitized through use presumably on second or third degree burns. So how do we get to that? I mean, we eventually got rid of the damaged skin because we show that it was on burn patients, and when you tape stripped the skin you weren't seeing these effects, but we don't have any of that data for this molecule.

DR. BERGFELD: So what are you suggesting?

DR. BELSITO: You know, I hesitate to say damaged skin because I think the skin was probably more damaged, but that would probably be the safest thing to say. And pending some ability of industry to show us that when you tape strip the skin you're not getting urticarial reactions as they did for the PEGs. But I clearly think we need a defined NESIL and QRA, and right now that looks to be 25 micrograms per sonometer squared, which is going to be low, but this is used in very low concentrations by and large.

DR. BERGFELD: So are you suggesting we go out as a tentative insufficient?

Or are you suggesting table and requesting this?

DR. BELSITO: I think that we can do, you know, one or two things. We can go as insufficient and ask industry to provide us data on, you know, urticarial reactions on tape stripped skin like they did for the pegs or we can say not to be used on damaged skin and, you know, apply a QRA based upon a NESIL that currently exists of 25 micrograms per sonometer squared.

DR. BERGFELD: Jim and then --

DR. MARKS: Yeah, I'll retract my motion. So I think the issue to me is do we just table this to get more or do we do a second insufficient data notice? And we're going to suggest that on another ingredient.

I can go either way. It doesn't matter to me. Your points are very well taken, Don, and I agree with all the points you make about sensitization about anaphylactic reactions and we still have the lung to get the margin of safety. So it's just a matter of, I would say either table it or do another insufficient data announcement. I don't think we need at this point to issue a tentative report because there's a lot of things still hanging.

DR. BERGFELD: Jay?

DR. ANSELL: Yeah. We'll leave up to the staff to decide which of those two makes more sense. I think our position is this should not proceed to the next step of development. These are new questions and we would like an opportunity to address them, many of which are very straightforward and some of which may be a little more complicated. So whichever as long as we don't proceed to the next step.

DR. MARKS: Right. Jay, which do you think has a greater potential for getting response from industry, a tabling or another insufficient data notice?

DR. ANSELL: You know, I don't know that industry would respond differently to either.

DR. MARKS: Okay.

DR. ANSELL: Yu know, we are committed to the support and analysis and assessment of the material. I just don't want this to start a development clock because these are new questions. So we should, you know, go back to wherever the last step was.

DR. MARKS: We concur. That's why I withdrew my motion about a tentative report. And the decision --

DR. BELSITO: I guess the question becomes what are we asking for? I mean, the margin of calculation could be done from the data we already have; right?

DR. MARKS: Right.

DR. BELSITO: The NESIL, we currently have an acceptable NESIL of 25 micrograms per sonometer squared. If industry doesn't like it, if that's too low when they do run a QRA or whatever method of risk assessment they want to use to address the sensitization hazard, they can come back to us with new information. I don't think we are going to get within a reasonable period of time the kind of information that would allow us to fully understand the situations under which these urticarial reactions occur and whether they could IGE mediated sensitization type one could occur by using the products on damaged skin.

My bigger concern is that if we, I mean, Europe's along this at.1 and they haven't said you need to use QRA. I mean, it's across the board. And if.1 is going to start creating problems in underarm deodorants and wet wipes, then we're going to lose another preservative. So, I mean, I feel inclined, only because I just -- you know what will happen, if there is a mini epidemic, polyaminopropyl biguanide will just be banned in Europe. They won't look at any risk assessment at that point. I would prefer to move ahead and just, I mean, you know, say that this should not be used on, you know -- how did we handle the PEGs where it was clear that it caused renal issues in burn patients when it was -- when the skin was completely --

DR. ANSELL: The confusion in that for us was that damaged skin was undefined.

DR. BELSITO: Right. But, I mean --

DR. ANSELL: And so --

DR. BELSITO: -- how do we handle finally saying, okay, we could get rid of that but in the discussion that we said, okay, you know, it caused renal effects because it was used on second and third degree burns where it essentially went into the bloodstream.

DR. BERGFELD: Carol?

DR. EISENMANN: The dermal penetration study, the in vitro dermal penetration study that was tape stripped skin that didn't go through the skin so it doesn't -- so it wouldn't get to cause the renal issues.

DR. BELSITO: Right.

DR. EISENMANN: So this is a little different if the facts are right in the skin.

DR. BELSITO: Right.

DR. MARKS: It's in the skin and it's systemic. Contact urticants is not concerning, really. It's the anaphylactic reaction, the systemic reactions which are really concerning. And the contact urticaria is just a harbinger of what potentially can occur.

DR. BELSITO: But the sensitization can occur initially in the skin as happened with latex gloves.

DR. MARKS: Oh, yeah, absolutely.

DR. BELSITO: So Carol has a point; that just doing a penetration study showing that it doesn't get through the skin doesn't help us.

DR. MARKS: Correct.

DR. BERGFELD: I'd like to have Bart tell us what the administration or staff would like us to do here.

DR. HELDRETH: Our preference, of course, would be to not table it simply because it leaves it to languish out there. As Dr. Belsito said, this is something that needs to be acted on sooner rather than later. So we would support either continuing with a TR with some sort of insufficiencies, or if you don't feel that that will get you the data that you need, we could issue a second IDA with the preface that there's a clock to that and we plan to come back and continue this report in the near future. But we're just afraid if we table it, it's going to sit there and wait.

DR. MARKS: I've already withdrawn my motion.

DR. BELSITO: Okay. So then I think what I'd like to do is simply go forward and say that, you know, we let industry know we will be doing a margin of exposure calculation for aerosol exposure that we will be suggesting that this be formulated to be nonirritating, nonsensitizing using risk assessment methods such as the QRA. And ask for one data request for the clarification on the urticarial issue. And you know, maybe that can give me a little more time and perhaps we can get more articles to suggest that it really occurred only in settings of, you know, where there was obvious systemic absorption as occurred because, I mean, one guy it was instilled into the trachea and the other two patients, it was applied -- the sensitization historically occurred with a wound dressing for a second degree burn. And I can give Ann Goossens a call or an email and find out details about what she thought about her patient that she reported.

DR. MARKS: Was this -- were all these reports -- was not familiar with the subsequent ones other than this index, two cases in 1998. Is it still the same commercial product?

DR. BELSITO: Yeah. It was all with this European product Lavasept.

DR. SNYDER: Lavasept.

DR. BELSITO: Lavasept.

DR. SNYDER: Lavasept. And there is -- because I looked into that, too, because there's the Baquacil that's used in the U.S., and there's no associated issues with that.

DR. MARKS: So what's interesting to me is, why is it still on the market if it's that dangerous? And then actually, the authors of the 1998 report referenced similar reactions to chlorhexidine.

DR. BELSITO: Yes.

DR. MARKS: Which is still widely used, and even though they occur, I mean, it's used daily widespread chlorhexidine is. So again, if it's still being used in Europe, why is it still being used if they've had these severe reactions with wound exposure?

So there are a lot of questions. I think, Don, I think it sounds like the question now is do we do an insufficient -- a second insufficient data announcement, which I'm fine with, or if you want to propose a tentative report with restrictions.

DR. BELSITO: Well, I mean, you know --

DR. MARKS: Hearing industry, obviously --

DR. BERGFELD: And do just that request.

DR. MARKS: -- the tentative report is moving forward and there are a lot of

questions. I think I'd prefer a second insufficient data announcement. That alerts industry what's going on and puts the onus to get some of those questions answered. And obviously, we're going to get calculations done and that way it doesn't languish.

DR. BELSITO: Yeah. And I would like to actually see a copy of that 2 percent HRIPT to look at all -- a detailed copy with all the reactions. And then I'll call Ann and maybe we can actually get the SECS document to see whether they noted these urticarial reactions, and since it did occur in Europe, perhaps they have further data on them.

The Oliveri paper, several of the papers did look because structurally this is similar to chlorhexidine. They did look to see if these individuals were also allergic to chlorhexidine and they were not. So the sensitization did not occur to be from chlorhexidine and it occurred to be from the Lavasept product.

DR. MARKS: And then I'll just comment. We had this discussion yesterday in our team, is we'd really like to avoid a conclusion that says formulate to be nonsensitizing. We know we do that with botanicals a lot but, you know, the ultimate absurdity is formulate to be nontoxic.

DR. BELSITO: But I think in cases where you have moderate to strong sensitizers and the area where you use it can significantly affect the outcome in terms of sensitization, as has been shown by methylisothiazolinone where, you know, at 100 parts per million in most rinse-offs it was perfectly fine. What caused the issue was wet wipes.

DR. MARKS: Yep.

DR. BERGFELD: I'd like to ask Bart again what he'd like us to do, whether we move forward with another insufficient or we ask for the data request and then take this up in September again. Would you comment?

DR. HELDRETH: Sure. I mean, of course, that's the panel's prerogative how we move forward, but if you feel that we are going to have all these needs met in time to prepare the reports again in September, then certainly, you could go forward with a tentative report. If you think it's going to take a little bit more time than that, then we could go forward with another IDA, meaning that this report would get finalized most likely in December instead of September. So if you feel that the extra time is needed to make sure we get everything collected, by all means we could do that second IDA. But if you feel that it's just some small calculations and some contacts with some of these authors, then you might want to move forward with the tentative.

DR. BELSITO: How quickly can you get the full SECS document? Is it publicly available?

DR. HELDRETH: Typically, we can download the SECS documents right away.

DR. BELSITO: Okay. So, I mean, I think, why don't we just move ahead and look at it in September? I mean, I would -- I think that having a little bit more opportunity to pursue the urticarial reactions I'll have a better sense and they're probably on burn patients, which we can then put into the discussion that, you know, significant mucosal exposure is not the situations under which these would be -- the consumer would be exposed to in cosmetic products. And we'll satisfy Ron Shank's issues with the calculation which should be fairly quickly, and we'll give industry a chance to calculate the NESIL for the best data they have and, you know, we can always move ahead with a conclusion. And then if they don't like it in terms of restriction, you know, they can live without restriction until they can provide data to show us it can go higher. Again, I don't want to -- I mean, this is a good preservative. I just don't want to see it removed from the marketplace like MI has been.

DR. BERGFELD: So I'm going to entertain another motion.
Yes?

DR. HILL: Yeah, you're going to make a motion here in a moment. I just wanted to point out, I'm not sure of Baquacil in swimming pools is still on the U.S. market for swimming pool use. Does anybody know?

DR. BELSITO: I tis.

DR. HILL: It is still? Okay. The other thing we want to point out with the chlorhexidine versus the Lavasept is chlorhexidine is not a polymer, so it's a defined length. The poly PHMB that we're considering here has long chain -- is long enough that I presume could crosslink IGE, so that's a different scenario than chlorhexidine. I just wanted to

point that out. If we're worried that urticaria is a sentinel for type ones, then I think there's an unknown there that doesn't exist with chlorhexidine or the isothiazlesinone.

DR. BERGFELD: Jim, do you want to propose a motion?

DR. MARKS: Yes. I propose that Don issue a motion for the tentative report with all the issues you suggested, Don.

DR. BERGFELD: Will you propose a motion?

DR. MARKS: Since you want to move forward. Although, I see Jay over there with nonverbal communication.

DR. BERGFELD: Jay? Okay, I'm sorry. Jay?

DR. ANSELL: Wholly separate from the data discussion which I think has all been entirely reasonable, you know, I, again, would urge that we go for a second report with insufficiency. These are, you know, new items that we haven't had a chance to discuss, and to proceed with the thought that we might catch up or not in this report or future reports I think would be troubling. The panel has the right to ask all sorts of new questions and request new data at any time, but I think we also have the obligation to have some time to be able to respond.

DR. MARKS: So my motion would be a second IDA. I hear you loud and clear, Don. It isn't a huge amount of time. We've heard from Bart that for sure we'll have it by December. We may have it before for September and we'd urge that to occur. But it gives you a little bit more time in industry.

So I don't know, Don, I can go either way. If you feel strongly --

DR. BELSITO: I don't know what the rules are. Once we go insufficient, can we go with a second IDA again? I mean, is that -- is the next step to do a tentative final?

DR. SHANK: There are no new data needs identified.

DR. BELSITO: Okay. I mean, I'm fine. I just don't want to table it because --

DR. MARKS: Right.

DR. BELSITO: -- otherwise, it's not going to move along.

DR. BERGFELD: So is there a second to the IDA motion? And the list, again

--

DR. BELSITO: Calculation, margin of exposure for inhalation based upon the 14 or 28 day study we have and the current use in hairsprays -- probably even better, deodorant sprays because they're said to have smaller particles, or a combination. Further clarification on the urticarial reactions. I've read the papers and I think I'm fairly certain those were significant burns. I'll find out from Ann whether she has a clue as to where her patient was sensitized. And we'll get a look at the hard data on the 2 percent study, and if industry has any HRIPTs that would give us a higher NESIL, hopefully they would provide those and go from there.

DR. BERGFELD: So it's been moved and seconded that we go out for an insufficient data announcement with the list that you've heard.

Any further comments?

DR. MARKS: No, it's just a clarification of all three points that we had in the first time insufficient data announcement.

DR. BELSITO: Right.

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

All those in favor of IDA? Unanimous. Thank you.

(The motion passed unanimously.)

DR. BERGFELD: Thank you. Very good discussion. Thank you.

DR. BELSITO: Can we just have one little further discussion?

DR. BERGFELD: Sure.

DR. BELSITO: In reading this report, we understand very clearly Dr. Shank's desire to point out that polyaminopropyl biguanide is actually polyhexamethylene biguanide hydrochloride, but it became very confusing for me and even for the writer because at one point they called it polyhexamethylene biguanide twice when one was one and one was the other, to keep doing this with parentheses. And what we suggested be done is that the INCI name, polyaminopropyl biguanide be defined up front as polyhexamethylene biguanide hydrochloride and indicate that it would be represented in cap letters throughout and that the chemical ingredient polyhexamethylene biguanide hydrochloride would be in lower case. So when you saw the caps you knew it was actually polyhexamethylene biguanide and when you saw the regular you knew it

was probably polyaminopropyl biguanide. But putting the parentheses there I thought was extremely, extremely confusing in trying to read the data.

DR. BERGFELD: Ron Hill?

DR. HILL: Could you just define it as PHMB somewhere near the beginning and just keep using that all the way through except when you needed to explicitly refer to the polypropyl as the actual polypropyl, which is only maybe in two spots? I mean, I don't know. At least give consideration to that.

DR. LIEBLER: Just to clarify, I think what we were suggesting, if we are indeed on the same page, Don, is that in the first paragraph, the introduction, when this discrepancy between the INCI name and the chemical substance name is explained, we thereafter in the report just use the INCI name, which always begins with a capital letter for the name throughout the report, indicate up front what the difference is, not use the abbreviation since we normally don't do that throughout our reports. We use the INCI name throughout the report. You know, we don't normally, but this is a very exceptional circumstance where the chemical name is -- and I think there is a purpose in using the parentheses and reminding people that it's not polypropyl --

DR. LIEBLER: Well, you don't need to remind them 10 times on every page. So I think that, you know, basically what I'm suggesting is that we stick to our standard practice. We define the discrepancy up front but we then don't beat the reader over the head with it repeatedly throughout the report because it's just unnecessary. My two cents. Our team's two cents.

DR. MARKS: Fine.

DR. BERGFELD: That's agreed.

DR. HELDRETH: And would you keep the parentheses in the title or would they go there, too?

DR. SHANK: Definitely in the title.

DR. BELSITO: You could keep it in the title.

DR. SNYDER: No objection.

DR. BERGFELD: Any other comments before we move on? Seeing none, let's move on then. The next one is plant-derived proteins. Dr. Belsito presenting.

DR. HILL: I'll just make a mention while they're getting settled on that particular issue because it's come up with me about this use of caps versus not caps is when you have toxicology data that's testing a chemical and we don't know that it is, in fact, the cosmetic ingredient, just that chemical, then frequently people are using -- our staff are using capital letters inappropriately in my opinion. If the material that's being tested in the toxicology study is not actually known to be the cosmetic ingredient, then why are we going to capitalize it in the report? So, I mean, I think this is a bigger issue than just that PHMB that we just talked about. We really need to discuss that practice. Because if you have a journal article from an academic group or from whatever source and they've tested something that has the same chemical name as the cosmetic ingredient but we have no idea if it is, in fact, purchased from a source that's the cosmetic ingredient, then I object to putting capital letters there in the report. So that's my issue with that.

DR. HELDRETH: It's very common that the data sources we get, whether they're published or unpublished, do not relate, whether or not that chemical tested was necessarily the same as what's in a cosmetic product. However, we only include those ingredients under the INCI ingredient name when we, to the best understanding, believe that it is the same chemical. When we do have a question about it, we point that out at the data set. So we'll put in parens, within the summary for that data point, that it was reported as this so that it gives the panel an inclination that this may not be exactly the same.

DR. HILL: So in a discussion of a chemistry section where you're talking about the chemical, that at least the chemical is the same as the ingredient, you think it's perfectly appropriate to capitalize all the way through. I mean, for generic drug names, for example, you don't ever capitalize those unless they appear at the beginning of a sentence or in a table heading or title.

DR. HELDRETH: Yes, but we're not dealing with a drug name.

DR. HILL: I know that.

DR. HELDRETH: The common practice in the cosmetic industry is to follow the format of the nomenclature dictionary. And their standard process is to capitalize the first letter of each name. So we're trying to keep that as a consistent thing so that the name that is in our report is the same exact name that the consumer or any stakeholder will find on a label.

DR. HILL: I don't disagree with that, but I think if it's capitalized, it should be referring to the ingredient -- clearly referring to the ingredient and not just a chemical purchased from Aldridge and tested in a lab. And that's where the gray area is for me.

DR. HELDRETH: Yeah. I mean, we would certainly like to see more of that direct relationship there, but I think that's beyond means.

DR. HILL: Unfortunately, we don't have cosmetic grade or product like we might have with food grades. That's -- I don't know if it's unfortunate or not but the point is that makes it more of a gray area than it might otherwise be.

DR. BERGFELD: All right. Than you.

DR. BELSITO: Okay. Just to point out, Dan, capitalizing only the first letter will make it confusing when the first word of the sentence is the material. So I really think we have to capitalize all the words -- all the letters, rather.

DR. HELDRETH: In this case, however, it's a two -- a two-word name. So the polyaminopropyl and the b in biguanide will be capitalized in each case.

DR. BELSITO: Okay, fine. As long as there's some way of differentiating it. Yeah, good. Okay.

Safety Assessment of Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride) as Used in Cosmetics

Status: Draft Tentative Report for Panel Review
Release Date: August 18, 2017
Panel Date: September 11-12, 2017

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Interim Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Ivan Boyer, Ph.D., Toxicologist.

ABSTRACT: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride), which functions as a preservative in cosmetic products. The Panel reviewed relevant data relating to the safety of this ingredient, and a conclusion will be determined.

INTRODUCTION

The safety of Polyaminopropyl Biguanide¹ as used as a preservative in cosmetics is reviewed in this assessment. Polyaminopropyl Biguanide is an International Nomenclature of Cosmetic Ingredients (INCI) name; it refers to the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (polyhexamethylene biguanide hydrochloride (PHMB HCl)). This chemical does not actually contain polyaminopropyl biguanide (a 3-carbon chain in each monomeric repeat unit), but instead applies exclusively to polyhexamethylene biguanide (a 6-carbon chain in each monomeric repeat unit; always supplied as the hydrochloride salt). Indeed, the chemical polyaminopropyl biguanide is not a cosmetic ingredient. However, throughout the safety assessment report, the capitalized INCI name Polyaminopropyl Biguanide is used to represent polyhexamethylene biguanide hydrochloride (appears in lower case), which is the ingredient with reported uses in cosmetics and is the subject of this safety assessment. The names of similar chemicals (e.g., polyhexamethylene guanidine phosphate) that are mentioned in the report text also appear in lower case. Furthermore, most of the safety test data included in this report are on polyhexamethylene biguanide hydrochloride. The only exception to the exclusive use of the INCI name Polyaminopropyl Biguanide in this safety assessment relates to the summary of the cytotoxicity study, in which results for Polyaminopropyl Biguanide (i.e., polyhexamethylene biguanide) and polyaminopropyl biguanide are compared.

In 2017, the Scientific Committee on Consumer Safety (SCCS) issued a final opinion stating that the use of Polyaminopropyl Biguanide as a preservative in all cosmetic products at concentrations up to 0.1% is safe and that its use in sprayable formulations is not advised.²

Additionally, Polyaminopropyl Biguanide has been reviewed by the United States Environmental Protection Agency (EPA), with the conclusion that its use as a pesticide has very low aggregate risk of adverse health effects to the public or environment.³

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

CHEMISTRY

Definition and General Characterization

Polyaminopropyl Biguanide is the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (polyhexamethylene biguanide (PHMB HCl)). The definition of this ingredient is also presented in Table 1.¹

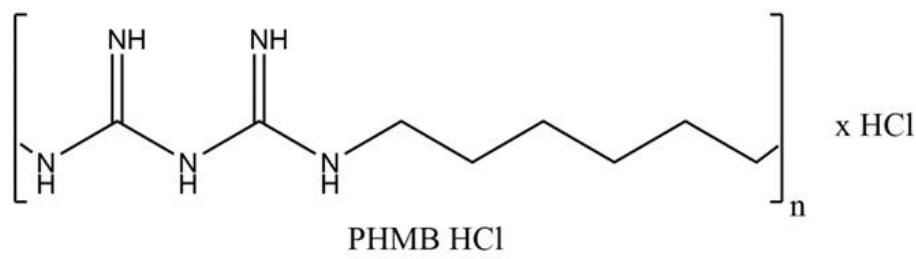


Figure 1. Polyhexamethylene biguanide hydrochloride (PHMB HCl), which is the chemical represented by the ingredient name Polyaminopropyl Biguanide in the Dictionary.

However, the current wINCI (online version of the *International Cosmetic Ingredient Dictionary and Handbook*) monograph for Polyaminopropyl Biguanide has recently been updated to define this ingredient as the chemical, PHMB HCl,

as depicted by the structure presented in Figure 1 and in the CAS File corresponding to the CAS No. in the wINCI monograph (32289-58-0).¹

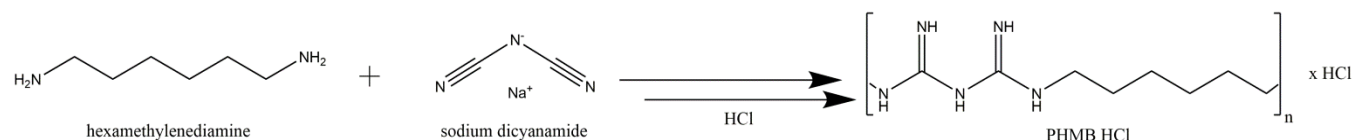
Comments on the identity of Polyaminopropyl Biguanide were received from a chemical supplier, which stated that, effectively, all Polyaminopropyl Biguanide is polyhexamethylene biguanide HCl (i.e., C6 alkyl chains linked together by biguanide groups), and no propyl biguanide groups are present (the INCI name, Polyaminopropyl Biguanide, is an artifact of arbitrarily choosing the middle of the C6 alkyl chains to identify the polymer repeating units of the ingredient).⁴

Chemical and Physical Properties

Polyaminopropyl Biguanide is a polymer that, in its neat form, is a solid/powder with purity > 94.2 %, and is often marketed as an approximately 20% aqueous solution.² Chemical and physical properties are summarized in Table 2.

Method of Manufacture

One of the current methods for manufacturing Polyaminopropyl Biguanide is through the polycondensation of sodium dicyanamide and hexamethylenediamine.⁵



Scheme 1. Synthesis of Polyaminopropyl Biguanide via the polycondensation of hexamethylenediamine and dicyanamide.

Impurities

The following chemicals have been reported as possible impurities of Polyaminopropyl Biguanide: *N*-(6-aminohexyl)-*N'*-(6-(6-guanidinohexyl)guanidine), *N*-cyano *N'*-(6-*N*-cyanoaminohexyl)guanidine, *N*-Cyano *N'*-(6-aminohexyl)guanidine), *N*-cyano-*N'*-6-(6-guanidinohexyl)guanidine hydrochloride, and 1,6-diguanidinohexane dihydrochloride.²

The trace metals content (in ppm, w/w) of 5 different batches of Polyaminopropyl Biguanide has been reported as follows: cadmium (< 0.25), chromium (< 0.25-0.7), cobalt (< 0.25), iron (14-40), lead (< 2), zinc (370-540), arsenic (< 2), and mercury (< 0.2).² The concentrations reported are from 5 different batches of technical grade Polyaminopropyl Biguanide (solid).

USE

Cosmetic

The safety of Polyaminopropyl Biguanide is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.⁶ Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.⁷

According to 2017 VCRP data, Polyaminopropyl Biguanide is being used in 147 cosmetic products, mostly leave-on products.⁶ The results of a concentration of use survey provided in 2017 indicate that Polyaminopropyl Biguanide is being used at concentrations up to 0.1% in rinse-off products and concentrations up to 0.2% in leave-on products (Table 3).⁷

Cosmetic products containing Polyaminopropyl Biguanide may be applied to the skin and hair or may come in contact with the eyes (at maximum use concentrations up to 0.2 %) and mucous membranes. Polyaminopropyl Biguanide is being used in a lipstick product, the application of which may result in incidental ingestion. It is also being used in baby products at maximum use concentrations up to 0.1%. Products containing Polyaminopropyl Biguanide may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Polyaminopropyl Biguanide is used in a fragrance preparation, which may result in incidental ingredient inhalation exposure. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters $> 10 \mu\text{m}$, with propellant sprays yielding a greater fraction of droplets/particles below $10 \mu\text{m}$, compared with pump sprays.^{8,9,10,11} Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.^{8,9}

The SCCS originally concluded that Polyaminopropyl Biguanide is not safe for consumers in all cosmetic products when used as a preservative up to the maximum concentration of 0.3%.¹² In 2017, the SCCS issued a final opinion stating that the use of Polyaminopropyl Biguanide as a preservative in all cosmetic products at concentrations up to 0.1% is safe and that its use in sprayable formulations is not advised.²

Polyaminopropyl Biguanide is currently listed in Annex V (entry 28) of the European Commission (EC) Regulation No. 1223/2009 (Cosmetic Regulation) as a preservative to be used in all cosmetic products at up to a maximum concentration of 0.3%.^{2,13} Additionally, Polyaminopropyl Biguanide is classified as CMR 2 (Carc. 2) according to the Commission Regulation (EU) No. 944/2013. CMR substances are classified as carcinogenic, mutagenic, or toxic for reproduction. A substance is placed in carcinogen Category 2 (Carc. 2, suspected human carcinogens) when the evidence obtained from human and/or animal studies is not sufficiently convincing to place the substance in Category 1A (substances known to have carcinogenic potential for humans) or Category 1B (substances presumed to have carcinogenic potential for humans). The Carc. 2 classification was effective as of January 1, 2015 and, according to Article 15 (1) of the Cosmetics Regulation, the use of Polyaminopropyl Biguanide as a cosmetic ingredient is considered to be prohibited as of this date.² However, Article 15 (1) of the Cosmetics Regulation also states that a substance classified in Category 2 may be used in cosmetic products if the substance has been evaluated by the SCCS and found safe for use in cosmetic products.

According to the Consumer Council Thinking Chemistry (Danish consumer chemistry watchdog), Polyaminopropyl Biguanide has been banned from personal care products in Denmark since January of 2015, based on the European Commission's classification of this ingredient as a CMR substance.¹³ Reportedly, a representative of the Association of Danish Cosmetics, Toiletries, Soap and Detergent Industries (SPT) has stated that the organization does not find the use of Polyaminopropyl Biguanide to be illegal, because CMR substances may be used in cosmetic products if a risk assessment shows that the use of the substance is safe. Reference was made to the SCCS's conclusion specifying a safe level of Polyaminopropyl Biguanide in cosmetics. It should be noted that "the use in cosmetic products of substances classified as CMR substances of category 2, under Part 3 of Annex VI to Regulation (EC) No. 1272/2008, shall be prohibited; however, a substance classified in category 2 may be used in cosmetic products where the substance has been evaluated by the SCCS and found safe for use in cosmetic products."¹⁴

Noncosmetic

Polyaminopropyl Biguanide is reported to be the most frequently used antiseptic in traumatic and orthopedic surgery.¹⁵ According to another source, Polyaminopropyl Biguanide has the following uses: fungicide, algicide, sanitizer in swimming pools, preservative for cut flowers, materials preservative, bacteriostat in industrial processes, and water systems, and hard surface disinfectant (food and non-food contact surfaces).³

Polyaminopropyl Biguanide is a broad-spectrum antimicrobial agent used in a variety of products, including contact lens cleaning solutions, skin disinfectant solutions, and wound dressings.¹⁶ Solid wound dressings are composed of various synthetic or naturally-derived materials, and typically contain added antimicrobials, such as silver, bismuth, chlorhexidine, bacitracin, or Polyaminopropyl Biguanide. Wound dressings are regulated by FDA as Class 1 medical devices (i.e., the device is exempt from premarket notification procedures). However, this classification does not apply to wound dressings that contain added drugs, such as antimicrobial agents.¹⁷

In Australia, Polyaminopropyl Biguanide is listed in the Poisons Standard – the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP) in Schedule 6.¹⁸ Schedule 6 chemicals are described as "Substances with a moderate potential for causing harm, the extent of which can be reduced through the use of distinctive packaging with strong warnings and safety directions on the label." Schedule 6 chemicals are labeled "Poison." According to this standard, Polyaminopropyl Biguanide can be used in preparations containing concentrations of 5% or less and when packed and labeled for therapeutic use.

TOXICOKINETICS STUDIES

Dermal Penetration

The dermal penetration studies summarized below are presented in Table 4.

In Vitro

In one study, skin penetration experiments were performed using both rat (skin disks in solutions; 5-day equilibration phase) and human skin (receptor fluid in diffusion cell collected up to 15 days) in vitro. At 0.4%, 1.4%, 5%, and 20% concentrations of Polyaminopropyl Biguanide, absorption rates (ng/cm²/h) through human epidermis were 8.13, 22.8, 350, and 1005, respectively. At 0.4%, 20% (early phase), and 20% (late phase) [¹⁴C]-Polyaminopropyl Biguanide, absorption rates (ng/cm²/h) in rat whole skin were 131, 3695, and 11940, respectively. Another study involved the application of Polyaminopropyl Biguanide (5% solution) to rat skin biopsies from newborn hairless rats and human epidermal skin in diffusion chambers. In rat skin, no absorption was detected up to day 5 of exposure. In human epidermal skin biopsies, a low rate of penetration (~0.09 %) was noted after 24 h. Polyaminopropyl Biguanide solutions (0.1% aqueous micellar solution, 0.1% oil-in-water emulsion, 0.3% aqueous micellar solution, and 0.3% oil-in-water emulsion) were applied to human split-thickness skin in a 2-part dermal penetration study. In Part 1, penetration of the 0.1% aqueous micellar solution and 0.1% in oil-in-water emulsion was determined directly after the 24 h exposure period. In Part 2, 24 h exposure to the 0.3 % aqueous micellar solution and to 0.3% in an oil-in-water emulsion was followed by an additional 72 h period to determine whether the test compound that was absorbed into the skin during the previous 24 h period would move from the skin into the receptor fluid after the washout [At 24-h post-dose, the skin was washed with an aqueous solution of polysorbate 20 (2% w/v) and water. The skin was then dried and removed from the diffusion cells, after which the skin was dried and the upper stratum corneum removed by tape stripping (5 tape strips). The remaining skin was divided into exposed and unexposed skin. The exposed epidermis was then separated from the dermis by heat separation. All samples were analyzed by liquid scintillation counting.].

In the 24-h study, 48.43% (from aqueous solution) and 52.35% (from oil/water emulsion) of [¹⁴C]-Polyaminopropyl Biguanide-derived radioactivity was removed during the washing procedure (dislodgeable dose at 24 h). At 24 h post dose, the absorbed dose was 0.03% (0.58 ng equiv/cm², from aqueous solution) and 0.04% (0.72 ng equiv/cm², from oil/water emulsion) of the applied dose. The epidermis + lower layers of stratum corneum contained 11.47% (238 ng equiv/cm², from aqueous solution) and 14.20% (291 ng equiv/cm², from oil/water emulsion) of the applied dose. The dermis contained 1.56% (32.3 ng equiv/cm², from aqueous solution) and 1.02% (20.9 ng equiv/cm², from oil/water emulsion) of the applied dose. The mass balance was complete (90.93% (from aqueous solution) and 98.96% (from oil/water emulsion) of the applied dose).²

In the 72-h study, 53.33% (from aqueous solution) and 58.10% (from oil/water emulsion) of [¹⁴C]-Polyaminopropyl Biguanide-derived radioactivity was removed during the washing procedure (dislodgeable dose at 24 h). At 72 h post dose, the absorbed dose was 0.02% (1.29 ng equiv/cm², from aqueous solution) and 0.03% (1.94 ng equiv/cm², from oil/water emulsion) of the applied dose. The epidermis + lower layers of stratum corneum contained 14.54% (972 ng equiv/cm², from aqueous solution) and 14.45% (921 ng equiv/cm², from oil/water emulsion) of the applied dose. The dermis contained 1.23% (82.0 ng equiv/cm², from aqueous solution) and 1.46% (93.4 ng equiv/cm², from oil/water emulsion) of the applied dose. The mass balance was complete (92.71% (from aqueous solution) and 99.25% (from oil/water emulsion) of the applied dose). There was a negligible increase (0.01% of applied dose) in Polyaminopropyl Biguanide concentration observed in the receptor fluid between 24 h and 72 h.²

The results of this dermal penetration study indicated that the residual stratum corneum + epidermis fractions were not considered as contributing to the systemic exposure dose (mg/kg/day) that is being used in the SCCS margin of safety (MOS) calculation (See Risk Assessment subheading in Chronic Toxicity Studies section). Study results also indicated that absorption through the skin equaled 1.56% (dermis contained 1.56% of applied dose) + 0.03% (absorbed dose = 0.03% of applied dose). Based on SCCS Notes of Guidance, one standard deviation (2.5%) was added to the absorbed amount, yielding a calculated dermal absorption value of 4.09% (1.56% + 0.03% + 2.5% = 4.09%) that is being used in the SCCS MOS calculation.²

Absorption, Distribution, Metabolism, and Excretion

The toxicokinetics studies (oral exposure) summarized below are presented in Table 5.

Animal

Oral

In rats, radiolabeled Polyaminopropyl Biguanide was excreted principally in the feces. In one study, rats were dosed orally with 20 mg/kg/day for 10 days and elimination after dosing was described as follows: $5.6\% \pm 0.35\%$ in urine, $93.1\% \pm 1.58\%$ in feces and 0.2 % exhaled. In another animal study (species not specified) of the distribution of radioactivity after dosing, the greatest amounts of radioactivity were detected in adipose tissue, followed by the kidneys and liver. No radioactivity was detected in brain. Small amounts of Polyaminopropyl Biguanide oligomers with 2 cyanoguanidino end groups were found in the urine, together with trace constituents, 3,3-dicyano-1,1-hexamethylenediguanidine and a compound considered to be 1-(6-aminohexyl)-3-cyanoguanidine.^{2,19,20}

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute toxicity data summarized below are presented in Table 6 (dermal studies), Table 7 (oral studies), and Table 8 (inhalation studies).

Dermal

There was no mortality or other signs of systemic toxicity in rats that received a single dermal dosage of 5000 mg/kg aqueous Polyaminopropyl Biguanide, but hemorrhage of dermal capillaries at the application site was observed. In an acute dermal toxicity study of 20% aqueous Polyaminopropyl Biguanide on rabbits, the LD₅₀ was reported to be > 400 mg/kg.^{2,12,19}

Oral

An LD₅₀ of > 1000 mg/kg was reported for rats dosed orally with aqueous solutions of up to 25% Polyaminopropyl Biguanide. A median lethal dosage of 25.6 mg/kg was reported for rats dosed orally with a 0.4% Polyaminopropyl Biguanide solution.^{2,18,19,21,22}

Risk Assessment

The EPA conducted a screening-level acute dietary human health risk assessment for Polyaminopropyl Biguanide in food.³ Risk estimates were calculated for females 13 to 50 years old, the only population subgroup with an acute toxicity endpoint (not stated) that was of concern. "Risk estimates for the use with the highest exposures were 9% of the acute Population Adjusted Dose (aPAD = 0.2 mg/kg/day) and, therefore, were not of concern." The EPA defines an aPAD as a dose at which an individual could be exposed on any given day and no adverse health effects would be expected.

Inhalation

An LC₅₀ was reported to be > 0.36 mg/l in acute inhalation toxicity studies in which rats were exposed (most for 4 h) to Polyaminopropyl Biguanide solutions (concentrations up to 500 mg/m³ in air). Dark/red lungs were observed at necropsy. A concentration-related depression of respiratory rate was reported in a study in which mice were exposed to Polyaminopropyl Biguanide at concentrations up to 208 mg/m³.¹²

Short-Term Toxicity Studies

The short-term dermal, oral, and inhalation toxicity studies summarized below are presented in Table 9.

Dermal

There were no mortalities or signs of systemic toxicity in rats that received dermal applications of Polyaminopropyl Biguanide at dosages up to 200 mg/kg daily over a 30-day period (21 applications total; NOAEL = 200 mg/kg/day). In a 21-

day dermal toxicity study involving rabbits, there was no evidence of toxic effects on the skin after 20% aqueous Polyaminopropyl Biguanide (12,000 ppm solution (1 ml)) was applied daily.^{12,19}

Oral

A lowest-observed-adverse-effect-level (LOAEL) of 0.1 mg/ml for Polyaminopropyl Biguanide was reported in 28-day oral toxicity studies involving rats and mice.^{12,19,21} In a 60-day oral toxicity study on Polyaminopropyl Biguanide involving rats, mild toxicity in the liver or kidneys was observed (by microscopic examination) at 2 mg/kg/day (dose equivalent to 0.2 mg/l of 0.4% solution of test substance), 8 mg/kg/day (dose equivalent to 0.4 mg/l of 0.4% solution of test substance), and 32 mg/kg/day (highest dose, equivalent to 1.2 mg/l of 0.4% solution of test substance). None of the animals died.

Inhalation

In 21-day and 28-day inhalation toxicity studies on Polyaminopropyl Biguanide involving rats, no-observed-adverse-effect-concentrations (NOAECs) of 0.025 mg/m³ and 0.0239 mg/m³ were reported, respectively. The animals were exposed (nose-only, concentrations up to 26 mg/m³) to the test substance 5 days per week, 6 h/day.¹²

Subchronic Toxicity Studies

The subchronic oral toxicity studies summarized below are presented in Table 10.

Oral

The following results were reported in 90-day oral toxicity studies on Polyaminopropyl Biguanide involving rats: no mortalities, but iron pigment/deposits observed in Kupffer cells (at 12500 ppm and 5000 ppm in diet) and a NOAEC of 1000 ppm. There were no treatment-related macroscopic post-mortem findings in mice in a 90-day drinking water study of 20% aqueous Polyaminopropyl Biguanide, and a NOAEC of 1000 ppm was reported for this ingredient in a 90-day feeding study in which mice received concentrations up to 4000 ppm in the diet. A NOAEC of 5500 ppm was reported for Beagle dogs fed Polyaminopropyl Biguanide at concentrations up to 11000 ppm in the diet for 90 days.^{12,19}

Chronic Toxicity Studies

The chronic dermal and oral toxicity studies summarized below are presented in Table 11.

Dermal

In an 80-week chronic toxicity study involving mice (dermal applications 5 days/week), a mortality rate of 75% was reported for the highest dose group (10% Polyaminopropyl Biguanide; 30 mg dose). The exophthalmos observed throughout the study was more severe in this group, compared with the other groups, but the results of histological examination of the eyes and gross and microscopic examination of the thyroids were negative. A NOAEL of 0.6 mg/mouse/day was reported.¹⁹

Oral

In a 104-week oral toxicity study involving rats, a NOAEL of 2000 ppm (highest concentration tested in diet) was reported for Polyaminopropyl Biguanide. This concentration corresponded to 36 mg/kg/day in male rats. A no-observed-effect-level (NOEL) of 200 ppm for histopathologic changes was reported in a 122-week oral toxicity study involving rats fed Polyaminopropyl Biguanide at concentrations up to 2000 ppm in the diet. Increased adrenal weight was reported for males and females at concentrations of 1000 ppm and 2000 in the diet. In a study involving mice, Polyaminopropyl Biguanide (concentrations up to 1000 ppm) in diet for 97 weeks did not cause any macroscopic changes in the spleen or liver. A NOAEC of 1500 ppm for Polyaminopropyl Biguanide was reported in a 1-year feeding study involving dogs; treatment-related histopathological findings in the liver and kidneys were reported at dietary concentrations of 3000 ppm/4500 ppm. In this study, groups of animals were fed test-substance concentrations of 300 ppm, 1500 ppm, and 4500 ppm for up to weeks 11/12. The 4500 ppm concentration was reduced to 3000 ppm for the remainder of the study because high dose males exhibited unexpected signs of toxicity, including marked reddening/peeling of scrotal skin, loss of appetite, body weight loss, and/or indications of liver impairment in the form of elevated plasma alanine transaminase and/or aspartate transaminase activities. In a 26-week feeding study involving dogs, dietary concentrations of 1500 ppm and 4500 ppm Polyaminopropyl Biguanide produced concentration-related hepatotoxicity and nephrosis.^{12,19,22}

Risk Assessment

In a chronic oral toxicity study that is summarized in Table 11, Polyaminopropyl Biguanide (20.2% aqueous) was administered in the diet daily for 104 weeks at concentrations of 0, 200, 600, and 2000 ppm (corresponding to 0, ~12.1, ~36.3, and ~126.1 mg/kg/day in male rats and 0, ~14.9, ~45.3, and ~162.3 mg/kg/day in female rats). The NOAELs for male and female rats in this study were 36 mg/kg/day and 45 mg/kg/day, respectively. The following assumptions were used to calculate a margin of safety (MOS): all cosmetics contain 0.3% Polyaminopropyl Biguanide, the NOAEL is 36 mg/kg/day, and dermal penetration is 7.65%. The estimated systemic exposure dose (SED) was 0.0666 mg/kg/day and the MOS was calculated to be 46 for Polyaminopropyl Biguanide (based on cosmetic exposure estimate).¹² In this calculation, the value for dermal penetration was determined based on dermal penetration data on one type of cosmetic formulation (oil/water emulsion; specific cosmetic product categories not mentioned). However, it was noted that the dermal penetration data are being used to support the safety of Polyaminopropyl Biguanide in all types of cosmetic products. Also, in this calculation, 17.4 g/day was considered the amount of cosmetic product that was applied daily; the assumed exposure duration was not stated. In more recent MOS calculations (assuming that all cosmetics contain 0.1% Polyaminopropyl Biguanide), an SED of 0.012 mg/kg/day was based on the assumption that the residual stratum corneum + epidermis fractions do not contribute to the SED. The new MOS values (assuming dermal absorption = 4.09%) are 258 (based on cosmetic exposure estimate) and 227 (based on cosmetic exposure estimate + noncosmetic exposure estimate). Thus, the MOS is lower when additional exposure from non-cosmetic use is incorporated. The SCCS was responsible for the margin of safety calculations.²

EPA assessed the human health risks associated with residential-handler and post-application pesticide exposure scenarios (including pesticides containing Polyaminopropyl Biguanide) using surrogate exposure data, maximum application rates (specified on the product labels), and standard assumptions.³ The agency determined that all margins of exposure (MOEs) from dermal and inhalation exposure for residential handlers are above the target 100 target and, therefore, were not concerning. For post-application dermal and incidental ingestion (oral exposures) scenarios, MOEs calculated based on an oral NOAEL of 20 mg/kg/day were also above the Agency's level of concern. Residential handler exposures may occur when individuals mix, load, or apply a pesticide. Individuals could incur post-application exposure either as bystanders affected by exposures during the application of the pesticide or when they enter a treated site after the application.

Chronic dietary risk estimates were provided for the general U.S. population and all population subgroups.³ These estimates were below EPA's level of concern for the general U.S. population (i.e., <10% of the chronic Population Adjusted Dose [cPAD]) and all population subgroups (i.e., <37% of the cPAD for children). The cPAD is the level of exposure (mg/kg/day) that the EPA determines should not be exceeded.³

The aggregate risk assessment integrates the assessments that were conducted for dietary and residential exposure. Aggregate calculations were performed for adults and children using the Aggregate Risk Index (ARI) method. ARIs were greater than 1.2 for children and greater than 5.4 for adults, and these risks were determined to be above the EPA's level of concern (ARI of 1).³

Inhalation

Risk Assessment

The ConsExpo Web Spray Model (Consumer Exposure Model, Web version 1.0.1)^{23,24,25,26,27} was used to estimate the inhalation exposure concentrations of Polyaminopropyl Biguanide during the use of cosmetic spray products containing the maximum concentrations of use reported in the PCPC Industry survey (submitted to CIR on April 11, 2017) in propellant hair sprays (0.0004%) and pump hair sprays (0.53%).²⁸ It should be noted that more recent PCPC Industry survey data (submitted to CIR on July 18, 2017) indicate that Polyaminopropyl Biguanide is no longer being used in hair sprays.⁷ Conservative default values published by RIVM were used in all of the calculations (Table 12).²⁵ One exception is that the room ventilation rate was assumed to be 0.2 room-air exchanges per hour, which is the default value specified in REACH guidance, rather than 2 exchanges per hour indicated by RIVM guidance for bathrooms.²⁷ The more conservative value (0.2/h) appears to be more appropriate to represent low-end air-exchange rates in homes in the US, in which ventilation fans may not be used routinely. No default values were available specifically for pump hair spray products. Thus, the spray duration assumed for propellant hair sprays (14.4 sec) and default values for pump toilet-water sprays were used in the calculations for pump hair sprays.

The use of conservative default values for multiple exposure parameters ensures that high-end, "reasonable worst-case" exposures are calculated.^{25,27} Generally, the exposure concentrations predicted by the ConsExpo Model increase with

increasing spray durations and decrease with increasing exposure durations/event (i.e., the time over which the exposure concentrations are averaged after each spraying event).

The average PHMB inhalation exposure concentrations over the 5-min default exposure duration/event were 0.00012 mg/m³ for propellant hair sprays and 0.0022 mg/m³ for pump hair sprays (Table 12).

The no observed adverse effect concentration (NOAEC) was approximately 0.024 mg/m³ in a 28-day inhalation study in which rats were exposed, nose only, to PHMB in an aerosolized water solution, 6 h/day, 5 days/week.² Margins of safety (MOSs) were calculated by dividing the NOAEC by the average inhalation exposure concentrations/event estimated using the ConsExpo model. The MOSs were 200 for propellant hair sprays and 11 for pump hair sprays (Table 12).

An MOS of 100 may be considered to be adequate to allow for the uncertainties associated with using the NOAEC from a short-term rat study to evaluate potential chronic human exposures (i.e., 10 for short-term to long-term exposure extrapolation x 10 for inter-species extrapolation = 100). Accordingly, the ConsExpo Web model was used to calculate concentrations of use that would yield an MOS of 100 for PHMB in pump and propellant hair spray products and propellant deodorant products. The results indicate that use concentrations of 0.0058% in pump hair sprays, 0.00084% in propellant hair sprays, and 0.000055% in propellant deodorant sprays would each be associated with an MOS of 100 (Table 12).

The daily exposure duration in the rat study (6 h) from which the NOAEC was derived (i.e., 6 h/day or 360 min/day) is 72 times greater than the exposure duration of a person using a hair spray once a day (1 event/day x 5 min/event = 5 min/day) 5 days per week and 24 times greater than the exposure duration of a person using a hair spray 3 times a day 5 days/week.

The daily exposure duration in the rat study is about 7 times greater than the exposure duration would be for a beautician applying hair spray to customers an average of 10 times a day 5 days/week. The beautician's occupational exposure may be reduced by workplace ventilation systems and larger room volumes, as well as the direction of the spraying (i.e., away from the beautician).

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

The developmental and reproductive toxicity studies summarized below are presented in Table 13.

NOAECs of 1,000 ppm and 1300 ppm have been reported in oral reproductive and developmental toxicity studies on Polyaminopropyl Biguanide (in the diet) involving rats. In an inhalation study, degeneration of seminiferous tubules in the testis of 1 male rat was observed after exposure to 0.25 mg/m³ (6 h/day, 5 days/week for 3 weeks), but this was not observed in any other group, including the group exposed to the highest concentration (26 mg/m³). No-observed-adverse-effect-levels (NOAELs) of 10 mg/kg/day and 40 mg/kg/day for developmental toxicity were reported in studies involving mice, and the 40 mg/kg/day dose was also classified as non-teratogenic in mice in another study. A NOAEL of 40 mg/kg/day for developmental toxicity has also been reported in a study involving rabbits. Polyaminopropyl Biguanide has been classified as embryotoxic at oral dosage rates of 32 mg/kg/day (animal strain not stated) and 100 mg/kg/day (rats), and as teratogenic in rats at an intraperitoneal dosage rate of 10 mg/kg/day.^{12,2,19,22}

GENOTOXICITY STUDIES

The genotoxicity studies (in vitro and in vivo) summarized below are presented in Table 14.

In the Ames test, Polyaminopropyl Biguanide was non-genotoxic at doses up to 5000 µg/plate with and without metabolic activation. At the highest dose evaluated (333,300 µg/plate) in the Ames test, Polyaminopropyl Biguanide was weakly genotoxic in *Salmonella typhimurium* strain 1538 without metabolic activation. Polyaminopropyl Biguanide was non-genotoxic in the mouse lymphoma assay at concentrations up to 2000 µg/ml with and without metabolic activation, or in the in vitro micronucleus test at concentrations up to 50 µg/ml (without metabolic activation) and up to 250 µg/ml (with metabolic activation). In the in vivo micronucleus test, Polyaminopropyl Biguanide was non-clastogenic in polychromatic erythrocytes from mice that received single oral dosages up to 400 mg/kg. In the in vivo unscheduled DNA synthesis assay, there was no induction of unscheduled DNA synthesis in hepatocytes from rats that received single oral doses up to 1500 mg/kg.¹²

CARCINOGENICITY STUDIES

The carcinogenicity studies (in vitro, dermal, and oral) summarized below are presented in Table 15.

In Vitro

Polyaminopropyl Biguanide was evaluated at concentrations up to 3000 µg/ml in the cell transformation assay (using baby hamster kidney fibroblasts); there was no difference in the number of transformed cell colonies between test and negative control cultures. In another assay involving RAW 264.7 mouse macrophages (a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice), Polyaminopropyl Biguanide tested at concentrations up to 1 ppm had no direct effect on liver cell proliferation and did not potentiate cell proliferation induced by activated macrophages.^{2,12}

Dermal

Polyaminopropyl Biguanide was classified as a hepatic tumorigen in mice at the highest dose tested in a study in which 30 mg of 10% Polyaminopropyl Biguanide in ethanol was applied to the skin daily (5 days/week) for 80 weeks. The doses administered in this study were 0, 0.6, 6, and 30 mg/mouse/day in ethanol (0, 25, 150, or 750 mg/kg/day). The NOAEL was 0.6 mg/mouse/day (15 mg/kg/day). An increase in the incidence of liver tumors was observed at the 30 mg/day dose; the increase was statistically significant only for liver tumors of endothelial origin. High mortality (76% to 78% of the animals) was noted in this group. The highest dose was clearly above the maximum tolerated dose (MTD) because of excessive mortality and reduced body weight gain in both sexes. Incidences of mortality in the other dose groups were not reported. A variety of inflammatory hepatic changes was observed in all groups, including the controls. However, at 30 mg/mouse/day, severe hepatitis was observed in some of the animals. These hepatic changes appeared to have been mainly responsible for causing increased numbers of deaths in the high dose group.^{12,19} The source of these results is the chronic oral toxicity study that is summarized above. A scientific advisory panel advising the SCCS indicated that the hepatitis observed in this study may be attributable to the *Helicobacter hepaticus* infections, which may also be responsible for the increased incidence of hepatocellular neoplasms in these animals.

Oral

A statistically significant increase in the incidence of hemangiosarcomas and hemangiomas was reported in male mice (C57B1/10J/CD-1 strain) that received Polyaminopropyl Biguanide at a dietary concentration of 4000 ppm daily for 2 years. In a 97-week study in which mice were fed Polyaminopropyl Biguanide at dietary concentrations up to 1000 ppm prior to and during mating, and their offspring were fed the same concentrations, there were no treatment-related (non-neoplastic or neoplastic) increases in histopathologic findings. Hemangiosarcomas or hemangiomas in the liver or other sites and a high mortality incidence (80%) were reported by week 97. A concentration-related increase (100 to 1000 ppm) in tumor-bearing mice was reported in a similar 97-week dietary study. In a 14-day feeding study, increased cell proliferation was noted in mice fed 1200 ppm Polyaminopropyl Biguanide in the diet. Polyaminopropyl Biguanide was classified as non-carcinogenic in rats fed dietary concentrations up to 2000 ppm for 122 weeks. At 124 weeks, 80% mortality was reported. A low incidence of hemangiomas and hemangiosarcomas was reported in a study in which rats were fed Polyaminopropyl Biguanide at a dietary concentration of 2000 ppm for 2 years.^{12,2,19,29}

OTHER RELEVANT STUDIES

Effect on Lung Cells

A study was performed to characterize the inflammatory responses, include the mechanism of action, induced in lung cells exposed to Polyaminopropyl Biguanide.³⁰ A549 cells that were exposed to Polyaminopropyl Biguanide showed concentration-dependent (0 to 80 µg/mL) decreased viability, significant reactive oxygen species (ROS) generation (at 20 µg/mL), inflammatory cytokine secretion (statistically significant increase in TNF-α release (at 20 µg/mL), and nuclear factor kappa B (NF-κB) activation (expression of IκB-α protein significantly degraded at concentrations >10 µg/mL). Statistically significant cytotoxicity to A549 cells was observed at concentrations >10 µg/mL. Polyaminopropyl Biguanide triggered inflammatory cytokine secretion and NF-κB activation by modulating the degradation of IκB-α and through the accumulation of nuclear p65. It was noted that TNF-α plays important roles in interleukin 8 (IL-8) expression as well as in NF-κB activation. IL-8 production induced by Polyaminopropyl Biguanide was completely suppressed by an NF-κB inhibitor, but not by an ROS scavenger. The authors suggested that Polyaminopropyl Biguanide induces inflammatory responses via the NF-κB signaling pathway.

Other Cellular Effects and Antimicrobial Activity

Polyaminopropyl Biguanide (polyhexamethylene biguanide; C6) was compared to the (structurally) closely related polyaminopropyl biguanide (C3) with respect to antiseptic efficacy and cytotoxicity in vitro.³¹ Antimicrobial efficacy tests were performed via determination of the minimum bactericidal concentration (MBC). Polyaminopropyl Biguanide (polyhexamethylene biguanide; C6) exhibited high antimicrobial activity against *Staphylococcus aureus* and *Echerichia coli*, whereas polyaminopropyl biguanide (C3) proved to be ineffective in bacterial eradication. These results suggest that even small differences in the chemical structure of related agents, such as Polyaminopropyl Biguanide (polyhexamethylene biguanide; C6) and polyaminopropyl biguanide (C3), can substantially affect their efficacy.

Cytotoxicity was evaluated in human keratinocytes (HaCaTs) and murine fibroblasts (L929). In fibroblast or keratinocyte cultures, concentrations for both test substances ranged from 0.005% to 1% v/v and, for polyaminopropyl biguanide (C3) only, also at concentrations ranging from 0.25% to 3% v/v. Cultures were incubated for up to 72 h. For all tested concentrations, Polyaminopropyl Biguanide (polyhexamethylene biguanide; C6) was highly cytotoxic to human HaCaT and L929 murine fibroblast cell after 24 and 72 h of incubation, never exceeding a survival rate of 27 %. Polyaminopropyl biguanide (C3) displayed significantly lower cytotoxicity at concentrations ranging from 0.005% to 0.1% v/v. At concentrations up to 0.1 %, no cytotoxic effect could be detected in L929 cells after 24 h, whereas, for HaCaT cells, moderate and high cytotoxicity was evident at 0.05% and 0.1% polyaminopropyl biguanide (C3). After 72 h, only a weak cytotoxic effect on L929 cell at 0.05% and 0.1% polyaminopropyl biguanide (C3) could be observed, while, for HaCaT cells, concentrations up to 0.1% were classified as non-cytotoxic. However, concentrations $\geq 0.25\%$ polyaminopropyl biguanide (C3) were highly cytotoxic to cells of both cell lines after 24 h of incubation. When compared directly, polyaminopropyl biguanide (C3) consistently resulted in a significantly higher cell survival rate than Polyaminopropyl Biguanide (polyhexamethylene biguanide; C6), irrespective of concentration and incubation time ($P \leq 0.0006$).³¹

It has been hypothesized that exposures to Polyaminopropyl Biguanide may have epigenetic effects, including non-genotoxic DNA base modifications (e.g., changes in DNA-base methylation) and altered mitogenic cytokine production.³² These effects have been assessed in vitro using 3 cell types: Caco-2 cells (from a human colon adenocarcinoma) with non-functional p53 genes ($\Delta p53$: mut p53), N2-A (Neuro-2A cells, mouse neural cells), because the brain is a possible target organ in rodents, and HepG2 cells (human hepatocellular carcinoma) with functional p53 genes. At Polyaminopropyl Biguanide concentrations of 1 $\mu\text{g/mL}$ to 20 $\mu\text{g/mL}$, neither a growth stimulatory effect nor a growth inhibitory effect was observed. Viability testing using neutral red resulted in an IC_{50} of 20–25 $\mu\text{g/mL}$ after treatment with Polyaminopropyl Biguanide for 3 h, whereas the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability test led to IC_{50} of 80 $\mu\text{g/mL}$, 160 $\mu\text{g/mL}$ and 160 $\mu\text{g/mL}$ for HepG2 cells, Neuro-2A cells and Caco-2 cells, respectively. Polyaminopropyl Biguanide does not induce significant oxidative stress (as determined by measuring production of malondialdehyde (MDA) or lipoperoxidation, nor does it induce hydroxylation of DNA (8-hydroxy-2'-deoxyguanosine [8-OH-dG]) and/or its hypermethylation (5-methylcytosine [m5dC] content), the latter being strongly implicated in DNA replication and regulation and cell division.

Additional results from this study indicated that Polyaminopropyl Biguanide did not induce significant production of mitogenic cytokines, such as TNF- α (tumor necrosis factor-alpha), interleukins (IL-1 alpha), and NF- κB , which can cause either apoptosis or stimulate the growth of transformed cells or tumors. Instead, concentrations of 20 to 100 $\mu\text{g/mL}$ Polyaminopropyl Biguanide killed cells of all types in less than 3 h. The expression of genes involved in the mechanisms of cell death induced by Polyaminopropyl Biguanide, including p53, the pro apoptotic gene bax and others, and the anti-apoptotic bcl-2 and caspase-3 genes, has been evaluated using reverse transcription polymerase chain reaction (RT-PCR) methodology. Finally, there was no apparent inhibition of GAP-junctions (i.e., gap junctional intercellular communication (GJIC)) in the presence of Polyaminopropyl Biguanide. Taken together, the data indicate that Polyaminopropyl Biguanide did not exhibit clear or remarkable epigenetic effects, except for a slight increase in the levels of some cytokines and a transcription factor at concentrations that cause rapid cell lysis.³²

DERMAL IRRITATION AND SENSITIZATION STUDIES

The skin irritation, sensitization, and phototoxicity/photosensitization studies summarized below are presented in Table 16.

Irritation

Polyaminopropyl Biguanide (single 4-h application) was classified as a mild skin irritant in rabbits. Single applications (24 h) of 20% aqueous Polyaminopropyl Biguanide to rabbits indicates that this compound is non-corrosive,

moderately irritating to intact skin, and severely irritating to abraded skin. Repeated applications of Polyaminopropyl Biguanide (12,000 ppm; 1.2%) to the skin of rabbits for 21 days were not irritating. Severe skin irritation was observed in all rats that received a single 24-h application of 25% aqueous Polyaminopropyl Biguanide, at dosages of 2.5 ml/kg and 5 ml/kg. Polyaminopropyl Biguanide (0.04%) was classified as a non-irritant when applied to the skin of rats for 24 h. Repeated applications of 20.2% aqueous Polyaminopropyl Biguanide to rats for 21 days resulted in slight skin irritation (at 60 mg/kg/day) and moderate irritation (at 200 mg/kg/day). Slight to moderate erythema was observed in guinea pigs that received repeated applications of 25% aqueous Polyaminopropyl Biguanide for 3 days. In a study involving mice, the highest dose of Polyaminopropyl Biguanide (10% concentration in ethanol, 30 mg dose) caused hyperkeratosis and, occasionally, ulceration extending into the dermis when applied repeatedly for 80 weeks. Polyaminopropyl Biguanide (up to 1.5% active) was not classified as a primary skin irritant when applied for 24 h to the skin of human subjects.^{2,12,19,33}

Sensitization

Results were positive for Polyaminopropyl Biguanide in the local lymph node assay (LLNA). In maximization tests on Polyaminopropyl Biguanide, moderate skin sensitization was observed in guinea pigs induced with 0.06% active ingredient (intradermal injection) and 20.2% active ingredient (occlusive application) and challenged with Polyaminopropyl Biguanide (20.2 % active ingredient) and a 30% solution of the ingredient (6% active ingredient) in deionized water, and moderate to strong sensitization was observed in guinea pigs induced with 0.2% active ingredient (intradermal injection) and 20.2% active ingredient (topical application) and challenged with Polyaminopropyl Biguanide (20.2% active ingredient). In another guinea pig maximization test, sensitization was not observed in guinea pigs induced with 0.15% Polyaminopropyl Biguanide (intradermal injection) and 20% (topical application) and challenged with Polyaminopropyl Biguanide (10% or 20%). In one Buehler test on Polyaminopropyl Biguanide, guinea pigs were induced with 2% active ingredient (topical application), challenged with 2% active ingredient, and rechallenged with 0.2%, 2%, and 4% active ingredient. The initial challenge with 2% active ingredient and rechallenge with 2% and 4% active ingredient resulted in faint erythema; rechallenge with 0.2% active ingredient produced negative results. Polyaminopropyl Biguanide (2% active ingredient) was classified as a moderate sensitizer. In another Buehler test, it was determined that the threshold for eliciting sensitization in guinea pigs was ~ 1%. Induction concentrations ranged from 0.3% to 5% and challenge concentrations ranged from 0.075% to 15%. Results from a study evaluating the possible cross-reactivity of Polyaminopropyl Biguanide (challenge with 20%) with chlorhexidine (challenge with up to 4% chlorhexidine gluconate) in guinea pigs were negative. In a human repeated insult patch test (HRIPT, 191 subjects), it was determined that Polyaminopropyl Biguanide (2% active ingredient) was not capable of causing primary skin irritation, but was capable of causing sensitization. When a leave-on product containing 0.1 % Polyaminopropyl Biguanide (0.5% of a trade name material containing 20% Polyaminopropyl Biguanide) was evaluated in an HRIPT involving 207 subjects, it was concluded that the product did not induce dermal sensitization. In another HRIPT on a neck cream containing 0.2% Polyaminopropyl Biguanide, the product did not cause clinically meaningful irritation or sensitization. Sensitization reactions were observed in the following patient populations tested: In a population of 1975 patients, sensitization was observed in 10 patients patch tested with 0.5% aqueous Polyaminopropyl Biguanide and in 16 Patients patch tested with 1% aqueous Polyaminopropyl Biguanide. Sensitization was also observed in 2 of 374 patients patch tested with 2.5% aqueous Polyaminopropyl Biguanide and in 6 of 1554 patients patch tested with 2.5% aqueous Polyaminopropyl Biguanide.^{12,34,35,36,37,38,39, 40, 41}

Risk Assessment

According to one source, data from predictive testing showed that Polyaminopropyl Biguanide is a weak sensitizer, and the results from an initial risk assessment indicated that the use of this antimicrobial at lower concentrations (<0.2%) could be extended to include underarm deodorants.⁴⁰ Neither details relating to this risk assessment nor the reference is identified in the secondary source of this information. Additional information from this source is stated as follows: To consolidate the specific risk assessment supporting the use of Polyaminopropyl Biguanide in underarm deodorants, a strategy was also deployed to monitor the ongoing frequency of Polyaminopropyl Biguanide sensitization and to determine whether the use of Polyaminopropyl Biguanide in these products could be identified as a likely causal exposure in any sensitized individuals. Two studies (both summarized in Table 15) provided a baseline frequency of Polyaminopropyl Biguanide sensitization; 2 of 374 patients in the United Kingdom study and 6 of 1554 patients in the German study had positive patch test reactions to 2.5% aqueous Polyaminopropyl Biguanide. It was noted that this initial series of data suggested that the baseline frequency of Polyaminopropyl Biguanide sensitization was very low (0.5% and 0.4% in United Kingdom and German studies, respectively). The majority of positive reactions were considered weak. It was noted that these data suggested that Polyaminopropyl Biguanide may not be a relevant contact allergen.

In a subsequent German multicenter study (summarized in Table 16) involving 1974 patients, 9 (0.5%) had positive reactions to 2.5% aqueous Polyaminopropyl Biguanide. The majority of the positive reactions were considered weak. When results of the 3 studies were considered together, it was noted that the frequency of sensitization reactions to

Polyaminopropyl Biguanide remained low and stable, in spite of the use of Polyaminopropyl Biguanide in underarm deodorants.

Photosensitization/Phototoxicity

Animal

Very strong irritation potential, but no significant photoirritancy, was reported in a study in which male rats were tested with Polyaminopropyl Biguanide at concentrations of 2% and 5%.

Human

When tested at a concentration of 1% (dose = 1 mg/cm²) in 26 subjects, Polyaminopropyl Biguanide was essentially non-irritating and did not induce sensitization, phototoxicity, or photoallergenicity.² The dose (1 mg/cm²) used in this study was specified by the Cosmetics Europe Consortium in response to a CIR request for additional information.⁴²

OCULAR IRRITATION STUDIES

The ocular irritation studies summarized below are presented in Table 17.

Undiluted Polyaminopropyl Biguanide was a severe ocular irritant/corrosive agent when instilled into the rabbit eye. The instillation of 25% aqueous Polyaminopropyl Biguanide into the eyes of rabbits resulted in severe inflammation and corneal damage in unrinsed eyes and slight inflammation in rinsed eyes. Moderate and mild ocular irritation were observed in unrinsed and rinsed rabbit eyes, respectively, after 20% aqueous Polyaminopropyl Biguanide was instilled. In another study involving rabbits, the instillation of Polyaminopropyl Biguanide (20% aqueous) into the eyes induced slight inflammation, but no corneal ulceration. Ocular irritation was not observed when Polyaminopropyl Biguanide (0.04% active ingredient) was instilled into the eyes of rabbits. In a study in which 20% aqueous Polyaminopropyl Biguanide (100 µl) was instilled into human eyes (from cadavers) and the eyes of rabbits in a temperature-controlled chamber (32-36°C), normal corneal morphology was observed at histological examination.^{12,19,43}

CLINICAL STUDIES

The patient multicenter studies summarized below are presented in the Human Sensitization Studies section of Table 16.

Retrospective and Multicenter Studies

The results of patient multicenter studies (study populations ranging from 374 to 1975) have indicated a low incidence of skin sensitization reactions to Polyaminopropyl Biguanide.^{34,35,36,40}

Case Reports

An itchy rash on the hand was observed over a 2-year period in a non-atopic patient with a history of retinal detachment surgery.⁴⁴ The patient had regularly used a rinse-off contact lens cleaning solution containing 0.001% Polyaminopropyl Biguanide twice daily. A patch test chamber containing the undiluted contact lens cleaning solution was applied to the skin for 2 days, and doubtful results were reported on day 4. A patch test chamber containing a 10% dilution of the product (0.0001% Polyaminopropyl Biguanide tested) was subsequently applied to the skin, and positive results (+ reaction) were observed on day 7. Additionally, semi-open tests of the undiluted product yielded a weak positive reaction on day 7. In other tests, the individual ingredients (obtained from the manufacturer) of the contact lens cleaning solution were diluted to different concentrations in water. There were no reactions to 2% aqueous Polyaminopropyl Biguanide, but a weak, late reaction (1+ reaction) to 5% aqueous Polyaminopropyl Biguanide was observed on day 7. However, stronger and earlier reactions were observed after the application of 10% aqueous Polyaminopropyl Biguanide (+? reaction on day 2; 2+ reaction on days 5 and 7) and 20% aqueous Polyaminopropyl Biguanide (2+ reaction on day 2; 3+ reaction on days 5 and 7). Patch test results for 20% aqueous Polyaminopropyl Biguanide in 10 control subjects were negative.

In a case report on a non-atopic patient with a history of bilateral leg ulcers and multiple contact allergies, mild hand dermatitis was observed after repeated use of a wound irrigation solution that contained Polyaminopropyl Biguanide and a wound gel containing the same disinfectant.⁴⁵ The composition of the disinfectant (liquid and gel) was as follows: 0.1% Polyaminopropyl Biguanide, 0.1% undecylenamiopropyl betaine, and water; the gel also contained glycerol and hydroxyethyl cellulose. In a repeated open application test, a positive reaction was observed after the gel was applied twice daily (in elbow fold) for 10 days. The patient was also patch tested (patch test chamber) with 5% aqueous Polyaminopropyl Biguanide (a dilution of a 20% aqueous solution). The solution was applied to the upper arm for 2 days; reactions, scored according to International Contact Dermatitis Research Group (ICDRG) guidelines were negative on day 2, but were positive on day 4. The patch test (same procedure) was repeated at concentrations of 2.5% and 5% aqueous Polyaminopropyl Biguanide. Positive reactions to the 5% concentration were observed on day 2 (+) and day 4 (++), with partially pustular morphology). Results for the gel and liquid were negative in patch tests.

A chronic, recurrent and itchy dermatitis was observed in a male patient who used wet wipes.⁴⁶ Polyaminopropyl Biguanide, an ingredient of the product, was tested at different concentrations (20%, 2% and 0.2% aqueous). Scoring was performed in accordance with International Contact Dermatitis Research Group guidelines. On day 2 and day 4, respectively, + and ++ reactions to 20% Polyaminopropyl Biguanide (with a papulovesicular reaction, extending outside of the test chamber) were observed; +? and + reactions to 2% Polyaminopropyl Biguanide were observed on days 2 and 4, respectively. No reactions to 0.2% Polyaminopropyl Biguanide were observed.

No adverse effects were noted following the exposure of 29 patients to a pre-operative antiseptic for cataract surgery that contained 0.2 % Polyaminopropyl Biguanide.⁴⁷

Two cases of severe anaphylaxis were reported following contact of a surgical wound with a hospital disinfectant containing 0.2 % Polyaminopropyl Biguanide. Immediate-type hypersensitivity to Polyaminopropyl Biguanide was suggested by positive skin prick tests in both patients and by negative skin tests in control individuals. Skin tests involving chlorhexidine were negative.⁴⁸

Contact Urticaria

A female patient experienced grade III anaphylaxis (IgE-mediated mechanism confirmed) with palmar pruritus, flush, swelling of lips, swallowing difficulties, hypotension and loss of consciousness while using a new brand of wet toilet paper containing Polyaminopropyl Biguanide as a disinfectant.^{18,49} The detailed allergy history of the patient indicated 3 prior anaphylactic episodes (grade II) during wound care of a leg ulcer. One of the episodes occurred after the use of a wound dressing that contained Polyaminopropyl Biguanide. The other 2 episodes occurred after wound cleansing with 2 different Polyaminopropyl Biguanide disinfectants, one of which contained Polyaminopropyl Biguanide, polyethylene glycol (PEG) 4000, and no other additives. The composition of the other disinfectant that contained Polyaminopropyl Biguanide was not detailed. However, according to another publication, the composition of that disinfectant (liquid and gel) is as follows: 0.1% Polyaminopropyl Biguanide, 0.1% undecylenamiopropyl betaine, and water; the gel also contains glycerol and hydroxyethyl cellulose.⁴⁵ The patient had no known allergies or atopic diseases. Skin prick tests were positive for the disinfectant of known composition, which was tested in a 1:10 dilution, corresponding to 20 µg/ml Polyaminopropyl Biguanide. Positive skin prick test results were also reported for chlorhexidine in different commercial preparations. Skin prick test results for PEG 4000 were negative, and the same was true for the 5 healthy volunteers who were prick tested with the disinfectant of known composition. Whether or not the other disinfectant containing Polyaminopropyl Biguanide was evaluated in prick tests was not mentioned. Other results reported in this case report indicated that there was limited in vitro cross-reactivity between Polyaminopropyl Biguanide and chlorhexidine. The author noted that patients with known chlorhexidine allergy could be at risk for anaphylactic reactions to Polyaminopropyl Biguanide.

A male patient (atopic and diabetic) had a history of angioedema and pruritus after using wet wipes.¹⁵ Patch test results for an ingredient of the wipes, Polyaminopropyl Biguanide (tested at 1:10 in water), and the wipe itself were negative. However, prick tests resulted in strong positive reactions to the wipe and this ingredient after 15 minutes, and the reactions continued to increase in intensity during the following 2 h.

The prick test (protocol and test concentration not specified) was used to diagnose immediate contact urticarial reactions in 44 patients with eczematous dermatitis. A positive reaction to Polyaminopropyl Biguanide was observed in 1 patient.⁵⁰

Other Clinical Reports

Based on medical surveillance information obtained between 2004 and 2007 on employees who came in contact with Polyaminopropyl Biguanide in the workplace, no cases of skin sensitization to this chemical were reported.¹² All

manufacturing and laboratory employees were offered complete medical evaluations on a regular basis depending on their age. These were conducted every one to two years.

In a clinical trial (106 dialysis patients) in which patients were treated for infections, Polyaminopropyl Biguanide was well-tolerated and there were only two cases of transient local skin erythema.⁵¹ Four of 28 patients were excluded from a cohort study because of adverse effects related to a Polyaminopropyl Biguanide dressing.⁵²

Reportedly, the application of very high doses of Polyaminopropyl Biguanide can trigger fever and a generalized exanthema.²²

Polyhexamethylene Guanidine Phosphate (PHMG)

Beginning in 2006, epidemics of a fatal lung injury were observed in Korea every spring.⁵³ It was subsequently demonstrated that this type of children's interstitial lung disease (chILD), characterized by rapid progression and high mortality, was associated with humidifier disinfectant use. These disinfectants contain oligo (2- [2-ethoxy] ethoxyethyl) guanidium chloride, polyhexamethyleneguanidine (PHMG), 5-chloro-2-methylisothiazol-3 (2H)-one/2-methylisothiazol-3-one, and didecyldimethylammonium chloride. PHMG (not the ingredient that is under review in this safety assessment) is chemically similar to Polyaminopropyl Biguanide. The 2 chemical structures are presented below. PHMG contains guanidine as part of its chemical structure, whereas Polyaminopropyl Biguanide contains biguanide. The 2 chemical structures are different enough not to be the same chemical.

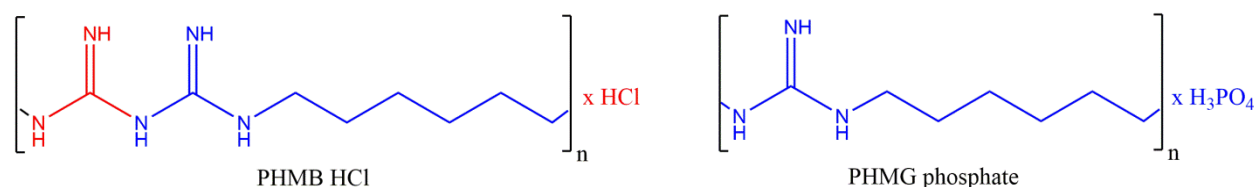


Figure 2. PHMB HCl vs PHMG phosphate.

The clinical characteristics of suspected cases between 2006 and 2011 were determined by a nationwide retrospective epidemiological study. The potential causal relationship with humidifier disinfectants was examined by a prospective surveillance study after humidifier disinfectant sales were suspended. One-hundred thirty-eight children (average age = 30.4 months) were diagnosed with chILD. The annual incidence increased in 2011 and then decreased to zero in 2012. At the time of hospital admission, the most frequent symptoms were cough and dyspnea. Disease progression resulted in spontaneous air leak and 80 children (58%) died. No new cases were found 2 years after the sale of humidifier disinfectants was suspended. The authors noted that the results of this study suggest that humidifier disinfectant inhalation causes an idiopathic type of chILD that is characterized by spontaneous air leak, rapid progression, lack of response to treatment, and high mortality.

A case-control study, with community-dwelling controls, was performed to validate the preceding study's findings and to confirm the exposure-response relationship between humidifier disinfectant and lung injury.⁵⁴ This study was based on re-examination of lung CAT scans and medical records at a hospital in Korea where many of the cases appeared. The purpose of the re-examination was to identify all cases of lung injury that fit certain criteria (i.e., criteria for the type of lung injury that was associated with the use of humidifier disinfectants in the previous studies). Each case of lung injury was matched with 4 community-dwelling controls, according to age (± 3 years), sex, residence, and history of childbirth since 2006 (for women). Using a questionnaire, environmental risk factors, which included the humidifier (type and use) and the humidifier disinfectant, were investigated in August of 2011. Exposure to the humidifier disinfectant was calculated for both cases and controls, and the corresponding risks of lung injury were compared. Sixteen patients who were among the 28 eligible cases agreed to participate. Sixty matched controls (selected from the community that the hospital serves) were considered eligible for participation in the study.

Study results indicated a statistically significant, exposure-response relationship between humidifier disinfectant exposure and lung injury. The cases were significantly more likely to have been exposed to humidifier disinfectants, compared to controls (odds ratio (OR): 116.1; 95% confidence interval (CI): 6.5 to 2,063.7). The OR for an association between use of a humidifier disinfectant in which the active ingredient was specifically PHMG and lung injury was even greater (OR: 203.8; 95% CI: 11.1 to 3,724.1), suggesting that the lung injuries observed in people who used humidifier

disinfectants were attributable to the use of humidifier disinfectants containing PHMG. All cases used several liquid humidifier disinfectant formulations that contained the same proportion of PHMG phosphate. The concentration of PHMG phosphate in the humidifier mist was not stated. Further examination of associations between exposure (number of bottles of disinfectant used per month x duration of exposure as number of months used x volume per bottle of disinfectant/days/month) and lung injury indicated a clear relationship between the magnitude of daily exposure to disinfectants containing PHMG and the magnitude of the ORs. There was no association between lung injury and use of humidifier disinfectants in which the active ingredient was a combination of isothiazolinone derivatives (5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one [CMIT/MIT]) or a guanidinium derivative (oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride [PHG])).⁵⁴

An analysis of patients and fatalities attributed to inhalation exposure to PHMG indicates that this chemical mainly causes lung diseases, such as pulmonary fibrosis.⁵⁵ Of the known main components of the humidifier disinfectants, PHMG has been identified as the chemical substance that caused the most deaths. In surveys conducted to identify victims of the humidifier disinfectant, 22% of the research participants answered that they had used the humidifier disinfectant, and 21% complained of side effects.

For the refined risk assessment presented in the publication by Lee et al. (2013), the time-weighted average (TWA) PHMG concentration in the bedroom air was 0.06 mg/m³ for this scenario, averaged over 8 hours.⁵⁶ This concentration in air 27 times greater than the 0.0022 mg/m³ inhalation exposure concentration of Polyaminopropyl Biguanide estimated for the use of a pump hair spray containing the highest maximum reported concentration of use (0.053%) Polyaminopropyl Biguanide (See Table 12 in the safety assessment report). Further, the exposure duration of 8 h for PHMG in the humidifier use scenario is 96 times greater than the conservative 5-min exposure duration/event assumed for Polyaminopropyl Biguanide in the consumer spray scenarios evaluated in the safety assessment.

SUMMARY

The safety of Polyaminopropyl Biguanide as used as a preservative in cosmetics is reviewed in this assessment. Polyaminopropyl Biguanide is an INCI name; it refers to the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (polyhexamethylene biguanide hydrochloride (PHMB HCl)). Most of the safety test data included in this safety assessment are on polyhexamethylene biguanide hydrochloride.

Polyaminopropyl Biguanide, in its neat form, represents a solid/powder of > 94.2 % purity, and is usually marketed as an approximately 20% aqueous solution. One method for manufacturing Polyaminopropyl Biguanide is via the polycondensation of sodium dicyanamide and hexamethylenediamine.

The following chemicals have been reported as possible impurities of Polyaminopropyl Biguanide: *N*-(6-aminoethyl)-*N'*-(6-(6-guanidinoethyl)guanidine, *N*-cyano *N'*-(6-*N*-cyanoaminoethyl)guanidine, *N*-Cyano *N'*-(6-aminoethyl)guanidine), *N*-cyano-*N'*-(6-(6-guanidinoethyl)guanidine hydrochloride, and 1,6-diguanidinoethane dihydrochloride.

According to 2017 VCRP data, Polyaminopropyl Biguanide is being used in 147 cosmetic products, mostly leave-on product. The results of a concentration of use survey provided in 2017 indicate that Polyaminopropyl Biguanide is being used at concentrations up to 0.2 % in rinse-off products and concentrations up to 0.1% in leave-on products

In 2016, the SCCS issued a revised opinion (preliminary opinion) stating that the use of Polyaminopropyl Biguanide as a preservative in all cosmetic products at concentrations up to 0.1% is safe. The opinion also states that, because no new safety data on inhalation are available on Polyaminopropyl Biguanide, its use in sprayable formulations is not advised.

The safety of Polyaminopropyl Biguanide has been reviewed by the United States Environmental Protection Agency (EPA), and the Agency concluded that this pesticide has very low aggregate risk of adverse health effects to the public or environment.

The results of a dermal penetration study on Polyaminopropyl Biguanide indicated that absorption through the skin equaled 1.56% (dermis contained 1.56% of applied dose) + 0.03% (absorbed dose = 0.03% of applied dose). Based on SCCS Notes of Guidance, one standard deviation (2.5%) was added to the absorbed amount, yielding a calculated dermal absorption value of 4.09% (1.56% + 0.03% + 2.5% = 4.09%).

The principal route of excretion of radioactivity from orally administered Polyaminopropyl Biguanide (radiolabeled) was in the feces in rat studies. The following components have been detected in the urine of rats fed Polyaminopropyl

Biguanide in the diet: oligomers with 2 cyanoguanidino end groups, as well as the trace constituents, 3,3-dicyano-1,1-hexamethylenediguanidine and a compound that was considered to be 1-(6-aminoethyl)-3-cyanoguanidine.

There was no incidence of mortality or systemic toxicity in rats that received a single dermal dose of 5000 mg/kg aqueous Polyaminopropyl Biguanide; but, hemorrhage of dermal capillaries at the application site was observed. In an acute dermal toxicity study on 20% aqueous Polyaminopropyl Biguanide involving rabbits, an LD₅₀ > 400 mg/kg was reported.

The LD₅₀ was reported to be > 1000 mg/kg for rats dosed orally with aqueous solutions (up to 25% aqueous) of Polyaminopropyl Biguanide. A median lethal dose of 25.6 mg/kg was reported for rats dosed orally with a solution of 0.4% Polyaminopropyl Biguanide.

An LC₅₀ of > 0.36 mg/l was reported in acute inhalation toxicity studies in which rats were exposed to Polyaminopropyl Biguanide solutions (concentrations up to 0.5 mg/l). Dark/red lungs were observed at necropsy. A dose-related depression of respiratory rate was reported in a study in which mice were exposed to Polyaminopropyl Biguanide at concentrations up to 208 mg/m³.

In a study involving A549 lung cells in vitro, it was noted that Polyaminopropyl Biguanide induces inflammatory responses via the NF-κB signaling pathway.

There were no mortalities or signs of systemic toxicity in rats that received dermal applications of Polyaminopropyl Biguanide at doses up to 200 mg/kg daily over a 30-day period (21 applications total; NOAEL = 200 mg/kg). In a 21-day dermal toxicity study involving rabbits, there was no evidence of toxic effects on the skin after 20% aqueous Polyaminopropyl Biguanide was applied.

A LOAEL of 0.1 mg/ml (lowest concentration in drinking water) for Polyaminopropyl Biguanide was reported in the two 28-day oral toxicity studies involving rats and mice, respectively.

In 21-day and 28-day inhalation toxicity studies on Polyaminopropyl Biguanide involving rats, NOAEL values of 0.025 mg/m³ and 0.0239 mg/m³, respectively, were reported. In a 60-day oral toxicity study on Polyaminopropyl Biguanide involving rats, mild toxicity in the liver or kidneys (at microscopic examination) was observed at daily doses of 2 mg/kg (equivalent to 0.2 mg/l of 0.4% solution of test substance), 8 mg/kg (equivalent to 0.4 mg/l of 0.4% solution of test substance), and 32 mg/kg (highest dose equivalent to 1.2 mg/l of 0.4% solution of test substance). None of the animals died.

In 90-day toxicity studies on rats and mice, 4000 to 5000 ppm Polyaminopropyl Biguanide or more in the diet was associated with iron pigment deposits in Kupffer cells in the rats, but no mortalities; the NOAEL was 1000 ppm in both species. In a 90-day study, 20% Polyaminopropyl Biguanide in drinking water yielded no treatment-related macroscopic findings in rats. A NOAEL of 5500 ppm was reported for Beagle dogs fed up to 11000 ppm Polyaminopropyl Biguanide in the diet for 90 days.

In an 80-week chronic toxicity study (dermal applications 5 days/week), a mortality rate of 75% was reported for the highest dose group (10% Polyaminopropyl Biguanide, 30 mg dose). The exophthalmos observed throughout the study was more severe in this group, but the results of histological examination of the eyes and gross and microscopic examination of the thyroids were negative.

In a 104-week oral toxicity study involving rats, a NOAEL of 2000 ppm (highest concentration fed in diet) was reported for Polyaminopropyl Biguanide. This concentration corresponded to a NOAEL of 36 mg/kg/day in male rats, used to calculate a margin of safety. MOS calculations were performed, assuming that all cosmetics contain 0.1% Polyaminopropyl Biguanide and a dermal absorption value of 4.09%, and using the NOAEL of 36 mg/kg/day and a SED of 0.012 mg/kg/day; MOS values of 258 (based on cosmetic exposure estimate) and 227 (based on cosmetic exposure estimate + noncosmetic exposure estimate) were determined. The SCCS was responsible for the margin of safety calculations. A NOEL (for histopathologic changes) of 200 ppm was reported in a 122-week oral toxicity study involving rats fed Polyaminopropyl Biguanide at concentrations up to 2000 ppm in the diet. In a study involving mice, feeding with Polyaminopropyl Biguanide (concentrations up to 1000 ppm in diet) for 97 weeks did not cause any macroscopic changes in tissues examined. A NOAEL of 1500 ppm for Polyaminopropyl Biguanide was reported in a 1-year feeding study involving dogs, though treatment-related histopathological findings in the liver and kidneys were reported at dietary concentrations of 3000 ppm and 4500 ppm. In a 26-week feeding study involving dogs, dietary concentrations of 1500 ppm and 4500 ppm Polyaminopropyl Biguanide produced dose-related hepatotoxicity and nephrosis.

In oral reproductive and developmental toxicity studies on Polyaminopropyl Biguanide involving rats, NOAEL values of 1000 ppm and 1300 ppm have been reported. In an inhalation study, degeneration of seminiferous tubules in the

testis of 1 male rat was observed at a concentration of 0.25 mg/m³, but this toxic effect was not observed at any other concentration, including the highest concentration (26 mg/m³). NOAELs of 10 mg/kg/day and 40 mg/kg/day for developmental toxicity were reported in studies involving mice, and the higher dosage rate was also classified as non-teratogenic in mice in another study. A NOAEL of 40 mg/kg/day for developmental toxicity has also been reported in a study involving rabbits. Polyaminopropyl Biguanide has been classified as embryotoxic at oral dosage rates of 32 mg/kg/day (animal strain not stated) and 100 mg/kg/day (rats), and as teratogenic in rats at an intraperitoneal dosage rate of 10 mg/kg/day.

In the Ames test, Polyaminopropyl Biguanide was non-genotoxic at doses up to 5000 µg/plate with and without metabolic activation. At the highest dose evaluated (333,300 µg/plate) in the Ames test, Polyaminopropyl Biguanide was weakly genotoxic in strain 1538 without metabolic activation. Polyaminopropyl Biguanide was non-genotoxic in the mouse lymphoma assay at concentrations up to 2000 µg/ml with and without metabolic activation, and in the in vitro micronucleus test at concentrations up to 50 µg/ml (without metabolic activation) and up to 250 µg/ml (with metabolic activation). In the in vivo micronucleus test, Polyaminopropyl Biguanide was non-clastogenic in polychromatic erythrocytes from mice that received single oral dosages up to 400 mg/kg. In the in vivo unscheduled DNA synthesis assay, there was no induction of unscheduled DNA synthesis in hepatocytes from rats that received single oral doses up to 1500 mg/kg.

Polyaminopropyl Biguanide was evaluated at concentrations up to 3000 µg/ml in the cell transformation assay (using baby hamster kidney fibroblasts), and there was no difference in the number of transformed cell colonies between test and negative control cultures. In another assay involving RAW 264.7 mouse macrophages, Polyaminopropyl Biguanide, tested at concentrations up to 1 ppm, had no direct effect on liver cell proliferation and did not potentiate cell proliferation induced by activated macrophages.

Except for a slight increase in some cytokines and transcription factor at concentrations at which cell lysis occurs rapidly, Polyaminopropyl Biguanide did not exhibit clear and remarkable epigenetic properties.

Polyaminopropyl Biguanide was classified as a hepatic tumorigen in mice, i.e., at the highest dose (30 mg of 10% Polyaminopropyl Biguanide (in ethanol) that was applied to the skin daily (5 days/week) for 80 weeks. An increase in the incidence of liver tumors was observed at the 30 mg/day dose; the increase was statistically significant only for liver tumors of endothelial origin. High mortality (76 to 78% of animals died) was noted in this group.

A statistically significant increase in the incidence of hemangiosarcomas and hemangiomas was reported for only male mice that received Polyaminopropyl Biguanide at a dietary concentration of 4000 ppm daily for 2 years. In a 97-week study in which mice were fed Polyaminopropyl Biguanide at dietary concentrations up to 1000 ppm prior to and during mating and their offspring were fed the same concentrations, there were no treatment-related (non-neoplastic or neoplastic) increases in histopathologic findings. Hemangiosarcomas or hemangiomas in the liver or other sites were reported in this study along with the high mortality incidence (80%) by week 97. A concentration-related increase (100 to 1000 ppm) in tumor-bearing mice was reported in a similar 97-week dietary study. In a shorter-term feeding study (14 days), increased cell proliferation was noted at a concentration of 1200 ppm Polyaminopropyl Biguanide in the diet. Polyaminopropyl Biguanide was classified as non-carcinogenic in rats fed dietary concentrations up to 2000 ppm for 122 weeks. At 124 weeks, 80% mortality overall was reported. A low incidence of hemangiomas and hemangiosarcomas was reported in a study in which rats were fed Polyaminopropyl Biguanide at a dietary concentration of 2000 ppm for 2 years.

Polyhexamethylene biguanide exhibited high antimicrobial activity against *Staphylococcus aureus* and *Echerichia coli*, whereas, though chemically closely related, Polyaminopropyl Biguanide proved to be ineffective in bacterial eradication. When compared to Polyhexamethylene Biguanide, Polyaminopropyl Biguanide displayed significantly lower cytotoxicity at concentrations ranging from 0.005% to 0.1% v/v; both chemicals were cytotoxic.

The results of animal studies indicate that the skin irritation potential of Polyaminopropyl Biguanide may be concentration-dependent as well as dependent upon the duration of application. For example, the skin irritation potential of Polyaminopropyl Biguanide (single 4-h application) was classified as a mildly irritating in rabbits. Single applications (24 h) of 20% aqueous Polyaminopropyl Biguanide to rabbits were non-corrosive, moderately irritating to intact skin, and severely irritating to abraded skin. Repeated applications of Polyaminopropyl Biguanide (12,000 ppm) to the skin of rabbits for 21 days were classified as non-irritating. Polyaminopropyl Biguanide (up to 1.5% active) was not classified as a primary skin irritant when applied for 24 h to the skin of human subjects. In a human repeated insult patch test (HRIPT, 191 subjects), it was determined that Polyaminopropyl Biguanide (2% active ingredient) was not capable of causing primary skin irritation, but was capable of causing sensitization. In an HRIPT on a neck cream containing 0.2% Polyaminopropyl Biguanide, results were negative for clinically relevant skin irritation and there was no evidence of allergenicity. When a leave-on product containing 0.1 % Polyaminopropyl Biguanide was evaluated in an HRIPT involving 207 subjects, it was concluded that the product did not induce dermal sensitization.

Positive results were reported for Polyaminopropyl Biguanide in the local lymph node assay. In maximization tests on Polyaminopropyl Biguanide, moderate skin sensitization was observed in guinea pigs induced with 0.06% active ingredient (intradermal injection) and 20.2% active ingredient (occlusive application) and challenged with Polyaminopropyl Biguanide (20.2 % active ingredient) and a 30% solution of the ingredient (6% active ingredient) in deionized water, and moderate to strong sensitization was observed in guinea pigs induced with 0.2% active ingredient (intradermal injection) and 20.2% active ingredient (topical application) and challenged with Polyaminopropyl Biguanide (20.2% active ingredient). In another guinea pig maximization test, sensitization was not observed in guinea pigs induced with 0.15% Polyaminopropyl Biguanide (intradermal injection) and 20% (topical application) and challenged with Polyaminopropyl Biguanide (10% or 20%). In one Buehler test on Polyaminopropyl Biguanide, guinea pigs were induced with 2% active ingredient (topical application), challenged with 2% active ingredient, and rechallenged with 0.2%, 2%, and 4% active ingredient. The initial challenge with 2% active ingredient and rechallenge with 2% and 4% active ingredient resulted in faint erythema; rechallenge with 0.2% active ingredient produced negative results. Polyaminopropyl Biguanide (2% active ingredient) was classified as a moderate sensitizer. In another Buehler test, it was determined that the threshold for eliciting sensitization in guinea pigs was ~ 1%. Induction concentrations ranged from 0.3% to 5% and challenge concentrations ranged from 0.075% to 15%.

Very strong irritation potential, but no significant photoirritancy, was reported in a study in which male rats were tested (dermal application) with Polyaminopropyl Biguanide at concentrations of 2% and 5%. When tested at a concentration of 1%, in 26 subjects, Polyaminopropyl Biguanide was essentially non-irritating and did not induce sensitization, phototoxicity, or photoallergenicity.

Case reports with sensitization reactions to Polyaminopropyl Biguanide (reported as an ingredient of wet wipes, contact lens cleansing solutions, wound irrigation solutions, and pre-operative antiseptics) have been reported. The prick test was used to diagnose immediate contact urticarial reactions in 44 patients with eczematous dermatitis. A positive reaction was observed in 1 patient.

Undiluted and 25% aqueous Polyaminopropyl Biguanide were severe ocular irritants when instilled into unrinsed rabbit eyes. Polyaminopropyl Biguanide (20% aqueous) induced slight inflammation, and Polyaminopropyl Biguanide (0.04% active ingredient) was non-irritating to the eyes of rabbits. In a study in which 20% aqueous Polyaminopropyl Biguanide was instilled into human eyes and the eyes of rabbits in a temperature-controlled chamber, normal corneal morphology was observed at histological examination.

DISCUSSION

The safety of Polyaminopropyl Biguanide as used as a preservative in cosmetics is reviewed in this assessment. Polyaminopropyl Biguanide is an INCI name; it refers to the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (polyhexamethylene biguanide hydrochloride (PHMB HCl)). This ingredient does not actually contain the chemical polyaminopropyl biguanide, which has a 3-carbon chain in each monomeric repeat unit. Rather, the INCI name, Polyaminopropyl Biguanide, applies exclusively to chemical polyhexamethylene biguanide, which has a 6-carbon chain in each monomeric repeat unit, and is always supplied as the hydrochloride salt. The chemical polyaminopropyl biguanide is not a cosmetic ingredient. However, in this safety assessment, the INCI name Polyaminopropyl Biguanide is used to represent the chemical polyhexamethylene biguanide hydrochloride (a preservative), which is the ingredient with reported uses in cosmetics and is the subject of this safety assessment.

Dermal toxicity was not observed at 0.4%, which is greater than the 0.1% maximum reported cosmetic use concentration of Polyaminopropyl Biguanide. Furthermore, the Panel noted that the dermal penetration of Polyaminopropyl Biguanide is minimal, considering that most of the compound remains in the epidermis and its distribution systemically is not a concern.

Overall, the available in vivo and in vitro genotoxicity data on Polyaminopropyl Biguanide in bacterial and mammalian cells are negative. The Panel noted that in vitro genotoxicity assays are difficult to interpret for microbial toxins such as the cytotoxic preservative Polyaminopropyl Biguanide. However, after reviewing the available data, the Panel determined that genotoxicity is not a concern. A low incidence of hemangiomas and hemangiosarcomas was reported in a study in which rats were fed Polyaminopropyl Biguanide at a dietary concentration of 2000 ppm for 2 years. The Panel noted that the vascular tumors observed in rats and mice was likely attributable to sustained hepatotoxicity (i.e., a non-genotoxic mechanism), from high exposures (near the MTD) that the Panel considered not toxicologically relevant to cosmetic use. Furthermore, the carcinogenicity study results reviewed are equivocal.

Results were classified as positive for Polyaminopropyl Biguanide in the local lymph node assay. However, interpreting the study results is hampered by the absence of a reported EC3. Additionally, the Panel noted that Polyaminopropyl Biguanide is a sensitizer at 2%, and that elicitation occurs at a much lower concentration (0.2%) in animal studies?. However, when Polyaminopropyl Biguanide was diluted to a concentration of 1% and applied repeatedly to the skin (dose = 1 mg/cm²) of human subjects, the test substance was essentially non-irritating and did not induce sensitization, phototoxicity, or photoallergenicity. The Panel agreed that a NESIL needs to be determined and also expressed concern over the existence of case reports of anaphylaxis attributable to the use of Polyaminopropyl Biguanide in wound dressings.

Regarding the issue of inhalation exposure, the Panel noted the availability of clinical studies relating to child deaths in South Korea associated with inhalation exposure from humidifiers that had been disinfected with a humidifier disinfectant containing polyhexamethylene guanidine phosphate (often referred to as polyhexamethylene guanidine; PHMG). PHMG is structurally related to the cosmetic ingredient, though it is not the same chemical as Polyaminopropyl Biguanide.

Finally, the Panel discussed the issue of incidental inhalation exposure, as Polyaminopropyl Biguanide is used in other fragrance preparations and could possibly be inhaled.

CONCLUSION

To be determined.

Table 1. Definition, idealized structure, and function of the ingredient in this safety assessment. ^(1; [CIR Staff])

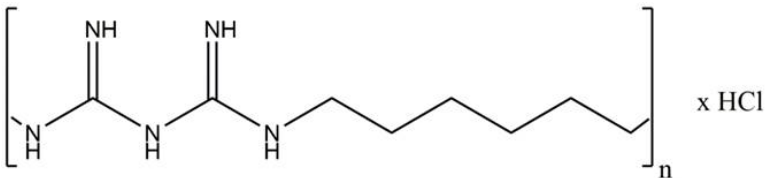
Ingredient CAS No.	Definition & Idealized Structure	Function
Polyaminopropyl Biguanide 32289-58-0 [PHMB HCl] [27083-27-8 (PHMB HCl)] [28757-47-3 (PHMB)]	Polyaminopropyl Biguanide is the organic compound that conforms to the formula. [Polyaminopropyl Biguanide is the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (polyhexamethylene biguanide (PHMB HCl).]	Preservatives
 <p style="text-align: center;">PHMB HCl</p>		

Table 2. Physical and Chemical Properties of Polyaminopropyl Biguanide

Property	Value	Reference
physical form (at 20°C and 101.3 kilopascals (kPa)) and/or color	pale yellow powder	²
average molecular weight (Daltons (Da))	3686-4216. Molecular weight distribution in commercially used mixture: 6% is < 500, 14.1% is between 500 and 1000, and 75.8% is > 1000	²
water solubility (g/100 ml)	41 ± 1 %	²
other solubility (g/100 ml)	ethanol: 0.5 ± 0.08% methanol: 41 ± 1 %	²
relative density (at 20 ± 0.5°C)	1.20 ± 0.0025	²
melting point (°C)	78.9-136.3	²
boiling point (°C)	decomposes at 205-210, before boiling	²
vapor pressure (Pa at 20°C)	1.32 x 10 ⁻⁷	²
log P _{ow} (at 25 ± 1°C)	-2.3	²
UV absorption (λ) (nm)	236	²

Table 3. Frequency and concentration of use according to duration and type of exposure

	# of Uses ^d	Max Conc of Use (%) ^{e,28}
Polyaminopropyl Biguanide		
Totals*	147	0.000023-0.2
Duration of Use		
Leave-On	102	0.000023-0.2
Rinse-Off	45	0.00025-0.1
Diluted for (Bath) Use	NR	NR
Exposure Type		
Eye Area	28	0.01-0.2
Incidental Ingestion	1	NR
Incidental Inhalation-Spray	1; 29 ^a ; 31 ^b	0.000023-0.1% ^a
Incidental Inhalation-Powder	31 ^b	0.00001-0.2 ^c
Dermal Contact	116	0.00001-0.2
Deodorant (underarm)	NR	0.003
Hair - Non-Coloring	16	0.000023-0.1
Hair-Coloring	NR	0.1
Nail	2	NR
Mucous Membrane	10	0.006
Baby Products	NR	0.1

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may or may not equal the sum of total uses.

^aIt is possible these products are sprays, but it is not specified whether the reported uses are sprays

^bNot specified these products are sprays or powders, but it is possible the use can be as a spray or powder, therefore the information is captured in both categories

^c It is possible these products are powders, but it is not specified whether the reported uses are powders

NR – not reported

Table 4. Dermal Penetration Studies

Ingredient	Animals/Protocol	Results
[¹⁴ C]- Polyaminopropyl Biguanide (20.2% aqueous; specific activity = 0.88 mCi/ml)	Various concentrations applied to human skin (epidermis from abdominal skin) in diffusion cell. (dose volume = 1 ml). Receptor fluid samples collected daily for up to 15 days. Also, uptake experiment whereby 2 cm ² rat skin disks (whole skin from flank and dorsum of male and female Wistar-derived, Alderley-Park rats) bathed in different concentrations; 5-day equilibration phase.	At concentrations of 0.4%, 1.4%, 5%, and 20%, absorption rates (ng/cm ² /h) through human epidermis were 8.13, 22.8, 350, and 1005, respectively. At concentrations of 0.4%, 20% (early phase - not defined), and 20% (late phase - not defined) [¹⁴ C]- Polyaminopropyl Biguanide, absorption rates (ng/cm ² /h) through rat whole skin were 131, 3695, and 11940, respectively. ¹²
[¹⁴ C]- Polyaminopropyl Biguanide (5% solution)	Applied to skin biopsies of newborn, hairless rats and to human epidermal skin in diffusion chamber.	For rat skin biopsies, no skin absorption was detected up to day 5 of exposure. For human epidermal skin biopsies, low rate of penetration of ~0.09 % was noted after 24 h, and this penetration rate was from 0.11 % up to 0.81 % after adding dimethylsulfoxide (DMSO). ¹²
[¹⁴ C]-Polyaminopropyl Biguanide (0.1% w/w in aqueous micellar solution); [¹⁴ C]-Polyaminopropyl Biguanide (0.1 % w/w in oil-in-water emulsion)	0.1% in aqueous micellar solution and 0.1% in oil-in-water emulsion, respectively, applied (24-h exposure study) to human split-thickness skin from 4 donors (dose = 200 µl/cm ² ; ≈ 2 mg/cm ²) in diffusion cell. Penetration was determined directly after exposure.	Total dislodgeable dose (skin wash + tissue swab + pipette tip + donor chamber wash): 48.43% (for test substance in aqueous micellar solution) and 52.35% (for test substance in oil-in-water emulsion) of radioactivity removed during skin washing. At 24 h post-dosing, absorbed (fraction of applied dose that was measured in receptor fluid) dose was 0.03% (for test substance in aqueous micellar solution) and 0.04% (for test substance in oil-in-water emulsion). The epidermis + lower layers of stratum corneum contained 11.47% (for test substance in aqueous micellar solution) and 14.20% (for test substance in oil-in-water emulsion) of the applied dose. The dermis contained 1.56% (for test substance in aqueous micellar solution) and 1.02% (for test substance in oil-in-water emulsion) of the applied dose. Mass balance was complete: 90.93% (for test substance in aqueous micellar solution) and 98.96% (for test substance in oil-in-water emulsion) of the applied dose. Based on SCCS Notes of Guidance, one standard deviation (2.5%) was added to the absorbed amount, yielding a calculated dermal absorption value of 4.09% (1.56% + 0.03% + 2.5% = 4.09%). ²

Table 4. Dermal Penetration Studies

Ingredient	Animals/Protocol	Results
$[^{14}\text{C}]$ -Polyaminopropyl Biguanide (0.3 % w/w in aqueous micellar solution); $[^{14}\text{C}]$ -Polyaminopropyl Biguanide (0.3 % w/w in oil-in-water emulsion)	Polyaminopropyl Biguanide solutions applied to human split-thickness skin from 4 donors (dose volume = 200 $\mu\text{l}/\text{cm}^2$; application rate $\approx 2 \text{ mg}/\text{cm}^2$) in diffusion cell. In Part 1, penetration of the 0.1% aqueous micellar solution and 0.1% in oil-in-water emulsion determined directly after 24 h exposure period. In Part 2, 24 h exposure to 0.3 % aqueous micellar solution and to 0.3% in oil-in-water emulsion followed by additional 72 h period to determine whether test compound absorbed into the skin during previous 24 h period would move from skin into the receptor fluid after the washout. All samples analyzed by liquid scintillation counting.	In 24-h study, 48.43% (from aqueous solution) and 52.35% (from oil/water emulsion) of $[^{14}\text{C}]$ -Polyaminopropyl Biguanide-derived radioactivity removed during washing procedure (dislodgeable dose at 24 h). At 24 h post dose, absorbed (fraction of applied dose measured in receptor fluid) dose was 0.03% (0.58 ng equiv/ cm^2 , from aqueous solution) and 0.04% (0.72 ng equiv/ cm^2 , from oil/water emulsion) of the applied dose. Epidermis + lower layers of stratum corneum contained 11.47% (238 ng equiv/ cm^2 , from aqueous solution) and 14.20% (291 ng equiv/ cm^2 , from oil/water emulsion) of applied dose. Dermis contained 1.56% (32.3 ng equiv/ cm^2 , from aqueous solution) and 1.02% (20.9 ng equiv/ cm^2 , from oil/water emulsion) of applied dose. In the 72-h study, 53.33% (from aqueous solution) and 58.10% (from oil/water emulsion) of $[^{14}\text{C}]$ -Polyaminopropyl Biguanide-derived radioactivity removed during washing procedure. At 72 h post dose, absorbed dose was 0.02% (1.29 ng equiv/ cm^2 , from aqueous solution) and 0.03% (1.94 ng equiv/ cm^2 , from oil/water emulsion) of applied dose. Epidermis + lower layers of stratum corneum contained 14.54% (972 ng equiv/ cm^2 , from aqueous solution) and 14.45% (921 ng equiv/ cm^2 , from oil/water emulsion) of applied dose. Dermis contained 1.23% (82.0 ng equiv/ cm^2 , from aqueous solution) and 1.46% (93.4 ng equiv/ cm^2 , from oil/water emulsion) of the applied dose. Absorption through skin = 1.56% (dermis contained 1.56% of applied dose) + 0.03% (absorbed dose = 0.03% of applied dose). Based on SCCS Notes of Guidance, one standard deviation (2.5%) added to absorbed amount, yielding calculated dermal absorption value of 4.09% (1.56% + 0.03% + 2.5% = 4.09%). ²
$[^{14}\text{C}]$ -Polyaminopropyl Biguanide (19.2% aqueous; specific activity = 38.9 mCi/g)		At 24 h, the absorbed dose (mean: 0.17 %) was the sum of the receptor fluid (0.171 %) and the receptor wash (definition not provided, 0.01 %). Dermal delivery (3.49 %) was the sum of the absorbed dose and the portion in the epidermis (3.18 %) and the dermis (0.14 %). ¹²
$[^{14}\text{C}]$ -Polyaminopropyl Biguanide (20.2% aqueous; specific activity = 1.85 GBq/732 mg)	Applied to human skin epidermal membranes in diffusion cell. Nominal concentrations up to ~200 g/l applied (not occluded) at 10 $\mu\text{l}/\text{cm}^2$. ~200 g/l also applied (occluded) at 200 $\mu\text{l}/\text{cm}^2$.	At ~200 g active ingredient/l (occluded), absorption rate $0.110 \pm 0.044 \mu\text{g}/\text{cm}^2/\text{h}$ (n = 4) and absorption percentage 0.001% over 24-h. At 197 g active ingredient/l (unoccluded), absorption rate $0.009 \pm 0.003 \mu\text{g}/\text{cm}^2/\text{h}$ (n = 5) and absorption percentage 0.012% over 24-h. ¹²
20.2% aqueous Polyaminopropyl Biguanide (20.2% aqueous; specific activity = 1.4 MBq/mg)	Test substance warmed to 40°C and nominal concentrations up to 200 g/l applied (at volume of 10 $\mu\text{l}/\text{cm}^2$, unoccluded and occluded) to human skin epidermal membranes in diffusion cell.	At a concentration of 200 g active ingredient/l (occluded for 0.5 h then unoccluded for 23.5 h), absorption rate was $< 0.002 \pm < 0.001 \mu\text{g}/\text{cm}^2/\text{h}$ (n = 6) and absorption percentage was < the limit of quantitation over a 24-h period. Other data for a dose of 200 g active ingredient/l (occluded) indicated an absorption rate of $0.118 \pm 0.012 \mu\text{g}/\text{cm}^2/\text{h}$ (n = 5) and an absorption percentage of 0.007% over a 24-h period. ¹²

Table 5. Toxicokinetics Studies

Ingredient	Animals/Protocol	Results
[¹⁴ C]-Polyaminopropyl Biguanide (20% aqueous in double deionized water; specific activity = 1.85 GBq/4 mmol)	Groups of Alpk:APfSD (Wistar-derived) rats (3 to 5/sex/group). Single oral dosage (20 mg/kg) administered by gavage. Labelled and unlabeled test substances fractionated into low, medium and high molecular weight (MW) fractions by centrifugation and also administered orally.	In bioavailability experiment (3 groups of 4 males), single oral dose of low, medium or high MW fraction: 94.9%, 101.4%, and 96% of radioactivity from low, mid, and high MW fractions, respectively, eliminated via feces. 5.2%, 0.2%, and 0.2 % excreted via urine. In biliary excretion experiment (3 rats), single oral dose of unfractionated test substance administered: Most of radioactivity excreted via feces over 48 h (96.8% in males; 98.9 % in females), < 3 % excreted in urine, and < 0.2% excreted in bile. In excretion and tissue retention experiments (5 males, 5 females), single oral dose of low MW fraction: Males excreted 7.8 % via urine and 94.1 % via feces; females excreted 2.6% via urine and 93.5% via feces. In tissues, highest amounts of radioactivity found in livers (0.18% of dose in males; 0.19 % of dose in females) and kidneys (0.03% of dose in males; 0.04 % of dose in females). Lower concentrations found in all other tissues investigated. Residual carcasses contained 0.22 and 0.28% of administered dose. It was noted that up to 8.5% of applied radioactivity might be considered bioavailable (sum of urinary excretion and radioactivity in tissues and residual carcass at study termination). ²
[¹⁴ C]- Polyaminopropyl Biguanide (20% aqueous in double deionized water; specific activity = 1.85 GBq/4 mmol)	Groups of Alpk:APfSD (Wistar-derived) rats (5/sex/group) fed diets containing either 200 ppm or 2000 ppm unlabeled ingredient for 14 days. Groups then fed single oral dose of diet incorporating [¹⁴ C]-labeled ingredient as 9 % suspension (4 ml/kg). High dose corresponded to 0.8 mg [¹⁴ C]-labeled ingredient /kg (2 MBq/kg) and, low dose, to 0.08 mg [¹⁴ C]-labeled ingredient/kg (0.2 MBq/kg).	Principal route of excretion of radioactivity was feces. At 200 ppm, fecal excretion of radioactivity amounted to 105 % and 109 % of administered dose for male and female rats, respectively. At 2000 ppm, percentages of fecal excretion were 106 % and 105% in male and female animals. Urinary excretion accounted for 2.1% and 2.2% of dose in males and females at the low dose and for 2.3 % and 1.8 % in males and females at the high dose. Conclusion: At 200 ppm, 4.7 % and 3.9 % of administered doses bioavailable in males and females, respectively. Bioavailability 3.0 % and 2.6 % in high dose males and females, respectively. ¹²
Radiolabeled Polyaminopropyl Biguanide	5 male Alderley Park rats. Oral dosage rate 20 mg/kg/day over 10 days.	5.6% ± 0.35 % excreted in urine, 93.1% ± 1.58% excreted via feces and 0.2 % exhaled. ²⁰
Radiolabeled Polyaminopropyl Biguanide	Rats fed diet containing 20 ppm.	Greatest amounts of radioactivity detected in adipose tissue, followed by kidneys and livers. No radioactivity detected in brain. Urinary polymer-related material consisted of small amounts of Polyaminopropyl Biguanide oligomers with 2 cyanoguanidino end groups, as well as the trace constituents 3,3-dicyano-1,1-hexamethylenediguanidine and compound that was considered to be 1- (6-aminoethyl)-3-cyanoguanidine. ²⁰

Table 5. Toxicokinetics Studies

Ingredient	Animals/Protocol	Results
[¹⁴ C]-20% Polyaminopropyl Biguanide (4.6 μ Ci)	5 male rats (strain not stated). Feeding with dosages of 100 mg/kg in the diet	93% of radioactivity excreted in feces within 5 days. Six percent of radioactivity found in urine, 0.6% found in bile, and 0.2% found in expired air. Findings suggested to the authors that test substance was poorly absorbed from gut and no evidence of enterohepatic recirculation. ¹⁹
[¹⁴ C]-20% Polyaminopropyl Biguanide	Groups of 3 male rats (strain not stated) maintained on diet that contained 100 ppm test substance	Concentration in abdominal fat peaked at 1.2 ppm after 3 weeks and was maintained at this level for another 2 weeks on diet. After returning to normal diet, concentrations in the abdominal fat reduced to 0.3 ppm after 5 weeks. Concentration in the liver did not exceed 0.6 ppm after 5 weeks of feeding, and was reduced to undetectable levels within 3 weeks of return to normal diet. Comparable concentrations (maximum) in the kidney and heart were 0.8 ppm and 0.1 ppm. Radioactivity not detected in brain. ¹⁹
[¹⁴ C]-Polyaminopropyl Biguanide	10 NMRI mice received single oral dose of 2.0 mL by gavage and were then frozen in acetone at up to 48 h post-dosing. Whole body autoradiography subsequently performed (additional details not provided).	No absorption detected ¹²

Table 6. Acute Dermal Toxicity Studies

Ingredient	Animals	Protocol	Results
Polyaminopropyl Biguanide (in distilled water)	10 Sprague-Dawley rats (5 males, 5 females).	OECD Guideline 402. Clipped skin of trunk treated with single dose of 5000 mg/kg. Application site covered with semi-occlusive dressing for 24 h. 14-day observation period.	No mortalities or systemic toxicity. Hemorrhage of dermal capillaries noted at treatment sites of 8 animals one and two days after dosing. ¹²
Polyaminopropyl Biguanide (20% aqueous)	2 groups of 20 (10 males, 10 females/group) specific pathogen free (SPF) albino rats.	Topical application of test substance at doses of 2.5 ml/kg and 5 ml/kg, respectively. Test substance applied to intact skin and spread over area of ~1 inch ² . Site covered with patch for 24 h. 7-day observation period. Necropsy not performed.	No mortalities. ¹⁹
Polyaminopropyl Biguanide (20% aqueous)	4 New Zealand White rabbits (2 males, 2 females).	OECD Guideline 402. Test substance (2 ml) applied to shaved area (~150 x 130 mm) of dorso-lumbar region and held in place with occlusive dressing for 24 h. 14-day observation period.	Dermal LD ₅₀ > 2 ml/kg, i.e., greater than 400 mg/kg (active ingredient). ¹²

Table 7. Acute Oral Toxicity Studies

Ingredient	Animals	Protocol	Results
Polyaminopropyl Biguanide (in distilled water)	6 female Sprague-Dawley rats	OECD Guideline 425. Dosed by gavage with 550 or 2000 mg/kg (dose volume = 20 ml/kg).	All 3 rats dosed with 2000 mg/kg died. No deaths at dose of 550 mg/kg. Signs of systemic toxicity in 1 animal dosed with 2000 mg/kg, but not at 550 mg/kg. Abnormalities noted at necropsy of rats that died were: hemorrhagic or abnormally red lung, dark liver, dark kidneys, hemorrhage or sloughing of the gastric mucosa, sloughing of the non-glandular epithelium of the stomach and hemorrhage of the small intestine. No abnormalities at necropsy of rats that survived 14-day observation period. LD ₅₀ = 1049 mg/kg. ¹²
25% aqueous Polyaminopropyl Biguanide	6 rats (3 males, 3 females; strain not stated)	Single oral dose of 4000 mg/kg (equivalent to 1000 mg/kg Polyaminopropyl Biguanide) by stomach tube. 7-day observation period.	1 female rat died. Necropsy findings included generalized congestion with gastric distention and hemorrhage, and lympholysis. LD ₅₀ > 1000 mg/kg Polyaminopropyl Biguanide. ¹⁹
25% aqueous Polyaminopropyl Biguanide	3 female rats (strain not stated)	Single oral dose (2 g/kg), followed by 7-day observation period.	No deaths and all organs appeared normal at necropsy. ¹⁹
25% aqueous Polyaminopropyl Biguanide	6 rats (3 males, 3 females; strain not specified)	Single oral dose of 40000 mg/kg	1 male rat died. Severe generalized congestion with dilatation of the stomach and mucosal hemorrhage were observed at necropsy. Microscopic examination revealed gastric inflammation, ulceration, and thymic lympholysis, but no other specific lesions. ¹⁹
20% aqueous Polyaminopropyl Biguanide	groups of Alderley Park rats (5 /sex/dose)	OECD Guideline 401. Doses up to 5000 mg/kg (dose volume = 10 ml/kg) administered by stomach tube. 14-day observation period. Necropsy not performed.	Signs of toxicity did not persist beyond day 7 or 8. LD ₅₀ of 2747 mg/kg (males) and 2504 mg/kg (females), corresponding to ~ 549 and ~501 mg/kg (active ingredient), respectively. ¹²
Polyaminopropyl Biguanide (in deionized water)	Groups of 10 Sprague-Dawley rats	Single dose by gavage (stomach tube). Dosages ranged from 2 mg/kg to 40 mg/kg.	Administration of 25.6 mg/kg dose, i.e. 1.6 mL of 0.4% Polyaminopropyl Biguanide solution (equivalent to 6.4 x 10 ³ mg/l of 0.1% solution) resulted in 50% mortality. LD ₅₀ = 25.6 mg/kg. Following signs observed at LD ₅₀ : inactivity, ataxia, diarrhea, hyperreflexia, and convulsive twitching. No histopathological lesions in heart and kidney samples. 30% of animals tested had mild hydropic changes in zone 1 of liver samples. ²¹

Table 8. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Polyaminopropyl Biguanide (purity 99.6%) in aqueous solution	Wistar CRL:(WI) rats (groups of 10; 5/sex/test concentration). OECD Guideline 403-compliant study. Exposure levels (nose-only): 0.1, 0.3 and 0.5 mg/l (10, 30, and 50 mg/m ³) for 4 h. Mass medium aerodynamic diameters: 1.49-2.20 µm, with GSD in 1.84-2.29 µm range.	<u>Note:</u> In preliminary test, 2 rats exposed to 1 mg/l died. At 0.1 mg/l, no deaths, but main clinical signs observed on day 0 and included: slight to moderately labored respiration, rhonchus, decreased activity, hunched back, and increased respiratory rate. At 0.3 mg/l, all animals with slight-to-moderately labored respiration. Slight-to-severe decreased activity also observed; moderate ataxia in one animal. At 0.5 mg/l, main clinical signs included: moderately -to-severely labored respiration with noisy respiration up to gasping, increased respiratory rate, and decreased activity. At necropsy, enlargement of dark/red discolored lungs and/or dark/red discoloration of the fur at the perinasal and/or white foamy material in the trachea in all animals found dead (only in 0.3 and 0.5 mg/l groups). LC ₅₀ = 0.37 mg/l (370 mg/m ³) for males and females combined. ¹²
20.6% w/w Polyaminopropyl Biguanide	Alpk:APfSC rats (10 rats; 5/sex). Exposed (nose-only) for 4 h to single dose of 1.76 mg/l (1760 mg/m ³) of formulation (corresponds to 0.36 mg/l (360 mg/m ³) of Polyaminopropyl Biguanide (mass medium aerodynamic diameters: 1.8-2.0 µm, with a geometric standard deviation [GSD] of 2 µm))	1 male died 3 h after exposure. Respiratory distress in all females and most males. Red mottled lungs in dead male and 2 other males on day 15. LC ₅₀ estimated at > 0.36 mg/l (360 mg/m ³) for Polyaminopropyl Biguanide. ¹²
Polyaminopropyl Biguanide (20% aqueous in spa water)	Groups of 5 mice of the Alpk:APfCD-1 strain exposed to aerosol. target concentrations 5, 50 and 200 mg/m ³ , corresponding to analyzed concentrations 11.7, 62.9 and 208 mg/m ³ , respectively; median aerosol sizes (MMAD) 2.52, 3.08 and 4.31 µm.	Mean respiratory rate depression was 12% ± 4%, 20% ± 7 % and 40 ± 15% for target concentrations of 5, 50 and 200 mg/m ³ , respectively, and RD ₅₀ (concentration causing 50 % depression in respiratory rate) 264 mg/m ³ (no sensory irritation) calculated. ¹² The SCCS noted that this RD ₅₀ is outside of investigated concentration range and is of questionable reliability. SCCS also stated that the results of this study indicate that test substance should be considered a respiratory irritant. ¹²

Table 9. Short-Term Toxicity Studies

Ingredient	Animals	Protocol	Results
Dermal Studies			
25% aqueous Polyaminopropyl Biguanide	3 female rats (strain not specified).	Test substance applied (amount per cm ² not specified) to intact skin of the back, under occlusive dressing, for 3 alternating 24-h periods; i.e., each application period followed by 24-h non-treatment period.	No specific systemic effects were observed. ¹⁹
20.2% aqueous Polyaminopropyl Biguanide	Groups of 10 (5 males, 5 females per group) rats of the Alpk:APfSD (Wistar-derived) strain	Three groups received applications (occlusive, on back) of 0 mg/kg, 20 mg/kg, 60 mg/kg, and 200 mg/kg, respectively, 6 h per day for 30 days (21 applications total). Fourth group served as the control.	No mortalities and no overt clinical signs of toxicity up to the highest dose tested. No substance-related effects on body weight, food consumption, organ weights, hematology or clinical chemistry. Gross pathology and histopathology revealed no evidence of systemic toxicity. NOAEL for systemic toxicity = 200 mg/kg/day. ¹²
20% Polyaminopropyl Biguanide (diluted with water to 0.04% active ingredient)	5 female rats of Alderley Park strain.	0.04% applied (0.1 ml) to back on alternate days for total of 6 applications. No covering or test site covered with polyethylene secured with an adhesive plaster for 24 h.	No evidence of systemic toxicity (with or without covering). ¹⁹
20% aqueous Polyaminopropyl Biguanide	female albino rabbits	12,000 ppm solution (1 ml) applied (unoccluded) to the back for 23 h. Re-applied, beginning at 1 h later, for total of 21 daily applications.	No evidence of toxic effects on the skin. ¹²
Oral Studies			
25% aqueous Polyaminopropyl Biguanide	14 rats (7 males, 7 females; strain not specified)	Administered orally for 21 days, initially at 1 g/kg and subsequently at 0.5 g/kg doses.	4 males and 2 females survived 21 days of dosing; toxic signs not reported. Necropsy findings: gastrointestinal irritation, severe gastric hemorrhage, ulceration, peritonitis, thymic atrophy, and generalized congestion. At microscopic examination of major organs, non-specific changes consistent with gastrointestinal inflammation. ¹⁹
20% aqueous Polyaminopropyl Biguanide	Groups of 16 (8 males, 8 females per group) rats of the Alpk:APfSD strain.	Four groups received concentrations of 0.1, 0.5, 1, and 2 mg/ml, respectively, in drinking water for 28 days.	Dose-related loss in bodyweight/body weight gain and reduced water and/or food consumption occurring predominantly during the first days of treatment (considered a palatability effect). Increased liver weight at 1 mg/ml, decreased liver weight at 2 mg/ml, and dose-related increase in kidney weight at all dose levels. NOAEL could not be derived. LOAEL = 0.1 mg/ml. ¹²

Table 9. Short-Term Toxicity Studies

Ingredient	Animals	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	Groups of 20 (10 males, 10 females per group) mice of the C57Bl/10JfAP/alkp strain	Four groups received concentrations of 0.1, 0.3, 0.6, and 1.2 mg/ml, respectively, in drinking water for 28 days.	One male in 0.3 mg/ml group found dead on day 13. Dose-related initial loss of body weight, reduction in food and water consumption, and continued reduction in body weight and water consumption (considered a palatability effect). Treatment-related decrease in liver weight for males given 0.6 and 1.2 mg/ml, probably associated with poor nutritional status. Because effects on body weight and water consumption at all dose levels, NOAEL could not be derived. LOAEL = 0.1 mg/ml. ¹²
Polyaminopropyl Biguanide (in deionized water)	Groups of 6 Sprague-Dawley rats	60-day study. Dosage (by gavage) rates: Group 1: 2 mg/kg (equivalent to 0.2 mg/l of 0.4% solution of test substance); Group 2: 8 mg/kg/day (equivalent to 0.4 mg/l of 0.4% solution of test substance); and Group 3: 32 mg/kg/day (equivalent to 1.2 mg/l of 0.4% solution of test substance). Control group received deionized water	No mortalities. Signs of systemic toxicity noted 2 days after dosing in 1 animal dosed with 32 mg/kg, exhibiting lethargy, ataxia, decreased respiratory rate, labored respiration, ptosis and tiptoe gait. 50% of rats dosed with 32 mg/kg had either mild hepatocyte cytolysis with or without lymphocyte infiltration and feathery degeneration. No visible gross pathological changes in heart, liver and kidney samples. At 2 and 8 mg/kg, mild toxicity in 50% of liver samples and 50% of kidney samples examined microscopically. At 32 mg/kg, mild toxicity in 50% of liver samples examined microscopically (mild kidney toxicity in 1 rat). In control group, mild toxicity (at microscopic examination) in kidneys of 30% of animals. ²¹
Inhalation Studies			
19.2% aqueous Polyaminopropyl Biguanide	Groups of 10 (5 males, 5 females per group) rats of the Alpk:APfSD (Wistar-derived) strain	Three groups were exposed (nose-only) to concentrations of 0.025mg/m ³ , 0.25 mg/m ³ , and 2.5 mg/m ³ , respectively, 6 h per day (5 days per week; 28 days total). For satellite groups (0, 0.025, and 2.5 mg/m ³) the recovery period was 13 weeks. Target air concentrations of aqueous Polyaminopropyl Biguanide were 0.0239 mg/m ³ (MMAD range: 0.32-1.30 µm), 0.257 mg/m ³ (MMAD range: 0.48-5.06 µm) and 2.47 mg/m ³ (MMAD range: 0.67-1.67 µm)	No treatment-related deaths or clinical signs up to 2.5 mg/m ³ . No toxicologically significant changes in hematology or blood clinical chemistry parameters. Lung weights slightly elevated for males and females exposed to 2.5 mg/m ³ ; thymus weights elevated in males only at 2.5 mg/m ³ . No macroscopic treatment-related findings observed at post-mortem examination. Squamous metaplasia seen in the larynx of males and females at 0.25 and 2.5 mg/m ³ , and tracheal inflammation in males and females at 2.5 mg/m ³ . Pneumonitis and bronchitis in the lung in males and females exposed to 2.5 mg/m ³ , at end of exposure period and recovery period. NOAEC = 0.0239 mg/m ³ . ¹²

Table 9. Short-Term Toxicity Studies

Ingredient	Animals	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	Groups of 8 (4 males, 4 females per group) SPF albino rats of the Alderley Park strain.	Five groups exposed (nose-only) to 0.025mg/m ³ , 0.25 mg/m ³ , and 2.75 mg/m ³ , 12.5 mg/m ³ , and 26 mg/m ³ , respectively, 6 h per day (5 days per week; 3 weeks total). Exposure to atmospheres of respirable particles (MMAD < 7 µm)	At 0.25 mg/m ³ : 1 rat died and signs of moderate nasal irritation and tachypnea in this group. Histopathological examination revealed: slightly-to-moderately severe pneumonitis; thymus glands of 3 male and 3 female rats with reduction in cortical thickness and depletion of lymphocytes. Patchy loss of cilia in tracheal epithelium of 3 rats. At 2.75 mg/m ³ , signs of nasal irritation and dyspnea. Histopathological examination revealed a moderate to severe pneumonitis. Thymus glands with severe depletion of lymphocytes and loss of normal architecture. At 12.5 and 26 mg/m ³ , all rats died. Severe nasal irritation and dyspnea at 12.5 mg/m ³ . NOAEC = 0.025 mg/m ³ . ¹²

Table 10. Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Oral Studies			
25% Polyaminopropyl Biguanide	Young adult SPF Wistar rats (25 males, 25 females/group)	90-day dietary study. Concentrations of 0 ppm, 2500 ppm, and 5000 ppm in diet.	No deaths during the 90-day feeding period. No gross abnormalities or abnormalities in hematological parameters. No remarkable changes in organ/body weight ratios. Microscopic examination revealed unusual degree of iron pigment in liver cells and in Kupffer cells for females fed 5000 ppm in the diet. Iron pigment not observed in liver of rats fed 2500 ppm in the diet (detailed histopathological results not included). Not possible to establish NOAEL. ¹⁹
25% aqueous Polyaminopropyl Biguanide	Alderley Park Wistar Rats (number of animals not stated)	90-day dietary study. Concentrations of 0, 625 and 1250 ppm active ingredient	No mortalities. At 1250 ppm, deposits of an iron-pigment in liver (in hepatocytes and Kupffer cells) observed in female rats. No toxicity findings after feeding with 625 ppm. ¹²
25% aqueous Polyaminopropyl Biguanide	Three groups of Beagle dogs (4 males, 4 females per group)	90-day dietary study. Concentrations of 0 ppm, 5500 ppm, and 11000 ppm	No adverse effects in treated or control animals. Results for hematological parameters and clinical blood chemistries unremarkable. Liver function test (for bromsulphthalein [BSP] retention) results indicated no test substance-related effect. No significant treatment-related variations in organ/body weight ratios or test substance-related gross pathology. Microscopic examination revealed slight hemosiderin deposits in 2 of 4 males fed 11000 ppm. NOAEL = 5500 ppm. ¹⁹
25% aqueous Polyaminopropyl Biguanide	Beagle dogs (inbred strain from Alderley Park, Cheshire). Groups of 4 dogs/sex/dose	90-day dietary study. Concentrations of 0, 1375 or 2750 ppm active ingredient as dietary admixture (no further explanation)	No mortalities or signs of clear systemic toxicity. ¹²
20.2% aqueous Polyaminopropyl Biguanide	Wistar -derived rats (Alpk:APfSD strain), 4 rats/sex/group	90-day dietary study. Concentrations: 0, 1000, 2000, 4000, and 6000 ppm active ingredient (corresponding to approximately 0, 83.9, 171.5, 373.0, 556.1 mg/kg/ day active ingredient in males and 92.3, 192.9, 409.8, 617.4 mg/kg /day active ingredient in females).	Beginning at 2000 ppm, increased hemoglobin and hematocrit in males. Kidney was target organ. Renal functional change in form of decreased urine volume and increased specific gravity at 2000, 4000 or 6000 ppm animals (more marked in males). Treatment-related increase in kidney weight apparent for males at 4000 ppm or 6000 ppm (toxicological significance not determined). NOAEL = 1000 ppm (corresponding to 83.9 mg/kg bw/day in male rats and 92.3 mg/kg /day in female rats). ¹²

Table 10. Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
20.2% aqueous Polyaminopropyl Biguanide	C57Bl/10JfCD-1 mice, 4 mice/sex/group	90-day dietary study. Concentrations: 0, 1000, 2000, 4000 ppm active ingredient (corresponding to about 0, 162, 328, 736 mg/kg/day active ingredient in males and 0, 224, 445, 963 mg/kg/day active ingredient in females) and 6000 ppm active ingredient	Marked toxicity at 4000 ppm. No treatment-related effects on liver and kidney weights and no gross or histopathological findings. NOAEL = 1000 ppm (corresponding to 162 mg/kg/day in male mice and 224 mg/kg/day in female mice) as NOAEL. ¹²
20% aqueous Polyaminopropyl Biguanide	Mice of the C57BL/10JfAP/Alpk strain. 2 groups of 10 males and 10 females (1 test and 1 control)	90-day drinking water study. Test group dosed with 0.1 mg/ml during 1 st week, 0.3 mg/ml during 2 nd week, and 0.3 mg/ml from 3 rd week until study termination.	Reduction in body weight gain and dose-related reduction in water consumption. No treatment-related macroscopic post-mortem findings. ²

Table 11. Chronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Dermal Study			
Polyaminopropyl Biguanide	Four groups of SPF Alderley Park mice (50 males, 50 females/group)	Test substance (0.3 ml) administered daily at following doses 5 days per week for 80 weeks: 0 (in ethanol), 0.6 mg (0.2% test substance in ethanol), 6.0 mg (20% test substance) and 30 mg (10% test substance in ethanol).	High mortality rate (75% in males and females) in 30 mg/day group at the end of the study, compared to ~30% in other groups. Exophthalmos observed throughout study; more severe in 30 mg group. Keratitis in many of affected animals. At week 80, exophthalmos incidence of 10% (6% for males and 13% for females). Clinical and histological examination of eyes and orbital contents revealed no evidence of pathological abnormalities. Gross and microscopic examinations of the thyroids normal in large majority of cases. Tissues from other organs were also examined microscopically. The SCCS noted that the highest dose administered in this study exceeded the maximum tolerated dose, and that the NOAEL was 0.6 mg/mouse/day (15 mg/kg/day). ^{2,19}
Oral Studies			
20.2% aqueous Polyaminopropyl Biguanide	Groups of 128 rats of the Alpk:APfSD (Wistar-derived) strain (64 males, 64 females per group)	Test substance administered in diet daily (for 104 weeks) at concentrations of 0 ppm, 200 ppm, 600 ppm, and 2000 ppm (corresponding to 0, ~12.1, ~36.3, and ~126.1 mg/kg/day (males) and 0, ~14.9, ~45.3, and ~162.3 mg/kg/day (females))	No treatment-related clinical signs, ophthalmoscopic findings, or effects on any hematological or urinalysis parameters throughout study. Slightly raised plasma alkaline phosphatase activity, predominantly in females receiving 2000 ppm, and a slightly increased incidence of hepatocyte fat and spongiosis hepatitis in males (at 2000 ppm). NOAEL = 2000 ppm., corresponding to 36 and 45 mg/kg/day for males and females, respectively. ¹²

Table 11. Chronic Toxicity Studies

Ingredient	Animals	Protocol	Results
20.2% aqueous Polyaminopropyl Biguanide	Groups of 8 Beagle dogs (4 males, 4 females per group)	Test substance administered daily (for 1 year) at dietary concentrations of 0 ppm, 300 ppm, 1500 ppm, and 4500 ppm (corresponding to 0, ~11, ~54, and ~169 or ~108 mg/kg/day) up to weeks 11 or 12, and the concentration was reduced to 3000 ppm thereafter.	Males dosed with 4500 ppm had marked reddening/peeling of scrotal skin, loss of appetite, body weight loss and/or indications of liver impairment in the form of elevated plasma alanine transaminase and/or aspartate transaminase activities. Low testes weight apparent in male survivor in 3000 ppm group. Treatment-related histopathological findings in skin (dermatitis of scrotum, chin and limbs) as well as in the liver, kidney (males only) and testes of animals that received 4500/3000 ppm. No treatment-related histopathological changes in dogs of 300 or 1500 ppm group. NOAEL = 1500 ppm. ¹²
20% Polyaminopropyl Biguanide	Groups of 30 male and 60 female SPF mice of the Alderley Park strain	Lifetime feeding study. 4 groups fed dietary concentrations of 0 ppm, 100 ppm, 200 ppm, and 1000 ppm, respectively, for 1 week prior to pairing and during mating. Feeding of females continued throughout pregnancy and lactation. All offspring were weaned at 3 weeks of age, and at 5 weeks of age, 50 males and 50 females were selected from each group. Offspring fed same diets as parents throughout study. Study terminated at 97 weeks after selection of the offspring, i.e., when the overall mortality had reached 80%.	After 18 months, mortalities in all groups comparable, though higher in males than in females. Increased liver weight in males and females fed 1000 ppm. For males fed 1000 ppm, mean spleen weight significantly higher when compared to controls; based on macroscopic examination of tissues, finding not test substance-related. Other non-neoplastic findings (specific findings not stated) also not test substance-related. ¹⁹
20% Polyaminopropyl Biguanide	Four groups of SPF rats of the Alderley Park strain (60 males, 60 females per group)	122-week study. Dietary concentrations of 0 ppm, 200 ppm, 1000 ppm, and 2000 ppm, respectively. Study terminated at 124 weeks, i.e., when 80% mortality occurred in control group and in experiment overall	Cumulative mortality comparable between control and treatment groups. Slight anemia at 104 weeks in female rats of 2000 ppm group. Other hematological parameters comparable among groups. At 52 weeks, females fed 2000 ppm had increased kidney weight. Increased adrenal weight reported for males and females of 1000 ppm and 2000 ppm groups. No treatment-related findings at necropsy. At 52 weeks, 104 weeks, and study termination, microscopic examination revealed increase in incidence of histiocyte conglomerates in mesenteric lymph nodes of female rats fed 1000 ppm and 2000 ppm. The NOEL (for histopathologic changes) = 200 ppm. ¹⁹

Table 11. Chronic Toxicity Studies

Ingredient	Animals	Protocol	Results
20% Polyaminopropyl Biguanide	Four groups of adult Beagle dogs (4 males, 4 females per group)	26-week study. Dietary concentrations of 0, 500, 1500, and 4500 ppm, respectively.	Treatment-related histopathological changes reported for sections of the liver and kidneys from dogs fed 4500 ppm: bile stasis, focal hepatocellular degeneration and necrosis, and focal proximal tubular nephrosis. Thus, feeding with dietary concentrations of 1500 ppm and 4500 ppm produced concentration-related hepatotoxicity and nephrosis. ¹⁹
Polyaminopropyl Biguanide	Strain not specified	Chronic toxicity study (protocol not described).	NOEL = 200 mg/kg/day. ²²
Polyaminopropyl Biguanide	Strain not specified	2-year chronic toxicity study (protocol not detailed). Dosage weight: 100 mg/kg/day	No adverse effects. ²²

Table 12. Exposure Concentrations and Margins of Safety (MOSS) for Hair Spray Products Calculated using the ConsExpo Web Model (ver. 1.0.1).²⁴

Exposure Scenario Assumptions (spraying towards person) and Spray Parameters not Specific to Spray Type ^a							
	Direction of spraying:	Towards exposed person	Room ventilation rate:	0.2/hr ^b			
	Exposure duration/event:	5 min	Cloud Volume:	0.0625 m ³			
	Room volume:	10 m ³	Density non-volatile:	1.5 g/cm ³			
	Room height:	2.5 m	Inhalation cut-off diameter:	15 µm			
Spray Parameters and estimates of Exposure Concentrations and MOSS Specific for Spray type							
Cosmetic spray type	Spray duration (sec)	Weight fraction of PHMB (%)	Mass generation rate (g/sec) ^e	Airborne fraction (g/g) ^e	Initial median aerosol droplet diameter (µm) (Coefficient of Variation) ^e	Mean event PHMB exposure concentration (mg/m ³) ^g	MOS (NOEC ^g /Mean event exposure concentration) ^h
Propellant hair spray	14.4 ^a	0.0004 ^d	0.4	0.2	46.5 (2.1)	0.00012	200
	14.4 ^a	0.00084	0.4	0.2	46.5 (2.1)	0.00024	100
Pump spray	14.4 ^c	0.053 ^d	0.1 ^f	0.02 ^f	2.7 (0.73) ^f	0.0022	11
	14.4 ^c	0.0058	0.1 ^f	0.02 ^f	2.7 (0.73) ^f	0.00024	100
Propellant deodorant spray	10.2 ^a	0.000055	0.45	0.9	8.3 (0.84)	0.00024	100

^adefault assumptions and values published by RIVM (Rijksinstituut voor Volksgezondheid en Milieu – Dutch National Institute for Health and Environment).^{25,27}^bdefault room ventilation rate specified in REACH guidance (Chapter R.15 Consumer exposure estimation, ECHA 2012), as noted in RIVM report.²⁷^cspray duration for pump hair sprays assumed to be the same as the default for propellant hair sprays^dconcentrations of use reported in PCPC Industry survey dated 11 April 2017.²⁸^emass generation rate, airborne fraction, and initial aerosol droplet diameters default assumptions published by RIVM.²⁶^fspray parameter default values developed for pump toilet water sprays assumed adequate for calculating conservative estimates of exposures from pump hair sprays^gexposure concentration averaged over the exposure duration^hno observed adverse effect concentration (NOEC) = 0.024 mg/m³ from study; rats exposed 6 h/day, 5 day/week for 28 days to aqueous aerosols (mass median aerodynamic diameter [MMAD] = 0.32-1.30 µm).²

Table 13. Developmental and Reproductive Toxicity Studies

Ingredient	Animals	Protocol	Results
20.2% aqueous Polyaminopropyl Biguanide	Groups of 52 rats (26 males, 26 females) of the Alpk:APfSD (Wistar-derived) strain.	Four groups received dietary concentrations of 0, 200, 600, and 2000 ppm (corresponding to 0, ~23-24, ~70-71, and ~239-249 mg/kg/day in (males), and to 0, ~25-26, ~77-79, ~258-270 mg/kg/day (females) through 2 successive generations (including a 10-week pre-mating period).	No evidence of an effect on reproductive parameters or on offspring growth and development at concentrations up to 2000 ppm. Systemic, parental NOAEL = 600 ppm. NOAEL for reproductive and offspring effects = 2000 ppm. ¹²
20.2% aqueous Polyaminopropyl Biguanide	Groups of 20 female New Zealand White rabbits	Four groups received oral dosages (by gavage) of 0, 10, 20, and 40 mg/kg/day on gestation days 8 through 20.	No effect on the number of fetuses, growth or survival in utero, except a slight increase in pre-implantation loss observed at 40 mg/kg/day (21.8 ± 25.6 vs 13.1 ± 15.2 in controls) and a significant increase in postimplantation loss at 20 mg/kg/day (11.4 ± 19.7 % vs 6.1 ± 8.4 % in controls) attributed to an increase in early intrauterine deaths. No evidence of teratogenicity. Percentage of fetuses with unossified 5 th sternebrae or with fused 4th and 5th sternebrae increased at 40 mg/kg/day, but results not considered test substance-related. Maternal NOAEL = 20 mg/kg/day. Developmental NOAEL = 40 mg/kg/day. ¹²
20% aqueous Polyaminopropyl Biguanide	Groups of 30 Sprague-Dawley rats (10 males, 20 females per group).	Four groups received dietary concentrations of 0, 200, 650, and 1300 ppm (dietary levels adjusted for 20% active ingredient) during the 9-week pre-mating period and until the 3 rd generation.	Evaluations of the various reproductive indices, sex ratios, and body weight data of fetuses taken by Caesarean section and the offspring maintained through weaning revealed no meaningful differences between the control and treated groups. Necropsy of weanlings did not reveal any compound-related gross pathology. No findings indicative of embryotoxicity or teratogenicity. NOAEC = 1300 ppm. ¹²
20% aqueous Polyaminopropyl Biguanide	Groups of 20 rats of the Alderley Park strain	Four groups received dietary concentrations of 0, 200, 1000, and 2000 ppm (expressed as active ingredient; corresponding to approximately 0, 13, 54, and 112 mg/kg /day) on gestation days 1 through 20 (mating day considered gestation day 0).	No mortalities and no adverse clinical effects in any group. No dose-related effects observed on fetal or litter weights. Increase in extra ribs at 2000 ppm considered consequence of maternal toxicity. No further test substance-related effect on fetal morphology, including ossification of the skeleton, in any of the test groups. Maternal NOAEC = 200 ppm. Developmental NOAEC = 1000 ppm. ¹²

Table 13. Developmental and Reproductive Toxicity Studies

Ingredient	Animals	Protocol	Results
20% aqueous Polyaminopropyl Biguanide (in 0.5% aqueous polyoxyethylene(20)sorbitan monooleate)	Groups of 47 to 49 mice of the Alderley Park strain. Group of 25 mice served as the control.	Four groups received (by gavage) 10, 20, or 40 mg/kg/day (expressed as active ingredient) on gestation days 6 through 15 (mating day considered gestation day 0).	<p>No mortalities or test substance-related adverse clinical signs. Gestational parameters such as implantation sites, pre- and post implantation loss, litter size and weight, resorptions not influenced by test substance at any dose. Indications of slight retardation of ossification from examination of forelimb and hindlimb digits and numbers of caudal vertebrae at 20 and 40 mg/kg/day. Maternal NOAEL = 40 mg/kg/day. Developmental NOAEL = 10 mg/kg/day.²</p> <p>Increased mortality (6 dams). No effect on number or growth or survival in utero, except slight increase, not statistically significant, in pre-implantation loss observed at 40 mg/kg/day (21.8 ± 25.6 vs. 13.1 ± 15.2 in controls) and significant increase in postimplantation loss at 20 mg/kg/day ($11.4 \pm 19.7\%$ vs. $6.1 \pm 8.4\%$ in controls), attributed to increase in early intrauterine deaths. Percentage of fetuses with unossified 5th sternebrae or with fused 4th and 5th sternebrae increased at 40 mg/kg/day, but not considered test substance-related. Maternal NOAEL = 20 mg/kg/day. Developmental NOAEL = 40 mg/kg/day.¹²</p>
20% aqueous Polyaminopropyl Biguanide	Four groups each of at least 21 pregnant mice of the Alderley strain	The following dosages were administered daily by gavage on gestation days 6 to 15: 0 (control), 10, 20, or 40 mg/kg.	<p>Litter and fetal parameters similar in all groups. Soft tissue anomalies unremarkable. Skeletal examinations revealed anomalies of the skull, sternebrae, and hindlimb. Incidences in the 3 dose groups were double those noted in control group. Based on these results, retardation of ossification observed in each dose group considered by the authors to be marginal. No effect-level for delayed ossification was not established. Test substance was classified as non-teratogenic.¹²</p>

Table 13. Developmental and Reproductive Toxicity Studies

Ingredient	Animals	Protocol	Results
20% Polyaminopropyl Biguanide	Four groups of Sprague-Dawley albino rats (10 males and 20 females/group).	Three-generation reproduction study. 4 groups received test substance at dietary concentrations of 0, 200, 650, and 1300 ppm for 9 weeks, through 3 successive generations.	No effects attributable to test substance observed in parental food consumption values, survival rates, clinical findings, pregnancy rates, or reproduction data. No meaningful differences between treated and control groups with respect to various reproductive indices, sex ratios, and body weight data for the fetuses. Necropsy of weanlings did not reveal test substance-related gross pathology. No findings indicative of embryotoxicity or teratogenicity. NOEL = 1300 ppm. ¹⁹
0.04% Polyaminopropyl Biguanide	Animal strain not specified.	Oral dosing (test protocol not included)	Embryotoxic at 32 mg/kg/day. ²²
Polyaminopropyl Biguanide	Rats (number and strain not specified)	Rats dosed orally with 100 mg/kg/day	Embryotoxic. ²²
Polyaminopropyl Biguanide	Rats (number and strain not specified)	Rats dosed intraperitoneally with 10 mg/kg/day	Teratogenic. ²²
20% aqueous Polyaminopropyl Biguanide	Groups of 8 (4 males, 4 females per group) SPF albino rats of the Alderley Park strain	In short-term toxicity study, 5 groups exposed (nose-only) to concentrations of 0.025, 0.25, 2.75, 12.5, and 26 mg/m ³ , respectively, 6 h per day (5 days per week; 3 weeks total).	At 0.25 mg/m ³ , degeneration of a few seminiferous tubules in testis of 1 male rat. ¹²

Table 14. Genotoxicity Studies

Ingredient	Strain/cell type	Assay	Dose/Concentration	Results
<i>In Vitro</i>				
20% aqueous Polyaminopropyl Biguanide	<i>Salmonella typhimurium</i> strains: TA98, TA100, TA1535, TA1537, and TA1538	Ames test, with and without metabolic activation	333.3 mg (333,300 µg) per plate	Toxic at 333.3 mg per plate, particularly in strains TA98, TA100, and TA1535. Weakly genotoxic in strain TA1538 without metabolic activation. ¹²
20% aqueous Polyaminopropyl Biguanide	<i>Salmonella typhimurium</i> strains: TA98, TA100, TA1535, TA1537, and TA1538	Ames test, with and without metabolic activation	Doses up to 500µg/plate	Non-genotoxic. ¹²
19.6% aqueous Polyaminopropyl Biguanide (in DMSO)	<i>Salmonella typhimurium</i> strains: TA98, TA100, TA1535, TA1537, and TA1538.	Ames test, with and without metabolic activation	Doses up to 5000 µg/plate	Non-genotoxic, with or without metabolic activation in all but one strain. In strain TA98, negative results without metabolic activation, but slight responses (2.1 x background) observed with metabolic activation. Non-genotoxic. ¹²
20% aqueous Polyaminopropyl Biguanide	L5178Y TK+/- mouse lymphoma cells	Mouse lymphoma assay, with and without metabolic activation	Concentrations up to 100 µg/ml	At 50 and 100 µg/ml, cytotoxicity higher than that of positive controls. Non-genotoxic. ¹²
20% aqueous Polyaminopropyl Biguanide	P388 (tk+/-) mouse lymphoma cell line	Mouse lymphoma assay, with and without metabolic activation	Concentrations up to 2000 µg/ml	2000 µg/ml was cytotoxic and clear cytotoxicity noted at 1000 µg/ml, with and without metabolic activation. Non-genotoxic. ¹²
19.6% aqueous Polyaminopropyl Biguanide	Cultured human peripheral blood lymphocytes from 2 volunteers	Micronucleus test	Concentrations up to 50 µg/ml without metabolic activation and concentrations up to 250 µg/ml with metabolic activation.	No chromosomal aberrations. Non-genotoxic. ¹²
<i>In Vivo</i>				
19.6% aqueous Polyaminopropyl Biguanide	1000 polychromatic erythrocytes (from C57BL/6JfCD-1/Alpk mice) scored for presence of micronuclei	Micronucleus test.	Groups of 10 mice. Test substance administered (single dose, by gavage) at 0, 250, and 400 mg/kg (dosage volume = 10 ml/kg).	Non-clastogenic. ¹²

Table 14. Genotoxicity Studies

Ingredient	Strain/cell type	Assay	Dose/Concentration	Results
19.6% aqueous Polyaminopropyl Biguanide	Alpk:APfSD (Wistar-derived) rat hepatocyte cultures exposed to [³ H]-thymidine	Unscheduled DNA synthesis assay	Test substance administered (single dose, by gavage) to 2 - 3 males per dose at 0, 750, and 1500 mg/kg (dosage volume = 10 ml/kg) for 4 h or 12 h.	No induction of unscheduled DNA synthesis. ¹²

Table 15. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
In Vitro Studies			
20% aqueous Polyaminopropyl Biguanide (in DMSO)	Baby hamster kidney fibroblasts (BHK21/C13)	Cell transformation assay, with metabolic activation. Test substances dose range of 0.25 - 2500 µg/ml and 25 -3000 µg/ml in separate experiments.	Cytotoxicity at 250 µg/ml and greater. No difference in number of transformed cell colonies between test and negative control cultures. Test substance did not induce cell transformation. ¹²
Polyaminopropyl Biguanide (up to 1 ppm)	RAW 264.7 mouse macrophages co-cultured with SVEC4-10 mouse endothelial cells.	Experiment 1: Preactivation of macrophages with Polyaminopropyl Biguanide (0, 0.75, and 1 ppm) or lipopolysaccharide (LPS) and/or co-culture in the presence of Polyaminopropyl Biguanide. Endothelial proliferation analyzed by incorporation of bromodeoxyuridine (BrdU). Experiment 2 summarized below.	Polyaminopropyl Biguanide had no direct effect on liver endothelial cell proliferation and did not potentiate cell proliferation induced by LPS-activated macrophages. ²
Polyaminopropyl Biguanide (up to 1 ppm)	RAW 264.7 mouse macrophages	Reactive oxygen species (ROS) assay. Macrophages cultured with Polyaminopropyl Biguanide (0, 0.75, and 1 ppm). Production of ROS in macrophages detected by measurement of fluorescence intensity after addition of dihydrorhodamine and by evaluation of tumor necrosis factor (TNF) α and interleukin (IL)-6 in cell culture medium, as quantified by the enzyme-linked immunosorbent assay (ELISA).	No activation of macrophages. ²
Dermal Studies			
Polyaminopropyl Biguanide (up to 20% aqueous)	Four groups of SPF mice (50 males, 50 females/group) of the Alderley Park strain (Alpk:APfCD-1 strain)	Test substance (0.3 ml) was administered dermally (non-occluded) at the following doses 5 days per week for 80 weeks: 0 (in ethanol), 0.6 mg (0.2% Polyaminopropyl Biguanide in ethanol), 6.0 mg (20% Polyaminopropyl Biguanide and 30 mg (10% Polyaminopropyl Biguanide in ethanol). The 0, 0.6, 6, and 30 mg doses corresponded to 0, ~15, ~150, and ~750 mg/kg/day.	Incidence of clinically-observed skin tumors: control (1 male, 6 mg of 20% concentration (2 males), and 30 mg/day of 10% concentration (1 male and 2 females). Liver + kidney tumors contributed more than 50% of total for the 30 mg/day group. Total number of kidney + liver tumors: control (5 males, 2 females), 0.6 mg/day group (4 males, 4 females), 6 mg/day group (5 males, 4 females), and 30 mg dose group (16 males, 7 females). Statistically significant increase in incidence of liver tumors (4 in controls and 10 in 30 mg/day group; statistically significant (Chi square, 1% level) only in case of liver tumors of endothelial origin (both benign and malignant; 2 in controls and 6 in 30 mg/day group). Many growths observed microscopically classified as moderate to severe hepatitis at histopathologic examination. Liver necrosis in all dose groups. Test substance classified as hepatic oncogene in mice dosed with 30 mg/day. ¹⁹

Table 15. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
			High mortality (76-78 % of animals died) in 30 mg group. Variety of inflammatory hepatic changes in all groups, including controls. Severe hepatitis in some animals (number not stated) of 30 mg/day group. Slight increase in incidence of liver tumors observed at 30 mg/day (4 in the control; 10 in 30 mg group); statistically significant only in case of liver tumors of endothelial origin (both benign and malignant; 2 in control and 6 in 30 mg group). NOAEL = 0.6 mg/day. ¹²
Oral Studies			
20.2% aqueous Polyaminopropyl Biguanide	Groups of 110 mice (55 males, 55 females) of the C57Bl/10J/CD-1 Alpk strain.	4 groups received dietary concentrations of 0, 400, 1200, and 4000 ppm (0, ~55, ~167, and ~715 mg/kg/day, respectively) for 2 years	Mortalities increased in the 3000 ppm group; hemangiosarcoma was most frequent factor causing death. At 4000 ppm, increases in squamous cell carcinomas of the recto-anal junction (5 males and 8 females); also, in 1 male, 1 adenocarcinoma at same site and a squamous cell carcinoma of the skin adjacent to the anus. Gall bladder papillomas in males at 4000 ppm. Highest incidence of treatment-related tumors at 4000 ppm was in neoplasms of vascular origin (i.e., hemangiosarcomas, common tumor in C57Bl/10J/CD-1 Alpk mice). Hemangiosarcoma and hemangioma incidences (in liver and other sites) at 4000 ppm were above control incidence; findings statistically significant in male mice only. Small increased incidence of hemangiosarcomas in 1200 ppm group. Some evidence of carcinogenicity. ¹²
20.2% aqueous Polyaminopropyl Biguanide	Groups of 30 male and 60 female Swiss-derived albino mice	Four groups fed diets containing 0, 500, 1000 or 5000 ppm (equivalent to 0, 100, 200 and 1000 ppm active ingredient, respectively) for 1 week prior to pairing and during mating. Offspring fed same diets as parents throughout experiment	Study terminated when overall mortality reached 80 % at 97 weeks. High mortality due to fighting of males. No treatment-related (non-neoplastic or neoplastic) increases in histopathologic findings. However, regarding vascular tumors of concern, there were some animals with hemangiomas or hemangiosarcomas in the liver or at other sites. According to the SCCS, these data were considered to be of low reliability due to high mortality. ¹²

Table 15. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
20.2% aqueous Polyaminopropyl Biguanide	Groups of 30 male and 60 female SPF mice of the Alderley Park strain	Four groups fed dietary concentrations of 0, 100, 200, or 1000 ppm for 1 week prior to pairing and during mating. Feeding of females continued throughout pregnancy and lactation; offspring fed same diet as parents throughout study	Study terminated at 97 weeks, when overall mortality reached 80%. Number of tumor-bearing animals: control (39 [18 males, 21 females]), 100 ppm (36 [16 males, 36 females]), 200 ppm (42 [17 males, 25 females]), and 1000 ppm (44 [23 males, 21 females]). Liver neoplasms observed only in male mice and incidence was: control (2/39 = 5.1%), 100 ppm (2/36 = 5.5%), 200 ppm (5/42 = 11.9%), and 1000 ppm (9/44 = 20.9%). Dose-related tumor incidence in liver. ¹⁹
20.2% aqueous Polyaminopropyl Biguanide	Groups of 60 male and 60 female rats of unspecified strain	4 groups fed at concentrations of 0, 200, 1000 and 2000 ppm	Study terminated at 124 weeks, due to 80% mortality. 2 outbreaks of infection noted. Long-term exposure unrelated to carcinogenic and other effects. Hemangiomas at week 52 in 1/12 male rats (mesenteric lymph nodes) fed 200 ppm and 1/12 male rats fed 200 ppm (cervical lymph nodes). Hemangiomas at week 104 in 2/12 males fed 1000 ppm (mesenteric lymph nodes) and in 1/8 females fed 200 ppm (uterus). Hemangiosarcoma at week 104 in 1/21 males fed 2000 ppm (mesenteric lymph nodes). Hemangiomas at week 124 (end of study) in 1/20 males fed 1000 ppm (mesenteric lymph nodes) and in 1/19 males fed 2000 ppm (spleen). No vascular tumors in controls. Study of questionable reliability due to infections and < 50% survival at end of study. ¹²
20.2% aqueous Polyaminopropyl Biguanide	Wistar rats (20 males, 20 females)	Oral dosage rates 100 mg/kg/day for 25 months	No findings of clinically apparent tumors. Testicular tumor in 1 male. Mammary tumor (benign adenofibroma) in 1 female. Classified as inadequate study for various reasons, including that only 20 rats per sex, no controls, and only 1 dose tested. ¹⁹
20% Polyaminopropyl Biguanide	SPF rats (60 males, 60 females per group) of the Alderley Park strain	Four groups fed dietary concentrations of 0, 200, 1000, and 2000 ppm, for 122 weeks.	Study terminated at 124 weeks, i.e., due to 80% mortality overall. Accumulative incidence of animals with suspected mammary tumors was comparable between control and treatment groups. Same was true for the number of tumor-bearing animals and the site and incidence of tumors. Non-oncogenic. ¹⁹

Table 15. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
Polyaminopropyl Biguanide	Groups of 5 male C57Bl mice	Concentrations of 0, 100, 200, 400, 1200, and 4000 ppm in diet for 7, 14, or 28 days. Immunohistochemical detection of BrdU in mouse liver used to quantify cell proliferation in liver endothelial cells. Liver hepatotoxicity assessed by measuring alanine aminotransferase and aspartate aminotransferase in plasma of animals killed	Polyaminopropyl Biguanide increased cell proliferation in concentration-related manner at 1200 ppm and 4000 ppm. Cell proliferation also increased at 1200 ppm after feeding for 14 days. Plasma endotoxin, known activator of macrophages, increased at 1200 and 4000 ppm (after feeding for 28 days) and at 100 and 200 ppm (after feeding for 14 days). ²
Polyaminopropyl Biguanide	Groups of Wistar-derived Alpk:ApfSD rats	Concentrations of 0, 200, 600 or 2000 ppm (approximately equivalent to 0, 12.1, 36.3 and 126.1 mg/kg/day in males and 0, 14.9, 45.3 and 162.3 mg/kg/day in females) in diet for 2 years.	Hemangioma (2/64 males and 2/64 females) and hemangiosarcoma (1/64 females) in the liver of one animal fed 2000 ppm. ²⁹

Table 16. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
Irritation Studies			
<u>Animal Studies</u>			
Polyaminopropyl Biguanide	5 male New Zealand White rabbits	Test substance (0.5 g, moistened with distilled water) applied to 3 sites on back (mg/cm ² not stated); sites covered with cotton gauze patch secured with adhesive tape. Patches removed after 3 minutes, 1 h, or 4 h.	Slight edema at 1 h after patch removal and very slight edema at 24 h and 48 h. After 4 h, very slight to well defined erythema; primary irritation index (PII) = 1. Mean values (at 24 h, 48 h, and 72 h) for erythema, eschar formation or edema formation calculated for each animal tested were ≤ 1 . No skin reactions after 7 days. Mild skin irritant. ¹²
Polyaminopropyl Biguanide (96%, as powder)	3 female rats (strain not specified)	Test substance (0.5 g moistened with 0.5 ml water) applied under occlusive patch to 3 sites on back of 1 rabbit; mg/cm ² not stated. Patches removed after 3 minutes, 1 h, or 4 h. For remaining 2 rabbits, patch remained in place for 4 h.	No irritation after 3-minute or 1-h application. After 4-h exposure, primary irritation index of 1 reported; very slight (at 1 h, 48 h, and 72 h after patch removal) to well-defined (at 4 h and 24 h) erythema observed. Slight edema (at 1h) and very slight edema (at 24 h and 48 h). No reactions at 7 days after patch removal. Mild skin irritant. ²
25% aqueous Polyaminopropyl Biguanide	3 female rats (strain not stated)	Test substance applied (dose not specified) under occlusive dressing to intact skin of back for 3 alternating 24-h periods, i.e., each application period followed by 24-h non-treatment period.	Focal ulceration observed after first 24-h application. Reaction increased in severity after 2 nd and 3 rd applications, by which time there was pronounced edema. ¹⁹
25% aqueous Polyaminopropyl Biguanide	2 groups of 20 (10 males, 10 females/group) healthy SPF albino rats	2 groups received a topical application of test substance to intact skin at dosages of 2.5 ml/kg and 5 ml/kg, respectively. Test substance spread over 1 inch ² area; site covered with dressing for 24 h.	Severe skin irritation in all animals. ¹⁹
25% aqueous Polyaminopropyl Biguanide	Albino guinea pigs (6 test and 4 control) of Porton strain	Both ears treated (patch application; 0.1 ml per ear) with 25% Polyaminopropyl Biguanide once per day for 3 consecutive days. Next, 0.2 ml of following concentrations (in dimethylformamide) applied to flank (1-cm diameter area): 25% , 12.5%, and 10%	Slight to moderate erythema (irritant effect) on ear at 25%. ¹²
20.2% aqueous Polyaminopropyl Biguanide	Groups of 10 rats (5 males, 5 females per group) of the AlpK:APfSD (Wistar-derived) strain	3 groups received applications (occlusive, on the back) of the test substance at doses of 20 mg/kg/day, 60 mg/kg/day, and 200 mg/kg/day, respectively, 6 h per day for 30 days (21 applications total).	Slight irritation at 60 mg/kg/day; in most animals, had regressed by end of study. Moderate irritation in all animals at 200 mg/kg/day; in most animals, persisted until end of study. Skin irritation observed was confirmed microscopically and considered test substance-related. ¹²

Table 16. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	9 (3 males, 6 females) New Zealand White rabbits	Test substance applied to 6 rabbits (0.5 ml, under occlusive dressing) for 24 h to ~ 6.25 cm ² area of intact and abraded skin of the flanks. Similar application to 3 male rabbits; animals then killed at 48 h or 72 h post-application for histopathologic examination of test site.	Moderately irritating to intact skin. Severely irritating to abraded skin. ¹²
20% aqueous Polyaminopropyl Biguanide	6 New Zealand White rabbits	Skin corrosivity test. Applied to intact and abraded skin (mg/cm ² and duration of application not stated).	Superficial scabbing and erythema around the abrasions. No signs of necrosis at intact skin sites. Non-corrosive. ¹²
20% aqueous Polyaminopropyl Biguanide	6 female albino rabbits	12,000 ppm solution (1 ml) applied to back for 23 h (mg/cm ² not stated; no occlusion). 21 daily applications.	Non-irritant. ¹²
20% aqueous Polyaminopropyl Biguanide	5 female rats of the Alderley Park strain	Test substance (0.04% active ingredient) applied (0.1 ml; mg/cm ² not stated) to the back on alternate days (6 applications total). Test site remained uncovered or was covered with polyethylene, secured with an adhesive plaster, for 24 h.	Non-irritant. ¹⁹
20% aqueous Polyaminopropyl Biguanide	3 rabbits (strain not specified)	Applied to skin for 24 h (mg/cm ² not stated).	Moderate erythema at 24 h post-application. Completely reversible within 8 days. No edema. ¹²
Polyaminopropyl Biguanide (0.2% in ethanol, 10% in ethanol and 20% [solvent not specified])	4 groups of SPF Alderley Park mice (50 males, 50 females)	Test substance (0.3 ml) was administered at the following doses 5 days per week for 80 weeks: 0 mg/day (in ethanol), 0.6 mg/day (0.2% Polyaminopropyl Biguanide in ethanol), 6.0 mg/day (20% Polyaminopropyl Biguanide and 30 mg/day [10% Polyaminopropyl Biguanide] in ethanol).	The highest dose (10% concentration; 30 mg/day) caused noticeable skin irritation in males and females immediately after application. Erythema observed during first few weeks. After 4 th week, hyperkeratosis became evident, especially in males. Also, occasionally, there was ulceration extending to the deeper layers of the dermis at the application site. ¹⁹

Table 16. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
<u>Human Studies</u>			
20% aqueous Polyaminopropyl Biguanide	45 volunteers (17 males, 28 females)	Following concentrations (in purified water) applied topically (Finn chamber) for 24 h to medial surface of upper arm: 0.3%, 0.6%, and 1.5% active ingredient.	Plaster dermatitis observed in all test groups, including vehicle controls. Skin irritation indices of 6.6, 5.5, 5.5 and 8.8 obtained for concentrations of 0 (vehicle control), 0.3, 0.6 and 1.5 % active ingredient. Not a primary skin irritant, given the similarity of skin irritation indices between test and control groups. ¹²
Bacterial nanocellulose dressing loaded with 1% w/v sericin and 0.3% w/v Polyaminopropyl Biguanide	105 healthy volunteers	Initially, skin randomly patched with dressings (2 cm x 2 cm area). After 3 days, new dressings patched onto same area. After an additional 3 days, dressings removed; removal followed by 7- to 10-day non-treatment period. Skin then patched (open and closed patch tests) with dressings on same area. After 3 days, dressings removed.	Majority of test sites did not show edema (more than 98 %) or papules (more than 97 %). Neither vesicles nor bullae were observed on the skin. Dressing classified as non-irritating to the skin. ³³
Sensitization Studies			
<u>In Vivo Assay</u>			
Polyaminopropyl Biguanide		Local lymph node assay (Unilever unpublished data, protocol details not provided).	Positive results. ^{37,41}
<u>Animal Studies</u>			
20.2% aqueous Polyaminopropyl Biguanide	20 female Alpk:Dunkin Hartley guinea pigs (test group) and 10 female guinea pigs (control group)	Guinea pig maximization test. Induction phase: intradermal induction (0.3 % of test substance as delivered [0.06 % active ingredient], 0.1 ml in shoulder region). One week later, dermal induction performed by occlusively applying neat substance (20.2 % active ingredient) to induction sites for 48 h. Challenge: occlusive epicutaneous application (24 h) of undiluted test substance (20.2% active ingredient) and a 30% solution in deionized water (6 % active ingredient) to previously untreated site	Scattered mild redness or moderate diffuse redness observed in 18/20 test animals at 24 h and 16/20 test animals at 48 hr. Moderate sensitizer. ¹²
20.2% Polyaminopropyl Biguanide (in saline)	Groups of 10 guinea pigs	Guinea pig maximization test. Intradermal induction with 0.15% Polyaminopropyl Biguanide and topical induction with 20%. Challenge with 20% or 10%	Moderate erythema at 10% and 20% (1 animal per concentration). Non-sensitizer. ¹²
20% aqueous Polyaminopropyl Biguanide	20 Alderley Park female guinea pigs (test animals) and 8 female guinea pigs (controls)	Guinea pig maximization test. Intradermal induction (in scapular region) with 1% of test substance as delivered (0.2% active ingredient). Topical induction and challenge with 20.2 % active ingredient	Mild to moderate erythema in 14 of 20 animals (at 24 h) and in 15 of 20 animals (at 48 h). Moderate to strong sensitizer. ¹²

Table 16. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	Female Dunkin Hartley guinea pigs (20 test and 8 control animals).	Guinea pig maximization test. Possible cross-reactivity with chlorhexidine also evaluated. Intradermal induction with 0.25%. Topical induction and challenge with 20% Polyaminopropyl Biguanide. Challenge with 0.05 %, 0.5 % and 4 % chlorhexidine gluconate	Challenge reactions to 20% in 8 of 20 animals. Reactions in 3 of 20 at rechallenge. No cross-reactivity with chlorhexidine. Test substance was mild sensitizer. ¹²
20% aqueous Polyaminopropyl Biguanide	10 Alderley Park guinea pigs (test animals) and 10 control guinea pigs.	Buehler test. Concentration of 10% (2% active ingredient, 0.4 ml) applied to scapular region (400 mm ²) during topical induction (occlusive dressing) for 6 h. Induction repeated 3 times/week for 3 weeks (10 applications total). Challenge exposures (2 % active ingredient) of 6 h performed 2 weeks after last induction exposure. Rechallenge with concentrations of 20%, 10% and 1% (4%, 2%, and 0.2% active ingredient, respectively).	Faint erythema in 6 of 10 test animals. Rechallenge yielded faint erythema at concentrations of 4% (8 of 9 animals) and 2% (3 of 10 animals) active ingredient. No reaction to 0.2% active ingredient. 2% active ingredient considered moderate sensitizer. ¹²
20% aqueous Polyaminopropyl Biguanide	Groups of 20 (10 males and 10 females per group) guinea pigs	Buehler test. Induction and challenge concentrations: induction (0.3%) and challenge (0.3%, 0.15%, 0.075%, and 0.03%); induction (0.8%) and challenge (0.8%, 0.4%, 0.2%, and 0.08%); induction (1.3%) and challenge (1.3%, 0.65%, 0.325%, and 0.13%); induction (1.8%) and challenge (1.8%, 0.9%, 0.45%, and 0.18%); induction (2%), challenge (2%), and rechallenge (2%); 1.2% induction, challenge (1.2%), and rechallenge (1.2% and 15%); and induction (5%), challenge (15%), and rechallenge (2% and 1.2%).	Threshold for eliciting sensitization in guinea pigs was approximately 1%. ¹²

Table 16. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
Human Studies			
20% aqueous Polyaminopropyl Biguanide	191 volunteers (49 on Panel 1, 114 on Panel 2, and 28 on Panel 3)	During induction, test substance applied (2 cm x 2 cm patches moistened with 0.5 ml aliquots) for 24 h to dorsal surface of upper arm at concentrations of 2% and 4% active ingredient. Repeated 3 times per week for 10 applications total. Applied at following concentrations during challenge phase: 0.05%, 0.1%, 0.2%, 0.5%, 1% and 2% active ingredient.	Panel 1: At challenge, 8 of 49 subjects (16%) had skin reactions to 2 %, 7 of 49 (14%) with reactions to 1% and 0.5 %, and 2 of 49 (4%) with weak reactions at 0.1%. Panel 2: 18 of 114 subjects (16%) with skin reactions to 0.5% and 7 of 114 (6%) with reactions to 0.2%. 2 other subjects with reactions during non-treatment period following 2% induction, characterized as likely allergic to 2%. Same true for 10 other subjects regarding reactions (described as weak) at late 2% induction. Panel 3: 1 of 28 subjects (3.6%) with reaction to 0.5%. Conclusion: 2% concentration not capable of causing primary skin irritation, but capable of causing skin sensitization humans. ²
Leave-on product containing 0.1 % Polyaminopropyl Biguanide (0.5% of a trade name material containing 20% Polyaminopropyl Biguanide)	207 subjects	In HRIPT, product (0.1 g on a 2 cm x 2 cm occlusive patch) applied to skin (48-h to 72-h application) at dose density of 25 mg/cm ² . Dose density of Polyaminopropyl Biguanide applied to skin calculated to be 0.025 mg/cm ² (25 µg/cm ²). 3-week induction period followed by 2-week non-treatment period. Challenge patch applied to a new test site. Reactions scored at 24 h, 48 h, 72 h, and 96 h.	Product did not induce dermal sensitization. ³⁹
Neck cream containing 0.2% Polyaminopropyl Biguanide	115 male and female subjects	During induction, product applied (2 cm x 2 cm occlusive patches containing 0.2 ml of product) for 24 h to upper back. Repeated 3 times per week for 3 weeks. Challenge patch applied for 24 h to new site on opposite side of upper back	Transient, barely perceptible to mild erythema in 43 of 115 subjects (37% of subjects tested) during induction and/or challenge phases. No evidence of clinically meaningful irritation, and no reactions allergic in nature. ³⁸
Patients			
20% aqueous Polyaminopropyl Biguanide	1554 male and female patients	Multicenter study. Patch tests (performed in accordance with recommendations of the International Contact Dermatitis Research Group [ICDRG] and the German Contact Dermatitis Research Group [DKG]) on 2.5% aqueous test substance (effective concentration = 2.5% x 20% = 0.5%). Applied to 389 patients for 1 day and to 1165 patients for 2 days.	6 patients (0.4%) with positive (+) reaction. One of the reactions in patient with atopic dermatitis may have been a false positive. Polyaminopropyl Biguanide sensitization considered extremely rare. ³⁵

Table 16. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	1975 patients	Multicenter study. Patch testing with 2.5% aqueous (effective concentration = 2.5% x 20% = 0.5%) and 5% aqueous (effective concentration = 5% x 20% = 1%). Frequencies of sensitization (as % of patients tested) calculated as crude proportions and additionally standardized for sex and age.	10 patients (0.5 %) with positive reaction 0.5% and 16 patients (0.8%) with positive reaction to 1%. Assumed that, probably, at least 4 reactions at 0.5% may have been doubtful or irritant, i.e. false positive, because were not confirmed by simultaneous reactions to higher concentrations. Probable cause of sensitization was occupational exposure. Other risk factors included leg dermatitis and old age. ³⁶
2.5% aqueous Polyaminopropyl Biguanide	374 patients (multicenter study in United Kingdom)	Patch test (protocol not described)	2 positive patch test reactions. Data series suggested that baseline frequency of Polyaminopropyl Biguanide sensitization was very low (0.5%) in United Kingdom. Majority of reactions were weak, and data suggested that Polyaminopropyl Biguanide may not be a relevant contact allergen. ^{34,40}
2.5% aqueous Polyaminopropyl Biguanide	1554 patients (multicenter study in Germany)	Patch test (protocol not)	6 positive patch test reactions. Data series suggested that baseline frequency of Polyaminopropyl Biguanide sensitization was very low (0.4%) in Germany. Majority of reactions were weak, and data suggested that Polyaminopropyl Biguanide may not be a relevant contact allergen. ^{35,40}
2.5% aqueous Polyaminopropyl Biguanide	1974 patients (multicenter study)	Patch tests (performed in accordance with recommendations of the ICDRG and the DKG)	9 patients (0.5%) with positive patch test reactions. Majority of reactions were weak. No evidence of axillary dermatitis. Occupational exposure considered most probable cause of sensitization. ^{36,40}
Phototoxicity/Photosensitization Studies			
<u>Animal Study</u>			
20% aqueous Polyaminopropyl Biguanide	10 male rats	2 concentrations of test substance (in distilled water) evaluated: 10% (effective concentration = 10% x 20% = 2%) and 25% (25% x 20% = 5%). Each test concentration (0.1 ml) applied to dorsal skin once daily for 4 days. Site irradiated with UVC (black lamp) for 3 h daily.	Very strong irritant potential, but no significant photoirritancy. ¹⁹

Table 16. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
<u>Human Study</u>			
20% aqueous Polyaminopropyl Biguanide	26 male and female subjects	Diluted test substance (1:20 in water; effective concentration = 1%; dose = 1 mg/cm ²) evaluated. Patches (20 mm x 20 mm square of Webril affixed to 40 mm x 40 mm adhesive square) moistened with 0.4 ml of the test substance. Patches applied to upper arm for 24 h, 3 times per week for 4 successive weeks. Immediately after patch removal, sites exposed to direct rays of mid-day sun for 1 h. Challenge application at week 6.	Test substance (at 1%) essentially non-irritating and did not induce sensitization, phototoxicity, or photoallergenicity. ^{19,42}

Table 17. Ocular Irritation Studies

Ingredient	Number of Animals	Test Protocol	Results
<u>In Vivo Studies</u>			
Polyaminopropyl Biguanide (powder form, 99.6% pure)	1 New Zealand rabbit	Test substance (0.1 g) instilled into 1 eye.	Moderate redness, chemosis, moderate corneal opacity, iridial congestion, and ulceration of the nictitating membrane and cornea. Severe ocular irritant. ²
Polyaminopropyl Biguanide (undiluted)	1 male New Zealand White rabbit	Test substance (0.1 ml) instilled into conjunctival sac of right eye; untreated eye served as control. Eye not rinsed after instillation.	Opalescent corneal opacity, iridial inflammation, and severe conjunctival irritation observed initially. Translucent corneal opacity, minimal conjunctival irritation and vascularization were noted at day 21 post-instillation and considered irreversible reactions. Test substance was corrosive to rabbit eye. ²
25% aqueous Polyaminopropyl Biguanide	3 rabbits (strain not specified).	Single instillation (volume not specified). Procedure repeated with saline rinse after instillation	Severe inflammation and corneal damage in all rabbits (unrinsed eyes). Condition partly resolved in 2 rabbits. 3 rd rabbit blinded in treated eye. In rinsed eyes, only slight inflammation observed; eyes normal by day 3. ¹⁹
20% aqueous Polyaminopropyl Biguanide	9 female New Zealand White rabbits	Test substance (0.1 ml) instilled into conjunctival sac of 1 eye; contralateral eye served as untreated control. Eyes of 6 animals not rinsed after instillation. Eyes of remaining 3 animals were rinsed.	Iritis and conjunctivitis in unrinsed eyes and 4/6 rabbits with transient corneal opacity. Conjunctivitis, but no corneal reaction, in rinsed eyes and slight iritis in 1 rabbit. Test substance was moderate eye irritant in unrinsed eyes and a mild irritant in rinsed eyes. ²
20% Polyaminopropyl Biguanide	3 rabbits (strain not stated)	Test substance (0.12 ml) instilled into 1 eye, followed by rinsing with saline	Slight inflammation, but no corneal ulceration. Changes resolved in 10 days. ¹⁹
20% Polyaminopropyl Biguanide	3 rabbits (strain not stated)	Test substance (diluted to 0.04% active ingredient; 0.1 ml) instilled into eyes	No immediate or delayed irritant effects observed. ¹⁹
<u>In Vitro Study</u>			
20% aqueous Polyaminopropyl Biguanide	Donated human eyes (41) and rabbit eyes	Applied (20 µl for 10 seconds; 100 µl for 1 minute) at superior limbus. Eyes situated in temperature-controlled chamber during application.	1-minute exposure did not cause change in corneal thickness. Normal corneal morphology at histological examination. ⁴³

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Final Report of the Cosmetic Ingredient Review Expert Panel on the Safety Assessment of Cocamidopropyl betaine (CAPB)

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Christina L. Burnett¹, Wilma F. Bergfeld², Donald V. Belsito²,
Ronald A. Hill², Curtis D. Klaassen², Daniel Liebler²,
James G. Marks Jr², Ronald C. Shank², Thomas J. Slaga²,
Paul W. Snyder², and F. Alan Andersen³

Abstract

Cocamidopropyl betaine (CAPB) and related amidopropyl betaines are zwitterions used mainly as surfactants in cosmetics. These cosmetic ingredients are similar in their chemistry, in particular with respect to the presence of 3,3-dimethylamino-propylamine (DMAPA) and fatty acid amidopropyl dimethylamine (amidoamine) impurities, which are known as sensitizers. The CIR Expert Panel concluded that because these ingredients present no other significant toxicity, when formulated to be nonsensitizing (which may be based on a quantitative risk assessment), these ingredients are safe for use as cosmetic ingredients in the practices of use and concentration of this safety assessment.

Keywords

cocamidopropyl betaine, CAPB, cosmetics, safety

Introduction

Cocamidopropyl betaine (CAPB) is a zwitterion used primarily as a surfactant in cosmetic products. A safety assessment for CAPB was published by the Cosmetic Ingredient Review (CIR) in 1991.¹ At that time, the CIR Expert Panel (the Panel) concluded that CAPB is safe for use in rinse off cosmetic products at the current levels of use, and the concentration of use for cosmetic products designed to remain on the skin for prolonged periods of time (leave-on products) should not exceed 3.0%. Because raw material CAPB is commonly supplied to product finishing houses as a 30% preformulation solution, a 3% solution would correspond to a 10% solution of a full-strength CAPB raw material solution. Frequently, these preformulation solutions are described as having an "activity" of the ingredient (eg, typical raw material CAPB has an activity of 30%). Accordingly, to prepare a 3% solution of a CAPB, from a CAPB preformulation solution with 30% activity, the preformulation solution would need to be diluted by a factor of 10.

Based on new published data that described sensitization in patients from use of rinse off products, new uses in aerosol products, and a substantial increase in the number of uses, the Panel reopened the final report on CAPB in 2007. The following report is a compilation of new data and summary data from the original safety assessment on CAPB and related amidopropyl betaines. Because of chemical similarities to CAPB, the

available data may be extrapolated to all of the following related aminopropyl betaines, in a process termed read across:

- almondamidopropyl betaine,
- apricotamidopropyl betaine,
- avocadamidopropyl betaine,
- babassuamidopropyl betaine,
- behenamidopropyl betaine,
- canolamidopropyl betaine,
- capryl/capramidopropyl betaine,
- coco/oleamidopropyl betaine,
- coco/sunfloweramidopropyl betaine,
- cupuassuamidopropyl betaine,
- isostearamidopropyl betaine,
- lauramidopropyl betaine,
- meadowfoamamidopropyl betaine,
- milkamidopropyl betaine,

¹ Scientific Analyst/Writer, Cosmetic Ingredient Review

² The 2011 Cosmetic Ingredient Review Expert Panel member

³ Director, Cosmetic Ingredient Review

Corresponding Author:

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

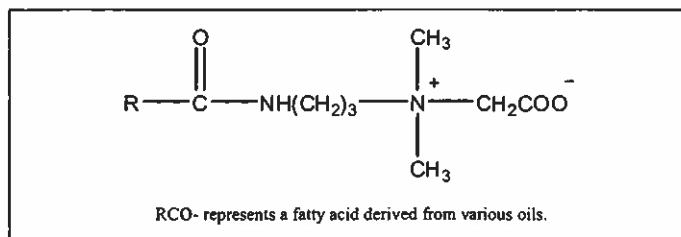


Figure 1. Amidopropyl betaine.

- minkamidopropyl betaine,
- myristamidopropyl betaine,
- oatamidopropyl betaine,
- oleamidopropyl betaine,
- olivamidopropyl betaine,
- palmamidopropyl betaine,
- palmitamidopropyl betaine,
- palm kernelamidopropyl betaine,
- ricinoleamidopropyl betaine,
- sesamidopropyl betaine,
- shea butteramidopropyl betaine,
- soyamidopropyl betaine,
- stearamidopropyl betaine,
- tallowamidopropyl betaine,
- undecyleneamidopropyl betaine, and
- wheat germamidopropyl betaine.

Chemistry

Definition and Structure

The general structure of amidopropyl betaines is as shown in Figure 1, where RCO- represents the fatty acids derived from various oils.² For example, for CAPB (CAS No. 61789-40-0), RCO- represents the fatty acids derived from coconut oil. Table 1 presents the definitions and structures of CAPB and related amidopropyl betaine ingredients.

Technical names for CAPB and its related amidopropyl betaines, as well as the functions these ingredients perform in cosmetics, are found in Table 2. There are numerous trade names and trade name mixtures containing CAPB and its related amidopropyl betaines.²

Physical and Chemical Properties

The CAPB is a clear, pale yellow liquid of medium viscosity (300-600 cps), with a slight fatty odor.^{3,4} The CAPB has a boiling point of 230°F, a specific gravity of 1.04 relative to water, and no flash point.⁵ The CAPB is soluble in water, ethanol, and isopropanol and insoluble in mineral oil.³

The CAPB is supplied as a solution in water and with sodium chloride (see Table 3). The concentration of CAPB in such supplied material is described by its activity.⁶ The concentration of cosmetic-grade CAPB (active concentration) is what is left in the supplied solution after water (62%-66%) and sodium chloride (4.6%-5.6%) have been accounted for, which

is ~30% of the supplied solution. In this report, unless a concentration has been reported as being active, a concentration of CAPB in solution will be calculated since it is unclear in some cases which is the true concentration that was tested. If, for example, a study reports the use of CAPB at 10% active, the assumption will be made that 10% active was tested. If a study reports use of 10% CAPB, concentrations will be calculated assuming both possibilities: (1) that it was 10% active or (2) it was 10% and only 30% of that was active, yielding 3% active.

Commercial grades containing concentrations of CAPB greater than 30% may contain solvents, such as propylene glycol. Although most commercial grades contain sodium chloride, low-salt products also are available. The concentration of sodium chloride in cosmetic grade CAPB ranges from 4.0% to 6.0%. Cosmetic grade CAPB may also contain a maximum of 3.0% glycerol.¹

The fatty acid compositions of the oils that are components of the additional amidopropyl betaines described in this report are presented in Table 4.

Method of Manufacture

Figure 2 depicts the formation of CAPB through the reaction of coconut oil fatty acids (coconut oil or hydrolyzed, glyceryl-free coconut acid) with 3,3-dimethylaminopropylamine (DMAPA), which yields cocamidopropyl dimethylamine (amidoamine or dimethylaminopropyl cococamide). The amidoamine, a tertiary amine, is then reacted with sodium monochloroacetate to produce CAPB. In Figure 2, R represents the coconut fatty acid chain that varies between C-8 and C-18.^{1,3,7-10}

Supplier information provided to the Personal Care Products Council (the Council) indicated that babassuamidopropyl betaine, coco/sunfloweramidopropyl betaine, cupuassuamidopropyl betaine, isostearamidopropyl betaine, lauramidopropyl betaine, meadowfoamamidopropyl (MF) betaine, oleamidopropyl betaine, ricinoleamidopropyl betaine, and wheat germamidopropyl betaine are manufactured in the same manner as CAPB.¹¹ Manufacturing data on the remaining amidopropyl betaines were not provided.

In cupuassuamidopropyl betaine, the intermediate is cupuassuamidopropyl dimethylamine, which can be found at a maximum level of 0.2% in the final product.¹¹ The DMAPA level in final cupuassuamidopropyl betaine product is 0.05%. In MF betaine, the intermediate is MF dimethylamine (MF-DMAPA), which can be found at less than 0.5% in the final product. The manufacturing process for MF betaine exhausts DMAPA. The levels of DMAPA and amidoamine were reported to be below 0.0002% (the detection limit) and <0.5%, respectively, in babassuamidopropyl betaine, coco/sunfloweramidopropyl betaine, isostearamidopropyl betaine, lauramidopropyl betaine, oleamidopropyl betaine, ricinoleamidopropyl betaine, and wheat germamidopropyl betaine.

The CIR accepts the US Food and Drug Administration (FDA) determination (21 CFR 700.27(a)) that tallow derivatives are not prohibited cattle materials.

Table 1. Definitions, Structures, and Functions for CAPB and Related Amidopropyl Betaine Ingredients²

Ingredient	Definition	Function	Related CIR Reviews and Conclusions
Cocamidopropyl Betaine (CAS Nos. 61789-40-0; 83138-08-3; 86438-79-1)	The zwitterion (inner salt) that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from coconut oil.	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Coconut oil & acid 1986, Safe; 2008 safe
Almondamidopropyl betaine (CAS no. not found)	The zwitterion (inner salt) that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from almond oil.	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Almond oil 1983, safe; 2005, not reopened
Apricotamidopropyl betaine (CAS no. 133934-08-4)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from <i>Prunus armeniaca</i> (apricot) kernel oil (qv)	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Avocadamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from <i>Persea gratissima</i> (avocado) oil (qv)	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Avocado oil 1980, safe; 2003, not reopened
Babassuamidopropyl betaine (CAS no. 147170 44 3)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from <i>Orbignya oleifera</i> (Babassu) Oil.	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Behenamidopropyl Betaine (CAS no. 84082 44 0)	The zwitterion that conforms generally to the structure in Figure 1	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Canolamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from canola oil	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Capryl/capramidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from caprylic and capric acids	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Coco/oleamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from coconut oil	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Oleic acid 1987, safe; 2006, not reopened coconut oil & acid 1986, safe; 2008 safe
Coco/sunfloweramidopropyl betaine (CAS no. 147170 44 3)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from a blend of coconut and sunflower seed oils	Slip modifiers; surfactants cleansing agents; surfactants foam boosters; surfactants solubilizing agents; viscosity increasing agents aqueous	NA

(continued)

Table 1. (continued)

Ingredient	Definition	Function	Related CIR Reviews and Conclusions
Cupuassamidopropyl betaine (CAS no. 657350 94 2)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from the pulp of the cupuassu tree (<i>Theobroma grandiflorum</i>).	Hair-Conditioning Agents; Skin-Conditioning Agents Miscellaneous; Surfactants Cleansing Agents; Surfactants Foam Boosters; Viscosity Increasing Agents Aqueous	None
Isostearamidopropyl betaine (CAS no. 63566 37 0)	The zwitterion that conforms generally to the structure in Figure 1	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Isostearic acid 1983, safe; 2005, not reopened
Lauramidopropyl betaine (CAS nos. 4292 10 8; 86438 78 0)	The zwitterion that conforms generally to the structure in Figure 1	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Lauric acid 1987, safe; 2006, not reopened
Meadowfoamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from meadowfoam seed oil	Humectants; skin protectants	None
Milkamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from milk	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Minkamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from mink oil	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Mink oil 2005, safe
Myristamidopropyl betaine (CAS no. 59272 84 3)	The zwitterion that conforms generally to the structure in Figure 1	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Myristic acid 1987, safe; 2006, not reopened; currently under review with the myr- istates group
Oatamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from <i>Avena sativa</i> (oat) kernel oil (qv)	Antistatic agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Oleamidopropyl betaine (CAS no. 25054 76 6)	The zwitterion that conforms generally to the structure in Figure 1	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Oleic acid 1987, safe; 2006, not reopened
Olivamidopropyl Betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from olive oil	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Palmamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from palm oil	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Palm oil 2000, safe

(continued)

Table I. (continued)

Ingredient	Definition	Function	Related CIR Reviews and Conclusions
Palmitamidopropyl betaine (CAS no. 32954 43 1)	The zwitterion that conforms generally to the structure in Figure 1	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Palmitic acid 1987, safe; 2006, not reopened
Palm kernelamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from palm kernel oil	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Palm kernel oil 2000, safe
Ricinoleamidopropyl betaine (CAS no. 71850 81 2)	The zwitterion that conforms generally to the structure in Figure 1	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Ricinoleic acid 2005, safe
Sesamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from sesame oil	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Sesame seed oil 1993, safe; currently under review.
Shea butteramidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from <i>Butyrospermum parkii</i> (shea butter).	Surfactants cleansing agents; surfactants foam boosters	None
Soyamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from soy	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Stearamidopropyl betaine (CAS no. 6179 44 8)	The zwitterion that conforms generally to the structure in Figure 1	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Stearic acid 1987, safe; 2006, not reopened
Tallowamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from tallow	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Tallow 1990, safe; 2006, not reopened
Undecylenamidopropyl betaine (CAS no. not found)	The zwitterion that conforms generally to the structure in Figure 1	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	None
Wheat germamidopropyl betaine (CAS no. 133934 09 5)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from wheat germ	Antistatic agents; hair-conditioning agents; skin-conditioning agents miscellaneous; surfactants cleansing agents; surfactants foam boosters; viscosity increasing agents aqueous	Wheat germ oil 1980, safe; 2003, not reopened

Table 2. Technical Names for CAPB and Related Amidopropyl Betaines²

Ingredient	Technical/Other Names
Cocamidopropyl betaine	CADG
	N-(carboxymethyl)-N,N-dimethyl-3-[(1-oxococonut)amino]-1-propanaminium Hydroxide, inner salt
	Cocamido betaine
	Cocamidopropyl dimethyl glycine
	Cocoyl amide propylbetaine
	Cocoyl amide propyldimethyl glycine
	Cocoyl amide propyldimethyl glycine solution
Almondamidopropyl betaine	1-Propanaminium, N-(carboxymethyl)-N,N-dimethyl-3-[(1-oxococonut)amino]-, hydroxide, inner salt
	Quaternary ammonium compounds (carboxymethyl)(3-cocoamidopropyl)dimethyl, hydroxides, inner salts
	Almond amide propylbetaine
	Almondamidopropyl dimethyl glycine
	N-(carboxymethyl)-N,N-dimethyl-3-[(1-oxoalmond)amino]-1-propanaminium hydroxide, inner salt
	1-propanaminium, N-(carboxymethyl)-N,N-dimethyl-3-[(1-oxoalmond)amino]-, hydroxide, inner salt
	Quaternary ammonium compounds (carboxymethyl)(3 almondamidopropyl) dimethyl, hydroxide, inner salt
Apricotamidopropyl betaine	Apricot amide propylbetaine
	Apricotamidopropyl dimethyl glycine
	N (carboxymethyl) N,N dimethyl 3 [(1 oxoapricot)amino] 1 propanaminium hydroxide, inner salt
	1 propanaminium, 3 amino N(carboxymethyl) N,N dimethyl, N apricot oil acyl derivs, hydroxides, inner salts
	1 propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxoapricot)amino], hydroxide, inner salt
	Quaternary ammonium compounds (carboxymethyl)(3 apricotamidopropyl) dimethyl, hydroxide, inner salt
Avocadamidopropyl betaine	Avocado amide propylbetaine
	Avocadoamidopropyl dimethyl glycine
	N(carboxymethyl) N,N dimethyl 3 [(1 oxoavocado)amino] 1 propanaminium hydroxide, inner salt
	1 propanaminium, N(carboxymethyl) N,N dimethyl 3 [(1 oxoavocado)amino], hydroxide, inner salt
	Quaternary ammonium compounds (carboxymethyl)(3 avocadoamidopropyl) dimethyl, hydroxide, inner salt
Babassuamidopropyl betaine	Babassu amide propylbetaine
	Babassuamidopropyl dimethyl glycine
	N (carboxymethyl) N,N dimethyl 3 [(1 oxobabassu)amino] 1 propanaminium hydroxide, inner salt
	1 propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxobabassu)amino], hydroxide, inner salt
	Quaternary ammonium compounds (carboxymethyl)(3 babassuamidopropyl) dimethyl, hydroxide, inner salt
Behenamidopropyl betaine	Behenamido propylbetaine
	Behenamidopropyl dimethyl glycine
	1 propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxobehenyl)amino], hydroxide, inner salt
	1 propanaminium, N(carboxymethyl) N,N dimethyl 3 [(1 oxodocosanyl)amino], hydroxide, inner salt
	Quaternary ammonium compounds (carboxymethyl)(3 behenamidopropyl) dimethyl, hydroxide, inner salt
Canolamidopropyl betaine	None found.
Capryl/Capramidopropyl betaine	None found.
Coco/oleamidopropyl betaine	None found.
Coco/sunfloweramidopropyl betaine	1 Propanaminium, 3 amino N(carboxymethyl) N,N dimethyl, N (C8 18 and C18 Unsats. Acyl) derivs, hydroxides, inner salts
Cupuassuamidopropyl betaine	1 Propanaminium, 3 amino N(carboxymethyl) N,N dimethyl N (Theobroma grandiflorum acyl) Derivs
Isostearamidopropyl betaine	N (Carboxymethyl) N,N Dimethyl 3 [(1 Oxoisooctadecyl)Amino] 1 Propanaminium Hydroxide, Inner Salt
	1 Propanaminium, N (Carboxymethyl) N,N Dimethyl 3 [(1 Oxoisooctadecyl)Amino], Hydroxide, Inner Salt
Lauramidopropyl betaine	Ammonium, (carboxymethyl)(3 lauramidopropyl)diemthyl, hydroxide, inner salt
	N (carboxymethyl) N,N dimethyl 3 [(1 oxododecyl)amino] 1 propanaminium hydroxide, inner salt
	N (dodecylamidopropyl) N,N diemthylammonium betaine
	Glycine, (3 lauramidopropyl)diemthylbetaine
	Lauroyl amide propyldimethyl glycine solution 1 propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxododecyl)Amino], hydroxide, inner salt
Meadowfoamidopropyl betaine	None found.
Milkamidopropyl betaine	None found.

(continued)

Table 2. (continued)

Ingredient	Technical/Other Names
Minkamidopropyl betaine	N (carboxymethyl) N,N dimethyl 3 [(1 oxomink)amino] 1 propanaminium hydroxide, inner salt Mink amide propylbetaine Minkamidopropyl dimethyl glycine 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxomink)amino], hydroxide, inner salt Quaternary ammonium compounds, (carboxymethyl)(3 minkamidopropyl) dimethyl, hydroxide, inner salt
Myristamidopropyl betaine	N (carboxymethyl) N,N dimethyl 3 [(1 oxotetradecyl)amino] 1 propanaminium hydroxide, inner salt Myristamidopropyl dimethyl glycine 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxotetradecyl)amino], hydroxide, inner salt
Oatamidopropyl betaine	None found.
Oleamidopropyl betaine	Ammonium, (carboxymethyl)dimethyl(3 oleamidopropyl), hydroxide, inner salt N (carboxymethyl) N,N dimethyl 3 [(1 oxooctadecenyl)amino] 1 Propanaminium hydroxide, inner salt Oleamidopropyl dimethyl glycine 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxooctadecenyl)amino], hydroxide, inner salt N (carboxymethyl) N,N dimethyl 3 [(1 oxoolive)amino] 1 propanaminium hydroxide, inner salt
Olivamidopropyl betaine	Olivamidopropyl dimethyl glycine Olive amide propylbetaine 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxoolive)amino], hydroxide, inner salt Quaternary ammonium compounds (carboxymethyl)(3 oliveamidopropyl) dimethyl, hydroxide, inner salt
Palmamidopropyl betaine	None found.
Palmitamidopropyl betaine	Ammonium (carboxymethyl)dimethyl(3 palmitamidopropyl), hydroxide, inner salt N (carboxymethyl) N,N dimethyl 3 [(1 oxohexadecyl)amino] 1 propanaminium hydroxide, inner salt Pentecamine (INN) 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxohexadecyl)amino], hydroxide, inner salt N (carboxymethyl) N,N dimethyl 3 [(1 oxopalm kernel)amino] 1 propanaminium hydroxide, inner salt
Palm Kernelamidopropyl betaine	Palm kernel amide propylbetaine Palm kernelamidopropyl dimethyl glycine Palm kernel oil amide propyl dimethyl glycine solution 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxopalm kernel)amino], hydroxide, inner salt Quaternary ammonium compounds, (carboxymethyl)(3 palm kernelamidopropyl) dimethyl, hydroxide, inner salt N (carboxymethyl) N,N dimethyl 3 [(1 oxoricinoleyl)amino] 1 propanaminium hydroxide, inner salt
Ricinoleamidopropyl betaine	1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxoricinoleyl)amino], hydroxide, inner salt Propyl betaine ricinoleate amide solution Ricinoleamidopropyl dimethyl glycine N (carboxymethyl) N,N dimethyl 3 [(1 oxosesame)amino] 1 propanaminium hydroxide, inner salt 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxosesame)amino], hydroxide, inner salt
Sesamidopropyl betaine	Quaternary ammonium compounds (carboxymethyl)(3 sesameamidopropyl) dimethyl, hydroxide, inner salt Sesame amide propylbetaine Sesamidopropyl dimethyl glycine
Shea butteramidopropyl betaine	None found
Soyamidopropyl betaine	N (carboxymethyl) N,N dimethyl 3 [(1 oxosoy)amino] 1 propanaminium hydroxide, inner salt 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxosoy)amino], hydroxide, inner salt Quaternary ammonium compounds (carboxymethyl)(3 soyamidopropyl) dimethyl, hydroxide, inner salt Soy amide propylbetaine Soyamidopropyl dimethyl glycine
Stearamidopropyl betaine	N (carboxymethyl) N,N dimethyl 3 [(1 oxooctadecyl)amino] 1 propanaminium hydroxide, inner salt 1 propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxooctadecyl)amino], hydroxide, inner salt Stearoyl amide propyl dimethyl glycine
Tallowamidopropyl betaine	N (carboxymethyl) N,N dimethyl 3 [(1 oxotallow)amino] 1 propanaminium hydroxide, inner salt 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxotallow)amino], hydroxide, inner salt Quaternary ammonium compounds (carboxymethyl)(3 tallowamidopropyl)dimethyl, hydroxides, inner salts N (carboxymethyl) N,N dimethyl 3 [(1 oxoundecylenyl)amino] 1 propanaminium hydroxide, inner salt 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxoundecylenyl)amino], hydroxide, inner salt
Undecylenamidopropyl betaine	Quaternary ammonium compounds (carboxymethyl)(3 undecylenamidopropyl) dimethyl, hydroxide, inner salt Undecylenamide propylbetaine Undecylenamidopropyl dimethyl glycine
Wheat germamidopropyl betaine	N (carboxymethyl) N,N dimethyl 3 [(1 oxowheat germ alkyl)amino] 1 propanaminium hydroxides, inner salts 1 Propanaminium, 3 amino N (carboxymethyl) N,N dimethyl, N wheat oil acyl derivs, hydroxides, inner salts 1 Propanaminium, N (carboxymethyl) N,N dimethyl 3 [(1 oxowheat germ)amino], hydroxide, inner salt

Table 3. Composition, Chemical, and Physical Characteristics of Batches of Cosmetic Grade CAPB⁵

Color	Clear pale yellow liquid
Odor	Faint
pH	4.6-5.6
Water content	62%-66%
NaCl	4.6%-5.6%
Active materials (100 - H ₂ O - NaCl, %)	29.5%-32.5%
Alkalinity	0.725-0.825 Meq/g
Boiling point	230°F
Specific gravity	1.04
Solubility at 25°C	
Water	2 g/10 mL
Alcohol	2 g/10 mL
Fatty acids	
C8	5.6%-6.0%
C10	5.4%-5.7%
C12	53.1%-53.2%
C14	16.1%-17.4%
C16	8.1%-8.3%
C18	10.0%-10.2%

Impurities

No *N*-nitroso compounds were detected in samples of commercially supplied CAPB.¹² CAPB samples with and without internal standards of *N*-nitroso compounds were analyzed using gas chromatography with a thermal energy analyzer (TEA). The CAPB has a secondary amido group that is susceptible to *N*-nitrosation to form an *N*-nitrosamide. Although a highly sensitive analytical method failed to detect traces of volatile *N*-nitrosamines in samples of commercial CAPB, this result does not exclude the possibility that in the presence of *N*-nitrosating agents CAPB gives rise to reactive and unstable nitrosamides. The TEA method does not detect nitrosamides.¹³

Coconut oil impurities may be present in CAPB, depending on the degree of refining to which the coconut oil is subjected, including free fatty acids and low concentrations of sterols, tocopherol, squalene, and lactones. Concentrations of pigments, phosphatides, gums, and other nonglyceride substances are usually low in coconut oil in contrast to other vegetable oils.¹⁴

Impurities associated with CAPB are the reactants and intermediates from production and include amidoamine, sodium monochloroacetate, and DMAPA.^{7,9,10} Depending on the manufacturer, residual amidoamine and DMAPA can range from 0.3% to 3.0% and from 0.0003% to 0.02%, respectively.⁹

In 2007, the Personal Care Products Council surveyed suppliers regarding the levels of DMAPA and amidoamine in CAPB. The limit of detection for DMAPA is 100 ppm in some analytical methods, but some methods may detect this impurity at concentrations as low as 2.5 ppm. Several companies reported DMAPA below the 100 ppm detection limit, with 1 supplier reporting a DMAPA below the limit of detection of 0.0002%. The survey found levels of amidoamine ranged from 0.5% to 5%, with 0.5% the typical value and 1.5% the

suggested maximum level. The variability in the amidoamine levels may be due to the differences in analytical methods.^{11,15}

Meadowfoam seed oil has been reported to have a typical value of <1 ppm for the heavy metal iron, copper, lead, mercury, cadmium, selenium, and chromium. The maximum value is 10 ppm.¹⁶

Use

Cosmetic

According to information supplied to the FDA by industry as part of the Voluntary Cosmetic Registration Program (VCRP), CAPB is used in a total of 2743 products (Table 5).²² A use concentration survey conducted by the Council showed CAPB use at concentrations ranging from 0.005% to 11%.^{23,24}

The VCRP also reported uses of babassuamidopropyl betaine, capryl/capramidopropyl betaine, coco/oleamidopropyl betaine, lauramidopropyl betaine, oatamidopropyl betaine, olivamidopropyl betaine, soyamidopropyl betaine, and undecylenamidopropyl betaine, with the highest total of uses reported for lauramidopropyl betaine at 187.²² Concentration of use ranges was reported for almondamidopropyl betaine, babassuamidopropyl betaine, capryl/capramidopropyl betaine, lauramidopropyl betaine, myristamidopropyl betaine, oatamidopropyl betaine, palm kernelamidopropyl betaine, shea butteramidopropyl betaine, soyamidopropyl betaine, and undecylenamidopropyl betaine, with the highest concentration of use reported for lauramidopropyl betaine at 13%.²³ For complete information on these ingredients, see Table 5. No uses or concentrations of uses were reported for: apricotamidopropyl betaine, avocamidopropyl betaine, behenamidopropyl betaine, canolamidopropyl betaine, coco/sunfloweramidopropyl betaine, cupuasamidopropyl betaine, isostearamidopropyl betaine, MF betaine, milkamidopropyl betaine, minkamidopropyl betaine, oleamidopropyl betaine, palmamidopropyl betaine, palmitamidopropyl betaine, ricinoleamidopropyl betaine, sesamidopropyl betaine, stearamidopropyl betaine, tallowamidopropyl betaine, and wheat germamidopropyl betaine.

The CAPB is primarily used as a pseudoamphoteric surfactant in hair shampoos.¹ Gottschalck and Bailey described the current functions of CAPB as antistatic agent; hair-conditioning agent; skin-conditioning agent—miscellaneous; surfactant-cleansing agent; surfactant-foam booster; and viscosity increasing agent—aqueous.²

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

There are no specific data for spray products containing CAPB. Jensen and O'Brien reviewed the potential adverse effects of inhaled aerosols, which depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system.²⁵ The aerosol properties associated with the location of deposition in the respiratory system are particle size and density. The parameter most closely associated with this regional deposition is

Table 4. Fatty Acid Compositions of the Oil Components of Amidopropyl Betaines (%)¹⁶⁻²¹

Fatty Acids	Coconut	Almond	Apricot	Avocado	Babassu	Canola	Cupuassu	Meadowfoam Seed
Caproic (C6)	0.008-1.2							
Caprylic (C8)	3.4-15				4-8			
Capric (C10)	3.2-15				4-8			
Lauric (C12)	41-51.3				44-47			
Myristic (C14)	13-23				15-20			
Palmitic (C16)	4.2-18	5.5-6.5	Small quantities	13-17	6-9	2.8-3	5.8	
Stearic (C18)	1.6-4.7	2-3			3-5	1.3	38.3	
Oleic (C18:1)	3.4-12	70-77		67-72	10-12	57.1-57.4	42.8	
Oleic/Linoleic			90-93					
Linoleic (C18:2)	0.9-3.7	17-20		10-12	1-3	20.1-22.1		
Arachidic (C20)	1.03						4.8	
Palmitoleic (C16:1)				3-5.1				
Linolenic (C18:3)						10.8-12.5	8.3	
Eicosenoic (C20:1)						2.5-3.1		52-77 ^a
Erucic (C22:1)						1-3.3		8-29 ^a
C22:2								7-20 ^a

^aNatural Plant Products, Inc. reports the fatty acid composition of meadowfoam seed oil to be 58%-64% C20:1 (▲5), 3%-6% C22:1 (▲5), 10%-14% C22:1 (▲13), and 15%-21% C22:2 (▲5▲13).

Table 4. Fatty Acid Compositions of the Oil Components of Amidopropyl Betaines (%) (Continued)¹⁶⁻²¹

Fatty Acids	Mink Crude	Olive	Palm	Palm Kernel	Sesame	Shea	Soybean	Sunflower	Tallow	Wheat Germ
Caprylic (C8)				3%-4%						
Capric (C10)				3-7%						
Lauric (C12)	0.1			46%-52%						
Myristic (C14)	3.5		1-6	15%-17%					3-6	
Myristoleic (C14:1)	0.9									
Pentadecanoic (C15)	0.1									
Palmitic (C16)	17.2	7.5-20	32-47	6%-9%	7%-10.9%	5-9		5.2-7.2	24-32	11-16
Heptadecanoic (C17)	0.4									
Heptadecanoic (C17:1)	0.5									
Stearic (C18)	2.5	0.5-3.5	1-9	1-3%	3.4-6%	30-41		2.7-6.5	20-25	1-6
Oleic (C18:1)	40.9	53-86	39-53	13%-19%	32.7%-53.9%	45-50	11.5-60	14.7-35	37-43	8-30
Linoleic (C18:2)	15.0	3.5-20	2-11	0.5-2%	37-59%	4-5	25-63.1	51.5-73.5	2-3	44-65
Arachidic (C20)					0.3%-8%			0.3-1		
Palmitoleic (C16:1)	17.0	0.3-3.5								
Linolenic (C18:3)	0.6	0-1.5					2.9-12.1	0.01-0.3		4-10
Eicosenoic acid (C20:1)										
Eicolenic (C20:1)	0.6									
Other							12-13.5 (unknown saturated acids)			0-1.2 (C20-C22 saturated acids)
Cholesterol, arachidonic acid, elaidic acid, and vaccenic acid									Small quantities	

the aerodynamic diameter, d_a , defined as the diameter of a sphere of unit density possessing the same terminal setting velocity as the particle in question. These authors reported a mean aerodynamic diameter of $4.25 \pm 1.5 \mu\text{m}$ for respirable particles that could result in lung exposure.²⁵

Bower reported diameters of anhydrous hair spray particles of 60 to 80 μm and pump hair sprays with particle diameters of

$\geq 80 \mu\text{m}$.²⁶ Johnsen reported that the mean particle diameter is around 38 μm in a typical aerosol spray.²⁷ In practice, he stated that aerosols should have at least 99% of particle diameters in the 10 to 110 μm range.

The CAPB was not restricted from use in any way under the rules governing cosmetic products in the European Union.²⁸

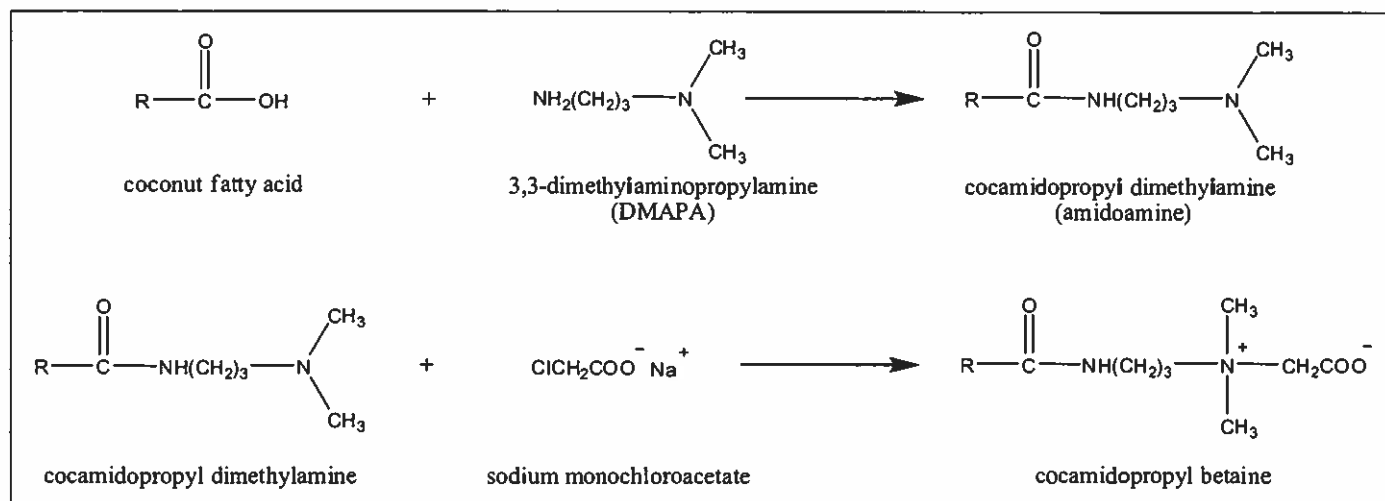


Figure 2. Reaction process of cocamidopropyl betaine (R represents the coconut fatty acid chain that varies between C-8 and C-18).

Noncosmetic

The CAPB is used in household cleaning products, including laundry detergents, hand dishwashing liquids, and hard surface cleaners.²⁹ A 30% active CAPB solution was tested for antibacterial and antimycotic activity using the agar cup plate method.³⁰ Zones of inhibition were measured for the bacteria and molds around agar cups containing 0.2 mL of the ingredient, which had been diluted with distilled water to 0.5% activity. No inhibition against *Escherichia coli* or *Pseudomonas aeruginosa* was observed. Bacteriostatic activity was detected in cultures of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus subtilis*. Fungicidal activity was observed in cultures of *Candida albicans*, *Trichophyton mentagrophytes*, and *Pityrosporum ovale*.

Toxicokinetics

No studies were found on the absorption, distribution, metabolism, and excretion of CAPB or other amidopropylbetaines. It is unclear whether the amide bond can be hydrolyzed to yield the fatty acids and 3-aminopropyl betaine. No metabolism data are available on the latter compound.

Toxicological Studies

Single-Dose (Acute) Toxicity

Oral. A full-strength CAPB solution, 30% active, was administered by gastric intubation to groups of 10 CFR mice of the Carworth strain, weighing 18 to 21 g. Mice were observed for 7 days following the administration. The oral LD₅₀ was 6.90 g/kg (calculated from volume per weight dosage units, based on a density of 1.07 g/mL). Confidence range is 6.06 to 7.86 g/kg.³¹

Undiluted CAPB, 30% active, with a pH of 5.5, was administered by gavage to groups of 10 (5 female, 5 male) Wistar rats.³² Dosage groups were 5.00, 6.30, 7.94, and 10.00 mL/kg. The rats were observed for 14 days. The oral LD₅₀ was 7.97 g/kg (calculated from volume per weight dosage units, based on a

density of 1.07 g/mL). Confidence range is 6.93 to 9.17 g/kg. Rats in all dosage groups had decreased motor activity, abnormal body posture, coordination disturbance, cyanosis, diarrhea, and decreased body temperature beginning approximately 20 minutes after dosage and persisting for 24 hours. Surviving rats in all groups had body weight gains of 36 to 45 g and were normal in appearance and behavior. Redness of the stomach and intestinal mucous membranes were observed at necropsy.

A full-strength solution of CAPB, 30% active, was administered by gavage to groups of 5 albino rats at single doses of 2.0, 4.0, 5.0, 6.3, 8.0, and 16.0 g/kg, and the rats were observed for 14 days.³³ Sluggishness, nasal hemorrhaging, diarrhea, and wetness around the hindquarters were observed, increasing in severity with dosage. The oral LD₅₀ for this full strength, 30% active CAPB solution was estimated at 4.9 g/kg, with a 95% confidence limit of 3.7 to 6.5 g/kg.

A full-strength solution of CAPB, 30% active, was administered by gavage to groups of 10 (5 female, 5 male) Sprague-Dawley rats at single doses of 2.0, 2.71, 3.68, 5.0, or 6.78 g/kg, and the rats were observed for 15 days.³⁴ At necropsy, a blood-like, viscous liquid was found in the intestines. Surviving rats gained an average between 20 and 130 g by day 15. Diarrhea was observed in rats of all treatment groups, and decreased motor activity was observed in rats of all treatment groups, except at the lowest dose. Dried blood around the nose and salivation were observed in male rats of the 5.0 g/kg dosage groups. The acute oral LD₅₀ for this full-strength CAPB, 30% active, was 4.91 g/kg within 95% confidence limits of 4.19 to 5.91 g/kg.

The American Chemistry Council summarized an acute oral toxicity study on 35.61% active CAPB.³⁵ Fasted Sprague-Dawley rats (5 female, 5 male; 220-294 g) received a single, oral dose via gavage of undiluted test material. The rats were weighed before dosing and at study termination, and they were observed frequently from the day of dosing and for 14 days. Animals that died during the study underwent gross necropsy. All of the female rats died on day 2 of the study. Prior to death,

Table 5. Current Cosmetic Product Uses and Concentrations for Cocamidopropyl Betaine and Its Related Amidopropyl Betaine According to Duration and Exposure^{22,23}

	Cocamidopropyl Betaine		Almondamidopropyl Betaine		Babassuamidopropyl Betaine		Capryl/Capramidopropyl Betaine		Coco/Oleamidopropyl Betaine		Laureamidopropyl Betaine		Myristamidopropyl Betaine	
	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)
Totals	3287	0.005-11	NR	3	25	0.9-4	3	0.3-2	10	NR	227	0.00006-13	1	0.3
Duration of use														
Leave-on	228	0.2-6	NR	NR	NR	NR	2	2	4	NR	9	0.00006-6	NR	NR
Rinse off	3059	0.005-11	NR	3	25	0.9-4	1	0.3	6	NR	218	0.6-13	1	0.3
Exposure type														
Eye area	8	0.005-3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Possible ingestion	NR	0.6-6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Inhalation	24	0.2-6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal contact	1859	0.005-11	NR	3	9	0.9-2	2	2	9	NR	148	0.7-13	NR	0.3
Deodorant (underarm)	NR	2	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair—nonColoring	1000	0.2-9	NR	NR	15	0.9-4	1	0.3	1	NR	48	0.00006-8	1	NR
Hair—coloring	426	0.6-6	NR	NR	1	NR	NR	NR	NR	NR	28	0.6	NR	NR
Nail	1	0.8	NR	NR	NR	NR	NR	NR	NR	NR	3	NR	NR	NR
Mucous membrane	1252	0.5-10	NR	NR	4	2	NR	NR	3	NR	87	2-13	NR	NR
Bath products	180	0.06-7	NR	NR	NR	NR	NR	NR	NR	NR	12	3-8	NR	NR
Baby products	106	2-6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Abbreviation: NR, not reported to the VCRP or Council; VCRP, Voluntary Cosmetic Registration Program.

Table 5. Current Cosmetic Product Uses and Concentrations for Cocamidopropyl Betaine and Its Related Amidopropyl Betaine According to Duration and Exposure (Continued)^{22,23}

	Oatamidopropyl Betaine			Palm Kernelamidopropyl Betaine			Shea Butteramidopropyl Betaine			Soyamidopropyl Betaine			Undecylenamidopropyl Betaine		
	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010	Conc. of Use 2010 (%)	No. of Uses 2010
Totals	1	0.3	1	NR	NR	0.9-5	11	0.6-4	4	1-2	1	2			
Duration of use															
Leave-on	1	0.3	NR	NR	NR	NR	NR	NR	1	NR	NR	NR			
Rinse off	NR	NR	1	NR	NR	0.9-5	11	0.6-4	3	1-2	1	2			
Exposure type															
Eye area	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR			
Possible ingestion	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR			
Inhalation	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR			
Dermal contact	1	0.3	1	NR	NR	0.9	9	0.6-4	4	2	NR	NR			
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR			
Hair—noncoloring	NR	NR	NR	NR	NR	5	2	NR	NR	NR	1	2			
Hair—coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR			
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR			
Mucous membrane	NR	NR	NR	NR	NR	0.9	9	2	NR	NR	NR	NR			
Bath products	NR	NR	NR	NR	NR	NR	NR	0.6	NR	NR	NR	NR			
Baby products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR			

Abbreviation: NR, not reported to the VCRP or Council; VCRP, Voluntary Cosmetic Registration Program.

the females exhibited salivation, diarrhea, ataxia, and/or decreased activity. Male rats exhibited similar clinical signs on day 1 (day of dosing) and day 2 but had recovered by day 3. Necropsy data were not reported. The acute oral LD₅₀ for 35.61% active CAPB was >1.8 g/kg for male rats.

The CAPB (31% active) was orally administered to male and female CD rats (5/sex; 110-150 g) at 5.0 g/kg body weight via gavage. Animals were observed daily until 14 days after dosing and were killed on day 15. Individual body weights were recorded on days 1, 8, and 15. Macroscopic postmortem examinations performed. Clinical signs of toxicity included piloerection, increased salivation, hunched posture, and diarrhea. Animals recovered by day 4. Slightly reduced body weight gains were recorded for 4 males and 3 females on day 8, but all animals achieved expected weight gains by day 15. No abnormalities were observed at necropsy. The acute oral LD₅₀ was greater than 5.0 g/kg.³⁵

In another acute oral toxicity study reported by the American Chemistry Council, fasted Wistar rats (5 rats per dose, sexes combined; 200-300 g) received a single oral gavage dose of CAPB (30% aqueous) at levels of 4.0, 8.0, 10.0, 12.5, 16.0, or 32.0 g/kg.³⁵ The rats were observed daily for 2 weeks after dosing. No postmortem or histopathology examinations were performed. Clinical signs included slight diarrhea and unkempt coats in the 4.0 g/kg dose group, and lethargy, diarrhea, nasal hemorrhage, and unkempt coats was observed in the dose group of 8.0 g/kg and above, with severity increasing proportionately. The acute oral LD₅₀ was 8.55 g/kg. (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 30% active or 30% aqueous, which equated to 9% active.)

Dermal

The American Chemistry Council summarized an acute dermal toxicity study of CAPB (31% active) using male and female CD rats (5/sex; 200-232 g).³⁵ The animals received 2.0 g/kg body weight on the clipped surface of the dorsolumbar region. The treated area was occluded. After 24 hours, the dressings were removed and the treated area was washed with warm water and blotted dry. The treated areas were examined daily for 14 days for signs of dermal irritation. The rats were weighed on days 1, 8, and 15. At day 15, the rats were necropsied. No unscheduled deaths occurred and no clinical signs of systemic toxicity were observed. No abnormalities were observed at necropsy. Slight or well-defined erythema was observed on day 2, with well-defined erythema persisting in 3 males and all females on day 3 and completely resolving by day 6. Sloughing or hyperkeratinization affected 6 rats on days 4 and 5 only. The acute lethal dermal dose of CAPB (31% active) was greater than 2.0 g/kg.

Repeated Dose Toxicity

Oral. Male and female Sprague-Dawley rats (8/sex/group) were treated with a full-strength (30.6% active) CAPB

solution.³⁶ Three dose groups (100, 500, and 1000 mg/kg body weight) were given the test material by gavage for at least 28 days. A control group of 16 animals received deionized water. Rats dying during the study and those killed on completion of dosing were necropsied, and tissues were collected for histopathological evaluation.

Mortality was increased in the treated groups as compared to controls, but mortality did not follow a dose-response relationship. The principal necropsy finding in the rats that died was congestion, noted in several tissues, with additional alterations in the lungs of some rats. The death of a high-dose female was ascribed to a dosing accident. It was considered possible that the 1 death of a male of the low-dose group and 1 female of the mid-dose group could be attributed to dosing accidents. The other deaths were related to compound administration. This conclusion was supported by the observation that deaths occurred later (3-4 weeks of study in the mid-dose group, as compared to the high-dose groups: deaths at 1-2 weeks of study). However, doubling of the dose of compound (from 500 to 1000 mg/kg) did not increase mortality, so a dose-response relationship with the mortality was not evident.

Lesions (subacute inflammation and epithelial hyperplasia) of the nonglandular portion of the stomach were suggestive of irritation by CAPB. Lesions were found in 1 of 5 stomachs examined from the high-dose males and in all 7 from high-dose females. The loss of 3 males during the first 2 weeks of dosing prevented adequate evaluation of the response of male rats to the compound. Both males and females of the 100 mg/kg dose group were comparable to concurrent controls.

The American Chemistry Council summarized a 28-day short-term oral toxicity of CAPB (concentration not stated) in Sprague-Dawley rats.³⁵ Male and female rats received 0, 250, 500, or 1000 mg/kg body weight of the test material once daily via oral gavage on 5 consecutive days per week. The number distribution of the rats per group was not described.

No treatment-related deaths or decreases in feed or water consumption were observed over the course of the study. Hematological evaluations, clinical chemistry, ophthalmic examinations, and absolute and relative organ weights also did not find any treatment-related effects. Head protrusion at the beginning of week 3 and salivation at the beginning of week 4 were observed in the 1000 mg/kg dose group. Compound-related edema of the mucosa of the nonglandular stomach was observed at macroscopic examination in the 1000 mg/kg dose group, which disappeared in the rats in the recovery group. Microscopic examination of the rats in the 1000 mg/kg dose group found acanthosis of the gastric mucosa, inflammatory edema of the submucosa, and multiple ulcerations. Effects were greater in the females than the males. These effects were considered to be the result of the irritating properties of CAPB and not of systemic toxicity, especially since the 1000 mg/kg recovery animals had complete and regular regeneration of the nonglandular mucosa. No other treatment-related effects were observed in the organs. The study concluded that the NOEL was 500 mg/kg per d and the LOEL was 1000 mg/kg per d for exposure to CAPB in this rat study.

Groups of 10 male and 10 female Crl:CF(SD)BR Sprague-Dawley rats received 0, 250, 500, or 1000 mg/kg per d CAPB (concentration not stated) in distilled water once daily via oral gavage at a dose volume of 10 mL/kg per d for 92 days.³⁵ Clinical signs were recorded daily and body weight and feed consumption were recorded once weekly. Ophthalmic examinations were performed on the control and 1000 mg/kg per d dose groups prior to dosing and to all groups during the final week of treatment. Blood and urine samples were collected from all rats during the final week of treatment. Complete necropsy was performed on surviving rats at study termination. Histopathology was performed on select tissues from the rats in the control group and the 1000 mg/kg per d dose group. Because treatment-related histopathological changes were observed in the stomachs of the 1000 mg/kg per d group, stomachs from the 250 and 500 mg/kg per d groups also were examined microscopically.

No treatment-related deaths or effects were observed during the course of the study for either sex. Necropsy revealed stomach ulcers at the fundic and cardiac regions in 1 male and 1 female in the high-dose group. Microscopic evaluations found nonglandular gastritis in 6 male and 3 female rats in the 1000 mg/kg per d group, and in 2 male and 2 female rats in the 500 mg/kg per d group. This effect was not observed in the 250 mg/kg per d dose group. No other treatment-related effects were observed. The study concluded that the NOEL for this subchronic study of CAPB in rats was 250 mg/kg per d.

Dermal Irritation

Animal. The available data on skin irritation studies are summarized in Table 6.³⁷⁻⁴³ These studies demonstrated that, while a full-strength CAPB solution, 30% active, was a mild irritant, a 50% dilution was nonirritating.

Human

Cocamidopropyl betaine. In a study of cumulative irritation, 0.3 mL of 2 soap formulations were applied to skin sites on the backs of 10 panelists using occlusive patches.³⁷ Each formulation contained 1.9% active CAPB. Daily 23 hour patches were applied for 21 consecutive days. The total irritation scores for all participants for all 21 applications of the 2 formulations were 588 and 581 (max 630), which indicated that these test formulations were primary irritants. The average irritation times for the formulations were 1.48 and 1.69 days, and the median irritation time was 2 days.

The CAPB at 0.06% (1.0% aqueous dilution of a product formulation containing 6.0% active CAPB) was tested for skin irritation using a single insult occlusive patch test and 19 panelists.¹ Fifteen panelists had no irritation and a + score was recorded for 4 panelists. The formulation was considered practically nonirritating.

Daily doses of 0.2 mL of 0.52% CAPB (an 8% aqueous dilution of a liquid soap formulation containing 6.5% active CAPB) were applied via occlusive patches to the forearms of

12 human participants for 5 days.¹ An erythema score of 0.48 (scale 0-4) was calculated.

Wheat germamidopropyl betaine. The irritation potential of 0.005% active wheat germamidopropyl betaine (a 0.5% aqueous solution of 1.0% wheat germamidopropyl betaine in a body polisher) was evaluated against a control shower gel in a single 24-hour insult patch test. Twenty participants completed the study. Two panelists had a \pm score and 4 panelists had a 1 score and the primary irritation index (PII) was calculated at 0.25. The control substance elicited a \pm score in 4 panelists, a 1 score in 2 panelists, and a + score in 2 panelists, yielding a PII of 0.35. The authors concluded that the test material containing 1.0% wheat germamidopropyl betaine was milder than the reference control.³⁸

Dermal Sensitization

Animal. Delayed contact hypersensitivity of 15 male Pirbright white guinea pigs (400 ± 50 g) to a commercial 10% active sample of CAPB was examined using a maximization test.³⁹ Test animals were administered 0.1 mL of a 50% aqueous solution of Freund complete adjuvant at the first pair of sites on the clipped, dorsoscapular region, 0.1 mL of 0.5% (v/v) dilution of the CAPB (0.05% active CAPB) sample in sterile isotonic saline at the second pair of sites, and 0.1 mL of 0.5% (v/v) dilution of the CAPB (0.05% active CAPB) sample in a 1:1 mixture of isotonic saline and Freund complete adjuvant at the third pair of sites. One week following the injections, a single occlusive 48-hour induction patch of 60% (v/v) dilution of the CAPB (6% active CAPB) sample in distilled water was applied to the same shaved interscapular area. Five control animals received intradermal injections and induction patches without the CAPB solution. All animals received a single occlusive 24-hour challenge patch of 10% (v/v) dilution of the CAPB (1% active CAPB) sample in distilled water on the left flank 2 weeks after the induction.

Well-defined irritation was observed at all sites receiving intradermal injections of Freund adjuvant. Temporary slight irritation was observed following injections of the 0.5% CAPB sample dilution in all test animals. Topical application of the 60% CAPB sample dilution resulted in slight dermal reactions. The barely perceptible erythema observed on the skin of 2 test animals after 24 hours was considered unrelated to CAPB treatment but was attributed to reactions to the elastic adhesive bandages used for site occlusion. With the exception of slight reactions to the bandages, no reactions were observed in controls throughout the 72-hour observation period. No evidence of delayed contact hypersensitivity was found.

A formulation containing 0.75% active CAPB was tested in a delayed contact hypersensitivity test.⁴⁰ Closed patches containing 0.4 mL of the test solution were applied to the shaved area on the left shoulder of 20 albino guinea pigs. After 6 hours, the patch was removed and the area was rinsed with warm water. This procedure was repeated at the same site for the following 2 weeks. The animals were left untreated for 2 weeks

Table 6. Animal Skin Irritation Studies on CAPB

Concentration	Number and Species	Results	References
50%, Diluted 1 part + 1 part (v/v)	3 albino rabbits	No erythema, eschar, or edema; not a primary skin irritant.	44
30% Active ^a	6 Albino rabbits	PII = 0.5. Very slight to well-defined erythema, no edema; mild primary irritant.	45
7.5% Active ^a solution	3 Albino rabbits	No irritation.	46
10% Active ^a solution, pH 6.1	1 Albino rabbit	PII = 0.25; nonirritating.	47
10% Active ^a solution, pH 4.5	6 NZW rabbits	PII = 0.3. Very slight erythema, no edema.	48
30% Active ^a	6 NZW rabbits	PII = 3.75. Eschar formation.	49
15% Active ^a solution	3 Albino rabbits	PII = 3.5. Well-defined erythema, slight edema; not a primary skin irritant.	50

^a Referenced as full strength.

before the primary challenge test, which used 0.01875% CAPB (a 2.5% solution of the 0.75% active CAPB) applied to a freshly clipped skin site not previously treated for 6 hours. Responses were graded after 24 and 48 hours. There was no evidence of sensitization following the exposure to the 3 dermal treatments or challenge dose.

A full-strength, 30% active CAPB sample was tested for skin sensitization using a maximization test and a modified Draize test.⁴¹ Albino guinea pigs (20 animals) received intradermal injections of (1) Freund complete adjuvant alone, (2) 0.1% aqueous dilution of the CAPB sample (0.03% active CAPB), and (3) 0.1% aqueous dilution of the CAPB sample (0.03% active CAPB) plus the adjuvant. One week later, a topical 48-hour occlusive induction patch containing the 10% aqueous dilution of the CAPB sample (3% active CAPB) was applied. Animals in the control group received intradermal injections and topical application of water alone. After 3 weeks, single 24-hour occlusive patches were applied to the clipped flanks of all animals. A 10% aqueous dilution of the CAPB sample (3% active CAPB) was applied to the left flank, and water was applied to the right. The lesions at necropsy were erythema and edema in 8 of the 20 test animals after the challenge application. Microscopic findings included epidermal acanthosis, inter- and intracellular edema, and massive infiltration of the superficial layers of the dermis with lymphocytes, monocytes, and a few eosinophils with a tendency to invade the epidermis in 2 of the animals. Less prominent microscopic lesions of acanthosis, mild intracellular edema, and a moderate lymphomononuclear infiltrate in the superficial dermis were found in 4 additional animals. Slight acanthosis was observed in the remaining 2 animals.

This same laboratory also tested 0.15% active CAPB for induction (0.015% for challenge) using the same assay. Slight erythema and edema were observed macroscopically in 6 of the 20 test animals. Slight acanthosis was observed microscopically. Control animals in the maximization and modified Draize tests had no dermatitis-type clinical or histological alterations. A few controls had moderate acanthosis with edema and vasodilation in the subjacent papillary layer of the dermis. The investigators concluded that the commercially supplied CAPB is capable of producing a delayed-type contact sensitization.

Basketter et al reported that CAPB was positive for sensitization in a local lymph node assay (LLNA).⁴² The EC₃ value was not reported.

Dermal Sensitization

Fisher contact dermatitis recommended that patch testing with CAPB should be performed at a concentration of 1% aqueous.⁴³ Care was advised for patch test readings since mild false-positive irritant reactions may occur.

de Groot, in a review of contact allergy literature, stated that CAPB in rinse off products such as shampoo, shower gel, bath foam, and liquid soap was linked to cosmetic allergy.⁷ Because patch testing for sensitization with these products may result in both false-positive and false-negative reactions, the author suggested that CAPB should be tested separately. The author also suggested that CAPB should be included in the hairdresser's series and the cosmetic series with the knowledge that commercial concentration of CAPB (1% in water, possibly 0.3% active) is a marginal irritant and not all positive patch test reactions indicate contact allergy to CAPB.

Another review of contact allergy literature by Mowad described CAPB as "contact allergen of the year" for 2004.¹⁰ Because impurities in CAPB may be responsible for allergic reactions, the author advised patch testing for amidoamine and DMAPA along with CAPB. The author further suggested that patients that test positive to amidoamine or DMAPA should be advised to avoid products that contain CAPB.

Historically, sensitization study results are reported as positive/negative for a particular concentration of the chemical tested. More recently, the dose per unit area is considered as the relevant parameter.⁵¹ CIR has performed calculations to determine dose per unit area where sufficient information was available.

The available data on clinical sensitization studies are summarized in Table 7.

Cocamidopropyl betaine. A repeated open application procedure was performed with 1.872% CAPB (a 10% w/v aqueous dilution of a shampoo containing 18.72% active CAPB), using 88 human volunteers to determine skin sensitization. [Estimated dose/unit area = concentration × amount × density × unit conversion × area⁻¹ = 2.6 × 10³ µg/cm²]. The disk was removed after 10 minutes. Induction applications were made 3 × a week for 3 weeks. Challenge patch strips were applied simultaneously to both the induction arm and the alternate arm,

Table 7. Clinical Sensitization Studies on CAPB and Related Amidopropyl Betaines.

Exposure	Subjects	Study Type	Result	Reference
Cocamidopropyl Betaine				
0.1872% active CAPB in a shampoo	88	Open application HRIPT	No sensitization	52
0.93% active aqueous sol. of CAPB	93	Open application HRIPT	No sensitization	53
0.3% active CAPB in formulation	100	HRIPT	No sensitization	54
1.5% active aqueous CAPB changed to 3.0% active CAPB	141	HRIPT	No sensitization	55
6% active CAPB in a cleansing cloth	210	HRIPT	No sensitization	56,57
0.018% active CAPB in a facial cleanser	27	HRIPT	No sensitization	58
1% aqueous CAPB or 0.3% active aq. CAPB	781	Patch test	56 positive (7.2%)	59
1% aqueous CAPB or 0.3% active aqueous CAPB	10,798	Patch test	29 positive (0.27%)	60
unknown % CAPB	12	Patch test	Irritation only	61
1% aqueous CAPB or 0.3% active aqueous CAPB	93	Patch test	4 positive reactions	62
1% aqueous CAPB or 0.3% active aqueous CAPB	210	Patch test	12 positive (5.75%)	63
Almondamidopropyl betaine and olivamidopropyl betaine				
1% active almondamidopropyl betaine and 1% active olivamidopropyl betaine in a body cleanser	103	HRIPT	No sensitization	64
Capryl/capramidopropyl betaine				
1.72% active capryl/capramidopropyl betaine in mousse with SLS cotreatment	26	Maximization test	No sensitization	65
Lauramidopropyl betaine				
14% active lauramidopropyl betaine in a shower gel with SLS co-treatment	25	Maximization test	No sensitization	66
0.042% active lauramidopropyl betaine in a shampoo	51	HRIPT	No sensitization	67
0.03955% active aq. sol. of lauramidopropyl betaine in a body cleanser	109	HRIPT	No sensitization	68
Shea Butteramidopropyl Betaine				
0.54% active shea butteramidopropyl betaine in a body wash	25	Maximization test	No sensitization	69
0.04% active aq. sol. of shea butteramidopropyl betaine in a body scrub	101	HRIPT	No sensitization	68

positioned between the shoulder and elbow, 18 days after the last induction application. The areas were scored 24, 48, and 72 hours following the removal of the patch after a 6-hour period. The same procedures were performed with another test substance containing an identical concentration of CAPB. No sensitization was seen in any of the 88 participants exposed to either of the test materials.⁵²

Another study was performed with a 0.93% active aqueous solution of CAPB. [Estimated dose/unit area = $7.7 \times 10^2 \mu\text{g}/\text{cm}^2$].⁵³ Ninety-three volunteers completed the study. Induction applications were made to the same site unless reactions became so strong that a first or second adjacent site had to be used for complete induction, and the sites were scored following a 48-hour period. An alternate site was used for the challenge test and was scored after 48 and 96 hours. Ten participants had slight responses to the test material. These responses were attributed to primary irritation, rather than sensitization, during both the induction and challenge tests.

In a similar study by Hill Top Research, Inc, a formulation containing 0.3% active CAPB was tested on 100 human volunteers.⁵⁴ The study had started out with CAPB at 0.6%, but due to several incidences of mild to moderate skin irritation early in the induction phase, the formulation was diluted. [Estimated dose/unit area = $2.5 \times 10^2 \mu\text{g}/\text{cm}^2$ at 0.3%]. No evidence of sensitization was observed in the formulation at 0.3% active CAPB.

CAPB was studied using 141 human participants. All applications contained a concentration of 1.5% active CAPB in

distilled water, until a protocol modification changed the concentration to 3.0% active CAPB. Participants who began the study a week earlier received 2 applications at a concentration of 1.5%, and all other applications of the test material at a concentration of 3.0%. [Estimated dose/unit area = $5.8 \times 10^1 \mu\text{g}/\text{cm}^2$ at 1.5%, $1.2 \times 10^2 \mu\text{g}/\text{cm}^2$ at 3%]. Induction applications were made to the same, previously untreated site on the back 3 times per week for 3 successive weeks. Patches were removed after 24 hours. Following a 10- to 15-day nontreatment period, the challenge application was applied to a previously untreated site for 24 hours, and the site was scored 24 and 72 hours after patch removal. No responses were observed during either the induction or challenge tests.⁵⁵

Clinical Research Laboratories, Inc performed an RIPT study on 6% active CAPB in cleansing cloths in 2 groups of participants (in phase I, 104 participants completed the study. In phase II, 106 participants completed the study).^{56,57} The test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material was cut to a ½ inch square and applied to the upper back under a semioccluded patch for 24 hours. There were a total of 9 induction patches. Induction sites were scored for irritation. Following a 2-week rest period, challenge patches were applied to a virgin site on the back. After 24 hours, the patches were removed and evaluated for dermal reactions. The test sites were scored again at 48 and 72 hours. No reactions were observed in either group of participants. It was concluded that 6% active CAPB in cleansing cloths did not demonstrate a potential for eliciting dermal irritation or sensitization.

In a study by KGL, Inc, 0.018% active CAPB (a 0.5% aqueous dilution of a facial cleanser containing 3.6% active CAPB) was tested on 27 participants to determine skin sensitization.⁵⁸ In the induction phase, the participants were pretreated with 0.05 mL of 0.25% aqueous sodium lauryl sulfate (SLS) under an occluded 15 mm Webril disc for 24 hours on the upper outer arm, volar forearm, or back. After 24 hours, the SLS patch was removed and 0.05 mL of the test material was applied to the same site and occluded. The induction patch was left in place for 48 hours and the site was scored for irritation. [Estimated dose/unit area = $5.1 \mu\text{g}/\text{cm}^2$]. If no irritation was present, the SLS patch followed by the test material patch procedure was repeated for a total of 5 induction exposures. If irritation developed at any time during the induction phase, the SLS treatment patch was eliminated and only the test material was reapplied after a 24-hour rest period. Following a 10-day rest period, the participants received 0.05 mL of 5% SLS for 1 hour prior to receiving the challenge patch of the test material to the opposite side of the body. The challenge patch was occluded and left in place for 48 hours. After patch removal, the site was scored 15 to 30 minutes later and again at 24 hours. No reactions were observed during the induction or challenge phases of this maximization study. It was concluded that 0.018% active CAPB in a facial cleanser was not likely to cause contact sensitivity reactions under normal use conditions.

Almondamidopropyl betaine and olivamidopropyl betaine. The irritation/sensitization potential of 0.005% almondamidopropyl betaine and 0.005% olivamidopropyl betaine in a body cleanser (a 0.5% dilution of 1% active almondamidopropyl betaine and 1% active olivamidopropyl betaine) was evaluated in a repeat insult patch test of 103 participants. [Estimated dose/unit area for each betaine = $2.5 \mu\text{g}/\text{cm}^2$]. After the induction phase ($3 \times$ per week for 3 weeks) and a 2-week rest period, the participants received a single challenge patch. No reactions were observed. It was concluded that a body cleanser containing 0.005% almondamidopropyl betaine and 0.005% olivamidopropyl betaine was not a primary sensitizer or irritant to the skin.⁶⁴

Capryl/capramidopropyl betaine. KGL, Inc evaluated the contact-sensitizing potential of a mousse (concentrate) containing 1.72% active capryl/capramidopropyl betaine in a maximization study.⁶⁵ Twenty-six adult participants completed the study. During the induction phase, ~ 0.05 mL of aqueous SLS (0.25%) was applied to test sites on the upper outer arm, volar forearm, or the back of each participant. After 24 hours, the SLS patch was removed and 0.05 mL of the test material was applied to the same site and occluded. [Estimated dose/unit area = $4.9 \times 10^2 \mu\text{g}/\text{cm}^2$]. The induction patch was left in place for 48 hours (72 hours if placed over a weekend). After patch removal, the site was examined for irritation. If no irritation was observed, the sequence of patching with SLS followed by patching with the test material was repeated for a total of 5 induction exposures. If irritation was observed during the induction phase, the SLS patch step was eliminated for that participant and only the test material was applied.

At the end of the induction period and a 10-day rest period, a single challenge application of 0.05 mL of the test material was made to a new skin site pretreated with ~ 0.05 mL of 5% SLS under occlusion for 1 hour. After 48 hours, the patch was removed and graded on a scale of 0 (*not sensitized*) to 3 (*strong sensitization*; large vesiculo-bullous reaction) 1 hour and 24 hours after removal. No adverse or unexpected reactions occurred, and no incidences of contact allergy were recorded. The study concluded that the mousse (concentrate) containing 1.72% capryl/capramidopropyl betaine did not have a detectable contact-sensitizing potential and was not likely to cause contact sensitivity reactions under normal use conditions.⁶⁵

Lauramidopropyl betaine. Consumer Product Testing Company performed a repeated insult patch test on a shampoo with 0.042% lauramidopropyl betaine (test material was prepared as a 1% dilution in distilled water of 4.2% active lauramidopropyl betaine).⁶⁷ [Estimated dose/unit area = $2.3 \times 10^1 \mu\text{g}/\text{cm}^2$]. Fifty-one participants completed the study. A total of 9 applications were made during the induction phase. Following a 2-week rest period, a challenge patch was applied to a virgin test site on the back. After 24 hours, the patch was removed and the site was scored 24 and 72 hours postapplication. No reactions were observed in any of the participants during the induction or challenge phases of this study. The study concluded that the shampoo containing 4.2% lauramidopropyl betaine, diluted to 1%, did not indicate a potential or dermal irritation or allergic contact sensitization.

In another human repeated insult patch test, the potential of a body cleanser with 0.03955% lauramidopropyl betaine (a 1% dilution of 3.955% active lauramidopropyl betaine) to cause dermal irritation and sensitization was studied.⁶⁸ One hundred and nine participants completed the study. Prior to patch application, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test solution was applied to the upper back and remained in direct skin contact for 24 hours. The induction period was comprised of a total of 9 applications on the same site. The sites were graded for dermal irritation 24 hours after patch removal. Following a 2-week rest period, a challenge patch was applied to a virgin test site on the back. After 24 hours, the patch was removed and evaluated for dermal reactions. The sites were reevaluated at 48 and 72 hours. Several participants had barely perceptible erythema and reactions were observed on 1 or 2 days of induction phase of the study. No incidences of dermal reaction were recorded during the challenge phase. The study concluded that the body cleanser with 3.955% lauramidopropyl betaine, diluted to 1%, did not demonstrate a potential for eliciting dermal irritation or sensitization.

A maximization study to evaluate the contact-sensitizing potential of a shower gel containing 14% active lauramidopropyl betaine was conducted by KGL, Inc.⁶⁶ The shower gel was tested as received, namely, 0.5% aqueous. Twenty-five adult volunteers completed the study. The study was conducted in the same manner as the capryl/capramidopropyl betaine maximization study described above, with the exception that

~0.1 mL of aqueous SLS (0.25%) and 0.1 mL of the test material were used during the induction and challenge phases. [Estimated dose/unit area = $2.8 \times 10^2 \mu\text{g}/\text{cm}^2$]. No adverse or unexpected reactions occurred, and no incidences of contact allergy were recorded. The study concluded that the shower gel containing 14% lauramidopropyl betaine did not have a detectable contact-sensitizing potential and was not likely to cause contact sensitivity reactions under normal use conditions.

Shea butteramidopropyl betaine. In a human repeated insult patch test, the potential of a body scrub containing 0.04% shea butteramidopropyl betaine (a 1% w/v dilution of 4.0% active shea butteramidopropyl betaine) to cause dermal irritation and sensitization was studied.⁷⁰ One hundred and one participants completed the study. The study followed standard RIPT methodology with a total of 9 induction applications of 24 hours in length and a single challenge application following a 2-week rest period. No adverse events were reported and no incidences of dermal reaction were recorded during the challenge phase. The study concluded that the body scrub with 4.0% shea butteramidopropyl betaine, diluted to 1%, was not sensitizing.

A maximization study to evaluate the contact-sensitizing potential of a body wash containing 0.0027% shea butteramidopropyl betaine (a 0.5% dilution of 0.54% active shea butteramidopropyl betaine) was conducted by KGL, Inc [Estimated dose/unit area = $7.6 \times 10^{-1} \mu\text{g}/\text{cm}^2$].⁶⁹ Twenty-five adult volunteers completed this RIPT study. The study was conducted in the same manner as the capryl/capramdiopropyl betaine study described above, with the exception that the patches were made only to the upper outer arm. No adverse or unexpected reactions occurred, and no incidences of contact allergy were recorded. The study concluded that the body wash containing 0.54% shea butteramidopropyl betaine did not have a detectable contact-sensitizing potential and was not likely to cause contact sensitivity reactions under normal use conditions.

Provocative Studies

In 706 patients studied for skin allergy, 93 (83 women and 10 men) were provisionally diagnosed with cosmetic contact dermatitis.⁷¹ Four of the 93 had positive reactions to CAPB 1% aqueous. Two participants had scalp itch and erythema on the forehead, ears, and neck following the use of shampoos with CAPB. The other 2 participants had eczema on the face and/or neck following use of face cleansers that contained CAPB. From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which would equate to 0.3% active.

Fowler studied 210 patients clinically suspected of having allergic contact dermatitis to cosmetics and toiletries.⁶³ Patch testing with CAPB (1% aqueous) in addition to the North American Contact Dermatitis Group (NACDG) series (70 allergens total) was performed. Twelve of the participants (5.7%) had positive reaction to CAPB in the patch test. Positive reactions were also observed for formaldehyde or formaldehyde releasers, neomycin, and nickel. All but 2 of the

participants had initially reported with head and neck dermatitis. The remaining 2 participants had hand dermatitis. Of the 12 participants, 7 were determined definitely relevant when the reported dermatitis cleared after cessation of use of products with CAPB. Specific case reports for 2 of the participants are detailed in the section on case reports. From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous.

de Groot et al studied 2 groups of patients for CAPB allergy.⁵⁹ The first group consisted of 781 patients that were patch tested with the European standard series, hairdresser's series, cosmetics series, and with other relevant allergens, including the patients' personal care products, and 1% aqueous CAPB from February 1991 to June 1994. Most of the patients in this group were suspected of having occupational contact dermatitis (217 patients were hairdressers). The second group was studied in approximately the same time period and consisted of 102 patients suspected of having cosmetic dermatitis. The patients were patch tested with 1% aqueous CAPB along with the cosmetic screening series. In both groups, relevance was only declared if the patients used products with CAPB and if their dermatitis cleared upon cessation of use of these products.

In the first test group, 56 patients (7.2%) had positive reactions to CAPB, and of these, 17 were classified as definite and all used shampoos and/or shower gels that contained CAPB. Eight of the 17 were hairdressers and had experienced dermatitis on their hands. In the second test group, only 3 patients (3%) had a positive reaction to CAPB. The patients had been using shower gels, shampoos, and/or body lotions containing CAPB. From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous.

Armstrong et al patch tested patients with suspected contact dermatitis (from January 1991 to September 1998) with a standard series that included 1% aqueous tegobetaine L7 (from 1991 to 1994) or 1% aqueous CAPB (from 1995 to 1998). The authors noted that the latter had significantly lower intermediate and reactant impurities.⁶⁰ Of the 10 798 patients tested, 29 (0.27%) had a positive reaction to CAPB (24 reactions to tegobetaine L7). Twenty-three of the 29 cases were deemed relevant and had reported dermatitis on the face, neck, hands, or widespread areas. The authors suggested that higher purity CAPB was linked to a diminished frequency of CAPB sensitization. From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous.

In a double-blind randomized controlled study to evaluate allergenicity to coconut oil derivatives, 10 control participants and 12 participants with previously diagnosed allergy to CAPB were patch tested with 11 coconut-derived surfactants, coconut oil, and lauric acid.⁶¹ Patch testing was performed in random order according to standardized procedures with readings at 48 and 96 hours. Three of the 12 participants had doubtful reactions to CAPB in the patch test and 1 control participant had a doubtful reaction to CAPB. The authors suggested that

Table 8. Eye Irritation Studies on CAPB

Concentration	No./strain of rabbit	Results	Reference
4.5% active ^b	6/albino	Slight conjunctival irritation in 3 unrinsed eyes. Very slight conjunctival irritation in 2 of 3 rinsed eyes.	86
30% active ^b	3/albino	Diffuse corneal opacity at day 3. Mild conjunctival erythema, chemosis, and discharge from day 1. Slight iritis on day 4.	87
6% active solution	3/albino	Mild conjunctival erythema and slight discharge, cleared by day 3.	88
7.5% active, pH 8.3	6/NZW	Mild to moderate conjunctival irritation after 24 h, disappearing by day 6.	89
10% active ^b , pH 6.1	1/albino	Max. unrinsed score = 30 after day 3, 7 by day 7.	47
30% active ^a	9/NZW	Max. mean score (unrinsed, n = 6) = 41.7 after 72 h, decreased to 27.2 after 7 days (scale 0 - 110). Minimal irritation in rinsed eyes (n = 3).	90
8.6% active ^a	9/NZW	Max unrinsed score = 25.7 after 24 h, 0 by day 7. Mean score rinsed (n = 3) = 2.0 after 24 h, 0 by 48 h.	91
5%	6/NZW	Draize score = 4.90. Not an ocular irritant.	92
10%	6/NZW	Draize score = 27.3. Moderately irritating.	93
3.0% active	6/albino	Corneal irritation day 3 - 7. Iritis and conjunctival irritation lessens in severity by day 7.	94
3.0% active	6/albino	No corneal irritation. Iritis and conjunctival irritation clear by day 7.	94
3.0% active	6/albino	Average ocular index = 41.6/110. Ocular irritant.	95,96
Soap formulation containing 2.3% active ^b CAPB	9/NZW	Max mean score (unrinsed, n = 6) = 18.7, primarily irritation of iris and conjunctiva. Max mean score (rinsed, n = 3) = 20.0.	97
Soap formulation containing 2.3% active ^b CAPB	9/NZW	Max mean score (unrinsed, n = 6) = 1.7. Max mean score (rinsed, n = 3) = 3.3. Primarily conjunctival irritation.	98
Soap formulation containing 6.5% active ^b CAPB	4/NZW	Max total score = 30.0 (max 110). Irritation of cornea, iris, and conjunctiva. Moderately irritating.	99
Formulation containing 6.0% active ^b CAPB	6/albino	Conjunctival irritation after day 1.	1

^a Reference cited as % solids.^b Referenced as full strength.

doubtful reactions to CAPB represent irritant reactions and not allergic reactions.

Photosensitization

An investigation of the potential of a 3.0% active aqueous solution of CAPB to induce contact photoallergy was tested using 30 human participants. The 11 participants who had mild to moderate erythemic responses at the irradiated sites during the induction testing were those that received both UVA and 2 MED of UVB irradiation (source spectrum not reported). These responses were expected from the UVB exposure alone. The CAPB was not a photosensitizer in this study.⁵⁵

Case Reports

Numerous case studies of allergic contact dermatitis reported positive patch tests to CAPB at concentration as low as 0.5%.⁷²⁻⁸⁴

Ocular Irritation

The available data on ocular irritation studies are summarized in Table 8. Two groups of 3 albino rabbits received 0.1 mL instillations of 4.5% active solution of CAPB into the conjunctival sac of 1 eye.⁸⁵ Treated eyes of one group were rinsed, but the treated eyes of the other group were not rinsed. Slight

conjunctival erythema and chemosis were noted in all treated, unrinsed eyes by day 2 following instillation and subsided by day 7. Slight conjunctival irritation was observed in 2 of the 3 treated, rinsed eyes on the first 2 days of observation. There was no corneal involvement or iris congestion.

The CAPB (30% active) was instilled (0.1 mL) into the conjunctival sac of 1 of the eyes of 3 albino rabbits using the Draize method.⁸⁷ Diffuse corneal opacity was observed by day 3 following instillation. Slight iritis was observed by day 4. Mild conjunctival erythema, chemosis, and discharge were noted from day 1.

Three albino rabbits received a 0.1 mL instillation of a 6% active CAPB solution into the conjunctival sac of the right eye.⁸⁸ Mild conjunctival erythema and slight discharge were observed in all treated eyes for the first 2 days after instillation, clearing by the third day.

Six NZW rabbits (body weight range 2.4-2.6 kg) received an instillation of 0.1 mL of 7.5% active CAPB with a pH of 8.3 into the conjunctival sac of the left eye.⁸⁹ Mild to moderate conjunctival irritation was observed in all treated eyes after 24 hours. The treated eye of 1 rabbit had moderate corneal opacity after the second day. These alterations disappeared by the sixth day after instillation.

One rabbit receiving a 0.1 mL administration of a 10% active CAPB solution (pH 6.1) had Draize scores of 28 after day 1, 25 after day 2, 30 after day 3, 14 after day 4, and 7 after day 7 of the observation period.⁴⁷

A full-strength sample of CAPB (30% active) was tested for ocular irritation using 9 NZW rabbits.⁹⁰ A volume of 0.1 mL was instilled into the conjunctival sac of one eye of each rabbit. Mean eye irritation scores for treated, unrinsed eyes were 32.5 ± 4.4 after 24 hours, 31.7 ± 3.3 after 48 hours, 41.7 ± 11.7 after 72 hours, and 27.2 ± 11.4 after 7 days (scale 0-110). Corneal opacity, slight iritis, and conjunctival irritation and necrosis were noted in treated, unrinsed eyes. Under these conditions, the sample was considered corrosive. Minimal irritation (mean score = 10.0 ± 2.0 after 24 hours), subsiding after 48 hours, was noted in treated eyes that had been rinsed.

An instillation of 0.1 mL of a sample of 10% active CAPB was made into the conjunctival sac of 1 of the eyes of 9 NZW rabbits.⁹¹ Mean eye irritation scores for treated, unrinsed eyes were 25.7 ± 8.3 after 24 hours, 16.7 ± 10.9 after 48 hours, and 9.3 ± 11.4 after 72 hours. No irritation was observed on day 7. Treated, rinsed eyes had a mean score of 2.0 ± 2.0 after 24 hours, returning to normal after 48 hours. The CAPB sample was considered moderately irritating to treated, unrinsed eyes and practically nonirritating to treated, rinsed eyes under these conditions.

In 2 ocular irritation studies by Hazelton Laboratories, 0.1 mL of either 5% or 10% CAPB was instilled into the left eye of groups of 6 NZW rabbits.^{92,93} The CAPB was not an ocular irritant in the 5% group (Draize score = 4.90) but was considered moderately irritating in the 10% group (Draize score = 27.3).

In a Draize test for ocular irritation, two 3.0% active CAPB samples were instilled into the conjunctival sac of 6 albino rabbits.⁹⁴ Scores for corneal irritation were 0 for the first 2 observation days, 1.66 for the third and fourth days, and 4.16 on the seventh day (max score = 80) for 1 of the CAPB samples. No corneal irritation was observed in eyes treated with the other sample. Both samples produced iritis by the first day (scores of 8.33 and 5, respectively, on a scale of 0-10), which decreased in severity by the seventh day (scores of 4.16 and 0, respectively). Both samples produced conjunctival irritation (scores of 15.37 and 14.33, respectively, on a scale of 0-20), which decreased in severity by the seventh day (scores of 6 and 0, respectively).

A 3.0% active CAPB sample was tested for ocular irritation using 6 male albino rabbits.^{95,96} The average ocular index was 41.6 (max = 110) 24 hours after instillation of 0.1 mL of the sample. The sample was considered an ocular irritant.

A volume of 0.1 mL of a liquid soap formulation containing 2.3% active CAPB was instilled into the conjunctival sac of each of 9 NZW rabbits.⁹⁷ An average irritation score of 18.7 (max 110) was calculated for unrinsed eyes, which compared with 20.0 for rinsed eyes. Irritation was observed primarily in the iris and conjunctiva. Under both sets of conditions, the liquid soap formulation was considered moderately irritating.

Another liquid formulation containing 2.3% active CAPB was tested for ocular irritation using 9 NZW rabbits.⁹⁸ The maximum average irritation score for the 6 treated, unrinsed eyes was 1.7 (max 110). Slight conjunctival erythema and chemosis were observed in 1 rabbit 2 days after treatment and in

the eye of another for the entire 7-day observation period. Slight discharge also was observed in the treated eye of the latter from 72 hours to 7 days following treatment. The formulation was considered minimally irritating to treated, unrinsed eyes of rabbits. The maximum average irritation score for the 3 treated, rinsed eyes was 3.3. Mild conjunctival erythema and chemosis were observed in all tested eyes 1 to 2 days following the instillation. The formulation was considered mildly irritating to treated, rinsed eyes of rabbits.

A liquid soap formulation containing 6.5% active CAPB was tested for ocular irritation by instilling 0.1 mL into the conjunctival sac of one eye of each of 4 NZW rabbits, followed by rinsing.⁹⁹ Mean corneal irritation scores were 13.8 after 1 hour, 18.8 after 24 hours, 11.3 after 48 hours, 5 after 72 hours, and 1.3 after 7 days (max 80). Mean iridial irritation scores were 3.8 after 1 hour and 24 hours, decreasing to 0 after 7 days. Mean conjunctival irritation scores were 11 after 1 hour, 7.5 after 24 hours, 4 after 48 hours, 3.5 after 72 hours, and 2 after 7 days. No irritation was observed 14 days after the instillation. With a total mean irritation score of 30.0 (max. total = 110.0), the formulation was considered moderately irritating.

A single 0.1 mL dose of a product formulation containing 6.0% active CAPB was instilled into the conjunctival sac of each of 6 albino rabbits in a Draize test.¹ Conjunctival irritation (mean score of 4; max = 20) was observed in all treated eyes on the first day following instillation, decreasing in severity on the second day. No corneal irritation or iritis was observed.

Mucous Membrane Irritation

Two soap formulations containing 7.5% CAPB were tested for vaginal irritation potential in Beagle dogs (7-10 months old; 8.2-10 kg). The formulations were tested in 3 dogs each. Prior to treatment and again before termination, hematology, clinical chemistry, and urinalysis were performed. A volume of 20 mL of the test material was administered into the vagina via a syringe once a day for 15 days (weekdays only). Vaginas and vulvas were examined 6 hours prior to and after each daily treatment. At termination of the study, the dogs were killed and necropsied. Tissue samples of the liver, kidney, and vulva/vagina were examined. Blood was found in the urine of 5/6 dogs. Gross necropsy revealed discoloration of the lining of the vagina in 5/6 dogs. Diffuse necrosis of vaginal mucosa occurred in 5/6 dogs and focal vaginal necrosis occurred in 1 dog (this dog was in estrus). There was corresponding inflammatory cell infiltration (mainly neutrophils) and often a fibrinopurulent membrane adherent to the injured surface. It was concluded that lesions were the result of test material application. Morphologic changes in the liver and kidneys in all dogs were not considered significant and were within normal parameters.^{100,101} (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 7.5% active or 7.5% aqueous, which equated to 2.25% active.)

Genotoxicity

Bacterial Assays

A commercial sample of CAPB (31.0% active) was tested using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, both with and without metabolic activation. The concentrations of CAPB solution tested were 0.004, 0.02, 0.1, 0.2, and 0.4 $\mu\text{L}/\text{plate}$. The CAPB is toxic above 0.3 $\mu\text{L}/\text{plate}$. The test material did not cause a significant increase in mutation frequency in any of the strains tested with or without metabolic activation.¹⁰²

CAPB (30% active) was tested using *S typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, with and without metabolic activation. Eight concentrations between 0.001 and 0.300 $\mu\text{L}/\text{plate}$ were used, based on CAPB solubility. The CAPB did not produce an increase in mutation frequency, with or without metabolic activation.¹⁰³

In a study summarized by the American Chemistry Council, CAPB (28.5–30.5% active) was tested using *S typhimurium* strains TA98, TA1535, TA1537, and TA1538, both with and without metabolic activation at 0, 50, 150, 500, 1500, or 5000 $\mu\text{g}/\text{plate}$.³⁵ Positive controls were *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (for TA100 and TA1535), 9-aminoacridine (for TA1537), 4-nitro-*o*-phenylenediamine (for TA1538), 4-nitroquinoline-1-oxide (for TA98), and 2-aminoanthracene (in all strains with metabolic activation only). Cytotoxicity was observed at 150 $\mu\text{L}/\text{plate}$ and above. The CAPB in this assay was found to be nonmutagenic.

The American Chemistry Council also summarized the findings of a CAPB (concentration not stated) mutagenicity assay using *S typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, with and without metabolic activation.³⁵ The test material was tested at 1, 4, 16, 64, or 256 $\mu\text{g}/\text{plate}$ without S-9 activation and at 4, 16, 64, 256, and 1024 $\mu\text{g}/\text{plate}$ with S-9 activation. The CAPB did not increase the mutation frequency, with or without metabolic activation.

Mammalian Cell Assays

The mutagenic potential of a 30.9% active sample of CAPB was tested in a L5178Y TK \pm mouse lymphoma assay with and without metabolic activation. The test substance was solubilized in water and diluted for testing at concentrations of 0.001, 0.01, 0.1, 1.0, 10, and 100 $\mu\text{L}/\text{mL}$. None of the treated cultures had a significant increase in mutation frequency over the average mutant frequency of the solvent controls.¹⁰⁴

Animal Assays

The American Chemistry Council summarized a mouse micronucleus test that studied CAPB (concentration not stated).³⁵ Groups of 5 male and 5 female OF1 mice received 2 doses of either 0.02 or 0.2 g/kg of the test material in sterile distilled water via intraperitoneal injection (dose volume 10 g/kg) at 24-hour intervals. Negative and positive controls received sterile distilled water and cyclophosphamide, respectively. The

rats were killed 6 hours after the second administration of the test material and bone marrow slides were prepared. One thousand polychromatic erythrocytes (PCEs) per animal were studied for the presence of micronuclei. In both dose groups, the number of micronucleated PCEs was not increased when compared to the negative control. The positive control group yielded expected results. The CAPB was not a mutagen under the conditions of this study.

Carcinogenicity

An aqueous preparation of a nonoxidative hair dye formulation containing an unspecified grade of CAPB at a concentration of 0.09% active CAPB was tested for carcinogenicity using groups of 60 male and female random-bred Swiss Webster mice from the Epplé colony.¹⁰⁵ The formulation also contained 5% propylene glycol, 4% benzyl alcohol, 0.6% kelzan (xanthan gum), 0.9% lactic acid, 0.04% fragrance, and less than 0.1% each of the disperse brown, red, yellow, and blue dyes. A dose of 0.05 mL per mouse was applied 3 times weekly for 20 months to interscapular skin that was clipped free of hair and shaved. Mortality, behavior, and physical appearance of the mice were observed daily. Dermal changes in particular were noted. Body weights were recorded weekly. Ten males and 10 females from each group were killed at 9 months for a hematological study, urinalysis, and necropsy. At termination, all mice were necropsied, and the tissues were examined microscopically. No adverse effects were noted on average body weight gains, survival, hematological or urinalysis values in any group. Varying degrees of chronic inflammation of the skin were seen in all groups, including controls. Other lesions occurred but were considered unrelated to hair dye treatment. The incidence of neoplasms in treated animals did not differ significantly from control groups.

Irritation/Sensitization Studies With Amidoamine, DMAPA, and Related Amines

Amidoamine is a term used for fatty acid esters of amidopropyl dimethylamine, intermediates in the synthesis of the amidopropyl betaines; DMAPA is also an intermediate in the synthesis of the amidopropyl betaines. These compounds can exist as impurities in cosmetic formulations containing amidopropyl betaines.

Animal Studies

Hill Top Research, Inc performed a delayed contact hypersensitivity study of stearamidopropyl dimethylamine in guinea pigs.¹⁰⁶ A pre-induction primary irritation study was conducted to determine the concentration for the induction phase of the study. Twenty Hartley outbred guinea pigs were treated with 1.0% w/v stearamidopropyl dimethylamine in 80% ethanol/20% distilled water. The test material was applied for 6 hours at a dose volume of 0.3 mL using 25 mm diameter occluded Hill Top chambers on clipped, intact skin on the left shoulder.

[Estimated dose/unit area = $6.1 \times 10^2 \mu\text{g}/\text{cm}^2$]. The exposure sites were rinsed after removal of chambers and re-exposed once a week for a total of 3 exposures. A control group of 10 guinea pigs received the vehicle alone. After a 2-week rest period, the animals received primary challenge patches of 0.25% w/v stearamidopropyl dimethylamine in acetone on naive skin. [Estimated dose/unit area = $1.5 \times 10^2 \mu\text{g}/\text{cm}^2$]. One guinea pig had delayed contact hypersensitivity to the test material. The control animals had no reactions. A rechallenge was conducted in 6 guinea pigs 13 days after the primary challenge with 0.25%, 0.125%, and 0.0625% w/v stearamidopropyl dimethylamine. An additional 5 animals were used as controls. One guinea pig had a positive response to the test material at 0.25%. No other reactions were observed.

Palmityl/stearylamidopropyl dimethylamine at a concentration of 25% active in 8.95% phosphoric acid and 66.05% water was studied for delayed contact hypersensitivity using albino Dunkin/Hartley guinea pigs.¹⁰⁷ A preliminary irritation test was conducted to determine the maximum concentration for the induction and challenge phases of the study. In the induction phase, 10 male and 10 female animals received 0.4 mL of test material on a 4 cm² patch on the clipped skin of the left shoulder for a period of 6 hours. [Estimated dose/unit area = $2.5 \times 10^4 \mu\text{g}/\text{cm}^2$]. The patches were occluded. An additional 5 male and 5 female animals were left untreated as the controls. A total of 3 induction patches were applied, once weekly, for 3 weeks. Following a 2-week rest period, all animals received primary challenge patches of 0.4 mL of test material on the right flank for 6 hours. The test sites were scored at 24 and 48 hours postapplication. All but 3 of the 20 guinea pigs had patchy to severe erythema at the 24- and 48-hour observation periods. Four control animals had slight to moderate patchy erythema during the observation periods. Rechallenges were conducted on 0.25% active and 0.5% active palmityl/stearylamidopropyl dimethylamine. No sensitization was observed with the 0.25% active material, but 0.5% active material elicited reactions in sensitized animals. The study concluded that palmityl/stearylamidopropyl dimethylamine had the potential to cause delayed contact hypersensitivity in guinea pigs.

Two guinea pig maximization studies to assess the skin sensitization potential of amidoamine were evaluated.⁷¹ In the first study, preliminary tests determined the maximum concentrations of intradermal injections, topical induction, and challenge applications. Ten albino Dunkin/Hartley guinea pigs (6 females and 4 males) received two 0.1 mL injections of 50% Freund complete adjuvant at the first pair of sites, two 0.1 mL injections of 0.1% amidoamine at the second pair of sites, and two 0.1 mL injections of amidoamine in DOBS/saline vehicle and Freund complete adjuvant (50/50 ratio) to yield a final concentration of 0.1% amidoamine at the third pair of sites. One week following the injections, a single occlusive 48-hour induction patch (2 × 4 cm) of 0.2 to 0.3 mL amidoamine 5% in acetone/PEG400 vehicle was applied to the same shaved area. Four male control animals received intradermal injections and induction patches using only the vehicles. Two weeks after the induction patch, all animals received a single

occlusive 24-hour challenge patch (8 mm diameter patch in a Finn chamber) saturated with 0.5% amidoamine in acetone/PEG 400 on a clipped and shaved flank. The treatment sites were examined 24 and 48 hours after patch removal. Two more challenges were made 1 and 2 weeks after the first challenge. Reactions were scored on a scale of 0 (*no reaction*) to 3 (*severe erythema and edema*).

At the first challenge, 7 animals had a reaction score of ≥ 0.5 at 24 hours after the removal of the patch. After 48 hours, 6 animals had a reaction ≥ 0.5 . Three out of 10 animals had a reaction score of 2. At the second challenge, 7 guinea pigs had a score of ≥ 0.5 at 24 hours after patch removal. These scores were consistent at the 48-hour reading. Five out of 10 animals had a reaction score of 2. At the third challenge, all 10 guinea pigs had a score ≥ 1 at 24 hours after patch removal. These scores remained largely consistent at the 48-hour reading. Eight of the 10 animals had a reaction score of 2. The study concluded that amidoamine was a moderate sensitizer.⁷¹

The second maximization study was conducted in the same manner as the first with the only changes being that 0.025% amidoamine was used in the intradermal injections instead of 0.1%, 1% amidoamine was used in the topical induction, only 2 challenges were made, and 4 female guinea pigs were used as controls.

At the first challenge, 3 animals had a reaction score of ≥ 1 at both the 24- and 48-hour readings, with 1 of the animals scoring a 2. At the second challenge, 3 animals had a reaction score of ≥ 1 at 24- and 48-hour readings, although 1 animal had no reaction at 48 hours had 1 at 24 hours, while another that had no reaction at 24 hours had 1 at 48 hours. The study concluded that amidoamine was a moderate sensitizer.⁷¹

Wright et al reported on the results of an LLNA study performed on 4 chemicals that are recognized human contact allergens, including DMAPA (99.0+ % pure).⁷² The chemicals were tested in 7 different vehicles: acetone, olive oil (4:1), dimethylsulfoxide, methethylketone, dimethyl formamide, propylene glycol, and 50:50 and 90:10 mixtures of ethanol and water. Groups of 4 female CBA/Ca mice were exposed topically on the dorsum of both ears to 25 μL of 0.5%, 1.0%, 2.5%, 5.0%, or 10.0% of the test material, or to an equal volume of the appropriate vehicle alone, daily for 3 consecutive days. Five days after the initial topical treatment, all animals were injected intravenously with 20 μCi of [³H] methyl thymidine. Approximately 5 hours after injection, the animals were killed and the auricular lymph nodes were excised. Single-cell suspensions were prepared from pooled lymph nodes, with the cells precipitated by trichloroacetic acid (TCA), and the radioactivity measured by liquid scintillation. The stimulation indices (SIs) were calculated, and at 10.0% DMAPA ranged from 2.2 in propylene glycol to 15.7 in dimethyl formamide. The estimated concentrations for a SI of 3 (EC₃) ranged from 1.7% (in dimethyl formamide) to >10% (in propylene glycol).

An LLNA study was performed using stearamidopropyl dimethylamine (TEGO AMID S 18).¹⁰⁸ A certificate of analysis reported that the DMAPA level conformed to the ≤ 20 ppm limit, the amine value was 150.8 mg KOH/g (limit

range = 148.0-152.0 mg KOH/g), and the melting point was 68.0°C (limit range 66.0°C-69.0°C).¹⁰⁹ CBA/Ca female mice were divided into 5 groups of 4 and received 0.1%, 0.5%, 1%, 2.5%, or 5% (w/v) of the test material in ethanol/water (7/3, v/v) on the dorsum of each ear lobe (25 µL per ear, diameter ~8 mm) once daily for 3 consecutive days. A control group of 4 mice was treated with the vehicle only. The positive control group received α -hexylcinnamaldehyde in acetone:olive oil (4:1, v/v). The mice were treated with [³H] methyl thymidine, killed, and the lymph nodes were prepared in the manner as described in the previous study.

No deaths occurred during the treatment period in any dose group. No clinical signs of toxicity were observed during treatment in the control group or in the 0.1% and 0.5% dose groups. Slight to moderate ear erythema was observed after the second or third application at both dosing sites in all mice in the 1%, 2.5%, and the 5% dose groups. This persisted for 2 days in the 1% dose group and until treatment end in the 2.5% and 5% dose groups. Body weight development was not affected in any of the animals. The SIs were 1.4, 2.1, 2.1, 5.8, and 3.9 for the 0.1%, 0.5%, 1%, 2.5%, and 5% dose groups, respectively. The EC₃ was calculated at 1.4%. The positive control group had expected results and validated the study. The study concluded that stearamidopropyl dimethylamine (TEGO AMID S 18) was a potential skin sensitizer in this LLNA test.¹⁰⁸

Calvert Laboratories, Inc performed an LLNA study using amidoamine (~99% C12-C18).¹¹⁰ A preliminary dose range study was performed. In the main study, groups of 5 mice received 0%, 0.1%, 0.5%, 1%, 2.5%, or 5% of the test material in ethanol/water, 7:3 (v/v) neutralized to pH 6.0 with citric acid monohydrate. An additional 5 mice received the positive control, 35% hexylcinnamaldehyde. The mice were treated on the dorsal surface of both ears (25 µL/ear) once daily for 3 days. On day 6, the mice were injected intravenously (iv) with 20 µCi of ³H-thymidine. Five hours later, the mice were killed and the draining auricular lymph nodes were removed, processed, and assessed for lymphocyte proliferation. No mortality or adverse effects were observed throughout the study. Very slight erythema was observed on day 3 and very slight erythema and edema were observed on days 4 to 6 of the 2.5% dose group. In the 5% dose group, 4 of the 5 mice treated had very slight erythema and very slight edema on day 2. On days 3 to 6, mice in this dose group had well-defined erythema and slight edema. The SIs were 1.8, 1.0, 3.1, 24.5, and 60.6 for the 0.1%, 0.5%, 1%, 2.5%, or 5% dose groups, respectively. The EC₃ for amidoamine was calculated at 0.98%. The positive control group had expected results and validated the study. This LLNA study concluded that amidoamine has skin-sensitizing activity.

Human Studies

Hill Top Research, Inc performed an investigation of the potential of stearamidopropyl dimethylamine to induce skin sensitization in 112 human participants.⁷³ Applications contained a concentration of 0.25% w/v of the test material in undiluted mineral oil. Induction applications of 0.3 mL were made to the

same site, with a Webril patch for a total of 9 applications. Challenge applications were made to naive alternate sites. Frequent incidences of slight to moderate irritation, including erythema, some edema, papules, glazing, and cracking, were observed during the induction period but were considered transient. Five participants had a reaction of grade 1 or greater during the challenge phase. The responses to stearamidopropyl dimethylamine were indicative of primary irritation rather than contact sensitization.

In a study by Inveresk Research International, the sensitization potential of a 4% aqueous liquid fabric softener formulation containing 0.5% stearyl/palmitylamidopropyl dimethylamine was investigated using 77 participants.⁷⁴ During the induction phase, the test material was applied at a dose volume of 0.5 mL with a ¾ inch square Webril pad to the dorsal surface of the upper arm. [Estimated dose/unit area = 6.9×10^2 µg/cm²]. Patches were applied for a duration of 24 hours, 9 times over a period of 3 weeks. The test material caused some degree of irritation in most volunteers. After a rest period of 2 weeks, the participants received challenge patches with the same concentration of test material on both arms. Patch sites were graded 48 and 96 hours after patching. Eight participants reacted at challenge, and 7 submitted to rechallenge with 4% and 0.4% aqueous formulations. No reactions indicative of sensitization occurred at rechallenge. The test formulation containing stearyl/palmitylamidopropyl dimethylamine had no significant sensitization potential.

Foti et al patch tested 285 consecutive dermatitis patients with the European standard series supplemented with oleamidopropyl dimethylamine (0.5% aqueous), CAPB (1% aqueous), and DMAPA (1% aqueous).⁷⁵ The standard patching technique was employed and test sites were scored on days 2, 3, 4, and 7. Twenty-three patients (8%) had allergic responses to DMAPA, 14 patients (4.9%) had allergic responses to DMAPA and oleamidopropyl dimethylamine, and 8 patients (2.8%) had allergic responses to all 3 of the supplemental chemicals. Analyses by thin-layer chromatography (TLC) of the oleamidopropyl dimethyl amine sample revealed contamination by DMAPA (6 ppm or 0.12% of the sample) and indicated that the allergic responses in the last group were not due to cross-reaction. (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

In a 2-year study by Pigatto et al, 1190 patients with eczema were patch tested with 1% aqueous CAPB using standard technique and grading according to the European Contact Dermatitis Group (ECDG).⁷⁶ From this patch test, 17 patients were diagnosed with allergic contact dermatitis to CAPB. Relevance was established with an additional positive patch test of 2+ or more to at least 1 personal care product containing CAPB used by the patients. Fifteen patients were further tested with CAPB 0.01%, 0.5%, 1% (from 2 different manufactures), and 2% in water; and DMAPA at 0.05%, 0.1%, and 1% in petrolatum; and, if possible, the patients' reported cosmetics diluted in water at 1:10, 1:100, and 1:1000.

In 12 patients tested with their own personal cosmetics, 9 had positive reactions to at least 1 dilution and 5 had irritant reactions. All except 3 patients, who were not tested, had 2 or 3+ reaction to DMAPA at concentrations as low as 0.05%. Only 1 patient had a positive reaction to CAPB. The presence of DMAPA was investigated via TLC in the personal cosmetics of 4 of the patients that had positive reactions. These positive reactions from DMAPA suggest that the positive reaction to CAPB-containing products was likely due to a certain concentration of DMAPA that was an impurity. The DMAPA was measured in the products at 50 to 150 ppm. The concentration of DMAPA was also measured in the 2 CAPB types: one had a concentration of DMAPA at 200 ppm and DMAPA was below detection level (level not reported) in the other type. The authors stated that the sensitizing agent in CAPB allergy is DMAPA, although their findings did not exclude the role of CAPB itself from causing allergic dermatitis.⁷⁶ (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

A study of sensitization to commercially available CAPB in patients with dermatitis was performed by Angelini et al.⁷⁷ Twelve hundred consecutive patients with dermatitis of various types were patch tested with the European standard series and CAPB 1% aqueous (30% active ingredient). Some of the patients that had allergic or irritant reactions to CAPB were then patch tested with the chemicals that were intermediates or reactants in the synthesis of CAPB (amidoamine, DMAPA, and monochloroacetic acid) along with a sample of CAPB of greater purity and Tego 103 G 1% aqueous.

Positive allergic reactions to CAPB were observed in 46 participants (3.8%), while irritant reactions were recorded in 15 participants (1.25%). Of these 46 participants, 30 had positive reactions to DMAPA 1% aqueous. In these 30 participants, 3 and 16 were positive to the purer grade of CAPB 0.5% aqueous and CAPB 1% aqueous, respectively. Patients with irritant reactions had negative reactions to the synthetic materials and to the purer grade of CAPB. No allergic or irritant reactions to DMAPA were observed in 50 healthy controls. No positive reactions to amidoamine 0.05% were observed. The authors concluded that the results suggested that DMAPA impurity was responsible for CAPB allergy.⁷⁷ (From the study documentation, it was not possible to determine whether the administered CAPB concentrations were 0.5% active and 1% active or 0.5% aqueous and 1% aqueous, which equated to 0.15% active and 0.3% active, respectively.)

A further study by Angelini et al was performed to determine whether CAPB or an impurity of CAPB was responsible for cases of contact dermatitis.⁷⁸ In this study, TLC was employed to analyze a sample of CAPB (Tego Betaine F 30% solution) and isolate and identify unknown impurities other than DMAPA, chloroacetic acid, and amidoamine found in the CAPB solution. An infrared spectrum analysis was used to confirm the presence of the sodium salt of *N,N*-dimethyl-propylene-diaminotriacetic acid.

Upon identifying the impurity, 30 patients with a history of contact allergy to 1% aqueous CAPB and 1% DMAPA were patch tested with pure CAPB and a blend containing sodium chloride and *N,N*-dimethyl-propylene-diaminotriacetic acid (both at 1%). None of the participants reacted to any of the chemicals. The authors suggested that pure CAPB, chloroacetic acid, amidoamine, and *N,N*-dimethyl-propylene-diaminotriacetic acid were not the components responsible for CAPB sensitivity and the involvement of DMAPA cannot be ruled out.⁷⁸ (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

In another study by Angelini et al, DMAPA was tested at varying concentrations with other tensioactive chemicals to determine whether they enhanced sensitivity to DMAPA.⁷⁹ Thirty-four participants with confirmed contact allergy to 1% aqueous DMAPA were patch tested with DMAPA in water, DMAPA in a SLES 2% aqueous solution, and DMAPA in a polysorbate 20 2% aqueous solution, all in decreasing concentrations from 0.1% to 0.00005%. The participants were also patch tested with CAPB and a series of 10 substances chemically related to DMAPA. Test sites were occluded for 2 days and the sites were measured for reactions on days 2, 3, 4, and 7.

Eighteen participants had positive reaction to DMAPA in water at 0.1%. No positive reactions were noted for DMAPA in water at 0.01% to 0.00005%. Positive reactions were observed in DMAPA in SLES, with 27 participants positive at the highest concentration, 10 participants positive at 0.01%, 5 participants positive at 0.005%, and 1 participant positive at 0.0001%. Positive reactions were also observed in DMAPA in polysorbate 20 in 21 participants at 0.1% and 4 participants at 0.01%. Patch tests for the chemically related structures were positive in 28 participants for *N,N*-dimethyl-2-ethylenediamine 1% aqueous, 12 participants for cocamidopropylamine oxide 1% aqueous (35% active material), and 18 participants for CAPB 1% aqueous (30% active material). No other reactions occurred. The authors concluded that tensioactives such as SLES and polysorbate 20 may enhance the risk of sensitization to DMAPA at low concentrations. They also concluded that the primary amine and the tertiary amine groups (dimethyl substituted) are the sensitizing chemical structures in DMAPA and related molecules when they are separated by 2 or 3 carbon atoms.⁷⁹

In another study by Angelini et al, 20 patients (ages 17-51 years, 13 females and 7 males) with confirmed contact allergy to DMAPA (1% aqueous) and CAPB (1% aqueous) were tested.⁸⁰ All the patients had intolerance to detergents and shampoos and none were sensitized through an occupation. The patients were patch tested using serial dilutions of DMAPA (100 ppm) in surfactant solutions (1% or 2% w/w surfactants) that included purified CAPB (DMAPA <1 ppm), SLES, polysorbate 20 (Tween 20), lauryl polyglucoside (APG), SLES/CAPB 3:1 (w/w), and APG/CAPB 3:2 (w/w). The test sites were scored on days 2, 3, 4, and 7. (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

Positive reactions were observed in serial dilutions of DMAPA in 1% CAPB at 1 ppm and higher (1 reaction each to 1 ppm and 5 ppm DMAPA, 3 reactions to 10 ppm DMAPA, and 4 reactions to 50 ppm DMAPA). Similar positive observations were made in serial dilutions of DMAPA in 1% SLES/CAPB 3:1. No positive reactions were observed when DMAPA (100 ppm) was tested in water, but 7 positive reactions were recorded when the material was tested in 2% CAPB. A greater number of reactions were observed when 100 ppm DMAPA was mixed with 2% SLES/CAPB (5 reactions) than when mixed with 2% APG/CAPB (2 reactions). The authors noted that CAPB and SLES/CAPB 3:1 act as carriers for DMAPA when applied under occlusion at 1%, and that surface activity in more concentrated surfactant solutions may be responsible for allergic reactions by DMAPA. The authors concluded that the concentration limit for DMAPA in 1% CAPB or 1% SLES/CAPB 3:1 should be 0.5 ppm (corresponding to 15 ppm and 60 ppm, respectively) and that betaine should be blended with nonionic surfactants to reduce allergy risks.⁸⁰ (From the study documentation, it was not possible to determine whether the administered CAPB concentrations were 1% active and 2% active or 1% aqueous and 2% aqueous, which equated to 0.3% active and 0.6%, respectively.)

Uter studied 80 participants (mainly hairdressers) with dermatitis from 1996 to 1999.⁸¹ During this period, the participants were patch tested with the hairdresser's series supplemented with DMAPA (1% pet and 1% aq Uter). The hairdresser's series contained CAPB (1% aqueous) that had a maximum residual DMAPA of <15 ppm. Of the 80 participants, 6 had + to +++ reactions to CAPB, but none of the 6 had reactions to DMAPA. A housewife with scalp and neck dermatitis had a + reaction to DMAPA 1% aqueous and a +? reaction to DMAPA 1% pet. This participant had no positive reaction to CAPB. (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

McFadden et al studied 7 participants that had relevant dermatitis to CAPB.⁸² The dermatitis occurred after use of liquid soaps, and in one case an eye makeup remover that contained CAPB. Four of the 7 participants were patch tested with partially purified CAPB (1% aqueous) containing <0.5% cocamidopropylamine and 0.1% and 0.01% cocamidopropylamine. The patch sites were read at day 2 and day 4 after the initial patching. One participant had a positive reaction that appeared only with cocamidopropylamine. Another had a reaction only with CAPB; however irritancy could not be ruled out since the participant's patch sites were only read on day 2. The other 2 patients had positive reactions to cocamidopropylamine and CAPB. Control participants had negative patch results.

Six out of the 7 original participants with dermatitis were patched tested with DMAPA along with controls on normal and tape-stripped skin at 0 ppm to 10 000 ppm. The participants were also tested with DMAPA in the presence of 0.2% aqueous, SLS, or in the presence of 1.0% pure CAPB (<0.3% cocamidopropylamine, <10 ppm DMAPA). The patch sites

were again read on day 2 and day 4 after the patch applications. One of the 6 participants reacted to DMAPA on normal and tape-stripped skin at concentrations >1000 ppm. Three of the 6 participants reacted to DMAPA in the presence of SLS (1 at 10 000 ppm, 1 at 1000 to 10 000 ppm, and 1 at 100 to 10 000 ppm). None of the participants reacted to the 1.0% pure CAPB. The authors concluded that the sensitization experienced by the participants to the CAPB products was likely due to the residual intermediates from the CAPB production, with reaction to cocamidopropylamine more likely than DMAPA.⁸² (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

The impurities DMAPA and amidoamine in CAPB were further analyzed for sensitization potential in 10 participants with CAPB allergy.⁸³ The participants that had all tested positive to CAPB 1% aqueous (Firma type) were patch tested with CAPB 1% aqueous (Chemotechnique type), DMAPA 1% aqueous, and purified amidoamine at 0.5%, 0.25%, and 0.1% aqueous. All the participants had ++ reactions to DMAPA at 1% and purified amidoamine at 0.5%. Most participants also had ++ reactions to purified amidoamine at 0.25% and the remaining had + reactions to this concentration. Four patients had positive reactions (++) to the purified amidoamine at 0.1%. No reactions were observed to the CAPB from Chemotechnique, which was suggested to have a higher purity by the authors. Control patches in 20 volunteers were negative for amidoamine. The authors concluded that cross-reactivity between DMAPA and amidoamine causes CAPB allergy. They also suggested that DMAPA is the true sensitizing material and amidoamine aids in the trans-epidermal penetration of DMAPA. (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

Brey and Fowler performed a retrospective study of patients that had positive patch test results to 1.0% aqueous CAPB and/or 1.0% amidoamine in the year 2001.⁸⁴ Reactions to other allergens were also recorded. Out of 957 patients patch tested in 2001, 49 had positive reactions to CAPB, amidoamine, or both. A follow-up evaluation in 35 patients was performed to establish relevance of reactions to CAPB and amidoamine with the use of products containing these chemicals. Fifteen patients (42.9%) reacted to CAPB, 12 patients (34.3%) reacted to amidoamine, and 8 patients (22.8%) reacted to both. Of the 35 patients, 29 (83%) could identify products containing CAPB at home. (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

Fowler et al performed a retrospective study of patients with CAPB and/or amidoamine contact allergy in 2001.¹¹¹ Out of 975 patients, 15 had a positive patch test reaction to 1.0% CAPB only, 25 had a positive patch test reaction to 0.1% amidoamine only, and 18 had positive reactions to both (58 patients total). Definite and probable relevance (known exposure to CAPB) was determined in 16 patients that tested positive for amidoamine and in 16 that tested positive for

CAPB. This study also evaluated formaldehyde allergy. Of the 58 patients, 12.7% were also allergic to formaldehyde. This was compared to the 10.1% of the total 975 patients that had formaldehyde allergy. The authors suggested that there is no significant relationship between CAPB or amidoamine allergy and formaldehyde allergy. (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

The NACDG evaluated 4913 patients for allergic contact dermatitis with an extended screening series of 65 allergens from January 1, 2001 to December 31, 2002. CAPB (1% aqueous) and the by-product of CAPB production, amidoamine (0.1% aqueous), were both included in this screening series. Positive results for CAPB were observed in 2.8% of the patients, while 2.3% were positive for amidoamine. The relevance of the CAPB and amidoamine reactions (present and past) was 90.9% and 85%, respectively.¹¹² (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

In a study by Li to determine the sensitization rate of CAPB in China and to analyze the relationship between CAPB and DMAPA, 429 patients (105 male, 324 female; 9-81 years old) with suspected contact allergy were patch tested with 1% aqueous CAPB (purified) and 1% aqueous DMAPA.¹¹³ The patients were also tested with the European standard series.

Of the 429 participants tested, 9 had irritant reactions, 12 had questionable reactions, and 42 had + reactions to CAPB. No reactions to CAPB greater than ++ were observed. Also of the 429 patients, 76 were diagnosed with cosmetic allergic contact dermatitis. Twenty-seven of these participants and 15 (out of 353) of the participants with cosmetic allergic contact dermatitis had positive reactions to CAPB ($P < .05$). Only 25 of the former and none of the latter had relevant reactions. Ten of the 429 patients had positive reactions to DMAPA, 8 of which were considered relevant. Six of the 10 patients also had positive reactions to CAPB. Because the participants of this study had positive reactions to both CAPB (purified) and DMAPA, the authors recommended that patch tests in cases of suspected cosmetic allergic contact dermatitis contain both CAPB and DMAPA.¹¹³ (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

Provocative Use Studies

A provocative use study of products containing CAPB was performed by Fowler et al.¹¹⁴ Ten participants were identified through positive reactions to 1% aqueous CAPB in routine patch testing. Ten control participants negative to CAPB were also enrolled. The provocative use test was divided into 3 phases, with 3 different test products (shampoo, liquid hand soap, and body wash) used in each phase. The products were specially formulated with CAPB-F grade (active level of CAPB in shampoo was 5.0%; active level in hand soap and

body wash was 5.2%). Phase I was a forearm wash test with the shampoo diluted to 10% in tap water. If no allergic reaction occurred in Phase I, participants then entered Phase II of the study: daily use of shampoo as hair cleanser. Participants proceeded to phase III of the study if no allergic reactions to the shampoo occurred. In phase III, the participants used the shampoo, body wash, and hand soap for 3 weeks.

At least 2 months after the product use tests, the participants were patch tested with CAPB grades F and S (both 1% aqueous), DMAPA (0.1% pet), amidoamine (0.1% aqueous), sodium monochloroacetate (0.1% aqueous), a proprietary mixture of preservatives for CAPB, and other potential allergens (perfumes and preservatives) that were in the test product formulations. Control participants were patched with 1% CAPB.

Three participants completed the product use phases without experiencing an allergic reaction. Seven participants had erythema, scaling, and pruritus on the arms, face, and/or neck in either phase I or II of the study. One participant that experienced a positive reaction in the first phase was asked to repeat the forearm use test with the CAPB-containing shampoo on the left arm and with a CAPB-absent shampoo on the right arm. The participant experienced a positive reaction on both arms, which was likely caused by the preservatives in the shampoo products (as shown through patch testing). In phase III, 3 participants had scalp, face, and/or neck and body dermatitis.

Patch testing was performed in 9 of the 10 participants, with 6 participants reacting to 0.1% amidoamine. Five of these 6 participants had positive reactions during the product use phases. Two participants had reactions to the CAPB-F grade with preservative, 3 had reactions to CAPB-F grade without preservative, 1 reacted to the CAPB-S grade, and 1 reacted to the proprietary preservative mixture. Two participants had questionable reactions to DMAPA. No other adverse reactions were noted in the participants. (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

A follow-up patch test with 7 of the participants was performed using purified CAPB (containing only 1 ppm amidoamine), CAPB-F grade (with approximately 3000 ppm amidoamine), and 2 concentrations of amidoamine (0.1% and 0.01% aqueous). Two participants had questionable reactions to the purified CAPB, while there were 3 positive reactions to the CAPB-F grade, 4 positive reactions to the higher concentration of amidoamine, and 2 positive reactions to the lower concentration of amidoamine. The authors concluded that the impurity amidoamine may be the causative allergen in CAPB sensitivity and they recommend that cosmetics and personal care products should be formulated to minimize contamination with this impurity. In addition, the authors could not rule out the possibility that CAPB alone was not an allergen to presensitized individuals.^{114,115}

Another provocative use test was conducted by Fartasch et al.¹¹⁶ Participants with eczema were tested for CAPB allergy while undergoing patch testing for the standard allergen series. Out of 1063 patients, 13 were identified with a positive patch

reaction; however, relevance could only be established in 4 of the participants. Another 6 patients were referred to the study for eczematous eruptions of the scalp and/or hand dermatitis and had positive 1% aqueous CAPB patch test reactions. Twenty volunteers served as controls for the study.

The product use study consisted of 3 phases. In phase I, a 0.1 mL test sample of shower gel containing CAPB (25% dilution; DMAPA below 1 ppm) was applied, lathered for 1 minute, and rinsed on the participants' forearms twice daily for 7 days. The second phase of the study consisted of patch testing in order to differentiate irritant reactions from allergic reactions and to reconfirm the sensitivity to CAPB and DMAPA. The participants were patch tested with 0.1%, 0.3%, and 1.0% dilutions of CKKB (Tegobetaine CKKB5; 1.1 ppm DMAPA) and DMAPA, respectively. Patch sites were read on days 2, 3, and 4 following application. Participants that had no allergic reactions in phase I participated in phase III. In this phase, the participants used the shower gel as they would in normal daily hygiene practices for 4 weeks.

No skin irritation was observed in phase I of the study. One participant with a history of atopic dermatitis was removed from the study due to a flare. Another participant had an immediate "wheal like reaction" on days 3 and 6 that cleared within minutes. This participant continued the forearm test an extra week and had no further effect. In phase II, 1 control had an irritating reaction to 1% CAPB. In the study group, 5 out of the 10 participants had a positive reaction to 1% CAPB and another 3 had marginal and/or irritant reactions. One participant had a positive reaction to DMAPA but had no clear reaction to CAPB. Another participant that had a positive reaction to CAPB had a doubtful reaction to 1% DMAPA. Eight participants did not react to DMAPA. Only 7 participants participated in phase III of the study (the other 2 were not available), and no adverse reactions were observed in these participants. The authors concluded that CAPB as tested may be used safely in individuals with CAPB sensitivity.¹¹⁶ (From the study documentation, it was not possible to determine whether the administered CAPB concentration was 1% active or 1% aqueous, which equated to 0.3% active.)

Case Reports

Several case studies of allergic contact dermatitis reported positive patch tests to amidoamine and DMAPA, with 1 study reporting DMAPA elicited reaction at concentrations of 0.1% and greater.^{9,123-127}

Quantitative Risk Assessment

The Personal Care Products Council's Task Force on Sensitization Risk from CAPB Impurities used a quantitative risk assessment (QRA) approach developed by Api et al.⁵¹ and the Research Institute for Fragrance Materials (RIFM)¹¹⁷ to determine the levels of DMAPA and amidoamine impurities for which no sensitization should occur.¹¹⁸ Based on the findings of LLNA and human sensitization studies on DMAPA and

amidoamine described in this report, the Council's task force determined the conservative weight of evidence no expected sensitization induction levels (WoE NESIL) for DMAPA and amidoamine to be 425 $\mu\text{g}/\text{cm}^2$ and 180 $\mu\text{g}/\text{cm}^2$, respectively. When the level of impurities in raw CAPB materials is determined for product exposure (based on a typical exposure of 0.5% for amidoamine and 0.01% for DMAPA and estimated dose per unit area), a level of acceptable risk can be calculated for each cosmetic product category. These values are calculated based on sensitization assessment factors (SAFs), acceptable exposure levels ($\text{AELs} = \text{WoE NESIL} \times \text{SAF}^{-1}$), and consumer exposure level (CEL) that are appropriate for each product category. According to the QRA method, the ratio of $\text{AEL} \times \text{CEL}^{-1}$ must be equal to or greater than 1 to ensure no sensitization to consumers. See Tables 9 and 10 for the breakdown of the values used in the calculations for this QRA. The QRA found that all of the product categories had acceptable levels of risk for exposure to DMAPA.

Using this approach, a ratio of less than 1 may result using the parameters given above, for example, with amidoamine in underarm deodorants ($\text{AEL} \times \text{CEL}^{-1} = 0.15$). Such a finding could be addressed for such particular product applications by reducing the concentration of CAPB raw material in these finished products or choosing CAPB of higher purity when producing these products.

Summary

Cocamidopropyl betaine is a zwitterionic ammonium compound containing a moiety of either a saturated or unsaturated fatty acid ranging in length from 6 to 18 carbons in amide linkage with aminopropyl betaine. The source of these fatty acids, predominately lauric acid, is coconut oil. Other related ingredients are amidopropyl betaines with attached fatty acid moieties unique to the source, for example, sesame oil for sesamidopropyl betaine.

Cosmetic grade CAPB, an aqueous solution, normally contains 35% solids. The NaCl content of these solids ranges from 4.5% to 5.6%. The concentration, when expressed as activity, is determined by subtracting the percentage NaCl from the percentage total solids. Because of uncertainty in whether concentrations given are active or dilutions of an active cosmetic grade material, in some cases the actual concentration of CAPB or other tested material is not known, but it appears that any uncertainty would not be greater than a factor of 3. No *N*-nitroso compounds were detected in samples of commercially supplied CAPB analyzed by gas chromatography-thermal energy analysis.

CAPB is used primarily as an amphoteric surfactant in shampoos, conditioners, and other cleansing preparations. It was listed as an ingredient in 2460 cosmetic formulations voluntarily reported to FDA. Reported use concentrations range from 0.2% to 25%.

The oral LD_{50} of full-strength commercial samples of 30% active CAPB was 4.91 g/kg in CFR mice and 7.45 mL/kg in Wistar rats. Another study of 30% active CAPB in Wistar rats

Table 9. Quantitative Risk Assessment of Amidoamine (AA) in Cosmetic Products Containing CAPB^{a,b,118}

Product Category	% Max Concentration of Use (active)	% Activity of Raw Material	Product Exposure ^c (µg/cm ²)	CAPB Exposure (µg/cm ²)	AA CEL (µg/cm ²)	SAF	AA AEL	AA AEL/CEL
Baby shampoo	4	30	200	26.67	0.13	100	1.80	13.50
Other baby products	6	30	10	2.00	0.01	100	1.80	180.00
Bath oils, tablets and salts	7	30	10	2.33	0.01	100	1.80	154.29
Bubble baths	6	30	10	2.00	0.01	100	1.80	180.00
Bath capsules	0.9	30	10	0.30	0.00	100	1.80	1200.00
Other bath preparations	6	35	10	1.71	0.01	100	1.80	210.00
Eye shadow	2.5	35	2170	155.00	0.78	300	0.60	0.77
Eye makeup remover	0.005	1	900	4.50	0.02	100	1.80	80.00
Hair conditioners	4	35	200	22.86	0.11	100	1.80	15.75
Hair sprays (aerosol fixatives)	0.2	36	1390	7.72	0.04	100	1.80	46.62
Hair straighteners	0.7	36	4200	81.67	0.41	100	1.80	4.41
Permanent waves	2	35	4200	240.00	1.20	100	1.80	1.50
Rinses (noncoloring)	9	30	170	51.00	0.26	100	1.80	7.06
Shampoos (noncoloring)	9	38	170	40.26	0.20	100	1.80	8.94
Tonics, dressings and other hair grooming aids	4.5	30	990	148.50	0.74	100	1.80	2.42
Hair dyes and colors ^d	6	30	1000	200.00	1.00	100	1.80	1.80
Hair tints ^d	6	30	990	198.00	0.99	100	1.80	1.82
Hair rinses (coloring)	6	30	200	40.00	0.20	100	1.80	9.00
Hair color sprays (aerosol)	6	30	1390	278.00	1.39	100	1.80	1.29
Hair lighteners with color ^d	6	30	1000	200.00	1.00	100	1.80	1.80
Hair bleaches ^d	6	30	1000	200.00	1.00	100	1.80	1.80
Other hair coloring preparations	3	30	1000	100.00	0.50	100	1.80	3.60
Other manicuring preparations	0.8	39	970	19.90	0.10	100	1.80	18.09
Dentifrices (aerosol, liquid, pastes, and powders)	6	Not reported	1290	NA	NA	100	1.80	NA
Bath soaps and detergents	10	34	15	4.41	0.02	100	1.80	81.60
Deodorants (underarm)	1.6	31	7500	387.10	1.94	300	0.60	0.31
Douches	3.8	30	1380	174.80	0.87	100	1.80	2.06
Other personal cleanliness products	10	36	10	2.78	0.01	100	1.80	129.60
Shaving cream (aerosol, brushless, and lather)	9	35	70	18.00	0.09	300	0.60	6.67
Shaving soaps (cakes, sticks, etc)	9	30	70	21.00	0.11	300	0.60	5.71
Other shaving preparations	11	32	70	24.06	0.12	300	0.60	4.99
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	6.9	31	900	200.32	1.00	100	1.80	1.80
Body and hand creams, lotions, and powders	3	35	4200	360.00	1.80	300	0.60	0.33
Foot powders and sprays	4	30	2200	293.33	1.47	100	1.80	1.23
Paste masks (mud packs)	0.2	35	4200	24.00	0.12	100	1.80	15.00

^a Assumptions in table above: AA @ 0.5% of CAPB; AA NESIL = 180 µg/cm².

^b Shaded rows indicate the ratio of AEL × CEL⁻¹ is less than 1.

^c These data are derived from RIFM. It is advisable that formulators use experimentally determined exposure data when available.

^d Note that these product categories may be diluted prior to application, such that maximum CAPB activity in finished product is 3%.

found the acute oral LD₅₀ to be 8.55 g/kg. The oral LD₅₀ of 30% active CAPB in albino rats of an unspecified strain was 4.9 g/kg. The acute oral LD₅₀ for 35.61% active CAPB was >1.8 g/kg for male Sprague-Dawley rats. All female rats in this study died before study end. The acute oral LD₅₀ was greater than 5.0 g/kg and the acute lethal dermal dose was greater than 2.0 g/kg in studies of CAPB (31% active) with CD rats.

In a 28-day short-term study in which groups of 8 male and female animals received 0, 100, 500, or 1000 mg/kg of 30% active CAPB, treatment-induced lesions were produced in the nonglandular portion of the stomach in the high-dose groups. Both males and females of the low-dose (100 mg/kg) group were comparable to concurrent controls.

In another 28-day oral toxicity study, rats received 0, 250, 500, or 1000 mg/kg of an unknown concentration of CAPB. In the 1000 mg/kg dose group, compound-related edema of the mucosa of the nonglandular stomach was observed at macroscopic examination and acanthosis of the mucosa, inflammatory edema of the submucosa, and multiple ulcerations were observed during microscopic examination. These effects were thought to be the result of the irritating properties of CAPB and not of systemic toxicity. The NOEL and LOEL for this study were 500 and 1000 mg/kg per d, respectively.

A subchronic oral toxicity study of an unknown concentration of CAPB rats that received 0, 250, 500, or 1000 mg/kg per d CAPB concluded that the NOEL was 250 mg/kg per d. Gastritis

Table 10. Quantitative Risk Assessment of 3,3-Dimethylaminopropylamine (DMAPA) in Cosmetic Products Containing CAPB^{a,118}

Product Category	% Max Concentration of Use (active)	% Activity of Raw Material	Product Exposure ^b ($\mu\text{g}/\text{cm}^2$)	CAPB Exposure ($\mu\text{g}/\text{cm}^2$)	DMAPA CEL ($\mu\text{g}/\text{cm}^2$)	SAF	DMAPA AEL	DMAPA AEL/CEL
Baby shampoo	4	30	200	26.67	0.0027	100	4.25	1593.75
Other baby products	6	30	10	2.00	0.0004	100	4.25	10625.00
Bath oils, tablets, and salts	7	30	10	2.33	0.0005	100	4.25	9107.14
Bubble baths	6	30	10	2.00	0.0004	100	4.25	10625.00
Bath capsules	0.9	30	10	0.30	0.0001	100	4.25	70833.33
Other bath preparations	6	35	10	1.71	0.0003	100	4.25	12395.83
Eye shadow	2.5	35	2170	155.00	0.0310	300	1.42	45.70
Eye makeup remover	0.005	1	900	4.50	0.0009	100	4.25	4722.22
Hair conditioners	4	35	200	22.86	0.0046	100	4.25	929.69
Hair sprays (aerosol fixatives)	0.2	36	1390	7.72	0.0015	100	4.25	2751.80
Hair straighteners	0.7	36	4200	81.67	0.0163	100	4.25	260.20
Permanent waves	2	35	4200	240.00	0.0480	100	4.25	88.54
Rinses (noncoloring)	9	30	170	51.00	0.0102	100	4.25	416.67
Shampoos (noncoloring)	9	38	170	40.26	0.0081	100	4.25	527.78
Tonics, dressings and other hair grooming aids	4.5	30	990	148.50	0.0297	100	4.25	143.10
Hair dyes and colors ^c	6	30	1000	200.00	0.0400	100	4.25	106.25
Hair tints ^c	6	30	990	198.00	0.0396	100	4.25	107.32
Hair rinses (coloring)	6	30	200	40.00	0.0080	100	4.25	531.25
Hair color sprays (aerosol)	6	30	1390	278.00	0.0556	100	4.25	76.44
Hair lighteners with color ^c	6	30	1000	200.00	0.0400	100	4.25	106.25
Hair bleaches ^c	6	30	1000	200.00	0.0400	100	4.25	106.25
Other hair coloring preparations	3	30	1000	100.00	0.0200	100	4.25	212.50
Other manicuring preparations	0.8	39	970	19.90	0.0040	100	4.25	1067.98
Dentifrices (aerosol, liquid, pastes, and powders)	6	Not reported	1290	NA	NA	100	4.25	NA
Bath soaps and detergents	10	34	15	4.41	0.0009	100	4.25	4816.67
Deodorants (underarm)	1.6	31	7500	387.10	0.0774	300	1.42	18.30
Douches	3.8	30	1380	174.80	0.0350	100	4.25	121.57
Other personal cleanliness products	10	36	10	2.78	0.0006	100	4.25	7650.00
Shaving cream (aerosol, brushless, and lather)	9	35	70	18.00	0.0036	300	1.42	393.52
Shaving soaps (cakes, sticks, etc)	9	30	70	21.00	0.0042	300	1.42	337.30
Other shaving preparations	11	32	70	24.06	0.0048	300	1.42	294.37
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	6.9	31	900	200.32	0.0401	100	4.25	106.08
Body and hand creams, lotions and powders	3	35	4200	360.00	0.0720	300	1.42	19.68
Foot powders and sprays	4	30	2200	293.33	0.0587	100	4.25	72.44
Paste masks (mud packs)	0.2	35	4200	24.00	0.0048	100	4.25	885.42

^a Assumptions in table above: DMAPA @ 0.01% of CAPB; DMAPA NESIL = 425 $\mu\text{g}/\text{cm}^2$.

^b These data are derived from RIFM. It is advisable that formulators use experimentally determined exposure data when available.

^c Note that these product categories may be diluted prior to application, such that maximum CAPB activity in finished product is 3%.

of the forestomach was observed in rats in the 500 and 1000 mg/kg per d dose groups.

Topical administration of varying commercial grades of CAPB (7.5%-30% activity) in single insult occlusive patch tests involving rabbits resulted in PIs ranging from 0 to 3.75 (maximum score = 8). Slight edema was observed with CAPB with a 10% activity but not with CAPB with a 7.5% activity.

No evidence of delayed contact hypersensitivity was found in Pirbright white guinea pigs topically administered solutions of 10% active CAPB in a Magnusson-Kligman maximization test. Microscopic changes in the treated skin of albino guinea pigs indicated slight delayed-type contact sensitization by a 3.0% active CAPB solution in a maximization test and modified Draize test.

Maximum mean irritation scores for eyes of rabbits treated with 30% active CAPB and left unrinsed ranged from 26 to 42 (maximum score = 110). Score for rinsed eyes ranged from 2 to 10. Irritation was observed primarily in the conjunctivae of treated eyes. At 4.5% active CAPB, there was slight conjunctival irritation in unrinsed eyes and very slight irritation in rinsed eyes. Scores for product formulations containing 2.2% to 6.3% active CAPB ranged from 4 to 30 in unrinsed, treated eyes of rabbits and were 3.3 and 20.0 in rinsed, treated eyes of rabbits.

The mutagenic potential of 30.9% and 31.0% active CAPB formulations was tested in the *Salmonella*/mammalian microsome mutagenicity assay and the L5178Y TK +/- mouse lymphoma assay. CAPB was nonmutagenic in these assays.

CAPB was not mutagenic to the *S typhimurium* indicator organisms in Ames *Salmonella*/microsome reverse mutation assays and in a mouse micronucleus assay.

In a single insult occlusive patch test of a 1.0% aqueous dilution of a product formulation containing 6.3% active CAPB, no skin irritation was observed in 15 of 19 human participants; 4 of the participants had slight irritation. Slight erythema was observed after occlusive patching of 12 participants with an 8% aqueous dilution of a soap formulation containing 2.0% active CAPB daily for 5 days. Two soap formulations containing 2.25% active CAPB were considered primary irritants after a 21-day consecutive occlusive patch study.

A formulation containing almondamidopropyl betaine and olivamidopropyl betaine (both at 0.005% active concentration) was not a primary skin sensitizer or skin irritant in 103 participants. A formulation containing capryl/capramidopropyl betaine at 1.72% active concentration was not a skin sensitizer in 26 participants. No dermal irritation or allergic contact sensitization was reported in studies of formulations containing 0.42%, 0.7%, or 0.03955% active lauramidopropyl betaine. Formulations containing shea butteramidopropyl betaine were not sensitizing in studies of 0.04% or 0.54% active concentration.

An additional study investigated the potential of a 3.0% active solution of CAPB to induce contact photoallergy. There was no response to the challenge tests except for those exposed to both UVA and UVB radiation, who had mild to moderate erythemic responses that were not uncommon and were said to have resulted from the sunburn derived from UVB exposure.

CAPB was not a skin sensitizer at 1% in a study of 100 volunteers or in another study at 1.5% in 141 volunteers. Clinical sensitization studies and case studies show that persons already sensitized to CAPB react to concentrations of 1.0% of the material in water. Several case reports have found patients reporting contact allergy to multiple types of personal care products, including shampoos, contact lens solutions, eye makeup remover, bath gels, and toothpaste. Researchers have included the CAPB impurities, DMAPA and amidoamine, in the scope of sensitization and case studies and have found that one or both of the impurities may be the responsible agent for contact allergy to CAPB. QRAs of these impurities may be performed to ensure acceptable levels of risk in consumers.

Discussion

While very few toxicity studies were identified specifically for the additional amidopropyl betaines (with R groups representing fatty acids derived from a source other than coconut oil) that were added to this safety assessment, there is no reason to expect these ingredients to differ in toxicity from CAPB. The amidopropyl betaines appear to be manufactured in the same manner as CAPB, with the difference only being in the fatty acid composition of the oil that is the source of the R group. Some of these fatty acid compounds have already been reviewed by the Panel and have been found to be safe for use

in cosmetic ingredients. The Panel noted gaps in the available safety data for some of the amidopropyl betaines in this safety assessment. The available data on many of the ingredients are sufficient, however, and similarity between structural activity relationships and biologic functions in cosmetic concentrations of use and can be extrapolated to support the safety of the entire group. Therefore, the Panel determined that the toxicity data on CAPB could be read across to include:

- almondamidopropyl betaine,
- apricotamidopropyl betaine,
- avocadamidopropyl betaine,
- abassuamidopropyl betaine,
- behenamidopropyl betaine,
- canolamidopropyl betaine,
- capryl/capramidopropyl betaine,
- coco/oleamidopropyl betaine,
- coco/sunfloweramidopropyl betaine,
- cupuassuamidopropyl betaine,
- isostearamidopropyl betaine,
- lauramidopropyl betaine,
- meadowfoamamidopropyl betaine,
- milkamidopropyl betaine,
- minkamidopropyl betaine,
- myristamidopropyl betaine,
- oatamidopropyl betaine,
- oleamidopropyl betaine,
- olivamidopropyl betaine,
- palmamidopropyl betaine,
- palmitamidopropyl betaine,
- palm kemelamidopropyl betaine,
- ricinoleamidopropyl betaine,
- sesamidopropyl betaine,
- shea butteramidopropyl betaine,
- soyamidopropyl betaine,
- stearamidopropyl betaine,
- tallowamidopropyl betaine,
- undecyleneamidopropyl betaine, and
- wheat germamidopropyl betaine.

In reviewing studies involving CAPB and related ingredients, often the percentage of active material in the test material was clearly stated; but in other cases, it was not clear whether the test material was active material or a dilution of active material. Because the difference, at most, would be a factor of 3, the uncertainty was factored into the review process.

The Panel considered that the available acute, short-term, and subchronic animal toxicity studies were supportive of the safety of CAPB. In vitro genotoxicity studies supported the absence of mutagenic activity. The Panel noted the absence of reproductive and developmental toxicity and absorption data but also noted that CAPB did not produce systemic toxicity in a 92-day oral toxicity study in rats. Because these ingredients are very large molecular weight structures and water soluble, the Panel concluded that they would not be readily absorbed into the skin.

In the absence of inhalation toxicity data, the Panel determined that CAPB can be used safely in hair sprays, because the product particle size was not respirable. The Panel reasoned that the particle size of aerosol hair sprays ($\sim 38 \mu\text{m}$) and pump hair sprays ($>80 \mu\text{m}$) was large compared to respirable particulate sizes ($\leq 10 \mu\text{m}$).

In past ingredient safety assessments, the Panel had expressed concern over *N*-nitrosation reactions in ingredients containing amine groups. CAPB, and the other betaine ingredients in this assessment, contain secondary amides that may serve as substrates for *N*-nitrosation. Additionally, these ingredients may contain secondary amine impurities which may serve as substrates for *N*-nitrosation. Therefore, the Panel recommended that these ingredients should not be included in cosmetic formulations containing *N*-nitrosating agents.

The Panel expressed concern regarding pesticide residues and heavy metals that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulation.

The Panel considered the dangers inherent in using animal-derived ingredients, namely the transmission of infectious agents. While tallow may be used in the manufacture of some ingredients in this safety assessment and is clearly animal derived, the Panel noted that tallow is highly processed and tallow derivatives even more so. The Panel agreed with determinations by the FDA that tallow derivatives are not risk materials for transmission of infectious agents.

While CAPB and the related amidopropyl betaines were noted to be dermal irritants, the primary concern was related to the presence of impurities that were found to be dermal sensitizers. The Panel recognized that these ingredients can have the potential to induce skin sensitization, most likely due to the impurities DMAPA and fatty acid amidopropyl dimethylamine (amidoamine). Thirteen studies of CAPB and related amidopropyl betaines on normal human skin at use concentrations indicated no sensitization induced by these cosmetic ingredients. A QRA on DMAPA at a concentration of 0.01% in raw CAPB indicated no sensitization in finished cosmetic products; amidoamine at a concentration of 0.5% in raw CAPB may cause sensitization in certain finished cosmetic products. The Panel concluded that skin sensitization is not a concern with the use of CAPB and related amidopropyl betaines as currently used in cosmetic products when a QRA is performed to demonstrate that concentration, product type, and product usage will not produce exposures that could induce sensitization. The Panel advises industry to continue minimizing the concentrations of the sensitizing impurities.

Conclusion

The CIR Expert Panel concluded that the following ingredients are safe in cosmetics as long as they are formulated to be nonsensitizing, which may be based on a QRA

- cocamidopropyl betaine,
- almondamidopropyl betaine,

- apricotamidopropyl betaine*,
- avocadamidopropyl betaine*,
- babassuamidopropyl betaine,
- behenamidopropyl betaine*,
- canolamidopropyl betaine*,
- capryl/capramidopropyl betaine,
- coco/oleamidopropyl betaine,
- coco/sunfloweramidopropyl betaine*,
- cupuassuamidopropyl betaine*,
- isostearamidopropyl betaine*,
- lauramidopropyl betaine,
- meadowfoamamidopropyl betaine*,
- milkamidopropyl betaine*,
- minkamidopropyl betaine*,
- myristamidopropyl betaine,
- oatamidopropyl betaine,
- oleamidopropyl betaine*,
- olivamidopropyl betaine,
- palmamidopropyl betaine*,
- palmitamidopropyl betaine*,
- palm kernelamidopropyl betaine,
- ricinoleamidopropyl betaine*,
- sesamidopropyl betaine*,
- shea butteramidopropyl betaine,
- soyamidopropyl betaine,
- stearamidopropyl betaine*,
- tallowamidopropyl betaine*,
- undecyleneamidopropyl betaine, and
- wheat germamidopropyl betaine*.

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

Authors' Note

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

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2017 FDA VCRP Data**Polyaminopropyl Biguanide**

03C - Eye Shadow	2
03D - Eye Lotion	3
03E - Eye Makeup Remover	8
03F - Mascara	12
03G - Other Eye Makeup Preparations	3
04E - Other Fragrance Preparations	1
05A - Hair Conditioner	5
05F - Shampoos (non-coloring)	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	7
05H - Wave Sets	1
05I - Other Hair Preparations	2
07C - Foundations	4
07E - Lipstick	1
07H - Makeup Fixatives	1
07I - Other Makeup Preparations	6
08C - Nail Creams and Lotions	1
08G - Other Manicuring Preparations	1
10A - Bath Soaps and Detergents	2
10E - Other Personal Cleanliness Products	7
11A - Aftershave Lotion	1
12A - Cleansing	20
12C - Face and Neck (exc shave)	18
12D - Body and Hand (exc shave)	13
12F - Moisturizing	12
12G - Night	6
12H - Paste Masks (mud packs)	1
12I - Skin Fresheners	4
12J - Other Skin Care Preps	4
Total	147



Memorandum

TO: Bart Heldreth, Ph.D., Interim Director
COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: July 18, 2017

SUBJECT: Updated Concentration of Use by FDA Product Category: Polyaminopropyl Biguanide (PHMB)

Concentration of Use by FDA Product Category - Polyaminopropyl Biguanide (PHMB)

Product Category	Maximum Concentration of Use
Baby lotions, oils and creams Not powder	0.1%
Eye shadows	0.03%
Eye lotions	0.04-0.2%
Eye makeup removers	0.04-0.056%
Mascara	0.1%
Other eye makeup preparations	0.01%
Hair conditioners	0.00025-0.06%
Hair straighteners	0.01%
Shampoos (noncoloring)	0.008%
Tonics, dressings and other hair grooming aids	0.000023-0.1%
Other hair preparations (noncoloring)	0.002%
Hair dyes and colors	0.1%
Foundations	0.01%
Deodorants Not spray	0.003%
Other personal cleanliness products	0.006%
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.02-0.1%
Face and neck products Not spray	0.01-0.02%
Body and hand products Not spray	0.00001-0.009%
Moisturizing products Not spray	0.00075%
Skin fresheners	0.0.085%
Suntan products Not spray	0.002-0.1%

Information collected in 2016

Table prepared October 28, 2016

Updated April 5, 2017: eye makeup removers 0.028% changed to 0.056%; mascara 0.3% deleted; hair conditioners high concentration changed from 0.3% to 0.06%; pump hair sprays 0.27% changed to 0.053%; hair grooming aids changed from 0.5% to 0.1%; hair dyes and colors changed from 0.5% to 0.1%; skin cleansing changed from 0.5% to 0.1%; face and neck products changed from 0.24% to 0.02%; skin freshener changed from 0.43% to 0.085%.

Updated May 19, 2017: added 0.5% non-spray suntan product

Updated June 13, 2017: Suntan products high concentration changed from 0.5% to 0.1%

Updated July 18, 2017: Hair spray products removed



Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

A handwritten signature in black ink, appearing to read "Beth A. Jonas", is written over the printed name.

DATE: May 2, 2017

SUBJECT: Polyaminopropyl Biguanide (PHMB)

Reliance Clinical Testing Services, Inc. 2011. Human repeated insult patch test (HRIPT) of a neck cream containing 0.20% Polyaminopropyl Biguanide (PHMB).



RCTS, Inc. "Your Assurance of Quality in Clinical Testing"
FINAL REPORT

RCTS' STUDY NOS.: 2853/2854

TRA PROJECT NOS.: 99001-334 and 99001-337

HUMAN REPEATED INSULT PATCH TEST (HRIPT)

Sponsor:	Technikos Research Associates 7418 E. Helm Dr., Suite 207 Scottsdale AZ 85260		
Sponsor's Representative:	Name: Email:		
Sponsor's Test Article Code:	TRA 11-127	RCTS' Test Article Codes: 2853.6738, 2854.6747	
Testing Facility:	Reliance Clinical Testing Services, Inc. 3207 Esters Road Irving, TX 75062 Phone 972-871-7578 Fax 469-524-0714 Website: www.rctslabs.com		
Study Objective:	To determine the irritation and contact sensitization potential of a test article under occlusive patch test conditions after repeated applications to the skin of at least one hundred (100) human subjects. <i>Test material: Neck cream containing 0.20%</i>		
Method:	Modified Draize HRIPT Procedure Induction: Patches applied on the back, generally 3 times each week for 3 weeks. Patches worn for approximately 24-hours. Rest phase: 10-14 days. Challenge: One 24-hour patch on a virgin site. Skin Grading: Induction evaluation occurred approximately 24- to 48-hours after patch removal. Challenge evaluation occurred approximately 24- and 72-hours after patch application (additional readings were made, if warranted). <i>Poly aminopropyl Biguanide</i>		
Number of Subjects:	One hundred fifteen (115) subjects satisfactorily completed the test procedure.		
Panel Description:	Male and female subjects aged 18-69 years successfully completed the test procedure.		
Conclusions:	<input checked="" type="checkbox"/> Non-Sensitizing <input checked="" type="checkbox"/> Non-Irritating <input type="checkbox"/> Sensitizing <input type="checkbox"/> Irritation acceptable (normal) for product type <input type="checkbox"/> Additional Data Needed <input type="checkbox"/> Irritation higher than normal for product type		
Study Start Date:	4/18/2011	Study End Date:	6/3/2011
Document Status:	Final	Date:	7/12/2011

I, the undersigned, certify that this document accurately describes the conduct and results of this investigation and that the study was conducted in the spirit of GCP and ICH E6 guidelines.

7/12/2011
 Date of Final Report

Barry T. Reece, M.S., M.B.A.
 Principal Investigator
 Managing Partner, RCTS, Inc.

Raymond L. Garcia, M.D.
 Medical Investigator
 Board Certified Dermatologist

Ana Ho, B.S.
 Study Coordinator
 Clinical Research Coordinator

QUALITY ASSURANCE STATEMENT

This study was conducted in accordance with the spirit of Good Clinical Practice regulations described in CFR 21, Part 50 (Protection of Human Subjects - Informed Consent) and the International Conference on Harmonization - Good Clinical Practice Guidelines, May 9, 1997, Federal Register.

For purposes of this clinical study:

- ☒ Informed Consent was obtained
☐ Informed Consent was not obtained
☒ An IRB review was neither requested nor required
☐ An IRB was convened and approval to conduct the proposed clinical research was granted

The Quality Assurance Department conducted in-study inspections (audits) on a random sampling of subjects during the study. Written status reports of the inspections and findings were submitted to Management.

Date of Inspection	Type of Inspection	Date Reported to Management
04/18/2011	Day 1 procedures including study organization and management, qualification of subjects, consenting process and patching procedures.	04/22/2011
04/29/2011 05/09/2011 05/18/2011	Induction phase including patching procedures and scoring of the test sites.	04/29/2011 05/12/2011 05/31/2011
05/23/2011 05/31/2011	Challenge phase including patching procedures	05/23/2011 05/31/2011
05/24/2011 06/01/2011	24-Hour read of Challenge phase.	05/24/2011 06/01/2011
05/26/2011 06/03/2011	72-Hour read of Challenge phase.	06/02/2011 06/03/2011
05/27/2011 05/28/2011 05/31/2011 06/01/2011 06/03/2011 06/06/2011 06/07/2011 06/08/2011	96-, 120-, 144-, 168-, 192-, 216-, 240-, 264-, 288-, 312-, and 336-Hour read of Challenge phase.	05/31/2011 06/14/2011 05/31/2011 06/01/2011 06/03/2011 06/06/2011 06/07/2011 06/09/2011
06/22/2011	Final Review of Data Tables	06/22/2011
07/01/2011	Review of Draft Report	07/01/2011
07/12/2011	Final Review of Final Report	07/12/2011

This study report has been reviewed to ensure that it correctly describes the methods of testing and that the reported results accurately reflect the data obtained during this clinical study.

On the basis of the audits conducted, this report is considered to be a true and accurate reflection of the methods of testing and source data obtained.



Carolyn Rupe, M.O.T.
Quality Assurance

7.12.11
Date



Samatha Prema, M.S.
Manager, Quality Assurance

07/12/11
Date

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APPENDICES

APPENDIX I Study Protocol

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Clinical Safety Evaluation
Human Repeated Insult Patch Test (HRIPT)

1. SUMMARY

A Modified Draize procedure was conducted to determine the irritation and contact sensitization in a population of normal, healthy subjects.

Under the conditions of a Human Repeated Insult Patch Test Procedure (Modified Draize; occlusive patch conditions), Test Article: TRA 11-127 produced generally transient, barely perceptible (0.5-level) to mild (1-level) patch test responses (specific and non-specific) on forty-three (43/115 or 37% of the test population) test subjects during the Induction and/or Challenge phases of the study. The skin reactivity observed was considered neither evidence of clinically meaningful irritation nor allergic in nature.

2. OBJECTIVE

To determine the irritation and contact sensitization potential of a test article under occlusive patch test conditions after repeated applications to the skin of at least one hundred (100) human subjects.

3. STUDY PERSONNEL

Principal Investigator: Barry T. Reece, M.S., M.B.A.
Medical Investigator: Raymond L. Garcia, M.D., Derm.
Study Coordinator: Ana Ho, B.S.

4. SPONSOR

Scottsdale
AZ 85260

5. SPONSOR'S REPRESENTATIVE

Fax:
Email:

6. TESTING FACILITY

The study was conducted at and by RCTS, Inc. at 3207 Esters Road, Irving, TX 75062.

7. EXPERIMENTAL DESIGN

7.1 INFORMED CONSENT

The investigator (or his designee) explained the nature of the study, its purpose and associated procedures, the expected duration and the potential benefits and risks of participation to each subject prior to his/her entry into the study. Each subject was provided with a copy of the informed consent form, had ample opportunity to ask questions and was informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision. No subject entered the study before his/her informed consent form was obtained.

7.2 SUBJECT SELECTION

One hundred thirty-three (133) subjects, 68 females and 65 males, ranging in age from 18-69 years were empanelled in this study.

7.2.1 INCLUSION CRITERIA

Subjects included in the study:

1. Were male and female volunteers between the ages of eighteen (18) and seventy (70), in general good health based upon a study screener (no physical required);
2. Were of any skin type or ethnicity, provided their degree of skin pigmentation did not significantly interfere with evaluations;
3. Were free of any systemic or dermatological disorder including a known history of allergies or other medical conditions which, in the opinion of the investigator, might have interfered with the conduct of the study, interpretation of results or increased the risk of adverse reactions;
4. Agreed to refrain from swimming, using hot tubs/saunas and any type of tanning;
5. Were able to read, understand and provide written informed consent;
6. Agreed to complete the course of the study and to comply with instructions; and
7. Agreed to arrive without lotions, creams or oils applied to their back.

7.2.2 EXCLUSION CRITERIA

Subjects excluded from the study:

1. Were women who were pregnant, nursing or planning to become pregnant during the course of the study;
2. Were individuals with any visible dermatological condition that might have interfered with evaluations;
3. Were individuals with abnormal skin pigmentation at the test sites that might have interfered with subsequent evaluations of dermal responsiveness;
4. Were individuals who were taking medications that might have interfered with the test results, including any regimen of steroidal/non-steroidal anti-inflammatory drugs or antihistamines;
5. Were individuals with a known history of allergies to cosmetics or personal care products;
6. Were individuals who were under treatment for asthma or diabetes; and/or
7. Were individuals who were enrolled in a study or had participated in a patch test study within 14 days prior to the start of this study.

7.2.3 SUBJECT DEMOGRAPHICS

Demographic information is summarized in Text Table 7-1.

Text Table 7-1 Demographics of Subjects

		Enrolled N= 133	Completed N= 115
Age of Test Subjects (years)	Mean	41.8	42.3
	Standard Deviation	12.8	12.9
	Median	42.0	42.0
	Range	18 - 69	18 - 69
Gender of Test Subjects	Female	68 (51.1%)	64 (55.7%)
	Male	65 (48.9%)	51 (44.3%)
Ethnicity	Caucasian	49 (36.8%)	43 (37.4%)
	African American	67 (50.4%)	58 (50.4%)
	Hispanic	15 (11.3%)	13 (11.3%)
	Native American	N/A (N/A)	N/A (N/A)
	Asian/Pacific Islander	N/A (N/A)	N/A (N/A)
	Other	2 (1.5%)	1 (0.9%)


N/A = Not applicable

Discontinued subjects' data are shown, up to the point of discontinuation, but are not used in the Results and Discussion or Conclusions sections of this final report.

7.3 TEST ARTICLE

The test article was provided by

Text Table 7-2 Test Article Information

Sponsor's Test Article Code	RCTS' Test Article Code	Manufacturer	Date Received	Description of Material	Identity	Patch Conditions
TRA 11-127	2853.6738, 2854.6747		4/11/11	White cream	Personal care product	occlusive

The testing facility confirmed receipt of the test article and used the test article only within the framework of this clinical study and in accordance with the study protocol. Responsibility of the identity, purity, strength, composition and stability of the test article remained with the sponsor. The test article was stored at room temperature in a secured location until use.

8. METHOD

The Human Repeated Insult Patch Test (HRIPT) was conducted as follows:

8.1 INDUCTION PHASE

The Induction phase was initiated on 4/18/2011

8.1.1 Screening/Induction 1/Day 1

At the Screening/Day 1 visit, potential subjects received all necessary written and verbal information and signed an informed consent form prior to entering the study. Subjects who fulfilled all of the inclusion and none of the exclusion criteria outlined in the study protocol were allowed to participate in the study and received a unique subject number.

Prior to test article application the test site was evaluated to ensure no dermatological condition, or anything that would interfere with the evaluation of the test site, was present. The site was initially wiped with a cotton ball treated with 70% isopropyl alcohol after which approximately 0.2 mL, or enough to cover the entire patch, of the test article was placed onto a patch (according to the testing conditions specified in the table above) and the patch applied to the back of each subject above the waist, between the left scapula and the spinal mid-line. The subjects were instructed to remove the patch 24-hours after application.

*Patches used were
2cm x 2cm Parker-Davis
Readi-Bandages*

8.1.2 Inductions 2-9/Days 2-20

On Days 2-20, subjects arrived at the testing facility at which time they were queried as to any adverse events they may have experienced or any concomitant medications they may have taken since their last visit to the testing facility. The test site was then scored by a trained evaluator just prior to the next patch application using the following 6-point scale:

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

All other observed dermal sequelae (i.e., edema, dryness, papular responses, hypo- or hyperpigmentation) were appropriately recorded and described as mild, moderate or severe.

Following evaluation, the test site was cleansed with a cotton ball wet with 70% isopropyl alcohol and a fresh patch of the test article was applied to the subject's back. The subjects were instructed to remove the patch 24-hours after application. In general, this procedure was repeated every Monday, Wednesday and Friday until nine (9) applications of the test article had been made. A twenty-four (24) hour rest period followed the Tuesday and Thursday removals and a 48-hour rest period followed each Saturday removal.

Procedurally, if a subject developed a 2-level (moderate) erythema reaction or greater during the Induction phase, or if the skin responses warranted a change in site, the patch was applied to a previously unpatched, adjacent site. If a 2-level reaction (or greater) occurred at the new site, no further applications were made; however, all subjects were subsequently patched with the test material at a naïve site during the Challenge phase of the study unless, in the opinion of the Principal Investigator, it was unwise to do so.

8.1.3 Day 22 (read only)

On Day 22 subjects returned to the testing facility and a trained evaluator examined the test site and recorded the degree of erythema and any other dermal sequelae present. At the conclusion of the Day 22 visit no further patches were applied and the subjects began a 10-14 day rest period following the final Induction application.

8.2 CHALLENGE PHASE

The Challenge phase was initiated on 5/23/2011.

The final Challenge patch reading was made on 6/3/2011

8.2.1 Day 1 of Challenge Phase

Approximately 10-14 days following the application of the last Induction patch subjects returned to the testing facility for the Challenge phase of the study. The same test article evaluated in the Induction phase was applied in the Challenge phase under the same testing conditions. Application consisted of applying the test article to a patch and applying the patch to a naïve site located away from the original application site (opposite side of the upper back). During the challenge phase the test article remained in contact with the skin for a period of approximately 24 hours.

8.2.2 Days 2 and 4 of Challenge Phase (24 and 72 hours after patch application)

Subjects returned to the testing facility twenty-four (24) hours after Challenge patch application for supervised patch removal. The site was scored 24- and 72-hours after test article application (i.e., immediately after patch removal and again 48-hours after patch removal) using the same 6-point scale as used for the Induction phase. All subjects were instructed to report any delayed skin reactivity that might have occurred after the final Challenge patch reading. When warranted, selected test subjects returned to the testing facility for additional examinations and scoring to determine possible increases or decreases in Challenge patch reactivity.

9. PROTOCOL AMENDMENTS

No amendments were made to the original protocol.

10. ADVERSE EVENTS OR OTHER UNEXPECTED EVENTS

The following adverse events were reported during the course of the study:

One (1) subject [Subject no. 8 (Panel 2853)] reported having surgery during the course of the study to repair a tendon torn prior to study enrollment. The subject reported receiving only a local anesthetic (subject was unable to provide the name and dosage to the testing facility) and no other medications during or after the surgery. This adverse event is definitely unrelated to the test article.

Two (2) subjects [Subject nos. 12 and 57 (Panel 2853)] experienced mild scratches in the patch tape area during the induction phase of the study. No medications or actions were taken. These adverse events are definitely unrelated to the test article.

One (1) subject [Subject no. 31 (Panel 2853)] experienced edema on the upper back beginning after the 1st patch induction (total test article applied prior to adverse event: approximately 0.2 mL). The subject did not return to complete the study and contact could not be re-established to obtain further information. The subject is considered lost to follow up. This adverse event is considered to be possibly related to the test article.

One (1) subject [Subject no. 37 (Panel 2853)] experienced mild bruising on the back during the induction phase of the study. The subject used Biofreeze gel on the area once a day for four (4) days. The adverse event lasted five (5) days before subsiding. This adverse event is definitely unrelated to the test article.

One (1) subject [Subject no. 37 (Panel 2854)] experienced mild bruising on the back during the induction phase of the study. The adverse event lasted 3 days before subsiding. No medication or action was taken. This adverse event is definitely unrelated to the test article.

11. PROTOCOL DEVIATIONS

No protocol deviations were reported during the course of the study.

12. CHANGES IN THE CONDUCT OF THE STUDY

There were no changes in the conduct of the study.

13. RESULTS AND OVERALL CONCLUSIONS

(See Post-Text Table I for Individual Scores)

Under the conditions of a Human Repeated Insult Patch Test Procedure (Modified Draize; occlusive patch conditions), Test Article: TRA 11-127 produced generally transient, barely perceptible (0.5-level) to mild (1-level) patch test responses (specific and non-specific) on forty-three (43/115 or 37% of the test population) test subjects during the Induction and/or Challenge phases of the study. The skin reactivity observed was considered neither evidence of clinically meaningful irritation nor allergic in nature.

APPENDIX II

Sponsor's Test Article Code:

TRA 11-127

RCTS Panel Number: 2853

HRIPT

Patch Type: Occlusive

RCTS' Test

Article Code:

2853.6738

Subject's					Induction Exposure Number									Challenge Reading (hrs)						
No.	Initials	Age	Gender	Ethnicity	1	2	3	4	5	6	7	8	9	24	72	96	120	144	168	192
1	MB	49.3	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
2	DH	42.0	Male	Caucasian	Disc															
3	CC	48.6	Female	African American	0	0	0	0	0	0	0	0	0	0.5	0					
4	CS	48.7	Female	Caucasian	0	0	0	0	0	0	0	0	0	0.5	0					
5	DC	56.6	Female	African American	0	Disc														
6	TT	27.7	Female	Hispanic	0	0	0	0	0	0	0	0	0	0	0					
7	WD	48.6	Male	African American	0	0	0	0	0.5	0.5	0.5	0	0	0	0					
8	MG	52.5	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
9	AO	26.7	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
10	DC	59.9	Male	Hispanic	0.5	0.5	0	0	0	0	0	0	0	0.5	0					
11	PJ	37.1	Male	Caucasian	0	0	0	0	0	0	0	0	0	0.5	0					
12	JM	66.9	Female	Hispanic	0	0	0	0	0	0	0	0	0	0	0					
13	MM	49.4	Female	Caucasian	0	0	0.5	0	0	0.5	0.5	0	0	0.5	0					
14	SD	67.3	Female	Caucasian	0	0	0	0	0	0	0	0	0	0	0					
15	AG	30.8	Male	Hispanic	0.5	0	0	0.5	Disc											
16	MG	39.9	Female	Hispanic	0	0	0	0	0	0	0	0	0	0	0					
17	TA	19.0	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
18	HR	43.6	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
19	TR	31.5	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
20	AS	42.7	Female	Hispanic	0	0	0	0	0	0	0	0	0	0	0					
21	PM	29.1	Female	Hispanic	0	0	0	0	0	0	0	0	0	0	0					
22	RB	51.4	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
23	BD	18.3	Female	Hispanic	0	0	0	0	0	0	0.5	0	0	0	0					
24	JD	19.3	Male	Caucasian	0	0.5	0	0	0	Disc										
25	MM	55.1	Female	Caucasian	0	Disc														
26	KR	49.3	Female	Caucasian	0	0	0	Disc												
27	DE	36.3	Male	African American	0	0	0	0	0	0	0	0	0	0	0					

Sponsor's Test Article Code:

TRA 11-127

RCTS Panel Number: 2853

HRIPT

Patch Type: Occlusive

RCTS' Test

Article Code:

2853.6738

Subject's					Induction Exposure Number									Challenge Reading (hrs)						
No.	Initials	Age	Gender	Ethnicity	1	2	3	4	5	6	7	8	9	24	72	96	120	144	168	192
28	DC	30.8	Male	Caucasian	0	0	0	0	0	0	0	0	0	0	0					
29	AS	21.2	Male	African American	0	Disc														
30	EP	38.6	Male	Caucasian	0	0	0	0	0	0	0	0	0	0.5	0					
31	AD	48.7	Male	Caucasian	0	Disc														
32	SB	41.9	Male	Caucasian	0	0	0	0	0	0	0	0	0	0.5	0					
33	LR	32.7	Female	Hispanic	0	0	0	0	0	0	0	0	0	0.5	0					
34	AB	32.9	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
35	UM	44.9	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
36	BL	53.1	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
37	GC	62.8	Female	Caucasian	0	0	0	0	0	0	0	0	0	0	0					
38	DC	31.6	Male	Caucasian	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0					
39	MJ	50.1	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
40	AL	26.9	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
41	KR	35.6	Female	Caucasian	0	0.5	0	0.5	0	0	0.5	0	0	0.5	0					
42	RB	38.0	Female	Hispanic	0	0	0	0	0	0	0	0	0	0.5	0					
43	RP	19.9	Female	Caucasian	0	0	0	0	0.5	0	0	0	0	0	0					
44	KM	22.5	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
45	MP	41.9	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
46	VG	23.0	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
47	NS	29.2	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
48	AA	51.6	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
49	RA	58.0	Male	Caucasian	0	0	0	0	0	0	0	0	0	0.5	0					
50	GT	54.9	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
51	RH	54.8	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
52	SP	33.5	Female	Caucasian	0	1	0.5	0	0	0	0	0	0	0.5	0					
53	JB	42.6	Male	African American	0	0	0	0	0	0	Disc									
54	DC	52.5	Female	African American	0	0	0	0	0	0	0	0	0	0	0					

Sponsor's Test Article Code:

TRA 11-127

RCTS Panel Number: 2853

HRIPT

Patch Type: Occlusive

RCTS' Test
Article Code:

2853.6738

Subject's					Induction Exposure Number									Challenge Reading (hrs)						
No.	Initials	Age	Gender	Ethnicity	1	2	3	4	5	6	7	8	9	24	72	96	120	144	168	192
55	CC	54.4	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
56	MR	48.4	Male	Other	0	0	0	0	0	0	0	0	0	0	0					
57	ML	51.2	Male	Caucasian	0	0	0	0	0	0	0	0	0	0	0					
58	ES	60.8	Female	Caucasian	0	0	0	0	0	0	0	0	0	0	0					
59	CW	49.4	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
60	RW	29.3	Male	African American	0	0	0	0	0	0	0	0	Disc							
61	WN	33.5	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
62	AB	48.7	Male	African American	0	0	0	0	0	0	0	0	0	0	0					
63	MM	69.5	Female	Caucasian	0	0	0	0	0	0	0.5	0.5	0.5	0.5	0					
64	CR	43.9	Male	Caucasian	0.5	0	0	0.5	0	0	0	0.5	0	0.5	0					
65	SW	59.6	Female	African American	0	0	0	0	0	0	0	0	0	0	0					
66	NG	26.0	Female	Hispanic	0	0	0	0	0	0	0	0	0	Disc						
67	JW	49.0	Female	African American	0	0	0	0	0	0	0	0	0	0	0					

Clinical Score	Induction Exposure Number									Challenge Reading (hrs)						
	1	2	3	4	5	6	7	8	9	24	72	96	120	144	168	192
0	54	53	54	53	53	53	50	53	53	40	55	0	0	0	0	0
0.5	2	2	2	3	3	3	6	3	3	16	1	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	56	56	56	56	56	56	56	56	56	56	56	0	0	0	0	0

Disc = Discontinued

Clinical Observation Scoring Scale

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

Test article tested neat as received

Sponsor's Test Article Code:

TRA 11-127

RCTS Panel Number: 2854

HRIPT

Patch Type: Occlusive

RCTS' Test

Article Code:

2854.6747

Subject's					Induction Exposure Number									Challenge Reading (hrs)					
No.	Initials	Age	Gender	Ethnicity	1	2	3	4	5	6	7	8	9	24	72	96	120	144	192
1	SW	48.9	Female	Caucasian	0	0	0.5	0	0	0	0.5	0.5	0	0	0				
2	CM	40.3	Male	Caucasian	0	0	0	0	0	0	0	0	0	0.5	0.5				
3	DA	30.3	Male	Hispanic	0	0	0	0	0	0	0	0	0	0	0				
4	LT	37.6	Male	African American	0	0	0	0	0	0	0	Disc							
5	JM	29.6	Male	Caucasian	0	0	0	0	0	0	0.5	0.5	0.5	0.5	0				
6	RK	30.2	Male	African American	0	0	0	0	0	0	Disc								
7	MG	52.7	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
8	BM	56.8	Male	African American	0	0	0	0	0	0	0	0	0	0	0				
9	JP	49.1	Male	African American	0	0	0	0	0	0	0	0	0	0	0				
10	LM	35.3	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
11	CM	51.8	Male	African American	0	0	0	0	0	0	0	0	0	0	0				
12	WM	40.7	Female	Caucasian	0	0	0	0	0	0	0.5	0.5	0	0.5	0				
13	FW	37.7	Male	African American	0	0	0	0	0	0	0	0	0	0	0				
14	BS	54.5	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
15	CA	52.8	Male	African American	0	0	0	0	0	0	Disc								
16	BN	28.8	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
17	DG	19.1	Female	Caucasian	0	0	0	0	0	0	0	0	0	0.5	0				
18	GK	45.6	Male	African American	0	0	0	0	0	0	0	0	0	0	0				
19	CC	25.7	Male	Caucasian	0	0	0.5	0.5	0	0	0	0	0	0	0				
20	ES	44.9	Male	Caucasian	0	0.5	0.5	0	0	0.5	0	0	0	0	0				
21	JN	31.4	Male	Caucasian	Disc														
22	JT	28.9	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
23	SW	33.9	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
24	RD	20.5	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
25	JH	46.7	Male	African American	0	0	0	Disc											
26	AM	59.9	Male	Caucasian	0	0	0	0	0.5	0	0	1	0	0.5	0				
27	LL	52.7	Female	Caucasian	0	0.5	0.5	0	0	0	0	0	0	0.5	0.5				

Sponsor's Test Article Code:

TRA 11-127

RCTS Panel Number: 2854

HRIPT

Patch Type: Occlusive

RCTS' Test

Article Code:

2854.6747

Subject's					Induction Exposure Number									Challenge Reading (hrs)					
No.	Initials	Age	Gender	Ethnicity	1	2	3	4	5	6	7	8	9	24	72	96	120	144	192
28	JG	30.9	Female	Caucasian	0	0	0	0	0	0	0	0	0	0	0				
29	TK	53.1	Female	Caucasian	0	0	0	0	0	0	0	0	0	0	0				
30	JM	28.8	Male	African American	0	0	0	0	0	0	0	0	0	0	0				
31	RM	40.9	Male	African American	0	0	0	0	0	0	0	0	0	0	0				
32	DM	41.0	Female	Caucasian	0.5	0.5	0.5	0	0	0	0	0.5	0.5	0	0				
33	RL	56.5	Male	Caucasian	0	0.5	0.5	0.5	0	0.5	0.5	0.5	0.5	0.5	0				
34	LH	33.5	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
35	ER	24.7	Female	African American	0	0	0	0	1	0.5d	0	0	0	0	0				
36	DH	48.7	Male	Caucasian	0	0.5	0	0	0	0	0.5	0.5	0	0	0				
37	IJ	36.6	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
38	AR	33.4	Male	African American	Disc														
39	TO	31.0	Female	Caucasian	0	0.5	0	0	0	0	0	0	0	0	0				
40	HW	42.7	Male	African American	0	0	0	0	0	0	0	0	0	0	0				
41	SR	30.8	Female	Caucasian	0	0	0	0	0	0	0	0	0	0	0				
42	TH	39.6	Male	Caucasian	0	0	0	0	0	0	0	0	0	0	0				
43	TR	57.1	Female	Caucasian	0	0	0	0	0	0	0	0	0	0	0				
44	RN	51.2	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
45	AS	28.4	Male	African American	0	0	0	0	0	0	0	0	0	0	0				
46	JT	62.3	Male	Hispanic	0	0	0	0	0	0	0	0	0	0	0				
47	RC	27.8	Male	Hispanic	0	0	0	0	0	0	0	0	0	0	0				
48	CG	57.0	Male	Hispanic	0	0	0	0	0	0	0	0.5	0	0	0				
49	CC	38.5	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
50	SD	28.7	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
51	AJ	40.6	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
52	TC	47.2	Female	Caucasian	0	0	0	0	0	0	0	0	0	0	0				
53	TI	68.2	Male	Caucasian	0	0	0.5	0.5	0.5	0.5	0	0.5	0.5	0.5	0				
54	MM	51.2	Female	Caucasian	0	0	0	0.5	0.5	0	0	0	0	0	0				

Sponsor's Test Article Code:

TRA 11-127

RCTS Panel Number: 2854

HRIPT

Patch Type: Occlusive

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Article Code:

2854.6747

Subject's					Induction Exposure Number									Challenge Reading (hrs)					
No.	Initials	Age	Gender	Ethnicity	1	2	3	4	5	6	7	8	9	24	72	96	120	144	192
55	CK	44.9	Male	Caucasian	0.5	0	0	0	0	0	0	0	0	0.5	0				
56	CD	39.6	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
57	MS	32.8	Male	Caucasian	0.5	0.5	0.5	0.5	0	0	0.5	0	0	0	0				
58	SR	49.0	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
59	DD	23.7	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
60	PM	55.1	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
61	GS	69.8	Female	Caucasian	0	0	0	0	0	0	0	0	0	0	0				
62	TV	46.2	Male	Caucasian	0.5	Disc													
63	BM	69.8	Female	Caucasian	0	0	0	0	0	0	0	0	0	0.5	0				
64	DH	58.8	Female	African American	0	0	0	0	0	0	0	0	0	0	0				
65	JC	57.7	Male	Caucasian	0.5	0	0	0	0	0.5	0.5	0.5	0.5	0.5	0				
66	LT	24.8	Female	African American	0	0	0	0	0	0	0	0	0	0	0				

Clinical Score	Induction Exposure Number									Challenge Reading (hrs)					
	1	2	3	4	5	6	7	8	9	24	72	96	120	144	192
0	55	52	51	54	55	54	52	49	54	45	56	0	0	0	0
0.5	4	7	8	5	3	5	7	9	5	14	3	0	0	0	0
1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	59	59	59	59	59	59	59	59	59	59	59	0	0	0	0

Disc = Discontinued

d= Mild Dryness

Clinical Observation Scoring Scale

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

Test article tested neat as received



Memorandum

TO: COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: June 15, 2017

SUBJECT: Polyaminopropyl Biguanide (PHMB)

Anonymous. 2011. Summary of an HRIPT of a leave-on product containing 0.1% Polyaminopropyl Biguanide (PHMB).

Summary of an HRIPT of a Leave-on Product Containing 0.1% Polyaminopropyl Biguanide (PHMB)

Study, overseen by a dermatologist, was completed in 2011

%Polyaminopropyl Biguanide (PHMB)	0.1% (0.5% of a trade name material containing 20% PHMB)
Product Type	Leave-On
Occlusivity	Occlusive
Completed subjects	207
Did formula induce an allergic response	No
Number of subjects exhibiting low level reaction during induction	0
Number of subjects exhibiting high level reaction during induction	0
Number of subjects exhibiting low level reaction during challenge	0
Number of subjects exhibiting high level reaction during challenge	0
Pass/Fail	Pass
Comment	Did not induce dermal sensitization

Calculation of Amount of Polyaminopropyl Biguanide mg/cm ²	
Concentration of Polyaminopropyl Biguanide (PHMB) in %	0.1
Amount of Product applied to Skin during HRIPT in gms.	0.1
Patch Size	2cm*2cm
Dose density of product applied to patched skin in mg/cm ²	25
Dose Density of Polyaminopropyl Biguanide (PHMB) applied to patch skin in mg/cm ²	0.025
Conclusion: Amount of Polyaminopropyl Biguanide (PHMB) Applied to skin is 0.025mg/cm ² (or 25 µg/cm ²)	

Details of Test Methodology and Results			
0	panelist discontinued due to reactions		
48 -72 hrs	patch duration		
9	induction patches		
3	weeks induction		
2	week rest period		
virgin site	challenge		
24, 48, 72, 96 hr	challenge readings		

Induction Grading Scale		
Grade	Response	Score
0	No evidence of irritation	0
1	Minimal erythema, barely perceptible	1
2	Definite erythema, readily visible; or minimal edema; or minimal papular response	2
3	Erythema and papules	3
4	Definite edema	3
5	Erythema, edema, and papules	3
6	Vesicular eruption	3
7	Strong reaction spreading beyond test site	3

Challenge Grades	
Grade	Response
0	No evidence of erythema
1	Mild erythema (faint pink to definite pink)
2	Moderate erythema (definite redness)
3	Severe erythema (very intense redness)

Grading Scale interpretation	
Low Level Reactions	0 and 1
High Level Reaction	2 and above



WOMEN'S VOICES FOR THE EARTH

OUR HEALTH. OUR FUTURE. TOXIC FREE.

July 20, 2017

To the CIR,

I was interested and glad to see that there was an insufficient data announcement made at the June meeting for the ingredient Polyaminopropyl Biguanide (PHMB). My organization also works on cleaning product chemicals, and thus I am very familiar with the tragedy in Korea regarding the similar chemical PHMG in humidifier disinfectants. I am pleased to see that information about the humidifier disinfectants problem was considered in the discussions around the similarly structured chemical PHMB. It seems, however, that the CIR came to a conclusion that the PHMG is a significantly different chemical than PHMB, rendering the information mostly irrelevant to the safety assessment.

For your information, I have attached two risk assessments (Lee, 2012 and Lee, 2013) that were conducted in Korea (unfortunately in hindsight) on PHMG. It is important to note that the researchers did not have inhalation data for PHMG to input into their risk assessment, and thus used a subacute inhalation NOEC (no-observed-effect-concentration) for PHMB "because of structural analogy." I have also attached a third paper (Kim 2016) which discusses in greater detail the similarities between PHMB and PHMG with respect to chemical structure, toxicity and antimicrobial activity. So whereas, the CIR thus far has indicated that PHMB is a significantly different chemical from PHMG, these Korean researchers felt the two chemicals were analogous enough to use the data for one to assess the safety of the other.

This could be an important case study to look into as the CIR furthers its work on the read-across guidelines in development, as clearly there is a discrepancy of professional opinion with respect to how similar these two chemicals are. Given the disastrous outcome of incorrectly assessing the inhalation safety of the humidifier disinfectant products in Korea before they were brought to market, this is clearly a case that the CIR needs to get right.

Also, it is worth noting that the estimated exposures to PHMG from humidifier disinfectants were very, very small. The disinfectant products contained merely .125% PHMG. To use the product, one tablespoon is added to a four liter humidifier tank of water, which is then vaporized into the air of a bedroom and then finally inhaled. Even with this situation of dilution upon dilution upon dilution, the risk quotients for PHMG exposure ranged between 1,400 and 20,000 (best to worst case scenario) indicating significant potential harm. Apparently, using the inhalation NOEC for PHMB was a useful and appropriate surrogate. The assessed risk of potential harm proved to be true in reality as well, as inhalation of this chemical from these products have been linked to the death of over 150 people (84 children, numerous pregnant women and other adults), linked to damaged health in over 900 people (thus far certified by the Korean government) and there are more than 5,000 claims of additional harm from consumers who used these products.

I encourage the CIR to use the greatest precaution in establishing the safety of this chemical, particularly for any cosmetic products with the potential to be inhaled.

A handwritten signature in black ink, reading "Alexandra Scranton". The signature is written in a cursive, flowing style.

Alexandra Scranton
Director of Science and Research
Women's Voices for the Earth



Refined Exposure Assessment for Three Active Ingredients of Humidifier Disinfectants

Jong-Hyeon Lee¹, Hyun-Joong Kang^{2,3}, Hwi-Soo Seol¹, Chan-Kook Kim¹, Seung-Ki Yoon⁴, Jin Gwack⁴, Yong-Hwa Kim⁵, Jung-Hwan Kwon^{2,3†}

¹Institute of Environmental Safety and Protection, NeoEnBiz Co., Bucheon 420-806, Korea

²Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-701, Korea

³Department of Environmental Engineering, Ajou University, Suwon 443-749, Korea

⁴Division of Epidemic Intelligence Service, Korea Centers for Disease Control and Prevention, Cheongwon-gun 363-951, Korea

⁵Korea Institute of Toxicology, Daejeon 305-343, Korea

Abstract

Exposure assessment for three major active ingredients used for humidifier disinfectants, polyhexamethylene guanidine (PHMG), oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride (PGH), and 5-chloro-2-methylisothiazol-3(2H)-one/2-methylisothiazol-3(2H)-one (CMIT/MIT) mixture, was conducted in a bedroom using an air sampler for a refined risk assessment. The experimental site was selected to reflect consumer exposure conditions. Aerosols formed by a humidifier were sampled during 8 hr at 7.5 L/min. Absorbed PHMG and PGH by the sampler were quantified using a spectrophotometric method, and high performance liquid chromatography-ultraviolet detection was used for CMIT/MIT. Three exposure scenarios were assumed for adding humidifier disinfectants to the humidifier water at 1, 2, and 10 times the volume recommended by the product suppliers, and the humidifier was on at its maximum rate of producing aerosols in order to consider reasonable worst-cases. The sampled mass of PHMG and PGH ranged 200 to 2,800 µg and 140 to 1,900 µg, respectively, under different exposure conditions, whereas the absorbed mass of CMIT/MIT was barely detected at the detection limit of 0.11/0.29 mg/L, only at 10 times the recommended level. The resulting risk quotients for PHMG and PGH ranged 1,400 to 20,000 and 1,000 to 13,000, indicating that health risks could be significant. For CMIT/MIT mixture, risk quotients were much smaller than estimated by assuming that they are conservative in the indoor environment, probably due to oxidative reactions. The refined exposure assessment presented here may provide a useful tool for assessing risks posed by active ingredients in spray-type biocidal products.

Keywords: Air sampler, chloromethyl/methyl isothiazolinone (CMIT/MIT), Indoor air, polyhexamethylene biguanidine (PHMG), Risk assessment

1. Introduction

In 2011, the Korea Centers for Disease Control and Prevention reported that an unidentified fatal lung disease was likely to be caused by chemical disinfectants used with household humidifiers [1, 2]. The causative active ingredients were identified as polyhexamethyleneguanidine (PHMG) and oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride (PGH), based on the epidemiological studies and *in vivo* histopathological readings, after instillation of those active ingredients to rats [3]. Chloromethyl/methyl isothiazolinone (CMIT/MIT) evaluated at the same time was not likely to cause fatal lung disease [3].

Although epidemiological evidences have revealed that those polymeric chemical disinfectants could be fatal when inhaled, quantitative risk assessment for the inhalation of those chemicals have not been conducted, except for a screening-level health

risk assessment [4]. Lee et al. [4] assumed that the active ingredients of humidifier disinfectants are inert and homogeneously distributed in a bedroom (i.e., no chemicals react, precipitate, or were removed by other pathways). The resulting health risk quotients were calculated at 2,500, 10,500, and 9.41 for PHMG, PGH, and CMIT/MIT, respectively. These values at the screening level risk assessment are very high, indicating potentially significant health concerns, and requiring a refined risk assessment. Uncertainties with risk quotient at the screening-level lie in uncertainties with the predicted exposure concentration using simple steady-state modeling, as well as the reliability of the "read across" method used to predict the long-term toxicity data for the derivation of the reference concentrations.

In this study, we intended to measure the realistic exposure concentration, based on the human exposure scenarios under normal and excessive use conditions. Products of humidifier



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[†]Corresponding Author

E-mail: junhwankwon@korea.ac.kr

Tel: +82-2-3290-3041 Fax: +82-2-953-0737

disinfectants containing three active ingredients (PHMG, PGH, and CMIT/MIT) were added to the humidifier water at the level recommended by the product suppliers and released to a room using a household humidifier. Aerosols containing active ingredients were sampled at a pumping rate close to the average breathing rate for Koreans, to estimate the human intake rate according to the exposure scenarios. Refined health risk quotients were estimated, based on the measured intake rates.

2. Materials and Methods

2.1. Active Ingredients and Chemicals

Three active ingredients of the humidifier disinfectants used in this study were PHMG, PGH, and CMIT/MIT. Chemical structures of all three ingredients are shown in Fig. 1. Aqueous solutions of PHMG-phosphate (25% w/w) and PGH (25% w/w) were kindly provided by SK Chemical Industries, Inc. Analytical grade 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT, 98%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and analytical grade 2-methyl-4-isothiazolin-3-one (MIT, 98%) was purchased from Sigma-Aldrich (St Louis, MO, USA). Three commercial humidifier disinfectant products containing three active ingredients each were purchased from the market, before they were recalled after November, 2011.

Eosin Y solution (0.5% w/v), glycine (98.5%), magnesium nitrate ($\text{Mg}(\text{NO}_3)_2$, 99%) and magnesium chloride (MgCl_2 , 99%) were purchased from Sigma-Aldrich. Hydrochloric acid (35%) was purchased from Dae-Jung (Siheung, Korea). Methanol (high performance liquid chromatography [HPLC] grade) was purchased from Burdick & Jackson (Ulsan, Korea).

2.2. Sampling Site, Generation of Humidifier Aerosols, and Aerosol Sampling

A bedroom (area, 21 m²; volume, 47 m³) was rented for the experiments. A humidifier that uses a water boiling system combined with ultrasonic mist generation was used for the production of mists. This type of the humidifier was similar to those humidifiers used by patients who suffered the unidentified lung disease. The water tank size was 4.8 L and approximately 4 L evaporated during the 8 hr sampling period.

Air containing humidified aerosols was sampled using a custom-made air sampler, consisting of two serial 250 mL impingers containing 100 mL of aqueous solution and a constant flow sample pump (The QuickTake 30; SKC Inc., Eighty Four, PA, USA) (Fig. 2) [5]. Distilled water was used as the sampling medium for PHMG and PGH aerosols, and aqueous solution containing 2.5 g/L $\text{Mg}(\text{NO}_3)_2$ and 0.5 g/L MgCl_2 was used for sampling CMIT/MIT, because PHMG and PGH are cationic polymers, and CMIT/MIT have high water solubility. Preliminary studies using a sampler with four serial impingers showed that two impingers were sufficient for quantifying the disinfectants in the sampled aerosols, since the trapped amount from the third impinger was below the detection limit. Air was sampled at the rate of 7.5 L/min, the mean breathing rate for Koreans at rest [6] with all windows and doors closed. The room was ventilated by opening all windows and doors, between independent exposure measurements, to minimize any potential carry-over effects from the previous measurement.

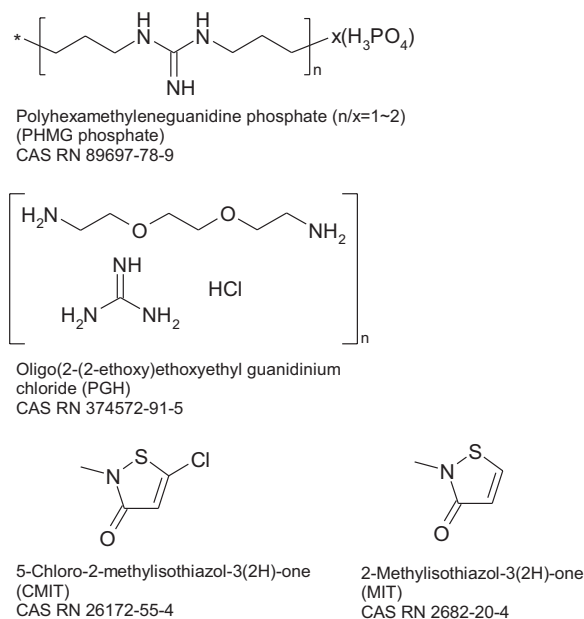


Fig. 1. Active ingredients of the humidifier disinfectants tested. CAS: Chemical Abstracts Service, RN: registry number.

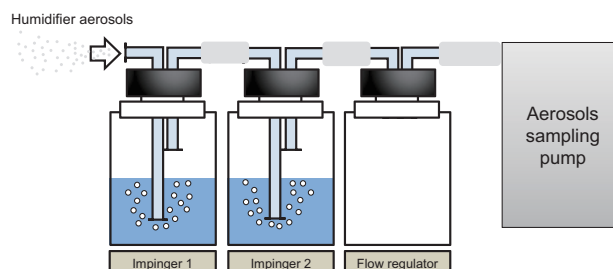


Fig. 2. Schematic diagram of the sampling apparatus for humidifier aerosols.

2.3. Instrumental Analyses of Active Ingredients

2.3.1. Spectrophotometric determination of PHMG and PGH

Quantitative analysis of polymeric active ingredients (PHMG-phosphate and PGH) containing guanidine group was conducted, using the color-changing reaction of guanidine with tetrabromofluorescein (Eosin Y) [7, 8]. Glycine buffer solution at pH 3.6 was prepared by adding 50 mL of 0.1 M glycine solution, and 2.5 mL of 0.2 M hydrochloric acid to 100 mL aqueous solution. Analytical standards were prepared at 1, 2, 4, 6, and 8 mg/L for PHMG-phosphate and PGH, for spectrophotometric determination of their concentration in the sampling solution. In case the sampled concentration exceeded the range of analytical standards, the solution was diluted appropriately before the analysis. Sample solution (10 mL) taken from the impingers was mixed with 10 mL of glycine buffer solution (pH 3.6) and 1.0 mL of 0.05% (w/v) Eosin Y solution. The mixture was vortexed briefly, and left for 5–10 min at room temperature for color development. Then, the absorbance of the mixture was measured

at 549 nm, using a DR/4000U UV/Vis spectrophotometer (Hach Company, Loveland, CO, USA).

2.3.2. HPLC analysis of CMIT/MIT

Aqueous samples containing CMIT/MIT mixture and stabilizers ($\text{Mg}(\text{NO}_3)_2$ and MgCl_2) were quantified, using an HPLC system equipped with a Waters 515 pump (Waters, Milford, MA, USA), an autosampler (Waters 717+), and a photodiode array detector (Waters 996). In order to exclude the potential degradation of CMIT and MIT by strong nucleophiles [9, 10], the collected samples were analyzed within 8 hr. Samples were separated on an ODS HYPERSIL C18 column (4.6×150 mm, $5 \mu\text{m}$ particle size; Thermo Scientific, Hampton, NH, USA) at ambient temperature, under an isocratic condition (methanol:water = 15:85, v/v) with a flow rate of 1 mL/min. Injection volume was 10 μL and both compounds were monitored at 280 nm. The chromatographic retention times were 11.3 and 3.3 min for CMIT and MIT, respectively.

2.4. Modeling Exposure Concentration

2.4.1. Emission scenarios

According to the suppliers of humidifier disinfectants, 20 mL of the liquid product should be added to approximately 4 L water in a tank. Thus, we set the normal exposure scenario (scenario 1) that the humidifier runs for 8 hr during sleeping, and almost all water in the tank (approx. 4 L) is consumed during the sampling time. In order to estimate the level of exposure considering the worst-case, the amount of humidifier disinfectants added to water was assumed 2 times (scenario 2) and 10 times (scenario 3) that suggested by the suppliers.

2.4.2. Modeling the uptake of humidifier disinfectants

The behavior of humidifier disinfectants in the room could be explained by a simple mass-balance model:

$$\frac{dC_{air}}{dt} = -\lambda C_{air} - \frac{Q}{V} C_{air} - k C_{air} + \frac{E}{V} \quad (1)$$

where, C_{air} is the ambient concentration of a humidifier disinfectant (mg/m^3), λ is the air change rate (h^{-1}), Q is the impinge pumping rate (m^3/h), V is the volume of the room (m^3), k is the pseudo-first-order decay constant including all possible linear processes, such as deposition to surfaces and decomposition (h^{-1}), and E is the emission rate by the humidifier (mg/hr). The emission rate (E) was estimated by the consumption of water in the water tank. The analytical solution of Eq. (1) for C_{air} is given by:

$$C_{air} = \frac{E}{\left(\lambda + k + \frac{Q}{V}\right)V} \left[1 - \exp\left(-\left(\lambda + k + \frac{Q}{V}\right)t\right) \right] \quad (2)$$

The total mass of humidifier disinfectant sampled (M) is

$$M = \int_0^t C_{air} Q dt = \frac{QE}{\left(\lambda + k + \frac{Q}{V}\right)V} + \frac{QE}{\left(\lambda + k + \frac{Q}{V}\right)^2 V} \left[\exp\left(-\left(\lambda + k + \frac{Q}{V}\right)t\right) - 1 \right] \quad (3)$$

Thus, $\lambda + k$ can be calculated, using the experimentally measured M .

The time-weighted average concentration of humidifier disinfectants ($C_{air,TWA}$) used for refined risk assessment is given by:

$$C_{air,TWA} = \frac{M}{Qt} \quad (4)$$

3. Results and Discussion

3.1. Quality Control

Method detection limits (MDL) of the analytical procedure were derived using an error distribution [11]. MDL for the spectrophotometric determination of PHMG-phosphate and PGH were 0.20 mg/L . For the quantification of CMIT and MIT using HPLC, MDL were 0.11 and 0.29 mg/L for CMIT and MIT, respectively. Calibration curves were obtained, using at least 5 levels of analytical standards. The relative standard deviation of the calibration factors was less than 15% for all measurements.

3.2. Estimation of Exposure Concentration

The masses of active ingredients collected by the sampler (M) are presented in Table 1. The time-weighted average concentrations in the bedroom were calculated using Eq. (4), as 0.06, 0.09, and 0.78 mg/m^3 for PHMG, and 0.04, 0.14, 0.53 mg/m^3 for PGH, according to the exposure scenarios 1, 2, and 3, respectively (Table 2). The concentration of CMIT/MIT in the absorbing solution was only measurable under exposure scenario 3. Because the value of $\lambda + k$ in Eqs. (2) and (3) is independent of the concentration in the air (C_{air}) and the emission rate (E), the mean value was obtained for each active ingredient. The value of $\lambda + k$ for PHMG was 1.0 ± 0.17 , whereas they were 5.0 ± 0.80 and 4.8 for PGH and CMIT/MIT, respectively. Because the air change rate λ is a variable that is not affected by chemicals, results indicate that degradation of PGH and CMIT/MIT is likely to occur in the indoor environment, although their reaction pathways in the indoor environment have not been reported. PGH and CMIT/MIT are reactive chemicals that may react with various surfaces in the indoor environment. Further evaluation is needed to quantify the effects of those heterogeneous reaction rates. Because oxidative reaction of CMIT/MIT with glutathione to form disulfides occurs, depending on pH [12], similar ring cleavage reactions may occur.

The possibility of potential deposition or decomposition of PHMG could not be excluded, although it has much lower

Table 1. Mass of active ingredients collected by the air sampler for 8 hr

Active ingredient	Measured mass (μg)		
	Scenario 1	Scenario 2	Scenario 3
PHMG	203 (n = 2)	334 \pm 49 (n = 6)	2,810 (n = 2)
PGH	145 (n = 2)	498 \pm 14 (n = 5)	1,910 (n = 2)
CMIT/MIT	ND (n = 2)	ND (n = 2)	74 (n = 2)

Values are presented as mean \pm standard deviation or number.

PHMG: polyhexamethyleneguanidine, PGH: oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride, CMIT/MIT: chloromethyl/methyl isothiazolinone, ND: not detectable.

Table 2. Derivation of health risk quotients for the use of humidifier disinfectants

Active ingredient	PHMG	PGH	CMIT/MIT
Effect assessment			
Subchronic inhalation NOEC (mg/m ³)	-	-	0.34
Subacute inhalation NOEC (mg/m ³)	0.024	0.024	-
Assessment factor	600	600	200
Reference concentration (mg/m ³)	0.00004	0.00004	0.0017
Exposure assessment			
Content of active ingredient in the product	0.125	0.5	0.019
Emission rate (mg/hr)	3.12	12.5	0.475
Time-weighted average concentration in the bedroom (mg/m ³)			
Scenario 1	0.056	0.040	-
Scenario 2	0.093	0.140	-
Scenario 3	0.780	0.530	0.021
Health risk quotient			
Initial screening [4]	2,500	10,500	9.41
Scenario 1	1,400	1,000	-
Scenario 2	2,300	3,500	-
Scenario 3	20,000	13,000	12

PHMG: polyhexamethyleneguanidine, PGH: oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride, CMIT/MIT: chloromethyl/methyl isothiazolinone, NOEC: no-observed effect concentration.

$\lambda + k$ than the other two active ingredients. The value of λ may vary significantly, depending on the ventilation. For example, the median value reported for 500 children's bedrooms in Denmark was 0.44 h⁻¹ [13], and that for 2,844 US single- and multi-family dwellings was 0.42 h⁻¹ [14] in the winter season. Newly constructed residences in Korea are regulated to secure a minimum air change rate of 0.7 since 2006 to improve indoor air quality [15]. For the experimental site, λ was less than 1.0/hr, although λ was not quantified in this study. Thus, the ventilation in the bedroom could be regarded as representative for a typical bedroom in the winter season.

3.3. Refined Health Risk Assessment Using Exposure Concentration and Indoor Air Modeling

Table 2 summarizes the steps in risk assessment for inhalation of the selected active ingredients of humidifier disinfectants. Whereas a subchronic inhalation no-observed effect concentration (NOEC) was available for CMIT/MIT (3:1) mixture [16], no values were reported for PHMG and PGH. Thus, we adapted a "read across" method, for the derivation of the reference concentrations for those chemicals. The EU harmonized classification and labeling report on polyhexamethylene biguanide (PHMB) reported the subacute inhalation NOEC of 0.24 µg/L using rats [17]. Assessment factors for the derivation of reference concentrations for the three active ingredients in air were 600 for PHG and PHMG and 200 for CMIT/MIT, according to the guideline of the European Chemical Agency [18]. The steady-state concentrations obtained in this study were used for the refined risk assessment. Experimental exposure concentrations for PHMG and PGH in this study were lower by factors of 1.8 and 10.5, respectively, than those calculated in the risk assessment at screening level using paper-and-pencil [4]. However, the corresponding risk quotients were higher than 1,000 for both PHMG and PGH under the normal use condition (scenario 1), indicating significant health concerns. For CMIT/MIT mixture, risk quotient was

not calculated under the normal-use condition (scenario 1). Based on the risk quotient of 12 in scenario 3, it could be extrapolated to about unity under the normal-use condition.

3.4. Implications for Risk Assessment

In spite of the uncertainties with inhalation toxicity data, the resulting risk quotients indicated that PHMG and PGH used as humidifier disinfectants are of significant concerns, because measured intake rates and exposure concentration significantly exceeded the reference concentrations for PHMG and PGH, under our exposure scenarios of normal and heavy uses of humidifier disinfectants. The experimental determination of the exposure levels in this study also strengthened the utility of a low-cost initial screening risk assessment [4], because measured intake rates did not decrease significantly for PHMG and PGH.

Because $\lambda + k$ values significantly varied among the three active ingredients, indicating the importance of deposition and decomposition reactions in the indoor environment, the processes determining the stability of biocides in the indoor environment need to be studied in the future, especially when they are applied as spray-types.

4. Conclusions

A refined risk assessment conducted for three active ingredients of humidifier disinfectants by measuring intake rates indicated that the exposure concentrations under plausible exposure scenarios were significantly higher, than the reference concentrations for PHMG and PGH. This confirmed that those chemicals may pose severe health concerns, when they are used as humidifier disinfectants. Unlike PHMG and PGH, low molecular weight biocides, CMIT and MIT seem to undergo significant decomposition in the indoor environment, resulting in the reduced exposure concentrations. Results in this study imply that

reactions with indoor surfaces may be important in the evaluation of the atmospheric concentration and corresponding risks for heavily applied spray-type biocides.

Acknowledgments

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Adverse health effects of humidifier disinfectants in Korea: lung toxicity of polyhexamethylene guanidine phosphate. Kim HR¹, Hwang GW, Naganuma A, Chung KH. *J Toxicol Sci.* 2016;41(6):711-717.

Fatal Misuse of Humidifier Disinfectants in Korea: Importance of Screening Risk Assessment and Implications for Management of Chemicals in Consumer Products. Jong-Hyeon Lee, Yong-Hwa Kim, and Jung-Hwan Kwon[§], [dx.doi.org/10.1021/es300567j](https://doi.org/10.1021/es300567j). *Environ. Sci. Technol.* 2012, 46, 2498–2500



Memorandum

TO: COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: June 6, 2017

SUBJECT: Draft Tentative Report: Safety Assessment of Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride) as Used in Cosmetics (draft prepared for the June 12-13, 2017 CIR Expert Panel Meeting)

Key Issues

Although the new summary of sensitization studies from Cosmetics Europe did not include any new studies, they did state that in the negative human photosensitization study, the dose used was 1 mg/cm². This dose should be added to the CIR report as it helps to address the CIR Expert Panel's request for data to help determine a no-effect level for dermal sensitization.

The maximum reported use concentration is incorrectly reported as 0.1% in the CIR report. It was 0.2% (eye lotion), and based on the most recent concentration of use table (wave 2) it is 0.5% (non-spray suntan product).

The SCCS opinion was finalized by written procedure on April 7, 2017. This needs to be corrected in several places in the report. Now that the SCCS opinion on Polyaminopropyl Biguanide (PHMB) has been finalized, the last paragraph of the cosmetic use section should be deleted. CMR materials with an SCCS opinion can be used in cosmetics. At a minimum, reference 13 (article from Kemi Taenk) should be deleted from the CIR report. This organization is a Danish consumer chemistry watchdog. If the reference is left in the report, the text should explain the source.

Sensitization - It should be made clear that the following sentence of the Sensitization section was a conclusion of the SCCS, and that the 0.2% concentration was in water not a formulation: "It was also determined that skin sensitization in humans can be elicited at concentrations beginning at 0.2% active ingredient." Among the references listed for this sentence only reference 5 is correct.

Information from reference 28 (Jowsey 2007) is not in the Sensitization paragraph and it is not included in Table 15. This is a surveillance study in which sensitization to Polyaminopropyl Biguanide (PHMB) was not increased even after use in underarm

deodorants at <0.2%. The CIR Expert Panel may find this paper useful as it also discusses surveillance in relationship to QRA.

References 29 (abstract), 30, 31 and 33 are about positive patch test reactions to Polyaminopropyl Biguanide (PHMB) and are not actually discussed in the sensitization paragraph.

Reference 32 should be associated with the LLNA.

Reference 34 is the HRIPT on the product containing 0.2% Polyaminopropyl Biguanide (PHMB).


Additional Considerations

Impurities - It should be made clear that the metal concentrations reported were for 5 batches of technical grade (solid) PHMB.

Dermal Penetration, In Vitro, Summary - The following sentence in the Dermal Penetration section, and a similar sentence in the Summary needs to be revised as the dermal penetration studies at 0.1% and 0.3% both used the aqueous micellar solution and the oil-in-water emulsion as vehicles. "Polyaminopropyl Biguanide solutions (polyhexamethylene biguanide hydrochloride as a 0.1% aqueous micellar solution and as a 0.3% oil-in-water emulsion) were applied to human split-thickness skin in a 2-part dermal penetration study."

It should be made clear that the margin of safety calculation was done by the SCCS.

Short-Term, Oral - There was only one 28-day drinking water study in rats and one 28-day drinking water study in mice. Therefore, it is not clear why the text implies that were multiple 28-day oral rat studies. Where does the dose of "~0.0002 mg/kg bw/day" come from? The LOAEL was 0.1 mg/ml in drinking water (which is not clearly stated in the text); if a 200 g rat drinks 25 ml of water/day, a more appropriate estimated dose is $0.1 \text{ mg/ml} \times 25 \text{ ml} \times 1/0.2 \text{ kg} = 12.5 \text{ mg/kg bw/day}$.

Short-Term, Inhalation - In the text, please state that the exposures were 6 hours/day, 5 days/week, nose-only. 

Chronic, Dermal - It is not clear why more details of the 80 week dermal study are not stated in this section. The SCCS opinion indicated that this study had a NOAEL of 0.6 mg/mouse (15 mg/kg/day).

Chronic, Oral - Please include the species used in the 104-week oral study. The high dose of the 1-year dog dietary study is not stated correctly. They started at a dietary concentration of 4500 ppm then at weeks 11/12 because of toxicity they reduced the dietary concentration to 3000 ppm. It should state that the SCCS completed the MOS calculations.

Carcinogenicity, Dermal - It should be made clear that the 80 week dermal study in mice is the same study that was described in the Chronic section. The doses used in this study (0, 0.6, 6 and 30 mg/mouse/day in ethanol or 0, 25, 150 or 750 mg/kg/day) should be clearly stated. It should also be stated that the NOAEL was 0.6 mg/mouse (or 15 mg/kg/day).

Cytotoxicity and Antimicrobial Activity - Perhaps when using the name polyaminopropyl biguanide for the actual compound, the first letters of this name should not be capitalized (capitalization should only be used when it is an INCI name for PHMB).

Epigenetic Effects - Please state the source of the classification scheme for which Polyaminopropyl Biguanide (PHMB) is considered a "category 3 carcinogen."

Photosensitization - Please add the dose (1 mg/cm² - provided in the most recent submission from Cosmetics Europe) used in the human photosensitization study of aqueous 1% Polyaminopropyl Biguanide (PHMB).

Ocular Irritation, Summary - Please make it clear that the human eyes into which Polyaminopropyl Biguanide was instilled were from cadavers.

Other Clinical Reports - Please add the subheading polyhexamethylene guanidine for the studies of the humidifier disinfectant. Adding the structure of this material would also be helpful.

Summary - It is not correct to state that the study results showed that dermal penetration was 4.09%. This was the value that was used by the SCCS - it includes one standard deviation added to the study results.

It should be made clear that the LOAEL of 0.1 mg/ml from the 28-day study is a concentration in drinking water. There was only one 28-day drinking water study in rats. There was also a 28-day drinking water study in mice.

The descriptions of the MOS calculations are not complete as they do not indicate that the lower value was calculated assuming products contained 0.3% Polyaminopropyl Biguanide (PHMB) and the higher value was calculated assuming products contained 0.1% Polyaminopropyl Biguanide (PHMB).

It should be made clear that the following sentence was a conclusion of the SCCS: "It was also determined that skin sensitization in humans can be elicited at concentrations beginning at 0.2% active ingredient." It should also be stated that it was an aqueous solution that was tested.

Discussion - It needs to be made clear that the maximum use concentration reported is now 0.5%.

The Discussion should mention that the photosensitization study was completed at a dose of 1 mg/cm².

Table 1 - Is this table needed for a single ingredient report?

Table 3 - The use table needs to be updated as there is a 0.2% eye lotion product and a 0.5% suntan (not spray) that are not yet included in this table.

Table 4 - The last 2 sentences in the results column for the 0.3% 72 hour post exposure study needs to be moved to the 0.1% study as it concerns the results of the 0.1% study not the 0.3% study.

Table 5, first study - It is not clear what the values (0.22 and 0.28%) in the carcasses represent.

Table 5, male rat, reference 19 - To be consistent with the other studies in this table, the Ingredient column should indicate that a radiolabeled compound was studied.

- Table 7, third study - The dose (2 g/kg) for this study from reference 19 needs to be added to this table.
- Table 10, last study - It does not make sense that they changed the drinking water concentration to 0.3 mg/ml during the 2nd week and it was also at 0.3 mg/ml from the 3rd week until study termination. Because of palatability issues, it is likely that they used a concentration between 0.1 and 0.3 mg/ml, e.g., 0.2 mg/ml, during week 2.
- Table 11, first study (dermal) - It should be stated that the SCCS indicated that the high dose exceeded the maximum tolerated dose and that the NOAEL was 0.6 mg/day (15 mg/kg/day).
- Table 11, 1 year dog study - Please review the doses, as it currently states: “at dietary concentrations of 0 ppm, 300 ppm, 1500 ppm and 4500 ppm (corresponding to 0 ppm, ~11 ppm, ~54 ppm, and ~169 or ~108 mg/kg/day)”. This does not make sense. Should all of the units of the “corresponding” values be mg/kg/day?
- Table 11, 26-week dog study - This study should not be included in the Chronic table.
- Table 12 - There was only one Alderly Park mouse developmental toxicity study completed. It is not clear why two studies with the same protocol are included in Table 12.
- Table 13, last study - The strain of rats used does not belong in the Dose/Concentration column as it is already stated in the Strain/cell type column.
- Table 14, dermal - There was only one 80-week study in Alderly Park mice. Please look at the references in CIR report reference 19 and CIR report reference 5. Reference 19 cites the 80-week study to Report No. CTL/P/331. Reference 5 cites this study to Central Toxicology Laboratory....Report No: CTL/P/331 - the same report as cited in reference 19. Therefore, it is the same study cited in two secondary references.
- Table 14, oral - Please check the two studies in 60 male and 60 female rats (terminated at 124 weeks) cited to references 5 and 19, as these are likely the same study cited in two sources.
- Table 15 - It is not necessary to present the 80-week study in mice in this table.
- Table 15, Sensitization - The LLNA is not an *in vitro* assay and should not be presented under an *in vitro* subheading.
- Table 15, Sensitization, last guinea pig study - It would be helpful to provide more details of the results of this study (see table 3 of reference 2). For example no sensitization was observed in guinea pigs at induction concentrations of 1.8% and lower.
- Table 15, Phototoxicity/Photosensitization, human - The dose of 1 mg/cm² from the new Cosmetics Europe summary of sensitization data needs to be added to the 26 subject study.
- Table 16 - It needs to be made clear that the last study is an *in vitro* study.
- Reference 19 - Please correct “recreational”